

THE LABORATORY RABBIT, GUINEA PIG,
HAMSTER, AND OTHER RODENTS

American College of Laboratory Animal Medicine Series

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AMSTERDAM • BOSTON • HEIDELBERG • LONDON
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32 Jamestown Road, London NW1 7BY, UK
225 Wyman Street, Waltham, MA 02451, USA
525 B Street, Suite 1800, San Diego, CA 92101-4495, USA

First edition 2012

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British Library Cataloguing-in-Publication Data

A catalogue record for this book is available from the British Library

Library of Congress Cataloging-in-Publication Data

A catalog record for this book is available from the Library of Congress

ISBN : 978-0-12-380920-9

For information on all Academic Press publications
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Typeset by MPS Limited, a Macmillan Company, Chennai, India
www.macmillansolutions.com

Printed and bound in United States of America

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Preface

Though the majority of animals used in biomedical research at present are mice and rats, a number of other species continue to serve as models in the effort to advance human and animal health. In this regard, *The Laboratory Rabbit, Guinea Pig, Hamster, and Other Rodents* is meant to be an authoritative summary of the basic biology, husbandry, veterinary perspective, and experimental use of these species.

While this is the first edition of *The Laboratory Rabbit, Guinea Pig, Hamster, and Other Rodents*, it should be recognized that this volume builds upon the work of previous volumes in the American College of Laboratory Animal Medicine Series. In particular, we wish to recognize the following works as providing the foundation for the present book:

Biology of the Laboratory Rabbit, 1st Edition (Edited by S. H. Weisbroth, R. E. Flatt, and A. L. Kraus)

Biology of the Laboratory Rabbit, 2nd Edition (Edited by P. J. Manning, D. H. Ringler, and C. E. Newcomer)

The Biology of the Guinea Pig (Edited by J. E. Wagner and P. J. Manning)

Laboratory Hamsters (Edited by G. L. Van Hoosier, Jr., and C. W. McPherson)

The editors and many authors of these books should feel gratified that their efforts have helped guide and inform veterinarians, scientists, students, and technicians with respect to the proper use of these species in work which has benefited many. It is our hope that

we have carried these earlier works forward in a way which will proclaim a similar contribution.

The Laboratory Rabbit, Guinea Pig, Hamster, and Other Rodents combines the content of several books into a single volume along with material on additional species such as the chinchilla, gerbil, and miscellaneous rodents other than laboratory rats and mice. It is meant to be relatively concise, yet sufficiently complete that readers will find ready access to information likely to be of practical use.

As part of the series sponsored by the American College of Laboratory Animal Medicine (ACLAM), *The Laboratory Rabbit, Guinea Pig, Hamster, and Other Rodents* is a component of the ACLAM mission to support the scientific community, in general, and expertise in laboratory animal medicine, in particular. All royalty income from this publication is assigned to support other continuing education activities of the College.

A work such as this represents the collective thoughts and efforts of many contributors and reviewers, and we wish to express our sincere gratitude to all. It is often a challenge to coordinate chapters and timelines with a multi-author work such as this, and we wish to further acknowledge the patience and support of the editorial staff at Elsevier. Though some variation in depth and style between authors in a book such as this is inevitable, it is our hope that readers will view this volume as a valuable reference to be consulted on a frequent basis.

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Ethical Considerations and Regulatory Issues

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“... by now it is widely recognized that the [most humane] possible treatments of experimental animals, far from being an obstacle, is actually a prerequisite for successful animal experiments.” **Russell and Burch, 1959**
The Principles of Humane Experimental Technique

INTRODUCTION

Like many aspects of life, involvement with animal research presents ethical challenges – areas where competing interests require use of an ethical decision-making

process, to provide guidance. Individuals in the laboratory may face basic competing interests between scientists, technicians, veterinary colleagues, an employing institution, the public, and concern for the animals themselves (e.g., individual health versus health of the colony). According to Tannenbaum, *normative veterinary ethics* refers to the search for correct principles of good and bad, right and wrong, and looks for the correct norms for veterinary professional behavior and attitudes (Tannenbaum, 1995). The intent of this chapter is to provide information which will be useful to all individuals involved in animal-based research, testing and teaching: scientists; technicians; laboratory animal veterinarians; and Institutional Animal Care and Use Committee (IACUC) members. This process might be considered an effort to define *normative laboratory animal use ethics*. The search for an appropriate ethical solution rarely leads to a complete and absolute answer, as science is a very dynamic field and new issues and new insights influence the outcome. This look at *normative laboratory animal use ethics* will examine some general ethical concepts within the context of Tannenbaum's definition of *descriptive [laboratory animal use] ethics*, where descriptive ethics is the study of the actual values or standards of a profession; that is, what members of a profession consider to be right and wrong regarding professional behavior and attitudes (Tannenbaum, 1995). In this chapter, reference will be made to relevant values and standards found in various principles and guidelines developed for use by individuals involved in animal research. Potential ethical challenges which might confront laboratory animal professionals using rodents and rabbits (e.g., challenges related to breeding colonies, genetically modified rodents, use of animals in cancer research, perinatal animal use, and use of animals in neuroscience and behavioral research) will be used as examples. Related ethical questions, and the appropriate principles which may pertain to the situation are discussed. The reader is challenged to test the general rules and principles against his or her own moral experience and intuition and thus create his or her own descriptive laboratory animal ethos. Further, the reader is encouraged to recognize that this is an ongoing process, that one's professional ethos will likely grow and mature as new challenges are encountered.

It is recognized that animal welfare is a "core concern of veterinary ethics ... [and] this subject has gained increased importance as society and the profession endorse ever more strongly the moral imperative to treat animals decently." When discussing laboratory animal ethics, one often uses the term "humane" (Tannenbaum, 1995). The *Guide for the Care and Use of Laboratory Animals (Guide)* states "Humane care means those actions taken to assure that laboratory animals are treated according to high ethical and scientific

standards" (National Research Council, 2011). The *Guide* uses the words 'ethics' or 'ethical' 59 times in either the text or references. The first place 'ethics' is mentioned in the *Guide* is the Preface: "The *Guide* is also intended to assist investigators in fulfilling their obligation to plan and conduct animal experiments in accord with the highest scientific, humane, and ethical principles" (National Research Council, 2011). Humane care, ethics, and animal welfare are closely linked and, for the purposes of this chapter, may be used interchangeably.

ETHICAL CONCEPTS

Moral Theories

Moral theory is an expansive field of study with its own vocabulary and competing points of view. It is beyond the scope of this chapter to provide a comprehensive discussion of the many theories that discuss the humane use of animals. Instead, focus is made on some principles which the authors have found helpful.

Consideration of ethics with respect to use of animals in research begins with the basic idea of where one feels animals (versus people) fit in the moral spectrum. In other words, what is the moral value of an animal relative to a human? Moral status or standing represents "the position or rank of an entity along a moral continuum from minimal to maximal moral significance" (Kraus and Renquist, 2000). Because animals lack moral agency, defined as a uniquely human capacity for making moral judgments (Kraus and Renquist, 2000), it may be concluded that animals, while having some moral status, fall below that accorded to humans. Even when examining the moral status of animals, there are many who accord different levels of moral status for different species. Such a continuum of moral status is essentially a sliding scale where moral status is based on a combination of cognitive and sensory capacities. This concept has been coined "speciesism" by some philosophers who then compare it to other concepts which foster differential treatment based upon a given trait, similar to racism or sexism (Singer, 1975). Regardless of where a species is placed on this Darwinian scale, a commonality shared by all vertebrates is that of sentience. Sentience is the capacity to perceive and process sensory input and thus the ability to feel pain and distress. Moral agents (humans) have obligations or are bound to do certain things out of a sense of duty, custom, or law and have responsibility toward other beings. It is from this obligation that most humans feel it is right to minimize the pain and distress felt by other sentient beings. This obligation may be called non-maleficence and is addressed in greater detail later in this chapter.

In the most general sense, there are two approaches to ethics: utilitarianism and deontology. Utilitarian theories look at the consequences of actions to determine which actions are good and which are bad. The goal is to maximize good consequences and minimize bad ones. In its most basic form, this is similar to what an IACUC protocol review does when it performs a cost or harm/benefit analysis. However, there are different views of the “good” that should be maximized. Utilitarianism is an example of *action-oriented ethical theories*, because it examines the consequences of actions. These theories tend to stress the concepts of duty and obligation. In contrast, deontological theories are also action-oriented ethical theories; however, there are some moral imperatives which are independent of how much “good” results from an action. Some deontologists advocate a set of “obligatory” moral principles but allow some compromise when different moral principles are conflicting (e.g., one principle trumps another). There may also be non-obligatory principles which are desirable but not mandatory to follow.

Other ethical approaches include those based on values and ethics. *Value-based ethics* center around basic values to be sought. These values tend to be hierarchical. There are also *virtue-oriented ethical theories*. A virtue contributes to a good moral life (e.g., honesty, kindness, generosity). These approaches tend to instill “attitudes, feelings, and states of mind central to the virtuous disposition” (Tannenbaum, 1995). It has been suggested that a satisfactory approach to normative ethics must include actions, values, and virtues (Tannenbaum, 1995).

Descriptive Laboratory Animal Use Ethics

Ethical Principles

Descriptive laboratory animal ethics represents an approach for determining appropriate moral behavior and attitude (Tannenbaum, 1995). Principles can be defined as “accepted generalizations about a topic that are frequently endorsed by many and diverse organizations” (National Research Council, 2011); and several sets of principles with relevance to ethical use of laboratory animals will be discussed. It is hoped that application of these principles in the discussion of example ethical challenges later in this chapter will serve as a basis for how other ethical challenges may be approached.

One set of principles, the concept of the “Five Freedoms,” was originally created by the United Kingdom Farm Animal Welfare Advisory Council (FAWC) in 1979 specifically to address issues related to the use of animals in agriculture. Today, the “Five Freedoms” are also often mentioned within the context of animals used in research. The “Five Freedoms” include: (1) freedom from malnutrition; (2) freedom

from thermal or physical discomfort; (3) freedom from injury and disease; (4) freedom to express normal social behavior; and (5) freedom from fear. The Five Freedoms were revised in 1993 to include: (1) freedom from hunger and thirst, by assuring ready access to fresh water and a diet sufficient to maintain full health and vigor; (2) freedom from discomfort, by providing an environment including shelter and a comfortable resting area; (3) freedom from pain, injury and disease, by preventive means or rapid diagnosis and treatment; (4) freedom from fear and distress, by ensuring conditions that avoid mental suffering; and (5) freedom to express normal behavior, by providing sufficient space, proper facilities, and company of the animal’s own kind (Webster, 2001).

In 1996, the United States National Aeronautics and Space Administration (NASA) developed basic principles, referred to as the Sundowner Principles (NASA, 1996). These principles were based upon the Belmont Report which had been written for the protection of human research subjects (National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research, 1979). These principles of bioethics offer a simple, yet elegant framework for looking at ethical questions:

“The use of animals in research involves responsibility – not only for the stewardship of the animals but to the scientific community and society as well. Stewardship is a universal responsibility that goes beyond the immediate research needs to include acquisition, care and disposition of the animals, while responsibility to the scientific community and society requires an appropriate understanding of, and sensitivity to scientific needs and community attitudes toward the use of animals.

Among the basic principles generally accepted in our culture, three are particularly relevant to the ethics of research using animals: respect for life, societal benefit, and non-maleficence” (NASA, 1996).

Respect for Life

Living creatures deserve respect. This principle requires that animals used in research should be of an appropriate species and health status, and should involve the minimum number required to obtain valid scientific results. It also recognizes that the use of different species may raise different ethical concerns. Selection of appropriate species should consider cognitive capacity and other morally relevant factors. Additionally, methods such as mathematical models, computer simulation, and in vitro systems should be considered and used whenever possible.

Societal Benefit

The advancement of biological knowledge and improvements in the protection of the health and well-being of both humans and other animals provide strong justification for biomedical and behavioral research.

This principle entails that when animals are used, the assessment of the overall ethical value of such use should include consideration of the full range of potential societal goods, the populations affected, and the burdens that are expected to be borne by the subjects of the research.

Non-Maleficence

Based upon the idea that vertebrate animals are sentient, this principle holds that the minimization of distress, pain, and suffering is a moral imperative. Unless the contrary is established, investigators should consider that procedures that cause pain or distress in humans may cause pain or distress in other sentient animals (*Interagency Research Animal Committee, 1985*).

The International Guiding Principles for Biomedical Research Involving Animals (*Table 1.1*) were developed by the Council for International Organizations of Medical Sciences (CIOMS) as a result of extensive international and interdisciplinary consultations spanning 1982–1984 (Bankowski, 1985; *Council for International Organizations of Medical Sciences, 1985*). These principles have a considerable measure of acceptance internationally. European Medical Research Councils (EMRC),

an international association that includes all the West European medical research councils, fully endorsed the CIOMS Guiding Principles in 1984. In the same year, the CIOMS Guiding Principles were endorsed by the World Health Organization (WHO) Advisory Committee on Medical Research. It should be noted that, at the time of writing this chapter, the CIOMS were undergoing revision.

During 1984–1985, the U.S. National Institutes of Health (NIH) convened the U.S. Interagency Research Animal Committee (IRAC) which created a similar set of principles, the U.S. Government Principles for the Utilization and Care of Vertebrate Animals used in Testing, Research and Training (*Table 1.2*) for research funded by the U.S. Public Health Service (PHS) (*Interagency Research Animal Committee, 1985*). These principles were, to a considerable extent, based on the CIOMS Guiding Principles.

As an indication of the wide acceptance of both the IRAC and CIOMS Principles, discussion of the IRAC Principles can be found under the heading “Ethics and Animal Use” in the *Guide* (*National Research Council, 2011*). NASA and CIOMS are also listed in Appendix A of the *Guide* under the heading “Ethics and Welfare”

TABLE 1.1 Council for International Organizations of Medical Sciences (CIOMS) *Basic Principles* (1985)

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- I. The advancement of biological knowledge and the development of improved means for the protection of the health and well-being both of man and of animals require recourse to experimentation on intact live animals of a wide variety of species
 - II. Methods such as mathematical models, computer simulation and in vitro biological systems should be used wherever appropriate
 - III. Animal experiments should be undertaken only after due consideration of their relevance for human or animal health and the advancement of biological knowledge
 - IV. The animals selected for an experiment should be of an appropriate species and quality, and the minimum number required to obtain scientifically valid results
 - V. Investigators and other personnel should never fail to treat animals as sentient, and should regard their proper care and use and the avoidance or minimization of discomfort, distress, or pain as ethical imperatives
 - VI. Investigators should assume that procedures that would cause pain in human beings cause pain in other vertebrate species, although more needs to be known about the perception of pain in animals
 - VII. Procedures with animals that may cause more than momentary or minimal pain or distress should be performed with appropriate sedation, analgesia, or anesthesia in accordance with accepted veterinary practice. Surgical or other painful procedures should not be performed on unanesthetized animals paralyzed by chemical agents
 - VIII. Where waivers are required in relation to the provisions of article VII, the decisions should not rest solely with the investigators directly concerned but should be made, with due regard to the provisions of articles IV, V, and VI, by a suitably constituted review body. Such waivers should not be made solely for the purposes of teaching or demonstration
 - IX. At the end of, or, when appropriate, during an experiment, animals that would otherwise suffer severe or chronic pain, distress, discomfort, or disablement that cannot be relieved should be painlessly killed
 - X. The best possible living conditions should be maintained for animals kept for biomedical purposes. Normally the care of animals should be under the supervision of veterinarians having experience in laboratory animal science. In any case, veterinary care should be available as required
 - XI. It is the responsibility of the director of an institute or department using animals to ensure that investigators and personnel have appropriate qualifications or experience for conducting procedures on animals. Adequate opportunities shall be provided for in-service training, including the proper and humane concern for the animals under their care
-

(National Research Council, 2011). The complete IRAC Principles are found on the cover of the PHS Policy (Office of Laboratory Animal Welfare, 2002) and Appendix B of the *Guide* (National Research Council, 2011).

There are really no points of conflict and, in fact, there are many points of consensus between the Sundowner, CIOMS, and IRAC principles all of which offer that animal-based research should:

- Acknowledge the importance of research with relevance to human or animal health, advancement of knowledge, or the good of society;
- Stress consideration of alternatives to reduce or replace the use of animals;
- Require avoiding or minimizing discomfort, distress, and pain.

Some items which are addressed in greater detail in the CIOMS and IRAC principles but are more generally under the concept of non-maleficence in the Sundowner Principles include:

- Use of appropriate sedation, analgesia, and anesthesia;
- Establishment of humane endpoints;

- Provision of adequate veterinary care;
- Assurance of appropriate training and qualifications of personnel using and caring for animals.

As previously mentioned, descriptive ethics is the study of actual values or standards of a profession. With respect to ethics governing laboratory animal use, the Sundowner, CIOMS, and IRAC principles could be considered major components.

Commonly accepted ethical principles result in development of professional guidelines. For example, in 1831 in the United Kingdom (U.K.), Marshall Hall, a leading British physiologist, developed guidelines for animal experimentation (Zurlo et al., 1993). The British Association for the Advancement of Science further refined these principles in 1871, 5 years before the first legislation in the U.K. In 1909, Walter B. Cannon developed guidelines for animal experimentation for the American Physiological Association. Many other scientific organizations have also created similar guidelines. Scientists are urged to seek those within their own professional societies. (e.g., Federation of American Societies for Experimental Biology at <http://www.faseb.org>).

TABLE 1.2 Interagency Research Animal Committee (IRAC) U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training (Interagency Research Animal Committee, 1985)

The development of knowledge necessary for the improvement of the health and well-being of humans as well as other animals requires in vivo experimentation with a wide variety of animal species. Whenever U.S. Government agencies develop requirements for testing, research, or training procedures involving the use of vertebrate animals, the following principles shall be considered; and whenever these agencies actually perform or sponsor such procedures, the responsible Institutional Official shall ensure that these principles are adhered to:

- I. The transportation, care, and use of animals should be in accordance with the Animal Welfare Act (7 U.S.C. 2131 et. seq.) and other applicable Federal laws, guidelines, and policies.
- II. Procedures involving animals should be designed and performed with due consideration of their relevance to human or animal health, the advancement of knowledge, or the good of society.
- III. The animals selected for a procedure should be of an appropriate species and quality and the minimum number required to obtain valid results. Methods such as mathematical models, computer simulation, and in vitro biological systems should be considered.
- IV. Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative. Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals.
- V. Procedures with animals that may cause more than momentary or slight pain or distress should be performed with appropriate sedation, analgesia, or anesthesia. Surgical or other painful procedures should not be performed on unanesthetized animals paralyzed by chemical agents.
- VI. Animals that would otherwise suffer severe or chronic pain or distress that cannot be relieved should be painlessly killed at the end of the procedure or, if appropriate, during the procedure.
- VII. The living conditions of animals should be appropriate for their species and contribute to their health and comfort. Normally, the housing, feeding, and care of all animals used for biomedical purposes must be directed by a veterinarian or other scientist trained and experienced in the proper care, handling, and use of the species being maintained or studied. In any case, veterinary care shall be provided as indicated.
- VIII. Investigators and other personnel shall be appropriately qualified and experienced for conducting procedures on living animals. Adequate arrangements shall be made for their in-service training, including the proper and humane care and use of laboratory animals.
- IX. Where exceptions are required in relation to the provisions of these Principles, the decisions should not rest with the investigators directly concerned but should be made, with due regard to Principle II, by an appropriate review group such as an institutional animal care and use committee. Such exceptions should not be made solely for the purposes of teaching or demonstration (Interagency Research Animal Committee, 1985).

org/Policy-and-Government-Affairs/Science-Policy-Issues/Animals-in-Research-and-Education/Statement-of-Principles.aspx; American Physiological Society at http://www.the-aps.org/pa/resources/policyStmnts/paPolicyStmnts_Guide.htm).

Examples of professional guidelines for laboratory animal veterinarians include the Principles of Veterinary Medical Ethics and Veterinary Oath of the American Veterinary Medical Association (at <http://www.avma.org/issues/policy/ethics.asp>, http://www.avma.org/about_avma/whoweare/oath.asp). Further, many laboratory animal science professionals refer to the Position Statements of the American Association for Laboratory Animal Science (AALAS; http://www.aalas.org/association/position_statements.aspx).

Central to these codes of conduct, principles, statements of ethics, and position statements is the commitment to the humane care and use of research animals.

The Three Rs (Replacement, Reduction, and Refinement)

Several sets of ethical principles and guidelines covering the use of animals in research, testing, and teaching have been mentioned but perhaps the simplest and the one with the greatest impact on animal research today is the ethical concept called “The 3Rs”, a call to apply whenever possible the alternatives of replacement of animals, reduction in the number of animals used, and the refinement in procedures used on animals in research (Russell and Burch, 1959).

The *Guide* was originally published in 1963 and has undergone numerous revisions, with the most recent edition being published in 2011. The Statement of Task of the latest revision committee begins with “The use of laboratory animals for biomedical research, testing and education is guided by the principles of the Three Rs... .” (National Research Council, 2011). The 3Rs are a common theme in the *Guide* which states “Throughout the *Guide*, scientists and institutions are encouraged to give careful and deliberate thought to the decision to use animals taking into consideration the contribution that such use will make to new knowledge, ethical concerns, and the availability of alternatives to animal use. A practical strategy for decision making, [is] the “Three Rs” (Replacement, Reduction, and Refinement) approach, ...”(National Research Council, 2011).

The concept of the 3Rs is also infused in U.S. regulations covering research using animals. Although the United States Department of Agriculture (USDA) Animal Welfare Act (AWA) regulations do not include the word “alternatives” in its section of definitions, the term is used several times in the regulations themselves. For example, in the section on IACUC review of protocols the regulations state protocols must indicate that

“(i) Procedures involving animals will avoid or minimize discomfort, distress, and pain to the animals; (ii) The principle investigator has considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animals, and has provided a written narrative description of the methods and sources, e.g., The Animal Welfare Information Center, used to determine that alternatives were not available” (Office of the Federal Register, 2002). The focus of USDA inspectors on adherence to this section of the regulations can be appreciated when looking at the USDA Research Facility Inspection Guide which instructs inspectors several times to evaluate institutional compliance in this area (USDA, 2009). In addition, the requirement for a search for alternatives is the subject of a specific Animal Care Policy – Policy 12 (Animal and Plant Health Inspection Service, 2000). Strategies to enhance electronic search efficiency using a search filter for PubMed have been published (Hooijmans et al., 2010b). A “Gold Standard Publication Checklist” has been proposed to help fully integrate the 3Rs into systematic reviews of the literature (Hooijmans et al., 2010a).

In a section on personnel qualifications, the AWA Regulations state that the institution should assure adequate training and qualifications and that this is fulfilled in part through the provision of training and instruction on the “concept, availability, and use of research and testing methods that limit the use of animals [Reduction] or minimize animal distress [Refinement]” (Office of the Federal Register, 2002). The AWA Regulations further indicate that research staff should be trained on the “utilization of services (e.g., National Agriculture Library of Medicine) available to find information: ... (ii) On alternatives to the use of live animals in research [Replacement]; ...” (Office of the Federal Register, 2002).

In addition to the specific references above, the AWA Regulations also refer to the use of anesthetics, analgesics, and sedatives, the availability of appropriate veterinary care, the use of appropriate housing; and timely, appropriate euthanasia, all of which demonstrate the practice of “refinement”.

The United States Public Health Service (PHS) Policy contains similar language regarding minimizing discomfort, distress pain, use of appropriate anesthesia, and the use of humane endpoints as examples of refinement. In addition, the PHS Policy refers to the IRAC Principles, requiring institutions receiving PHS funds to use the *Guide* as a basis for their animal care and use programs.

National and international agencies and organizations such as the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) (<http://iccvam.niehs.nih.gov/>), the European Centre for the Validation of Alternative Methods (ECVAM) (<http://ecvam.jrc.ec.europa.eu/>), and the National

Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) (<http://www.nc3rs.org.uk/>) are charged with helping to find and promote the use of alternatives.

With so much official emphasis on the 3Rs, there is sometimes a perception that the concept is not being adequately implemented in practice. Indeed, it has been suggested that scientists and IACUCs do not fully understand the concepts of the 3Rs (Graham, 2002; Schuppli and Fraser, 2005). In addition to incomplete understanding of the concepts, factors believed to negatively influence the full implementation of the 3Rs by IACUCs might include: (1) a belief that the scientists themselves would implement the 3Rs; (2) an assumption that funding agencies have reviewed the use of the 3Rs during proposal review; (3) confidence that sample size, rather than study design, is the sole criterion for reduction; and (4) focus upon potential harm from procedures without consideration for potential distress that animals might experience from husbandry and housing. Although these conclusions were based upon a relatively small number of IACUCs, these are troubling observations and indicate the need for greater emphasis on the 3Rs in training programs for scientists and IACUCs. The authors hope that this chapter can be a resource for that process.

It would be disingenuous to imply that, although well accepted, the concept of the 3Rs is universally accepted. In an article titled "Time to Abandon the Three Rs", Derbyshire wrote that the 3Rs "draw attention away from the value of experimentation and toward the importance of animal welfare" (Derbyshire, 2006). Although the article supports the concept of reducing animal stress for the sake of science, the authors do not clearly recognize the opportunity to balance facilitation of science and application of the 3Rs.

REPLACEMENT

Replacement refers to methods that avoid using animals. The term includes absolute replacements (i.e., replacing animals with inanimate systems such as computer programs) as well as relative replacements (i.e., replacing animals, such as vertebrates, with animals that are lower on the phylogenetic scale) (National Research Council, 2011). Relative replacement may be controversial to some people as it implies "speciesism", the idea that one species has greater moral standing than another (Singer, 1975).

Like many of the ethical considerations relating to animal use, relative replacement is essentially a continuum of moral standing. Society, in general, often differentiates between humans and non-human animals; however, with respect to the animal world, different opinions exist regarding our obligations to some species versus others. For example, non-human primates and

animals commonly kept as pets such as horses, dogs, and cats are often regarded differently than rats and mice, which in turn are regarded differently from fruit flies and worms, and so on.

Development of fully validated and accepted replacement alternatives can be a frustratingly slow process. However, there are significant examples of successful replacement of live animals. For example, one of the most criticized uses of animals for toxicity testing is the Draize test in rabbits. This test was developed to determine ocular toxicity and irritancy caused by products and chemicals. Ocular toxicity tests represent one of the four most commonly conducted product safety tests (Interagency Coordinating Committee on the Validation of Alternative Methods, 2010). The 3Rs were implemented by the development of three validated and accepted replacements for screening products for ocular toxicity: the bovine corneal opacity and permeability test using a cow eye or the isolated chicken eye test (both by-products of the meat industry); and the Cytosensor[®] microphysiometer (Molecular Devices, Inc., Sunnyvale, CA). A balanced preemptive pain management plan for rabbit Draize test studies, when the test is still required, has also been validated and accepted as a refinement (Interagency Coordinating Committee on the Validation of Alternative Methods, 2010).

A second example of implementation of the 3Rs involves the replacement of rabbits in the testing of pharmaceuticals and medical devices for pyrogens by use of an *in vitro* alternative, the Limulus Ameobocyte Lysate (LAL) Test. In the LAL, blood of horseshoe crabs is collected and the animals are returned, unharmed, back to the ocean. Previous tests required the injection of drugs, biologics, medical devices, or raw materials into rabbits to look for a febrile response as an indication of contamination with endotoxins.

REDUCTION

Reduction includes strategies for obtaining comparable levels of information from the use of fewer animals or for maximizing the information obtained from any given number of animals (without increasing pain or distress) so to ultimately require fewer animals to acquire the same scientific information. This approach relies on an analysis of experimental design, applications of newer technologies, the use of appropriate statistical methods, and control of environmentally related variability in animal housing and study areas" (National Research Council, 2011).

Strategies to reduce the numbers of animals needed include improved statistical design of a study (Dell et al., 2002) and improved selection of an animal model, including selection of animals with the most appropriate health and genetic status. Control of the genetic status is an advantage of using rats and mice. The use

of inbred strains of rats and mice allows scientists to control and investigate genetic variation, and to evaluate responses to treatments on specific areas of interest (Festing, 2004). The use of animals without confounding disease or genetic variation results in less variation, thus requiring fewer animals to determine a treatment effect.

Individuals involved with study design, study review, or those participating as a member of the research team, have the ethical imperative to ensure studies use the minimum number of animals necessary to achieve the scientific objective of the study. Scientists should design studies with particular attention to methodology, statistics, and choice of model. Veterinarians and facility staff should collaborate to minimize non-experimental variables in animal care. IACUCs should be diligent during review of the protocol, semiannual program and facility evaluations, and review of post-approval monitoring to assure that the appropriate number of animals have been used. Having a statistician on the IACUC is one strategy that may be helpful.

REFINEMENT

“Refinement refers to modifications of husbandry or experimental procedures to enhance animal well-being and minimize or eliminate pain and distress” (National Research Council, 2011). In the authors’ opinion, refinement is commonly employed by scientists in ongoing efforts to improve their science; that is, better animal welfare leads to higher-quality science. Many scientists do not recognize this as utilization of “alternatives”, even though it clearly falls within the 3Rs. However, this is also an area where scientists, veterinarians, and IACUCs can make significant strides to enhance animal welfare. Use of less invasive procedures (e. g., use of a blood pressure cuff instead of an implanted catheter for blood pressure monitoring) is one method of refinement. However, there are also situations where an invasive procedure, such as implantation of telemetry sensors to allow ongoing collection of real-time data, can result in much less stressful data collection (Stephens et al., 2002). Examples of other refinements include accurate recognition of pain and the use of analgesics and supportive care; implementation of humane endpoints; and enhanced housing and husbandry.

Carbone and Garnett (2008), state, “... the prime ethical concerns in laboratory animal welfare is what animals consciously experience: their pain, distress, fear, boredom, happiness and psychological well being.” The emotional dimension of pain, a characteristic of suffering, requires pain pathways to extend to higher levels of the cortex unique to humans and some other primates (such as apes) (Nuffield Council on Bioethics, 2005), but it has been stated that “... the absence of analogous structures cannot necessarily be taken to

mean that they [animals] are incapable of experiencing pain, suffering or distress or any other higher order states of conscious experience” (Nuffield Council on Bioethics, 2005).

Basic to minimization of pain is the ability to recognize the signs of pain in specific species. It has been suggested that some animals, particularly prey species, may try to mask pain to avoid displaying abnormal activity that might increase their risk of predation (Roughan and Flecknell, 2000). Further, many animals are most active during the dark cycle, when observations are more difficult. Since clinical indices of pain may be very subtle, it is important to be able to recognize a departure from normal behavior and appearance (Table 1.3; National Research Council, 2003). A short list of general signs and measurements that might indicate pain or distress includes: (1) vigorous attempt to escape; (2) changes in biological characteristics such as food and water consumption and body weight; (3) changes in blood levels of hormones and glucose; (4) increased adrenal gland mass; and (5) appearance, posture, and behavior (Moberg, 1985, 2000). Behavioral indicators of pain in mice and rats have also been described (Flecknell, 1999; Kohn et al., 2007; Roughan and Flecknell, 2000, 2001, 2003). In addition, guidelines for the assessment and management of pain in rodents and rabbits have been published by the American College of Laboratory Animal Medicine (ACLAM, 2006).

Scientists sometimes have concerns about the effect of perioperative analgesics on the research. Many studies have been done investigating analgesic effect on a wide variety of parameters (e.g., litter size, body weight,

TABLE 1.3 Indicators of Pain in Rodents and Rabbits

Species	General Behavior	Appearance	Other
Rodents	Decreased activity; excessive licking and scratching; self-mutilation; may be unusually aggressive; abnormal locomotion (stumbling, falling); writhing; does not make nest; hiding	Piloerection; rough/stained haircoat; abnormal stance or arched back; porphyrin staining (rats)	Rapid, shallow respiration; decreased food/water consumption; tremors
Rabbit	Head pressing; teeth grinding; may become more aggressive; increased vocalizations; excessive licking and scratching; reluctant to locomote	Excessive salivation; hunched posture	Rapid, shallow respiration; decreased food/water consumption

No single observation is sufficiently reliable to indicate pain; rather several signs, taken in the context of the animal's situation should be evaluated. The signs of pain may vary with the type of procedure (e.g., orthopedic versus abdominal pain) (National Research Council, 2003)

behavior, and hemodynamic parameters) (Bourque et al., 2010; Goulding et al., 2010; Lamon et al., 2008; McBrier et al., 2009; Valentim et al., 2008). These studies have demonstrated varying effects on parameters of interest, including no effect. Therefore, rather than assuming that analgesics cannot be given, it is recommended that a literature search be conducted to determine if studies have been done to validate the existence of an effect of analgesia on a given experimental parameter. If data do not exist, consideration should be given for conducting and publishing an appropriate study to indicate which analgesics are, or are not, a viable scientific option for future experiments. Investigators should be required to provide scientific justification for withholding analgesia when potentially painful procedures are to be conducted.

Defining distress in animals has proven to be a difficult task. The ILAR Committee on the Recognition and Alleviation of Distress in Laboratory Animals stated “Although most definitions of distress characterize it as an aversive, negative state in which coping and adaptation processes in response to stressors fail to return an organism to physiological and/or psychological homeostasis, philosophical differences center on the inclusion of emotions and feelings affected by this state of being.” (Committee on Recognition and Alleviation of Distress in Laboratory Animals, 2008).

Stress has been defined as “the biological response an animal exhibits in an attempt to cope with threats to its homeostasis” (Stokes, 2000). This response can involve immunologic, metabolic, autonomic, neuroendocrine, and behavioral changes (Moberg, 2000). The type, pattern, and level of the response depend upon the strength, severity, intensity, and duration of the stressor(s). The aversive state of distress results when an animal is unable to adapt. By limiting the frequency, strength, severity, intensity, and/or duration of the stressor(s), it may be possible to limit the level of distress in the animal. One method is to reduce the cumulative stress an animal experiences (allowing recovery or adaptation to a given stressful situation before adding additional stressors) or refining practices and procedures to make the individual stressors less severe or shorter.

The use of humane endpoints contributes to refinement by providing an alternative to experimental endpoints that result in more severe animal pain and distress. Scientific or experimental endpoints are defined as occurring when the objectives of the study have been reached. Humane endpoints occur at the point at which pain or distress is prevented, terminated, or relieved in an experimental animal. In most studies, the scientific and humane endpoints occur at the same time, in other words, the scientific endpoint occurs prior to the development of pain or distress. Studies that may result in severe or chronic pain or significant alterations in the animal’s ability to maintain normal physiology, or

adequately respond to stressors, should contain descriptions of appropriate humane endpoints or provide science-based justification as to why a particular, accepted humane endpoint cannot be employed. Veterinary consultation must occur when pain or distress is beyond the level anticipated in the protocol description or when interventional control is not possible (National Research Council, 2011). Most of the ethical principles guiding humane animal research mention the use of humane endpoints (Bankowski, 1985; Council for International Organizations of Medical Sciences, 1985; Interagency Research Animal Committee, 1985). Stokes (2000, 2002) provides an overview and reviews specific situations where endpoints are appropriately utilized.

The need, criteria, and timing for humane endpoints should be part of pre-study planning and are often best done as a research team including scientists, technicians, and veterinarians (Canadian Council for Animal Care, 1998; NRC, 2003; Organisation for Economic Co-operation and Development, 2000). Endpoints are an important element of IACUC protocol review. Therefore, it is essential that a protocol contain all appropriate information regarding the criteria for humane endpoints, observation schedules, and training of personnel to adequately observe for the agreed-upon criteria. Pilot studies may be useful for gathering this information if it is not known at the time of protocol submission. In addition, the criteria and other details may need to be modified when unexpected adverse events occur. The IACUC should be notified when this happens and the protocol amended as needed.

Development of criteria for humane endpoints may be general and applied to any study. Institutions often adopt standards or policies that cover situations when study-specific endpoints have not been determined. These documents often appear as Standard Operating Procedures (SOPs) or guidelines, and may be developed and instituted by the IACUC, the veterinary staff, the institutional administration, or any collaboration of the above. The policies should encompass generic clinical or behavioral conditions that potentially are associated with pain and distress, and should be widely recognized and accepted by the research staff. General clinical signs which may be monitored include: weight loss, inability to ambulate adequately to obtain food and/or water, and body condition scores (Figure 1.1) (Hickman and Swan, 2010). Anorexia or lack of appetite is a significant observation since parenteral supplementation in rodents is not commonly used. In some cases in these species, clinical abnormalities are only obvious when advanced illness, toxicity, or impending death (e.g., a moribund state) are reached (Toth, 2000). In these situations, decisions to initiate humane endpoints must be made promptly.

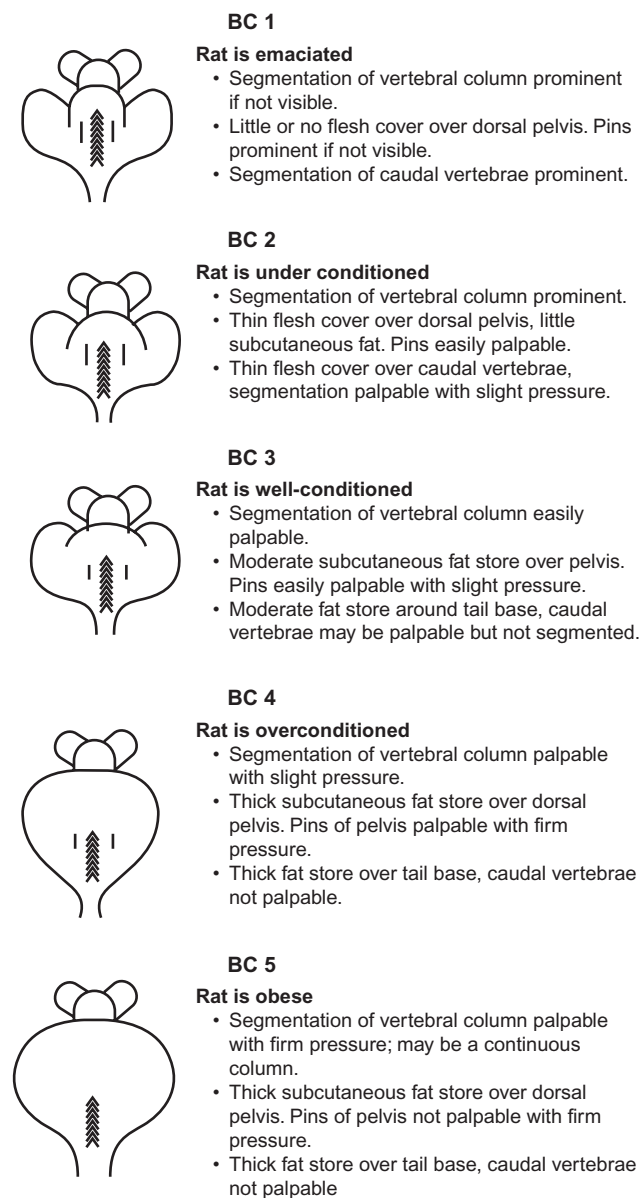


FIGURE 1.1 System for body condition scoring (reproduced with permission from Hickman and Swan, 2010).

In some cases, humane endpoints may be developed for a specific type of study (Dennis, 2000; Montgomery, 1987; Olfert, 1996; Olfert and Godson, 2000; Sass, 2000; Workman et al., 2010) or a specific individual study (Hickman and Swan, 2010; Singh et al., 2010). The use of scoring systems has been described and usually utilizes multiple observations which, in total, identify the humane endpoint (Lloyd and Wolfensohn, 1998; Medina, 2004; Morton, 2000). Other systematic approaches to determining humane endpoints have also been reported (Medina, 2004; Morton, 2000). There are excellent references available that review the establishment and use of humane

endpoints to avoid death as an endpoint (National Research Council, 2011).

To be prepared for situations when unanticipated pain and distress occur, the institution should have sufficient veterinary oversight in place to advise when alleviation of negative consequences from experimental procedures should be addressed. This may result in animals receiving veterinary medical care, removal from the experiment, or euthanasia to best align with the scientific objectives of the research. These decisions are ideally made through a collaborative discussion including animal care, scientific, and veterinary staff. However, in cases where the animals are significantly compromised, the veterinarian is obligated to take whatever actions are necessary for animal welfare. When multiple animals receiving the same treatment exhibit severe adverse effects, consideration should be given to adjusting the endpoint in the remaining animals or euthanizing an entire treatment group if experimental objectives can no longer be achieved.

Refinements in husbandry and handling (e.g., provision of species-appropriate enrichment, positive humane interaction and operant training to minimize stress in handling and sample collection), are also ways in which distress and other negative impacts can be minimized. Refinements of handling that may seem minor (e.g., picking up a mouse by using a plastic tunnel or cupped hand instead of the traditional method of grasping the base of the tail) (Hurst and West, 2010) can have significant impact, particularly when applied to a large number of animals.

Many refinements in husbandry rely on a good working knowledge of the ethology (study of animal behavior, generally under natural conditions) of the animal species used. The knowledge of normal species-specific behavior is necessary to recognize abnormal behavior and to create an appropriate environment. But consider an animal's natural environment – what is natural for mice and rats that live in forests, rural areas, and urban environments? Some species are highly adaptable, thus attempting to recreate a natural environment in the laboratory may not only be impractical, but difficult to even define. In addition, most of the rodents and rabbits used in research today are purpose-bred and have been for many generations. The ethograms for such animals would be expected to be different from their wild counterparts (e.g. it would be expected and desirable that rodents and rabbits habituated to humans would be less fearful and easier to handle). However, animals bred for research may retain, and attempt to exhibit, certain intrinsic behaviors. Knowledge of those behaviors can help the laboratory animal scientist develop practical strategies to maximize those behaviors and minimize stress.

When considering the stressors in the laboratory animals' environment, one must consider not only

individual stressors, but also the potential cumulative effect of multiple stressors and plan activities accordingly (Committee on Recognition and Alleviation of Distress in Laboratory Animals, 2008). For example, a laboratory mouse will have been exposed to multiple stressors, such as weaning, transport, and a new environment before it even reaches the laboratory. Allowing adequate time for animals to acclimate to their new environment and recover from these stressors is not only good for animal welfare but good for science (Conour et al., 2006).

Additional stressors may include husbandry and care. For example, most rodent and rabbit species are highly olfactory and loss of their olfactory cues when given a completely clean cage is a stressor (Duke et al., 2001; Sharp et al., 2002). Implementing methods that minimize this impact, such as transfer of the animal along with some nesting material, enrichment device, or at least the same cage mates has been suggested as a means to help mitigate the stress.

Most rodents are nocturnal, yet husbandry and experimental activities are usually performed during the sleep cycle. Reverse light cycles may be an alternative that could reduce the stress to animals of having procedures conducted during the sleep cycle, but most husbandry and research procedures are difficult to perform in a red light environment.

Laboratory rodents and rabbits originated as prey species and thus are not, by nature, comfortable with handling and restraint. Some degree of habituation may be desirable in some species (Moynihan et al., 1992). Positive reinforcement after such handling and restraint has been shown to aid tractability in some species, however there is little published about the use of this technique in rodents and rabbits, and so its use is based upon extrapolation and empirical observations.

The environment in which an animal is housed also impacts on its welfare, and assuring that the environment addresses the physical and behavioral needs of the animal can minimize negative impacts on welfare. Failure of the environment to meet the animal's needs can have a negative impact on both animal welfare and can affect scientific validity of the research (Garner, 2005; National Research Council, 2011; van Praag et al., 2000; Wurbel, 2001). An animal's environment consists of the physical environment and the social environment. Within the physical environment there are many variables, such as temperature, lighting, noise, and primary housing. Discussion here will be limited to that aspect of primary housing considered environmental enrichment. Within the animal's social environment the primary consideration is individual versus social housing.

The goal of environmental enrichment is to provide an animal with some choice, some degree of control over

its environment, and to promote species-specific normal behaviors (National Research Council, 2011). Logically, one must understand the specific normal behaviors and how important those behaviors are to the animal. Examples of environmental enrichment for rodents and rabbits include: (1) elevated shelves (Stauffacher, 1992); (2) shelters for guinea pigs, hamsters, and rats (Baumans, 2005); (3) nesting materials for rats, mice, hamsters, and gerbils (Baumans, 2005); and (4) gnawing material for rats, gerbils, hamsters, and rabbits (Baumans, 2005; Patterson-Kane, 2003). It is important to note that enrichment can, under some circumstances, lead to negative consequences such as increased aggression (Marashi et al., 2003). Further, the response to enrichment may be strain-specific (Nevison et al., 1999; van de Weerd et al., 1994).

To determine the most appropriate social environment for an animal, the species-specific social behavior must be known and the needs of the experiment must be accommodated. It has been demonstrated that group housing of social species such as most rodents and some rabbits is beneficial to the animal and minimizes stress (Hall, 1998; Spani et al., 2003; Van Loo et al., 2001, 2007). Some species and/or genders have a higher risk of incompatibility, such as with male mice of some strains (Jacoby et al., 2002), and adult male rabbits (Suckow et al., 2002). Risk of incompatibility can be reduced if animals are grouped and raised together from a young age, if group composition remains stable, and if the environment is designed to allow escape and minimize competition for resources (National Research Council, 2011). In addition, socially housed groups should be monitored for early signs of aggression and separated if necessary (National Research Council, 2011).

Some potential impacts of an enriched environment on research have been known for decades. For example, enhanced performance on complex learning was reported in rats housed in a more "stimulating" environment (Bingham and Griffiths, 1952; Forgays and Read, 1962). The effect of enrichment on variability of research results has been argued as both increasing variability (Mering et al., 2001; Tsai et al., 2002, 2003) and not affecting variability (Wurbel, 2000). Positive human interaction costs little and it is hard to imagine a negative effect on research outcomes. Therefore, this is only an ethical issue if it does not happen. Operant conditioning does require staff time and thus may only be appropriate for those studies in which procedures are going to be done frequently, or are potentially harmful enough to make the time investment worthwhile. When examining environmental enrichment, in addition to investigating real or potential effects on research, the impact on data obtained from an animal that is more highly stressed if unable to perform normal behaviors must also be considered.

Instituting changes in the environment may affect the ability to compare research results with historical data. In that case, more animals may be required; and this must be balanced against continuing to house animals in environments which do not maximize animal welfare. Who decides what is practical? Literature searches and pilot studies may be helpful in this decision. In summary, enrichment and social housing is not a “one size fits all” and it is important to balance the science with the well-being of the animals (Bayne, 2005; Weed and Raber, 2005). Decisions of where to balance such potentially conflicting interests benefit from consideration of the ethical principles discussed elsewhere in this chapter.

Once various forms of refinement have been applied, how can it be determined if animal welfare has been enhanced? In other words, how can it be determined if the refinement is successful? Assessment of animal welfare can involve examination of physiology, behavior, and gross appearance of the animals. Some physiologic measurements relevant to animal welfare involve indicators of stress, such as levels of corticosteroids and glucose. Blood sampling can cause elevations in stress indicators making interpretation difficult. Instead, the use of fecal corticoid has been reported as a non-invasive, less distressful alternative (Bamberg et al., 2001; Cavigelli et al., 2005). Assessment of reproduction parameters such as number of litters, pups per litter, and number and weight of pups weaned may provide indicators of the level of animal welfare for animals maintained as part of a breeding colony. Behavior and gross appearance are often assessed using scoring systems which, after appropriate training of the observers, can provide readily accessible and reproducible assessments of animal well-being (Lloyd and Wolfensohn, 1998; Lloyd et al., 2000; Morton, 2000). It is worth noting that body weight alone as a measure of animal well-being can be deceiving if the animal is young and in a growth phase, or bears an experimental tumor burden, as these situations often are characterized by changes in body weight that may mask those associated with well-being. Body condition scoring has been advocated as a non-invasive technique to assess health status and well-being (Figure 1.1) (Hickman and Swan, 2010).

ETHICAL CHALLENGES

Some studies, due to their potentially significant animal welfare costs, present a particular ethical challenge to research, husbandry and veterinary staff as well as IACUCs. In such cases, small pilot studies are an option and may help demonstrate whether the procedure will have adverse effects, allowing development of appropriate timelines for observation, treatments, or other interventions, and defined endpoints. Pilot studies are

often done in collaboration with the veterinary staff with reports back to the IACUC prior to approval of a full study.

When considering ethical challenges in the research environment, the authors suggest the following general approach:

- Determining what questions should be asked – some specific examples are found in this chapter. However, this chapter does not offer an exhaustive list but should help get the discussion started.
- Determining the the best approach to answer the questions. Often, solutions to ethical challenges emphasize the importance of a team approach. The collective judgment and cooperation of all members of the research team: scientist; veterinarians; technicians; and the IACUC are important (National Research Council, 2003; Organisation for Economic Co-operation and Development, 2000).
- Deciding who should make decisions with respect to the ethical merit of a study and experimental design. Sometimes regulations and guidelines will dictate who has responsibility for a final decision. For example, regarding the health and welfare of the animals, in the U.S., the attending veterinarian is responsible (Office of the Federal Register, 2002). The person responsible for the technical conduct of a study, is typically the Study Director (Organisation for Economic Co-operation and Development, 2000), though in other circumstances, it may be the Principal Investigator. In still other situations, it may be the enterprise conducting the study that makes the decision. However, in all these cases, the IACUC has the final authority to approve, disapprove, or suspend a study that has previously been approved (National Research Council, 2011; Office of the Federal Register, 2002; Office of Laboratory Animal Welfare, 2002).
- Identifying or developing specific policies that help the decision-making process. The purpose of a policy is to create and document a consistent response to a particular type of situation. Use of an existing policy is an approach to consistently apply defined criteria to all actions by personnel. The ethical principles discussed in this chapter should serve as a basis for development of policies.

To further illustrate this process and application of principles already discussed, examples of common situations presenting ethical challenges are described below.

Breeding Colonies

In addition to many technical challenges, maintenance of rodent breeding colonies can present ethical challenges. Among these are minimizing animal

numbers (prevention of overbreeding), breeding paradigms (monogamous pairs, trios, harem mating), and identification of pups.

Since the rationale for animal numbers is part of the ethical review of the IACUC (reduction) the IACUC should require a justification for the number of breeding animals that will be maintained based upon the numbers of offspring that will be used in the study. Examples of methods to calculate these numbers are available (White, 2007). There is an expectation that institutions will establish mechanisms to document and monitor the number of animals produced and used in research protocols (Office of Laboratory Animal Welfare, 2006, revised 2009). Monitoring these numbers will alert the IACUC if an overproduction will predicate excessive euthanasia of animals.

Ethical concerns may also arise in some breeding schemes where multiple females produce litters in the same cage. This may help pup survival since cross nursing often occurs; however, if multiple litters of various ages are in a cage, older, stronger pups may result in high mortality rates in newborns.

Methods of animal identification are sometimes a concern, particularly for breeding colonies where large numbers of animals need to be identified. There are several ways to identify a rodent if individual identification is necessary. For example, ear notching or application of ear tags, tattoos, and subcutaneous transponders are all relatively non-invasive. In contrast, toe-clipping is an invasive method of identification sometimes used for small rodents that would likely cause distress to the animal; thus, toe-clipping should be used only when no other individual identification method is feasible, although it may be the preferred method for neonatal mice up to 7 days of age, especially if dually used for genotyping, as it appears to have few adverse effects on behavior and well-being at this age (Castelhano-Carlos et al., 2009; Schaefer et al., 2010). If toe-clipping is used, the procedure can be refined by use of aseptic practices and use of anesthesia or analgesia appropriate to the age of the animals (Hankenson et al., 2008, National Research Council, 2011).

Genetically Modified Rodents

The use of genetically modified (GM) rodents (particularly mice) continues to increase. The use of GM rodents offers some unique opportunities to enhance animal welfare and the 3Rs. With careful phenotyping, early indicators of disease may replace the need for development of more serious clinical disease in animals (Brown and Murray, 2006). However, creation and use of GM rodents also raise some distinct ethical/animal welfare questions. Wells et al. (2006) summarized useful current practices, with recommendations for best practices.

Ethical issues may arise in the three phases of establishing and using genetically engineered rodents – the development, production, and/or research use (National Research Council, 2011). In the development of these models, one must consider if it is necessary to have a full spectrum of clinical disease versus the possibility that research questions might be answered with animals having a milder form of the disease or perhaps even having metabolic or biochemical changes in which there are no symptoms at all. The biotechnological procedures related to transgenesis (i.e., microinjection, in vitro culture, embryo transfer) have no significant effect on the normal development of the mice in the post-weaning period, suggesting that the techniques themselves would not be of great ethical concern related to animal welfare (van der Meer et al., 2001).

As the animals are phenotyped during the development phase, animal welfare assessments should be made at various ages. The welfare assessment should include a range of non-invasive indicators. In the neonate, these include skin color, activity, and presence of the milk spot; and, at weaning and in the adult these include appearance, size, coat condition, posture, gait, activity levels, interaction with the environment, and clinical signs. The welfare assessments may be carried out using a checklist as a prompt or reminder (Wells et al., 2006). Additional strategies to refine the development of these animals have been described (Brown and Murray, 2006).

Once the GM model has been developed, one should take what has been learned with respect to the phenotype to determine the optimal housing and breeding strategy. An example of a housing concern would be the need for husbandry modifications if the phenotype involved immunodeficiency, either anticipated or not. Development of breeding strategies for GM animals often requires balancing the animal welfare concerns with the detrimental phenotype and the numbers of animals being produced. (Dennis, 2000).

Both the development and production stages of genetically modified rodents need to assess genotype through DNA analysis. Although many protocols for collecting DNA for genetic testing have been proposed, a study using real-time quantitative polymerase chain reaction (qPCR) analysis to quantify DNA extracted from fecal pellets, hair, buccal swab samples, ear punch samples, and tail biopsy samples found that 2-mm tail biopsy samples yielded significantly more DNA than did the other sources (Garzel et al., 2010). Of course, obtaining the needed samples requires amputation of a terminal section of the tail, a procedure that could impact animal well-being, thus requiring thoughtful consideration. In a study of tail vertebral ossification and behavioral response to tail biopsy, the findings of Hankenson et al. (2008) support the collection of 2 mm

of tail tissue before 17 days of age unless anesthesia or analgesia is provided.

Most of the special considerations involving genetically modified animals are found in the development and production phases of model establishment (Brown and Murray, 2006). Once the animals are in actual use in a research protocol, the ethical questions (i.e., justification of animal numbers, minimization of pain and distress, etc.) would be the same as for any protocol for animal use.

The welfare of GM animals can be enhanced by sharing full documentation of all aspects of the phenotype with all scientists using the animals. Information should be used to create a “welfare profile”, specific to the particular GM line; and templates for the recommended welfare assessment scheme and a “rodent passport” have been developed (Wells et al., 2006). Such sharing of information allows optimal care of the GM animals and planning of experiments.

Cancer Research

The use of animals is essential in understanding the fundamental mechanisms of malignancy and to discover improved methods to prevent, diagnose, and treat cancer. An excellent summary of standards and recommendations for animal care and use in cancer research by Workman et al. (2010) will serve as a basis for this topic. The recommendations include: (1) consideration of study design, statistics, and pilot studies; (2) choice of tumor models (e.g., genetically modified, orthotopic, or metastatic); (3) therapy (including drugs and radiation); (4) humane endpoints (including tumor burden and site); and (5) publication of best practices.

As with other examples of ethical challenges, when designing studies with tumors of unknown *in vivo* growth characteristics, pilot tumor growth studies using small numbers of animals can establish reproducibility of local and metastatic growth. Such small-scale studies can also highlight adverse effects associated with tumor progression allowing development of observation and treatment strategies and allow identification of humane endpoints. For metastatic models, pilot experiments should define the extent and time course of dissemination to internal organs. The use of biomarkers, imaging, and measurement of circulating tumor cells using fluorescence and PCR-based techniques can facilitate determination of humane endpoints in these studies (Glinskii et al., 2003; Komatsubara et al., 2005). Use of aseptic technique, anesthetics, and post-implantation analgesia are recommended for implantation of tumor material as a means of refinement. For injection of cell suspensions, the minimum number of cells in the smallest volume should be used, consistent with the properties of the tumor. Implantation of tumor material into the muscle

requires special justification as it is more likely to affect mobility and cause pain. Footpad injection, which has been traditionally used to potentiate lymphatic dissemination, is generally considered to be unacceptable without exceptional scientific justification and should then only involve a single paw (Workman et al., 2010). When procedures are used to improve tumor take rate (e.g., moderate doses of whole-body irradiation), the added potential impact on animal welfare should be considered.

As a means to reduce the number of animals, studies designed to identify tolerable doses of experimental therapeutics to be used in subsequent efficacy trials, typically use two mice per dose level with a doubling dose-escalation or dose-halving de-escalation. One must balance the potential need for more animals, with reduced toxicity when the dose-escalation design versus the need for fewer animals but chances for increased toxicity when the de-escalation design is used. Animals should be closely monitored, and often they should be examined at least twice daily. When performing studies in which a combination of agents is used, care needs to be taken with respect to individual drug doses and scheduling, particularly if overlapping toxicities are likely.

In vitro and *in silico* pharmacokinetic methods are useful to predict absorption, distribution, metabolism, and elimination (ADME) properties and to help prioritize compounds for evaluation in animals (Singh, 2006). However, at present such methods are unable to predict accurately the full pharmacokinetic profile of an agent. Pharmacokinetic studies should use a validated and sufficiently sensitive detection method, ideally avoiding the need to pool separate blood samples, thereby minimizing animal usage (Workman et al., 2010). Repeat sampling of small volumes of blood from a superficial vein in mice over a series of time points has been established as a way to reduce animal numbers (Abatan et al., 2008; Barfield et al., 2008). In rats, a 5–8 time-point pharmacokinetic profile may be generated using 2–6 animals in total, through repeated blood sampling. “Cassette dosing”, which involves administration of low doses of compound mixtures, should also be considered initially as this can reduce animal usage (Watanabe et al., 2006). Wherever possible, computational compartmental kinetic modeling should be used to predict optimal doses or to design multiple dosing protocols which facilitate more limited sampling (Rowland and Tozer, 1995).

Initial studies of investigational therapies using tumor-bearing animals should aim to determine whether the target, or an appropriate downstream pathway or phenotype, is modulated by using suitably validated pharmacodynamic biomarkers (Collins and Workman, 2006). Simultaneous measurement of drug concentrations and mechanistic biomarkers is recommended to reduce animal numbers and establish

a pharmacokinetic–pharmacodynamic relationship (Workman et al., 2010).

Some cancer studies may involve radiation treatment. In such cases, there is potential for injury to the animal. For example, damage to the skin may occur. Should moist desquamation occur, this should not be allowed to persist for more than 24 hours (Workman et al., 2010). When ulceration occurs, if there is evidence of infection and/or no evidence of tissue repair the animal should be euthanized. A common outcome of radiation exposure is pulmonary fibrosis, and close monitoring of respiratory rate has been recommended as a means to detect symptoms before they become distressful to the animal (Jackson et al., 2010).

Commonly used in studies related to cancer research, intravital microscopic imaging uses a wide variety of optical imaging techniques, often incorporating fluorescent or bioluminescent genetic reporters or markers, including nano-particles (Hoffman, 2005). This method has particular animal welfare issues because it involves surgery to provide optical clarity and visualization on a microscope stage or by using fiber-optic light guides (Weissleder and Pittet, 2008). Surgical implantation of “window” chambers for tumor implantation enables imaging to be performed over days to weeks (Brown et al., 2001; Dewhirst et al., 1987; Lehr et al., 1993; Reyes-Aldasoro et al., 2008). When window chambers are used, general anesthesia is only essential for the initial surgery and imaging may be performed with restrained animals. Strict aseptic technique and good post-operative care and analgesia are essential to optimize animal welfare for all survival surgery (Flecknell, 2008; Richardson and Flecknell, 2005). The potential for development of hypothermia during anesthesia, maintenance of physiologic stability, and exposure to potential pathogens in imaging centers should be considered when developing methods for imaging of animals.

As with all studies, humane endpoints need to be defined for studies involving cancer models. Early endpoints reduce non-specific systemic effects of debilitation and so may increase the precision of the results obtained (Workman et al., 2010). Pilot studies, including autopsy to determine the full extent of tumor growth, will facilitate the definition of robust and refined endpoints. Workman et al. (2010) described general suggestions for endpoints appropriate to some commonly used animal cancer models. The intentional use of death as an endpoint is unacceptable and animals should not be allowed to become moribund unless strong scientific justification is provided.

The anatomic site for implantation of solid tumors will influence the maximum acceptable tumor load and the appropriate humane endpoints. Tumors or cancer cells implanted at sites such as the footpad, tail, eye, or bone are likely to be painful or distressful and require

special justification and earlier endpoints. Similarly, tumors that metastasize to sensitive sites need special consideration (Workman et al., 2010). For example, if tumors in the brain are to be studied, body weight loss is reportedly a sensitive endpoint (Redgate et al., 1991); and MRI or bioluminescent imaging (BLI) techniques can be very useful to assess tumor progression (McCann et al., 2009; Ragel et al., 2008; van Furth et al., 2003).

When determining endpoints, tumor size and burden should be considered. Tumor burden should always be limited to the minimum required to answer the scientific question (e.g., efficacy studies should be terminated once statistically significant therapeutic effects are demonstrated and minimal tumor loads for controls should be designed into therapeutic studies). When assessing the size of superficial tumors, measurement using calipers (usually of two diameters at right angles) is an easy and definable method. For example, when only a single tumor is present, the mean diameter should not normally exceed 1.2cm or 2.5cm in mice, or 1.5 and 2.8cm in rats. Where multiple tumors are present, for example in contralateral flanks, the size should be correspondingly less and the combined diameters should not exceed the maximum burden of a single tumor. Due to the difficulty in determining tumor burden of internal orthotopic cancers, systemic lymphoreticular tumors or metastatic disease, the data obtained in pilot studies allow characterization of the kinetics and patterns of spread, prediction of clinical signs and help define humane endpoints. The use of biomarkers or circulating cancer cells, as previously mentioned, also can be helpful to estimate tumor burden.

Perinatal Animal Use

Perinatal research has given science a better understanding of conditions such as autism, learning disabilities, and fetal alcohol syndrome. Fetal studies must consider the welfare of both the fetus and the mother. There are conflicting opinions on the ability of a rodent or rabbit fetus to experience pain. It has been reported that in precocial young, the fetal brain is too immature initially to support sentience of any state resembling consciousness and when sentience occurs, the animal is maintained in a sleep-like state of unconsciousness controlled by neuroinhibitory factors in the in utero environment (Mellor et al., 2005). The neurologic development necessary for the conscious perception of pain occurs at about 80% of the pregnancy time progression. After that point, it is likely that the perinatal animal can perceive pain if aroused from its unconscious state by noxious stimuli. In altricial young, neurologic immaturity persists throughout pregnancy. However, other studies report that rat fetuses at day E19 showed behavioral responses to an irritating substance (formalin) injected into one

paw which correlates to the expression of c-fos protein (an indicator of neuronal activation) in the spinal cord at day E20 (Yi and Barr, 1997). The *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research* (Committee on Guidelines for the Use of Animals in Neuroscience and Behavioral Research, 2003) advises anesthesia for potentially painful procedures in late-term fetuses (days 19–20 in the rat). However, consideration should be given to the potential effect of in utero drug exposure on physiologic and behavioral development of the fetus (Niesink et al., 1999; Rodier et al., 1986).

There are similar conflicting reports for neonatal rodents. Murrell et al. (2008) report that at 3–14 days of age, REM–non-REM differentiation occurs and there is EEG evidence of conscious wakefulness. In another study, rat pups exhibited behavioral arousal and withdrawal to noxious thermal and mechanical stimulation at 1 day of age (Barr et al., 1992). At 3 days of age, rats showed allodynia and hyperalgesia in response to induced inflammation (Abbott and Guy, 1995).

Neonatal exposure to noxious stimuli has been shown to cause alteration of the developing central and peripheral nervous systems (Bhutta et al., 2001; Mellor et al., 2005), and this may justify the use of anesthesia and/or analgesia even if the neonate is unconscious and unable to experience pain.

The many neonatal physiologic differences versus adults (such as blood–brain barrier permeability, immature hepatobiliary system impacting on drug metabolism, and hypoxia-induced bradycardia) affect the response to anesthetics, analgesics, and euthanasia. Inhalation anesthesia is recommended for neonates when possible. Hypothermia has been reported as safe and effective as the sole method of analgesia for altricial rodents up to about the age of 7 days (Danneman and Mandrell, 1997). However, rapid cooling of non-protected flesh is painful (Wolf and Hardy, 1941) and rats recovering from hypothermia (but not rats recovering from inhalation anesthesia) emit ultrasonic vocalizations during recovery (Hofer and Shair, 1992).

Pups should have regained their righting reflex prior to putting them back with their mother. Strategies such as smearing the pups with bedding and urine of litter mates and temporary fostering have been suggested to minimize chances of cannibalism and neglect when returned to the mother (Committee on Guidelines for the Use of Animals in Neuroscience and Behavioral Research, 2003).

Neuroscience and Behavioral Research

Food and Fluid Restriction

One of the “Five Freedoms” for animals as noted earlier is the freedom from hunger and thirst. Food or water deprivation (beyond that necessary for normal

presurgical preparation) is considered to be a procedure that may cause pain or distress (Animal and Plant Health Inspection Service, 1997).

Food and/or water may be restricted for the purpose of looking at homeostatic regulation, studying motivated behaviors and physiologic mediators of hunger or thirst and/or to motivate animals to perform novel or learned tasks (Toth and Gardiner, 2000). In “the case of conditioned-response research protocols, use of highly preferred food to fluid as positive reinforcement, instead of restriction, is recommended” (National Research Council, 2011). If restriction is necessary, it is recommended to use the “least restriction necessary to achieve the scientific objective while maintaining animal well-being” (National Research Council, 2011).

Sensitivity to food restriction is increased by single housing of animals and by feeding during the illumination phase of the light cycle (Hotz et al., 1987; van Leeuwen et al., 1997). The risk to animals on fluid restriction is reduced if periods of access and total amounts available match the animal’s typical watering schedules and circadian variable (Toth and Gardiner, 2000).

When designing or reviewing these types of studies, examples of questions which should be answered are: (1) What is the proposed schedule of food and water access? (2) Does the schedule take into account the circadian rhythm of feeding? (3) Will the diet provided avoid unintended nutritional imbalances? (4) How will the animals be acclimated to the reduced feeding/watering schedule? (Toth and Gardiner, 2000). Further, additional questions to be answered might include: (1) What is the proposed schedule for monitoring and what does it include (behavioral and physiologic monitoring) to assure that any adverse effects will be promptly recognized? (2) What are the plans for record keeping of daily food or water intake, hydration status, animal appearance, and routine weighing to help adverse effects? and (3) What are the endpoints for intervention (usually with supplemental nutrition and/or hydration)?

Neuroanatomic Studies

Anatomic studies are done for the purpose of studying cellular organization or chemical composition of specific brain lesions. These studies typically use tracer substances to label neural pathways or techniques to create lesions to destroy discrete areas of the brain using electrolytic and radiofrequency techniques as well as neurotoxins. Both approaches generally involve the use of a stereotaxic surgery.

Examples of questions which should be answered are: (1) What is the extent of the clinical impairment that the injection or lesion may cause? (2) What are the chemical properties of the substance injected (e.g., sterility, toxicity, inflammatory response)? (3) Will aseptic technique be used for all injection and lesioning procedures?

(4) What complications might arise from any surgery and how will they be monitored and treated? and (5) Will an implanted device (cranial implant or chamber) be required? If so, it is recommended that the animal be conditioned to the training prior to surgery to avoid surgery in animals that do not adapt to training ([Committee on Guidelines for the Use of Animals in Neuroscience and Behavioral Research, 2003](#)). Will multiple major survival surgeries be done? If so, why; under what circumstances; and when and by whom will it be determined that euthanasia is most appropriate?

Neural Injury and Disease

These types of studies include degenerative diseases such as Alzheimer disease, traumatic injury, infectious disease, pain, and neuroimmunologic neurodevelopmental and psychiatric disorders.

Some factors which will refine these studies include: easier access to food/water; provision of highly palatable, highly nutritious feed; promotion of urination and defecation through the use of massage and laxatives; maintenance of fluid balance; provision of additional bedding; and administration of analgesics or tranquilizers, particularly for studies involving injury or pain. The duration and severity of the condition should be limited to the least amount of time necessary to achieve scientific objectives. As with all studies using animals, intervention on behalf of the animal (i.e., palliative care and treatment, euthanasia) must be identified prior to the start of the study.

When induced lesions of the nervous system are used to create animal models, the clinical consequences can sometimes be refined through the use of reversible lesions. In this technique, the target structure can be temporarily deactivated during different states of the assessment of learning and memory. The clinical consequences of other lesions may be reduced by making unilateral, instead of bilateral, lesions ([Tolwani et al., 1999](#)). Some neurectomy and deafferentation lesions can result in autotomy (self-mutilation). This may be minimized through the use of local anesthetic at the time of lesioning ([Magnusson and Vaccarino, 1996](#)). When designing and reviewing these types of studies it is important to know the expected clinical course of the disease/injury and what intervention or clinical management will be implemented.

Behavioral Studies

These studies generally use appetitive (animal will voluntarily approach) or aversive (animal tries to escape or avoid) stimuli. The same stimuli may be appetitive to some species or individuals and aversive to others. For example, the same dose of alcohol can be aversive in rats but appetitive in mice ([Cunningham et al., 1993](#)). In addition to species, the effects may vary as a function

of genotype, sex, and past experience. These different types of stimuli are necessary because the brain mechanisms, which form the basis of different types of learning and behavior may differ. When deciding which type of stimulus to use, the response variability of species, individuals, and the individual over time must also be considered since there is generally a desire to minimize this variability. Such reduction in variability can serve to reduce the numbers of animals needed. When stimuli are aversive, they are generally considered acceptable if they are well tolerated by the animal and do not produce maladaptive behaviors. If the animal's behavior is dominated by escape-avoidance activity, the behavior is maladaptive, indicating unacceptable levels of pain or distress ([National Research Council, 2009](#)). In situations where the scientific rationale for a motivating stimulus is questioned, one approach may be to perform a pilot study with the proposed stimulus and carefully monitoring the response of the animal, followed by reporting to the IACUC for further consideration.

Behavioral research may call for the use of mazes to study cognitive function (spatial learning and memory) in rodents. Performance in mazes may involve food or water restriction or other stressors which should be considered in determining the overall cumulative stress that a research animal experiences. Due to sensory impairments, lesioned or genetically modified animals may be more likely to experience stress or injury.

Behavioral screening tests (often referred to as functional observational batteries or FOBs) are used in pharmacology, genetics, and health surveillance. These batteries are able to assess multiple behavioral measures. They are justified by the need to evaluate the effect of a drug or chemical on behavior in both humans and animals. This type of testing is sometimes required by regulatory agencies (e.g., FDA or EPA as described in greater detail later in this chapter) ([Weisenburger, 2001](#)). These batteries include sensorimotor and motor tests as well as assessment of anxiety. During these tests, animals should be carefully observed to assure their safety on mechanical devices and to assure minimum duration of the tests consistent with scientific objectives. The degree of anticipated animal impairment should be known, if possible, as tasks may need to be modified. If the level of expected animal impairment is not known, as with other studies mentioned in the chapter, pilot studies are encouraged. And, as with all studies, clear and humane endpoints need to be identified.

Prolonged Restraint and Anesthesia

Restraint of Awake Animals

Restraint of awake animals may occur in a variety of physiological and anatomic studies. To minimize

distress to the animal, restraint devices must be of suitable size and type for the animal involved. Animals must be monitored during restraint periods, and animals exhibiting maladaptive behavior or those which develop lesions should be removed from the restraint and provided veterinary attention.

Animals should be acclimated to the restraint. The duration of restraint should be for the minimal amount of time necessary. The need for prolonged restraint must be scientifically justified.

Prolonged Studies in Anesthetized Animals

Maintaining an appropriate physiological condition in an animal under prolonged anesthesia is a technical challenge. Careful monitoring is essential to assure well-being of the animal. Techniques such as hyperinflation, hydration of inhaled gas, and fluid replacement to help maintain physiologic homeostasis have been described ([Committee on Guidelines for the Use of Animals in Neuroscience and Behavioral Research, 2003](#)).

Some studies require that any self-generated movements by the animal be minimized. This is usually done with some type of neuromuscular blocking drug (NMBD). The primary animal welfare concern is that the usual behavioral indicators of pain and/or distress are then inhibited, thus requiring special considerations to assure adequate anesthetic depth. Adequate anesthesia must be established and verified before the administration of NMBDs to establish and validate the physiological signs that will be monitored in the paralyzed animal. Reducing the level of anesthesia after NMBD administration is strongly discouraged. Even in the absence of other signs, autonomic nervous system changes such as heart rate and blood pressure can be indicators of pain and inadequate anesthetic depth. A trained observer should be present at all times during a prolonged anesthetic procedure because the clinical condition of an animal can change quickly and require intervention ([Committee on Guidelines for the Use of Animals in Neuroscience and Behavioral Research, 2003](#)).

INSTITUTIONAL ANIMAL WELFARE OVERSIGHT

Effective oversight of animal welfare in research, testing and teaching relies on the expertise and collaboration of the senior management of an institution (generally referred to as the Institutional Official or IO in the United States), an appropriately qualified veterinarian with responsibility and authority for the health and well-being of all laboratory animals at the institution and an Institutional Animal Care and Use Committee (IACUC), or equivalent ethical oversight committee ([National Research Council, 2011](#)). In the U.S., the

IACUC's primary role is responsibility for assessment and oversight of the institution's animal care and use program as well as the related facilities. The committee membership is specified by regulations. It is important for research animal users to know which specific regulations apply to their given situation. In general, IACUC membership includes a veterinarian, a scientist, one individual from a non-scientific background, and a public member to represent general community interests. Large institutions with broad research programs may need to choose scientists from a number of disciplines to properly evaluate proposals.

The IACUC provides oversight of animal welfare through its review of animal use protocols and amendments, ongoing review of animal use (often referred to as post approval monitoring or monitoring outcomes), periodic assessment of the animal care and use program and inspection of facilities, assuring personnel working with animals are appropriately qualified and implementation of a process for review of animal welfare concerns. The functions of the IACUC are well described elsewhere ([Anderson, 2007](#); [National Research Council, 2011](#); [Silverman et al., 2007](#)) This section will focus on ethical considerations generated as a result of the protocol review process. The principles of protocol review would be pertinent to both a local review, as would occur in an IACUC, and a remote review, as might occur where regional or national protocol review is required.

The protocol submitted to the IACUC for review must include enough detail to allow a harm or cost/benefit analysis; that is, weighing the cost to the animal against the potential benefit of the study. Clearly, this is a utilitarian approach, with the cost to the individual weighed against the potential benefits to many. Of course, the first question which must be answered is whether animals need to be used at all, or if non-animal alternatives (replacements) can be used to answer the scientific question(s) of the study. There needs to be justification/rationale for the use of animals as well as the species of animal and number of animals. In the authors' experience, to better understand the cost to the animal (implications of the study on animal welfare), the IACUC must understand: the sequential description of the procedures; non-standard housing and husbandry requirements; anticipated adverse clinical effects on the animals; appropriate use of sedation, anesthesia, and analgesia; details of surgical procedures, post-procedural care and observation; a description and rationale for endpoints proposed; criteria and process for timely intervention, method of euthanasia or disposition; and adequacy of training for all personnel involved. Some IACUCs find the use of severity codes or invasiveness levels helpful in both initial and ongoing review.

As previously mentioned, the ethical principles mentioned above are interrelated with dynamic global

regulations, policies, and guidelines. One should bear in mind that regulations and guidelines are evolving and no one text can remain completely current. For example, as this text was being written, a revision of the *Guide for the Care and Use of Laboratory Animals* was released (National Research Council, 2011). The interpretations of this version of *Guide* will continue to progress over time. Compliance with the laws and regulations which govern the use of animals in research is mandatory.

There may conceivably be situations where the law or regulation would require one action but an individual's moral conscience might dictate a different action. In such a case, it may seem that the law is a "bad" law. Violation of a law could potentially lead to a more general disregard for the law and regulations, with resultant great harm. Indeed, some philosophers make the point that disobeying even "bad" laws leads to the tendency to think that one is above the law, leading to wholesale violation of the law (Tannenbaum, 1995).

REGULATIONS AND NON-REGULATORY CONSIDERATIONS

United States Regulatory Considerations

The ethical considerations discussed above have been the basis for many aspects of governmental oversight of the conduct of animal research. Historically, Western societal values have included concern for the welfare of animals. Animal welfare and animal rights organizations have stressed both the rationale and the necessity of laws and regulations that will ensure that animals are handled appropriately. The use of animals in research has been a particularly sensitive and emotional issue, and it is not surprising that extensive legislation has been enacted for the protection of research animals. An overview of the regulatory requirements is presented here.

United States Animal Welfare Act

In the United States, federal laws, regulations, and policies are a critical component influencing the use of research animals. Species covered by the Animal Welfare Act (AWA, 1990) include dogs and cats, non-human primates, rabbits, guinea pigs, and hamsters, aquatic mammals, and, with certain exceptions, any other live or dead warm-blooded animal designated by the Secretary of Agriculture. Birds, rats of the genus *Rattus*, and mice of the genus *Mus* bred for use in research, teaching, or testing, and horses and farm animals intended for use as food or fiber or used in studies to improve production and quality of food and fiber, are specifically excluded. The law clearly states, however, that nothing in the Act should be construed as authorizing the Secretary of

Agriculture to promulgate rules, regulations, or orders with regard to the design or performance of research protocols. It also mandates that the U.S. Department of Agriculture (USDA) may not interrupt the conduct of research during inspections.

The law authorized the USDA to develop and enforce regulations and standards based on the AWA. The regulations, standards, and policies are administered through the USDA, Animal and Plant Health Inspection Service (APHIS), Animal Care (AC) unit.

The complete set of USDA regulations and standards are published in the Code of Regulations (CFR), Title 9, Animals and Animal Products, Subchapter A, Animal Welfare (Office of the Federal Register, 2002). In addition, the USDA has issued the "Animal Care Policy Manual" (http://www.aphis.usda.gov/animal_welfare/policy.shtml) to further clarify its enforcement of the Animal Welfare Act. Each research institution is required to identify an Institutional Official (IO) who is authorized to legally commit on behalf of the institution that the USDA requirements will be met. All research facilities housing regulated species must be registered with the USDA.

The AWA was amended in 1985, specifically to "reflect the importance of the '3 Rs'" (Hamilton, 1991). The 1985 amendment to the AWA required every animal research facility to establish an IACUC as a means to provide oversight of the research facility's program of animal care and use. Congress mandated that the committee include at least three members, with no requirement that research scientists be represented. IACUC members are appointed by the Chief Executive Officer (CEO) of the research facility. The members must possess sufficient experience and expertise to assess animal care, treatment, and practices in experimental research as determined by the needs of the research facility and are to represent society's concerns regarding the welfare of animal subjects. At a minimum, membership must include one doctor of veterinary medicine and one member who is not affiliated in any way with the research facility other than as a member of the IACUC. This member, commonly referred to as the non-affiliated member, cannot be a member of the immediate family of a person who is affiliated with the facility and provides representation for general community interests in the proper care and treatment of animals. In instances where the IACUC consists of more than three members, not more than three members can be from the same administrative unit of the facility.

The IACUC is responsible for conducting a regular review of the animal care and use program. The review, based on USDA regulations, must also include an inspection of all animal study areas and animal facilities at least once every 6 months. An animal study area is defined as a location where animals are housed for more than 12 hours; however, the term is often used to

also include areas where survival surgery is conducted. After each program review and inspection, the committee must file reports of its evaluations with the IO of the research facility.

The IACUC is responsible to review and approve all proposed activities and significant changes in activities related to the care and use of animals, and ensure that all aspects of the research will be conducted in accordance with USDA regulations. All activities must be approved prior to the commencement of the activity. The committee may also suspend an activity if it determines that the activity is not being conducted in accordance with the IACUC-approved procedures. Any suspended activities must be reported to the USDA.

Proposals to conduct an activity or to make changes in an ongoing activity involving animals must provide adequate information to assure that the IACUC can conduct a thorough review. In addition to items previously mentioned, the regulations of the AWA specify that survival surgical procedures must be performed using aseptic technique; appropriate preoperative and postoperative care must be provided, major survival surgery on non-rodents may be conducted only in facilities intended for that purpose under aseptic conditions; any unrelieved pain or distress may continue only for the necessary period of time; and animals that would otherwise experience severe or chronic pain or distress that cannot be relieved must be euthanized during or at the end of the procedure, using methods consistent with the current Report of the American Veterinary Medical Association (AVMA) Panel on Euthanasia.

The regulations require that research activities be performed by appropriately qualified and trained personnel. Specific topics to be covered in the training program are described in the AWA regulations. Scientists are directed to the Animal Welfare Information Center (AWIC) to obtain information on alternatives (the 3Rs). Such database searches are required if painful/distressful procedures are to be conducted ([Animal and Plant Health Inspection Service, 2000](#); [Office of the Federal Register, 2002](#)).

The IACUC is also charged with reviewing, and, if warranted, investigating concerns involving the care and use of research animals through an established reporting procedure. The committee must also establish a mechanism for addressing such concerns.

Each research facility is required to have an attending veterinarian who is authorized to ensure the provision of adequate veterinary care and oversee the adequacy of animal care and use. The veterinarian must have training or experience in laboratory animal science and medicine and have direct or delegated program responsibility for activities involving animals at the research facility. Part-time or consulting veterinarians must provide a written program of veterinary care

and conduct regular scheduled visits to the facility. The veterinarian must be consulted, in advance, if potentially painful procedures are planned, and ensure sedatives, analgesics, or anesthetics are provided, unless withholding them is scientifically justified in writing and approved by the IACUC. The American College of Laboratory Animal Medicine (ACLAM) further defines adequate care, and establishes standards of education, training, experience, and expertise necessary for veterinarians to become qualified as a specialist through board certification ([ACLAM, 1996](#)).

Public Health Service Policy on Humane Care and Use of Animals

The Health Research Extension Act of 1985 (Pub. L. 99-158), Section 495, Animals in Research, establishes standards (Policy) for the proper care and treatment of animals used in biomedical and behavioral research. Any institution receiving support through the U.S. Public Health Service (PHS) for animal research, training, biological testing, or animal-related activities, is required to provide extensive written assurance of their compliance with PHS policy. The assurance statement documents the institution will comply with the PHS policy requirements and provisions of the *Guide* ([National Research Council, 2011](#)). The policy applies to all PHS-conducted or -supported activities involving animals regardless of where they are conducted. The PHS policy requires each IACUC to have a minimum of five members, including a veterinarian, a practicing scientist with experience in research involving animals, a member whose primary concerns are in a non-scientific area, and a non-affiliated member. It allows an individual who meets the requirements of more than one category to fulfill more than one role as long as the committee still has at least five members. The AWA and the 2002 edition of the PHS policy require research institutions to provide training for personnel similar to the AWA. The PHS policy was amended in 2002 regarding the timing of verification of IACUC approval of competing applications or research proposals subsequent to peer review but prior to award (PHS, 2002).

Food and Drug Administration Good Laboratory Practices

In June 1979, the U.S. Food and Drug Administration (FDA) implemented the Good Laboratory Practice (GLP) regulations (21 CFR, Part 58), effective June 1979, and amended in 1987 ([Food and Drug Administration, 2009](#)). Even though the goals of the FDA GLP regulations are intended to assure the quality and integrity of safety data submitted to the FDA in support of an application for a research or marketing permit, they have significant impact on appropriate procedures for the care and use of laboratory animals in safety testing.

An animal care and use program operating under the GLP regulation is a complex and highly regimented situation requiring communication, cooperation, and integrated problem-solving skills that involve multiple units (including quality assurance and pathology) in the facility. It is necessary to maintain a delicate balance to assure all aspects of the program follow both the intention and letter of both the FDA and USDA laws. In brief, the GLP regulations require certain activities and documentation of those activities in minute detail. A summary of the scope of the activities appears here.

Institutions seeking FDA approval of preclinical studies and data must include written protocols, standard operating procedures (SOPs); adequate facilities, equipment, and animal care; properly identified test substances; and meticulous comprehensive records of observations and results. The FDA relies heavily on the quality of documentation. Every study is overseen by a study director, an individual who has ultimate responsibility for implementation of the protocol and technical conduct of the study. The quality assurance unit monitors the conduct of studies and audits every aspect of documentation.

The FDA conducts routine surveillance inspections, data audits, and directed inspections to verify data of pending applications for approval. The FDA may also conduct inspections to investigate potential non-compliance issues, referencing the GLP Act in the animal care and use program. A data audit compares the protocol and amendments, raw data, records, and specimens against the final safety assessment report. Medical records for study animals, only more recently addressed in reporting of data collection, may also be scrutinized by the auditors. Inspection reports are very detailed and objective. There is cooperation with other federal agencies as described below.

Interagency Cooperation

In 1995, authorized representatives of the USDA, NIH, and FDA signed a "Memorandum of Understanding concerning Laboratory Animal Welfare", as instructed by Congress in the AWA ([Office of Laboratory Animal Welfare, 2006](#)). Each agency, operating under its own authority, has specific responsibilities for fostering proper animal care and welfare. This agreement sets forth a framework for reciprocal cooperation which will assist each agency in meeting its responsibilities in promoting proper laboratory animal care and welfare. Implementation of this agreement is intended to maintain and enhance agency effectiveness while avoiding duplication of efforts to achieve required standards for the care and use of laboratory animals.

Features of the Agreement include the promulgation of standards and policies aimed at promoting laboratory animal welfare, the maintenance of

registries/inventories of institutions and facilities subject to agency policies and regulations, the periodic conduct of routine and "for cause" inspections or site visits, efforts designed to promote compliance, and the application of a range of sanctions when necessary. The agreement is renewed every 5 years.

Environmental Protection Agency Good Laboratory Practices

Similar to the FDA, the Environmental Protection Agency (EPA) also has established Good Laboratory Practices (GLPs) for conduct of studies required to register a new pesticide product. The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), enacted in 1947, authorized the administrator of the EPA to register and control the use of pesticides. The EPA conducts a premarket review of potential health and environmental effects, and requires animal tests to be conducted in support of registration. EPA's GLPs differ in some respects from the standards of the FDA. According to the final rules published in 1983, the Toxic Substances Control Act (TSCA) also requires the use of GLP standards for conducting industrial chemical studies required by the TSCA (Environmental Protection Agency, 2002, 2007).

Non-Regulatory Considerations

Institute for Laboratory Animal Research

The Institute for Laboratory Animal Research (ILAR), is a private, non-governmental, non-profit organization that promotes high-quality science through humane animal research and implementation of alternatives. The mission of ILAR is to "evaluate and disseminate information on issues related to the scientific, technological, and ethical use of animals and related biological resources in research, testing, and education" ([Institute for Laboratory Animal Research, 2010](#)). Using reports of expert committees, the *ILAR Journal*, excellent web-based resources, and other means of communication, ILAR functions as a component of the U.S. National Academies to provide independent, objective advice to the federal government, the international biomedical research community, and the public.

The most widely distributed publication from ILAR is the *Guide for the Care and Use of Laboratory Animals* ([National Research Council, 2011](#)), which is recognized by the PHS and the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) as the accepted reference standard on laboratory animal care and use programs. ILAR also publishes the quarterly *ILAR Journal* and important reference documents, including *Occupational Health and Safety in the Care and Use of Research Animals* ([National Research Council, 1997](#)).

Guide for the Care and Use of Laboratory Animals (Guide)

The *Guide* (National Research Council, 2011), written under the auspices of ILAR, applies to all live vertebrate animals, including traditional laboratory animals, farm animals, wildlife, and aquatic animals used in research, teaching, or testing. It emphasizes performance standards, which are less prescriptive and more flexible than engineering standards. The *Guide* also encourages the application of professional judgment when addressing unique circumstances. Recommendations in the *Guide* are based on published data, scientific principles, expert opinion, and experience with methods and practices that are consistent with high-quality, technically and scientifically appropriate, humane animal care and use.

The most recent edition of the *Guide* includes references, and covers the ethics of laboratory animal use; components of effective animal care and use programs; and guidelines for the housing, environment, and enrichment of terrestrial and aquatic animals. Information on biosecurity is addressed, as is information on transportation, pain and distress, euthanasia, and veterinary clinical care. The guidelines regarding institutional policies and responsibilities, personnel and program management, occupational health and safety, and animal facility design and management are essential for developing an effective animal care and use program.

International Regulations, Policies, and Standards

AAALAC, International

The Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC) is a non-profit association that conducts voluntary, confidential, peer review, and accreditation of animal care and use programs. AAALAC provides advice and independent assessments to participating institutions and accredits those that meet or exceed applicable standards. AAALAC uses the *Guide for the Care and Use of Laboratory Animals* as a primary reference for program review and evaluation, but it also considers the laws and regulations for the country in which the institution is located. AAALAC also uses other widely accepted standards and references for animal care and use, occupational health, and biosafety. Assessment stresses application of performance standards and professional judgment, rather than inspection and enforcement of engineering standards. The AAALAC website provides information, resources, and links to numerous sources of information regarding accreditation, education, regulations, and IACUC support (AAALAC, 2011).

Canada

The Criminal Code of Canada – Cruelty to Animals Bill, of the federal Criminal Code prohibits anyone from

willfully causing animals to suffer from neglect, pain, or injury, and applies to all animals, regardless of their purpose (Parliament of Canada, 2008).

This legislation is more commonly applied to privately owned animals than those used in research. The Canadian system is primarily based upon a quasi-regulatory system, the Canadian Council for Animal Care (CCAC). Five provinces have enacted their own legislation making it mandatory to comply with CCAC policies. Any research facility in Canada receiving Tri-Council (Canadian Institutes of Health Research-CIHR, Natural Sciences and Engineering Research Council of Canada-NSERC, and Canadian Foundation for Innovation-CFI) funding must adhere to the CCAC Guidelines, including all academic institutions. Institutions importing dogs and non-human primates across the U.S./Canada border must also have a CCAC Good Animal Practice certificate. The Ontario Animals for Research Act is very similar to the U.S. AWA except it covers all vertebrate species, including fish. This is a minimum standards act with unannounced veterinary inspections, additional reporting, and registration requirements. All small companies and businesses must be registered as research facilities to conduct any type of animal research in Ontario (Animals for Research Act, RSO 1990).

The Canadian Council on Animal Care (CCAC), established in 1968, is the national peer review agency responsible for developing and maintaining standards for the care and use of animals used in research, teaching, and testing in Canada. Major Canadian agencies actively support the CCAC's programs to assure ethical and proper treatment of animals used in research. Institutions receiving funding from these agencies must monitor ongoing research involving vertebrates and cephalopods (squid and octopi) and assure compliance with federal and provincial laws, regulations, and guidelines. Participation in the CCAC's assessment program is required, and institutions are required to maintain a valid Certificate of Good Animal Practice confirming compliance with CCAC guidelines and policies. CCAC general guidelines are published in two volumes of *Guide to the Care and Use of Experimental Animals*, available from the CCAC (Canadian Council for Animal Care, 1984, 1993).

In addition, the CCAC published numerous other guidelines and policies which are available on their website, <http://www.ccac.org.ca/>.

European Union

The basis for national legislation on the protection of animals used for scientific purposes is European Union Parliament and Council Directive 2010/63/EU, revised in 2010 (European Union, 2010). One of the main goals of this legislation is to minimize the disparities between member states that arose from the original Directive of

1986. Although member states do have some flexibility, the 2010 Directive and accompanying Appendix, set minimum standards. Once the Directive is fully ratified, member states are given 2 years to develop and implement national legislation which is consistent with the Directive.

In addition to vertebrate animals, cephalopods are also covered, as are fetal forms of mammals in the last trimester of gestation. Consistent with many of the general principles mentioned earlier in this chapter, the principles of the 3Rs are stressed including strategies to use non-animal alternatives when possible, minimize animal numbers, and minimize pain and distress through refinement of procedures, use of humane endpoints, and appropriate euthanasia procedures. For transparency purposes, reporting is required based upon actual severity levels of procedures. These are spelled out in more detail in Annex IX of the Directive. Regular inspections of facilities are also required. There are requirements for adequate training of all personnel. Appropriate veterinary care and the use of an internal animal welfare body (AWB) to oversee animal welfare are also included. Review of projects, including a harm:benefit analysis is conducted by “competent authorities”, usually at the regional or national level.

Chapter II of the Directive covers the provisions on the use of certain animals in procedures. The pertinent items on rodents and rabbits would include those circumstances where animals would be taken from the wild and when use of rodents and rabbits bred for research must be used. Chapter III includes information on procedures such as how methods are chosen, anesthesia, severity classification, reuse, and endpoints. Chapter IV, Section 1 includes requirements for breeders, suppliers, and users and discusses such topics as competence of personnel, veterinary care, details of the AWB, record keeping and, through reference to Annex IV, care and accommodation requirements of animals. Section 2 includes further details on inspections and Section 3 covers requirements for project authorization, and retrospective assessment, often called post approval monitoring. Chapter V discusses avoidance of duplication and alternative approaches. Chapter VI, entitled, Final Provisions, provides information on implementation (EU, 2010).

Particularly important is Annex III, the requirements for the care and accommodation of animals. This Annex begins with a general section covering such things as facility design, environment, health care, handling, and husbandry. This is followed by Section B, the species-specific section, where tables provide details on enclosures, exercise where appropriate, weaning, and other topics. Annex IV covers methods of killing animals and includes a detailed chart of accepted methods by species.

Pacific Rim

AUSTRALIA

Australian animal welfare legislation is established by each state and territory, with guidance from the Australian National Health and Medical Research Council. The Council provides resources and oversight through the Animal Welfare Committee, regarding issues pertaining to the conduct and ethics of using animals for scientific purposes. A model code of practice, *The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* is prepared by the Animal Welfare Committee and is available from each state and territory to set minimum animal welfare standards (Australian National Health and Medical Research Council, 2004). The Australian and New Zealand Council for the Care of Animals in Research and Teaching Ltd (ANZCCART) was established in 1987 in response to particular concerns from scientific communities about the use of animals in research and teaching. The Council addresses scientific, ethical, and social issues associated with the use of animals for scientific purposes (Australian New Zealand Council for the Care of Animals in Research and Teaching, 1987).

NEW ZEALAND

In New Zealand, the Animal Welfare Act 1999, Part 6, controls animal use in research, testing, and teaching (Ministry of Agriculture and Forestry of New Zealand, 1999). The Ministry of Agriculture and Forestry (MAF) leads and facilitates the management of animal welfare policy and practice, and promotes policies for the humane treatment of animals. An Animal Ethics Committee (AEC) and measures to minimize animal pain or suffering are required.

JAPAN

Animal care in Japan is regulated by the Japan Law for the Humane Treatment and Management of Animals (1973), however, the actual conduct of research is not specifically regulated (Kurosawa, 2007). Guidelines compiled by relevant government agencies, such as The Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities developed by the Ministry of Education, Culture, Sports, Science and Technology (2006), the *Guidelines for Proper Conduct of Animal Experiments* developed by the Science Council of Japan (Science Council of Japan, 2006), and the *Policy for Animal Experimentation* published by the Japanese Association for Laboratory Animal Science (Japanese Association for Laboratory Animal Science, 2007) are used to provide guidance for animal research on a local level. Self-regulation is practiced and proper management of laboratory animals, and minimization of pain during experimental procedures are emphasized.

SOUTH KOREA

Korean Animal Protection Law provides legislation for the protection of animals. Sections 13 and 14 of this Law focus on experiments with animals and the formation of an Ethics Committee ([Korean Ministry of Agriculture and Forestry, 2007](#)). The 1996 ILAR *Guide* ([National Research Council, 1996](#)) has been translated into Korean and serves as a reference for Korean laboratory animal science practices.

PHILIPPINES

In the Philippines, the Animal Welfare Act known as *The Animal Welfare Act of 1998* covers animals and facilities used for breeding, maintaining, keeping, treating, or training of all animals as objects of trade or household pets ([Congress of the Philippines, 1998](#)). The Act does not specifically mention the use of animals in research.

THAILAND

The National Research Council of Thailand has developed *Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes* ([National Research Council of Thailand, 1999](#)). These *Guidelines* call for valuing the life of animals. The concepts of the 3Rs (although not mentioned by name) are covered. The *Guidelines* mention the necessity of users being adequately skilled and require an institutional Committee to manage and be accountable for animal use (including project review) as well as a national Committee to investigate and oversee animal use.

CHINA

Beginning in 1983, the People's Republic of China enacted the first legislation to regulate the care and use of laboratory animals. In 1988, the Ministry of Science and Technology (MOST) established primary administrative responsibility for laboratory animal science in China with the Statute on the Administration of Laboratory Animals used in research, education, and testing ([Ministry of Science and Technology, 1988](#)). The Statute was approved by China's State Council, and covers licenses for production of laboratory animals as well as regulation regarding control of epidemics, protection of wildlife, and biosafety management requirements for laboratories with microorganisms. The regulation focuses more on laboratory animal quality than welfare. Many provinces, municipalities, and autonomous regions have passed local legislation, creating a Provincial Department of Science and Technology (PDST) responsible for the local Administration Office of Laboratory Animal Science (AOLS). An example of this for Beijing includes the requirement for ethical reviews of animal experiments, specialized training for personnel using animals, and an occupational health and safety program for animal users. It covers both the production and utilization of laboratory

animals and provides for inspections by the Municipal Administrative Department of Science and Technology ([Ministry of Science and Technology, 1988](#)).

The Laboratory Animal Development Program Plan established a national laboratory animal quality control network, which is subject to the Regulation on the Management of Laboratory Animal Quality Control and determines requirements for lab animal quality monitoring ([Ministry of Science and Technology, 1997](#)). Regulation GB14925-2001, *Laboratory Animal—Requirement of Environment and Housing Facilities*, 2001 was issued by China's General Administration of Quality Supervision and mandates requirements for facility construction; separation of animals according to species and strain, disease status, and experimental use; food, water, and bedding quality; quarantine; preventative medicine; and animal transportation ([Conour et al., 2008](#)). In 2006, MOST issued the first broad laboratory animal welfare regulations with the *Guidelines for the Humane Treatment of Laboratory Animals* ([Kong and Qin, 2010](#); Ministry of Education, Culture, Sports, Science and Technology, 2006). The guidelines require each institution to establish an IACUC to oversee and evaluate all aspects of its animal care and use programs as well as covering general requirements, husbandry, experiments, transportation, and methods. The Chinese Association for Laboratory Animal Science (CALAS), established in 1987, is dedicated to the exchange and dissemination of information, expertise, and knowledge about laboratory animal science ([Kong and Qin, 2010](#)).

TAIWAN

In Taiwan, the Animal Protection Law, 1998, provides oversight by the Council of Agriculture (COA). Chapter III of this Act, "Scientific Application of Animals," mandates that the scientific use of animals will minimize pain and requires institutions to establish a management panel to supervise the scientific application of animal experiments. In addition, the central government must establish an ethics committee for laboratory animals to supervise and manage the use of animals in research ([Chen, 2007](#)).

SINGAPORE

In Singapore, the National Advisory Committee on Laboratory Animal Research (NACLAR) was established in 2003 to develop national guidelines for the care and use of animals for scientific purposes in Singapore ([NACLAR, 2004](#)). The NACLAR Guidelines on the Care and Use of Animals for Scientific Purposes were released in October, 2004. The scope of the Guidelines covers all aspects of the care and use of animals for scientific purposes including use in teaching, field trials, environmental studies, research, diagnosis, product testing, and the production of biological

products. The Guidelines promote humane and responsible care and use of animals for scientific purposes in Singapore. In addition, guidelines can be found through the Singapore Association of Laboratory Animal Science.

HONG KONG

In Hong Kong, the *Code of Practice for the Care and Use of Animals for Experimental Purposes* developed by The Hong Kong Animal Welfare Advisory Group of the Agriculture, Fisheries and Conservation Department was published in 2004 (Agriculture Fisheries and Conservation Department of Hong Kong, 2004). This comprehensive document emphasizes the responsibilities of investigators and institutions to assure animal welfare.

CONCLUSION

Using the concepts and principles outlined in this chapter, as well as the references provided for further information, it is hoped individuals will create an approach to develop reasoned arguments for ethical recommendations with respect to the use of animals in research. Because science is so diverse and unique, ambiguous situations may arise as science advances. This makes it important to have performance standards in place, as well as having an ethical framework for implementing those standards.

Tannenbaum (1995) recommends five tests of an acceptable approach to the use of animals in research. Such an approach:

1. Must be as clear as possible in terms of what it says and how it will be applied;
2. Must be internally consistent and coherent;
3. Must be as comprehensive as possible;
4. Must be as simple as possible to be applied without confusion;
5. Must be able to account for the whole range of moral experience.

Normative laboratory animal use ethics must build upon accepted principles yet challenge ordinary beliefs when appropriate.

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Anesthesia and Analgesia

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PRE-ANESTHETIC PREPARATION

Physical Examination

Every animal, no matter how large or small, should have their health assessed before being subjected to any anesthetic protocol. When assessing rabbits and rodents, things to consider include: changes in behavior, appearance, or weight; irregular respiratory patterns; ocular or nasal discharges; and examination of feces and urine in the cage. It is important to initially assess rabbits and rodents in their home cage prior to restraint/manipulation. In addition to the above-mentioned items, rabbits and larger rodents should have their body temperatures measured and their heart and lungs auscultated. Diagnostic tests such as complete blood counts, serum biochemistries, etc., may be warranted depending on the animal's history and/or the nature of the research protocol.

Fasting

Pre-anesthetic fasting for rodents is generally unnecessary unless the experimental procedure requires it. If fasting is warranted, it should be minimized (no more than 2–3 hours) due to the high metabolic rate of small rodents, and water should not be withheld prior to anesthesia. Though there is disagreement regarding pre-anesthetic fasting of rabbits, the recommendation is that rabbits under 3 kg should not have food withheld for greater than 12 hours due to the development of metabolic acidosis and a decline in blood glucose concentration (Bonath et al., 1982). In general, water should not be withheld from rabbits for more than several hours preoperatively (Lipman et al., 2008). Guinea pigs may benefit from a short period (2–3 hours) of pre-anesthetic fasting followed by rinsing of the oral cavity with water using a syringe. This can help decrease the amount of food present within the oral cavity and lessen the risk of aspiration, especially if endotracheal intubation is not being performed.

Stabilization

Transportation stress can affect many physiological systems in laboratory animals and though these changes are short-lived, they may confound research (Obernier and Baldwin, 2006). Therefore, rodents and rabbits should be allowed a minimum stabilization period of 48 h after shipping before being anesthetized and/or used as research subjects (Landi et al., 1982; Toth and January, 1990). This allows acclimation to new environments, restoration of homeostasis, and normalization of physiological status (Obernier and

Baldwin, 2006). Further, this affords the opportunity to evaluate the animals for any abnormalities or clinical signs of disease prior to undergoing anesthesia.

Regulatory Requirements

"The U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training" (1985), the Public Health Service Policy (IV.C.1.b) (PHS, 2002), and The Animal Welfare Regulations (9CFR 2.c.) (Code of Federal Regulations, 2002) require the administration of appropriate sedatives, analgesics, or anesthetics to animals undergoing procedures that cause more than momentary or slight pain or distress, unless withholding of such agents is scientifically justified. "The Guide for the Care and Use of Laboratory Animals" further emphasizes this point by stating "the proper use of anesthetics and analgesics in research animals is an ethical and scientific imperative." In addition to regulatory obligations, laboratory animal professionals have ethical obligations to avoid/minimize pain or distress in laboratory animals (Koch, 2006).

CONTROLLED SUBSTANCES

Due to their potential for abuse and risk of diversion, many drugs commonly used for anesthesia, analgesia, and sedation are classified as controlled substances. The federal government regulates the manufacture, importation, possession, use, administration, and distribution of controlled substances via the Federal Comprehensive Drug Abuse Prevention and Control Act of 1970 also known as the Controlled Substance Act (CSA). The CSA is enforced by the United States Department of Justice and administered through the Drug Enforcement Administration (DEA). State agencies have similar laws and regulations that must be followed. State laws regarding controlled substances may be stricter than their federal counterpart laws but cannot conflict with federal laws.

Controlled substances are classified into five schedules depending upon their medical use, abuse potential, and risk of physical and psychological dependence. As controlled substance schedules increase, the potential for their abuse and dependence decreases. Schedules are defined in the Controlled Substance Act, Chapter 21 U.S.C. 802, the Code of Federal Regulations, 21 C.F.R. 1308, and state controlled substance regulations. Drugs classified as controlled substances are marked with an uppercase C and the schedule number "C-I, C-II, C-III, C-IV, or C-V".

- **Schedule I (C-I):** No medical use accepted. Only authorized for research use (e.g. heroin, LSD, mescaline).
- **Schedule II (C-II):** Accepted medical use. High potential for addiction (e.g. morphine, cocaine, hydromorphone, pentobarbital, fentanyl).
- **Schedule III (C-III):** Accepted medical use. Low to moderate risk of physical dependence. High risk of psychological dependence (e.g. ketamine, tiletamine-zolazepam, buprenorphine).
- **Schedule IV (C-IV):** Accepted medical use. Limited potential for physical and psychological dependence (e.g. diazepam, midazolam).
- **Schedule V (C-V):** Accepted medical use. Very limited abuse potential (e.g. codeine preparations – 200 mg/100 ml or per 100 g).

The Controlled Substance Act creates a closed system to decrease abuse and diversion of controlled substances. A separate DEA registration is required for each principal place of business or professional practice where controlled substances are manufactured, prepared, distributed, stored, or dispensed. In addition, a state-controlled substance license is usually required, yet this varies between each state depending upon specific state laws and regulations. For those states requiring a controlled substance license, the DEA will not grant a registration until a state license is first acquired since the DEA registration is predicated upon the laws and regulations of the state in which the registrant resides. Once registered, an initial inventory must be performed and detailed records must be maintained for at least 2 years from the date of such records (Title 21 C.F.R. 1304) concerning inventory, usage, transfers, and disposal. In addition to an initial inventory, the DEA requires a biennial inventory to be completed. Depending upon the state, additional inventory and record-keeping requirements may apply. Acquisition of controlled substances can only occur between registered entities. Schedule II drugs require the completion of a DEA 222 triplicate form. One copy is sent to the DEA, the supplier, and a copy remains with the purchaser. All DEA 222 forms must be accounted for and returned to the DEA if unused. A prescription cannot be used to obtain controlled substances from a pharmacy for use in a clinic or research facility. Violations of controlled substance regulations are investigated and conducted by local law enforcement, DEA diversion investigators, and state diversion investigators.

When seeking information regarding controlled substance licensing or DEA registration requirements, one should contact the relevant state licensing board and the DEA for information. For additional

information, one can refer to the DEA website at: <http://www.dea.gov/diversion/index.html/>.

PHARMACOLOGY

Anticholinergics

Anticholinergic agents such as atropine and glycopyrrolate inhibit acetylcholine at muscarinic receptors by acting as competitive antagonists within the parasympathetic nervous system. Their main effects upon cardiac, secretory glands and smooth muscle include tachycardia, mydriasis, decreased intestinal motility, decreased salivation and respiratory secretions, decreased gastric acid secretion, and decreased urinary bladder and urethral tone. Their usefulness in anesthesia is aimed at reducing vagal-mediated bradycardia and decreasing salivation or secretions. Their use is not necessary in most rodent surgical procedures but they may be useful in rabbits and guinea pigs in order to decrease salivation and secretions. Although secretions may be diminished they may become more viscous, resulting in airway obstruction (Quesenberry and Carpenter, 2004). Atropine is a naturally occurring belladonna alkaloid tertiary amine, unlike glycopyrrolate, a synthetic anticholinergic agent. Atropine and glycopyrrolate can be administered intravenously, intramuscularly, intratracheally, and subcutaneously.

Atropine may lower the threshold for ventricular dysrhythmias. The route of elimination for atropine is via ester hydrolyzing enzymes, hepatic metabolism, and urinary excretion. The rabbit is a unique species in that some breeds have higher levels of circulating esterase enzymes in their plasma resulting in rapid inactivation and a resistance to toxicity (Ecobichon and Comeau, 1974). As a result, the duration of action of atropine may be reduced requiring more frequent re-dosing when utilized in rabbits (Sedgwick, 1986). High doses should be used cautiously due to the development of tachycardia and possible dysrhythmias. Alternatively, glycopyrrolate can be utilized in rabbits to elevate the heart rate (0.1 mg/kg IM) (Olson et al., 1994). The metabolism of glycopyrrolate is not affected to the same degree as atropine by rabbit esterase enzymes. The duration of action of glycopyrrolate is approximately 2–4 hours while that of atropine is approximately 60–90 minutes (Muir and Hubbell, 1989). Atropine and glycopyrrolate have minimal effects upon blood pressure unless cardiac output is highly dependent upon heart rate. Due to their ability to increase heart rate, thus increasing myocardial oxygen demand, anticholinergics should be used cautiously in cases or conditions where tachycardia is already present or expected such as excitement, hypotension, or shock.

Phenothiazines

Phenothiazine tranquilizers can be utilized as a premedicant to induce a state of tranquility, calmness, or to diminish anxiety or stress. Their main pharmacologic activity results from blockade of dopamine-1 (D_1) and dopamine-2 (D_2) receptors within the mesolimbic dopamine pathway. In addition to their effects upon the dopamine pathway, phenothiazines can block both histamine (H_1) and cholinergic muscarinic receptors leading to sedation, suppression of oral secretions, tachycardia, urinary retention, and constipation. Phenothiazines do not possess analgesic or muscle-relaxant properties. In addition, they are associated with minimal respiratory depression at therapeutic doses (Thurmon et al., 1996), can induce mild to moderate hypotension due to their blockade of peripheral vasculature α_1 receptors, and moderate hypothermia due to depressed temperature regulation (Flecknell, 1996). Phenothiazine agents have been associated with increased risk of seizure development in human medicine and should be used with caution in animal models prone to seizures (e.g. gerbils) or central nervous system research involving electroencephalogram recordings. Recently, several papers have documented no correlation between the use of acepromazine in dogs with a history of seizures and the recurrence of seizures (McConnell et al., 2007; Tobias et al., 2006). Additional studies will need to be performed in order to thoroughly evaluate the safety of phenothiazine use in animals prone to seizures. Phenothiazines are commonly combined with opioids to enhance sedation (neuroleptanalgesia). These agents are primarily metabolized in the liver with metabolites secreted in the urine. There are no available direct reversal agents for phenothiazines. Acepromazine is the most commonly utilized phenothiazine in veterinary medicine and the research setting. It can be used either alone or in combination with other agents for use as a sedative or as a premedicant prior to surgery. Acepromazine can potentiate the effects of other anesthetic agents so a lower dosage of that agent can be used to produce surgical anesthesia (Flecknell, 1996). Acepromazine is commonly used as a sedative and vasodilator to assist in rabbit phlebotomy. Decreased tear production was observed in New Zealand white rabbits administered acepromazine at 1 mg/kg IM (Ghaffari et al., 2009).

Benzodiazepines

Benzodiazepines are sedative-hypnotics used as single agents, in combination with opioid analgesics as a pre-anesthetic medication, or in conjunction with anesthetic induction agents to enhance sedation and muscle relaxation. The sedative effect varies by species and

marked sedation has been shown to occur in rabbits and rodents (Flecknell, 1996). However, some animals may experience excitement upon IV administration (Papich, 2007). In addition to their sedative and muscle-relaxant properties, benzodiazepines possess anticonvulsant properties. However, benzodiazepines are not effective anesthetics when used alone and possess no analgesic properties. Benzodiazepines used for anesthetic purposes include diazepam and midazolam. Both are available in parenteral dosage forms. Benzodiazepines augment the inhibitory actions of gamma-aminobutyric acid (GABA) by binding to the $GABA_A$ receptor thus enhancing chloride ion influx at neuronal synapses (Johnston, 1996). Benzodiazepines have minimal adverse effects upon the cardiovascular system (Kanto, 1985) making them useful agents in compromised or hemodynamically depressed animals. Guinea pigs administered a diazepam-fentanyl combination displayed a significant increase in mean blood pressure above resting level (Brown et al., 1989). Additive or synergistic sedative and muscle-relaxant effects are observed when benzodiazepines are used in conjunction with opioids (neuroleptanalgesia) or N-Methyl-D-Aspartic Acid antagonists such as ketamine (Dupras et al., 2001; Green et al., 1981). Lower doses of each agent in a neuroleptanalgesic combination can be used to decrease adverse effects observed with higher doses of single agents and to prolong the duration of anesthesia or sedation. Diazepam is dissolved in a non-aqueous solution containing propylene glycol, which can cause pain and irritation upon IM or SC injection, or phlebitis upon IV injection into small vessels (Papich, 2007). Hypotension has been reported when administered by a rapid intravenous bolus. Unlike diazepam, midazolam is water-soluble and can be mixed with other anesthetic agents such as ketamine. Due to its improved absorption properties and water solubility, midazolam is preferred when dosing by the subcutaneous or intramuscular route. The duration of action of midazolam is shorter than diazepam. Flumazenil is a specific inhibitor of benzodiazepines. Both midazolam and diazepam are Schedule IV controlled substances.

Alpha₂-Agonists

Alpha₂-agonists such as xylazine and medetomidine are sedative-hypnotic agents that provide sedation, muscle relaxation, and analgesia. Medetomidine is the racemic mixture of the active D-enantiomer (dexmedetomidine) and the inactive L-enantiomer. Medetomidine is no longer commercially available and has been replaced by its racemic isomer dexmedetomidine (Dexdomitor®). Other alpha₂-agonists include romifidine and detomidine, which are generally reserved for use in cattle and horses.

Alpha₂-agonists exert their clinical effects by binding to presynaptic alpha₂ receptors leading to a negative feedback and a reduction in norepinephrine from central and peripheral neurons. Alpha₂ receptors located in the dorsal spinal cord modulate and relay pain transmission (Pan et al., 2008). Alpha₂-agonists are located in the central nervous system, gastrointestinal tract, uterus, kidneys, and platelets (Paddleford, 1999). These drugs vary in their selectivity for the various alpha-receptors. A study by Virtanen et al. (1988) showed that the α_2/α_1 selectivity was as follows: medetomidine (1620:1), detomidine (260:1), clonidine (220:1), and xylazine (160:1). Therefore, medetomidine is the most potent and will require a lower dose for effect than clonidine or xylazine. There will also be fewer α_1 effects with the more selective α_2 agents. Peripheral α_1 adrenoreceptor activation can lead to vasoconstriction, salivation, piloerection, and mydriasis while central α_1 adrenoreceptor activation can lead to increased locomotor activity and arousal (Duteil et al., 1990).

Alpha₂-agonists can be used as single agents to produce sedation and muscle relaxation for facilitating short-term restraint or can be used to provide sedation and analgesia for minor diagnostic procedures.

In addition, alpha₂-agonists have been used as premedicants to anesthesia either as single agents or in combination with opioids or tranquilizers in healthy animals with minimal cardiovascular or respiratory dysfunction (Ko et al., 1992). When administered with opioids profound sedation and enhanced analgesia are common. The risk of bradycardia is increased with this combination due to enhanced effects upon vagal tone secondary to G-protein-coupled receptor synergy. Administration of anticholinergic agents to reduce the occurrence of bradycardia is generally not recommended as it has been shown to cause dysrhythmias (Short, 1991), hypertension, and increase myocardial oxygen demand and workload (Monteiro et al., 2009). If the bradycardia is severe and placing the patient at risk, the most appropriate course of action would be to reverse the α_2 agonist with an α_2 adrenoreceptor antagonist (Pypendop et al., 1998; Sinclair, 2003).

The sedative effects, analgesic, and muscle-relaxant properties of alpha₂-agonists make them useful adjuncts when combined with a N-methyl-D-aspartic acid (NMDA) antagonist such as ketamine for producing short-term immobilization and surgical anesthesia (Difilippo et al., 2004; Henke et al., 2005; Marini et al., 1992; Mero et al., 1989). In rabbits, the combination of ketamine and medetomidine produced surgical anesthesia of medium duration (Hellebrekers et al., 1997). Orr et al. (2005) showed that a medetomidine-ketamine dose of 0.25/15mg/kg with supplemental isoflurane provided sufficient anesthesia for orchiectomy or ovari-hysterectomy. Intramuscular administration was

associated with more rapid onset of anesthesia but a higher level of discomfort in the rabbit than subcutaneous administration. Due to the respiratory depression seen with this combination, the authors advised intubation so supplemental oxygen could be provided. The medetomidine was reversed with atipamazole but it should be noted that this will also reverse the analgesic properties as well. Hedenqvist et al. (2001) also showed that a medetomidine-ketamine dose of 0.25/15mg/kg would provide 15–30 minutes of surgical anesthesia. In contrast to rabbits, a medetomidine-ketamine dose of 0.5/40mg/kg only provided immobilization in guinea pigs and did not produce adequate surgical anesthesia (Nevalainen et al., 1989). Dang et al. (2008) compared the level of anesthesia provided by ketamine-xylazine (30/2.5mg/kg IM, SC, and IP), medetomidine (0.5mg/kg IM), and pentobarbital (37mg/kg IP) in guinea pigs. Ketamine-xylazine was found to produce reliable anesthesia for a 45-minute procedure, while medetomidine alone was found to induce sedation but not anesthesia. Henke et al. (2004) compared several anesthetic regimens in chinchillas and showed that ketamine-xylazine (40/2.0mg/kg IM) produced the longest period of surgical anesthesia followed by medetomidine-ketamine (0.06/5.0mg/kg IM), and finally midazolam-medetomidine-fentanyl (1.0/0.05/0.02mg/kg IM). However, recovery time was shortest in the latter two groups. The maintenance requirement for inhalant anesthetic agents is generally drastically reduced when used in conjunction with alpha₂-agonists.

Yohimbine and atipamazole are alpha₂-antagonists used to reverse the effects of xylazine and medetomidine or dexmedetomidine respectively (Lipman et al., 1987). A study by Virtanen et al. (1989) showed an α_2/α_1 receptor selectivity of 8526:1 for atipamazole and 40:1 for yohimbine. Therefore, atipamazole is a more potent α_2 receptor antagonist than yohimbine and is the preferred antagonist for medetomidine and dexmedetomidine. Administration of either atipamazole or yohimbine IV can induce hypotension and tachycardia (Maze et al., 1991) so IM administration is recommended.

Side effects of alpha₂-agonists include an initial increase in systemic vascular resistance in peripheral tissues leading to a reflex bradycardia followed by a centrally mediated decrease in cardiac output and subsequent hypotension. Due to potential cardiovascular adverse effects alpha₂-agonists should not be used in hemodynamic or cardiac compromised animals (Greene, 2002). However, in certain heart failure models (e.g. ventricular outflow obstruction due to hypertrophic cardiomyopathy), the bradycardia and increased afterload that occurs with the use of alpha₂-agonists may be beneficial to the animal (Lamont et al., 2002). Xylazine has variable effects on the respiratory system while medetomidine is associated with significant

respiratory depression when used at higher doses or in conjunction with other tranquilizers, opioids, or anesthetics (Paddleford, 1999). As a result, supplemental oxygen is advised with the use of α_2 -agonists. Chang et al. (2009) showed that dexmedetomidine produced less ventilatory depression in rabbits when combined with sevoflurane than propofol or midazolam but induced more hypotension and bradycardia. Henke et al. (2005) showed that in rabbits, medetomidine/ketamine administration resulted in a transient hypertension but the mean arterial pressure (MAP) decreased to pre-anesthetic levels. This was significantly higher than the MAP for the xylazine-ketamine group of rabbits.

Systemic Analgesics

Opioids

Opioid analgesics are useful agents for treating mild to severe pain. In addition to their analgesic properties, opioids can provide sedation and be used in balanced anesthetic techniques to augment injectable or inhalant anesthetics. As a result, lower anesthetic doses of each agent can be utilized, thus decreasing adverse side effects associated with higher doses of a single agent. Opioid analgesics mimic the response of endogenous opioid peptides such as endorphins and enkephalins and mediate descending inhibition of pain-modulating pathways within the brain and dorsal horn of the spinal cord.

Opioids act upon G-protein-coupled receptors, which share significant homology. Three opioid receptor types, mu, kappa, and delta, have been identified (Mansour et al., 1995). In addition, studies in receptor knockout mice have shown that all three opioid receptors can mediate antinociception (Kieffer and Gaveriaux-Ruff, 2002). In general, binding of an opioid analgesic agent to a G-protein-coupled receptor activates second messenger pathways causing hyperpolarization of neuronal cells, thus increasing their threshold for activation. Although complicated cell signaling events occur secondarily to the activation of opioid receptors, the end result is to provide analgesia, sedation, and modulate pain nociception.

Opioid analgesics differ in their opioid receptor affinity and intrinsic activity. Opioids are usually classified as agonists, partial agonists or agonist-antagonists, and antagonists. Full agonist agents are useful for treating moderate to severe pain. Partial agonists are best suited for conditions associated with mild to moderate pain or where extra sedation is needed. Opioids may differ in potency, yet may have similar intrinsic activity or analgesia activity. For example, the dose of hydromorphone is seven times less compared to morphine, yet both agents provide equivalent analgesia.

Moderate to severe pain is best treated with full agonist opioids that bind primarily to mu receptors. For most species, activation of these receptors provides

excellent analgesia, sedation, and euphoria, however, this can be species- and strain-specific. Commonly used opioid agents for treating moderate to severe pain that bind to mu opioid receptors include morphine, hydromorphone, fentanyl, and oxymorphone.

Buprenorphine is classified as a partial agonist or agonist-antagonist. It has partial agonist actions at the mu receptors and antagonist actions at the kappa and delta receptors. Buprenorphine has a slightly longer onset of action and longer duration of action compared to other opioids. As a partial agonist, buprenorphine has an analgesic and respiratory depression ceiling effect. As a result, it is useful for providing mild to moderate analgesia and sedation. Depending upon the species and type of surgery, severe pain may require treatment with a full agonist such as morphine, fentanyl, or hydromorphone. However, when buprenorphine is used prior to surgery it can prolong anesthesia and decrease the dosage of anesthetic that needs to be used. Murphy et al. (2010) demonstrated that surgical anesthesia with medetomidine-ketamine was prolonged when rabbits received 0.03 mg/kg of buprenorphine SC 1 hour before anesthesia. Shafford and Schadt (2008) showed that if given (0.06 mg IV) prior to colorectal distension in rabbits, it decreased the animal's response to this noxious stimulus. They therefore concluded that it is an acceptable agent for visceral analgesia if used preemptively. Its high affinity and long duration of action require potentially larger and more frequent dosing with an antagonist such as naloxone for reversal purposes. In addition, supplemental analgesia may require higher doses of a full agonist opioid to compete with the binding properties of buprenorphine. Side effects in rodents and rabbits can include ileus, pica, and loss of appetite (Flecknell et al., 2000).

Butorphanol is classified as an agonist-antagonist with antagonist actions at the mu receptors and agonist actions at the kappa receptors. As an agonist-antagonist it has an analgesic and respiratory depression ceiling effect. It is useful for providing mild to moderate analgesia and sedation. Butorphanol is a useful agent for providing partial reversal to reestablish breathing parameters, yet not completely reverse analgesia. Portnoy and Hustead (1992) determined the half-life of butorphanol in rabbits to be 1.64 hours after IV administration and 3.16 hours after SC administration. Therefore, this drug requires more frequent re-dosing if used for post-operative analgesia.

Fentanyl is a potent mu receptor agonist and has a rapid onset and short duration of action. It can be administered via a constant rate infusion during surgery and the post-operative period. Fentanyl patches can also be used in rabbits. Plumb (2005) recommends use of half of a 25 µg/hour patch for a 3-kg rabbit every

3 days for post-operative analgesia. The patch must not be cut but the half that is not in use should be covered with tape. The fur along the lateral thorax should be clipped close (the site should not be shaved and depilatory agents should not be used as this will alter fentanyl absorption) with a 1-cm margin and the patch applied to the skin. The patch should be held in place with a gloved hand to improve the adhesive bond to the skin and covered with a clear, adhesive bandage (Plumb, 2005). Weight and appetite should be carefully monitored in rabbits when using fentanyl patches (Foley et al., 2001). Along with inhalant anesthetics, constant rate infusions of fentanyl can be used in conjunction with propofol or ketamine infusions. Due to the rapid clearance of fentanyl, recoveries are generally rapid and smooth.

Morphine is an agonist at the mu, kappa, and delta receptors. It is the opioid most likely to cause GI side effects, especially in rabbits and guinea pigs. Therefore it is best to use this drug only once prior to surgery or to use it as an epidural agent. The kappa receptors are thought to be responsible for its efficacy as an epidural. If given as an epidural, the preservative-free (PF) solution should be used. This can be an excellent technique to provide analgesia for abdominal and hindlimb procedures in rabbits. The analgesia will last for about 12–24 hours and side effects are minimal. Morphine can be combined with a local anesthetic, such as lidocaine or bupivacaine (Gaynor and Muir, 2009). The rabbit should be placed in ventral or lateral recumbency and the coxofemoral and lumbosacral joints hyperflexed to open up the lumbosacral space. The landmarks for this technique include the wings of the ilium and the dorsal prominence of the first sacral vertebrae (Gaynor and Muir, 2009). The spinal cord in rabbits does continue through the sacral vertebrae so care should be taken when performing a lumbosacral epidural as the spinal cord can be punctured accidentally (Greenaway et al., 2001).

Opioids can be used in combination with inhalant anesthetics to provide additional analgesia and sedation. This usually allows for lower concentrations of inhalant agents to maintain anesthesia and reduces inhalant-associated hypotension.

Side effects of opioids vary depending upon the receptor activity and species. Main side effects include respiratory depression, mild hypoxemia, vagal-induced bradycardia, decreased intestinal motility, and urinary retention (Shafford and Schadt, 2008). Opioids can be combined with an α_2 -agonist or non-steroidal anti-inflammatory agents for additive analgesia effects. When combined with α_2 -agonists profound respiratory depression and bradycardia is commonly observed. As a result, lower doses of each agent should be utilized and α_2 -antagonists administered in emergency situations (i.e. severe bradycardia that is

life-threatening). Gut stasis is often mentioned, especially for rabbits and guinea pigs, as a side effect of opioid use but a study by Cooper et al. (2009) showed no difference in food consumption, weight, or fecal production between rabbits given buprenorphine (0.03 mg/kg IM q 12 hours for 48 hours) and meloxicam (0.2 mg/kg SC q 24 hours for 48 hours) post-operatively. However, when using these agents, close post-operative monitoring should be performed.

Depending upon their intrinsic activity and potential for abuse, the majority of opioids are controlled substances with schedules ranging from II–IV. Schedule II opioids used for anesthesia, sedation and analgesia include morphine, hydromorphone, fentanyl, and oxymorphone. Buprenorphine is classified by the DEA as a Schedule III agent, while butorphanol is classified by the DEA as a Schedule IV agent.

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

Non-steroidal anti-inflammatory drugs exert their effects via inhibition of cyclooxygenase-derived inflammatory eicosanoids known as prostaglandins and thromboxane. NSAIDs inhibit the enzyme cyclooxygenase (COX) which catalyzes the production of prostaglandins, prostacyclins, and thromboxane A_2 from arachidonic acid. There are currently two recognized isoforms of cyclooxygenase, COX-1 and COX-2. A third COX isoform (COX-3) has been described and found to be expressed by various tissues but its function remains unknown at this time (Hersh et al., 2005). COX-1 is a constitutive enzyme responsible for normal physiologic functions such as maintaining a gastro-protective mucosal barrier and renal blood flow, while the COX-2 enzyme is induced and responsible for producing prostaglandin mediators associated with swelling, fever, and pain. However, it has been shown that completely blocking COX-2 can be harmful to some normal physiologic functions. Although considered equally efficacious for treating pain, COX-2 inhibitors (COX-1 sparing) are generally preferred over COX-1 NSAIDs due to their potential gastro-renal protective effects. COX-1 versus COX-2 selectivity may vary depending upon species, enzyme location, and dose (Bergh and Budberg, 2005). The selectivity is assessed by studying the concentration of the drug that is needed to block each COX isoenzyme (Gaynor and Muir, 2009). Examples of non-selective COX inhibitors are aspirin, ketoprofen, and ketorolac. Examples of drugs with COX-2-selective inhibition include carprofen, etodolac, meloxicam, and deracoxib. Few studies have been done investigating the use of these medications in the species covered by this book, but clinical experience seems to suggest that the COX-2-selective inhibitors produce fewer side effects in rabbits, guinea pigs, and other small mammals. Several studies have investigated the use of meloxicam

in rabbits. [Cooper et al. \(2009\)](#) showed no significant difference in body weight, food consumption, or fecal production between the meloxicam-treated group (0.2mg/kg SC q 24 hours) and the buprenorphine-treated group (0.03mg/kg IM q 12 hours) after undergoing ovariohysterectomy. Both of these groups did better post-operatively than the group that received only incisional infiltration with bupivacaine. [Carpenter et al. \(2009\)](#) documented a half-life of approximately 8 hours in rabbits after giving meloxicam at 0.2mg/kg orally. However, [Leach et al. \(2009\)](#) showed that only high-dose oral meloxicam (1mg/kg initially, followed by 0.5mg/kg/day) induced some degree of analgesia after ovariohysterectomy in rabbits. This may indicate that parenteral administration (SC) of meloxicam is more efficacious post-operatively, possibly due to decreased appetite and therefore differences in absorption of the drug from the gastrointestinal tract. Further studies are needed to better assess dosages and routes of administration in rabbits and other small mammals.

The main side effects associated with NSAID usage are gastric ulceration, renal disease, and platelet inhibition. The COX-2-selective drugs show less of a tendency to cause renal disease and platelet inhibition than the non-selective COX inhibitors ([Gaynor and Muir, 2009](#)). Due to their inhibition of prostaglandins, which could alter vasodilation, NSAIDs should be avoided in patients with preexisting renal disease or dehydration. Furthermore, NSAIDs should be used with caution in patients with bleeding disorders, expected surgical hemorrhage, or gastric ulceration. Carprofen has been shown to be safe to administer pre-operatively to dogs, even those that had changes in renal function and hemostasis due to trauma ([Bergmann et al., 2005](#)).

Tramadol

Tramadol is a centrally acting analgesic that binds to mu receptors, but is not related to opioids, and inhibits uptake of norepinephrine and serotonin ([Gaynor and Muir, 2009](#)). It can be used for mild to moderate pain control and has increased efficacy when combined with an NSAID. Side effects are usually gastrointestinal in nature (e.g. constipation, diarrhea, nausea) but are relatively infrequent. [Souza et al. \(2008\)](#) examined the pharmacokinetics of orally administered tramadol (11mg/kg once) in rabbits. No adverse effects were observed in healthy animals and the half life was 145.4 minutes while the mean maximum plasma concentration was 135.3ng/mL. This is well below the level required to produce analgesia in humans so additional studies need to be performed to determine whether this dose or higher dosages would provide sufficient post-operative analgesia in rabbits as well as whether addition of an NSAID improved analgesic efficacy. A study by [Egger et al. \(2009\)](#) showed a significant

but clinically unimportant decrease in the minimum alveolar concentration (MAC) of isoflurane in rabbits given IV tramadol (4.4mg/kg over 1 minute). They caution against using higher IV doses during anesthesia as some cardiovascular depression was observed at this dosage. Finally, a study by [Herbert et al. \(2007\)](#) showed that the most potent metabolite of tramadol (O-desmethyltramadol) did inhibit peristalsis in the guinea pig small intestine. However, in vivo studies in guinea pigs using tramadol need to be performed to determine the relevance of this observation.

Gabapentin

Gabapentin is a structural analog of gamma-aminobutyric acid (GABA) and was originally used as an antiepileptic drug ([Gaynor et al., 2009](#)). Its mechanism of analgesic action is unknown as it does not appear to have activity at GABA, NMDA, or opioid receptors or modulate COX activity or dopamine, serotonin, or norepinephrine uptake ([Gaynor and Muir, 2009](#)). It seems to be most useful for pain related to neuropathic conditions, osteoarthritis, or neoplasia. Like tramadol, it seems to be most effective when used in a multimodal approach (e.g. gabapentin + NSAID). Side effects reported in humans include sleepiness, fatigue, and weight gain after long periods of use ([Gaynor and Muir, 2009](#)). A study by [Fox et al. \(2003\)](#) used oral gabapentin in guinea pigs following partial ligation of the left sciatic nerve. They demonstrated good reversal of neuropathic mechanical hyperalgesia when gabapentin was dosed twice daily but poor reversal when it was dosed once daily. Further studies are needed to determine optimum dose and dosing interval.

Amantadine

Amantadine was originally used as an anti-viral agent but has been shown to have antagonist effects at the NMDA receptor. Like gabapentin, it has been used in humans for treatment of neuropathic pain. In dogs and cats, it has been shown to reduce windup and allodynia, allowing a reduced dosage of opioids to be used while improving their analgesic action ([Gaynor and Muir, 2009](#)). It also increases the efficacy of NSAIDs in dogs with osteoarthritis. Side effects in dogs include anxiety, restlessness, and dry mouth. Caution should be used when administering to patients with renal impairment as it is excreted in the urine. There have not been any studies done on the use of amantadine in the species covered by this book but like gabapentin, it may prove useful in animals used in neuropathic pain studies.

Local Anesthetics

Local anesthetics are used peripherally in order to block transmission from nerve endings or fibers via inhibition of sodium channels within the nerve membrane

(Gaynor and Muir, 2009). The susceptibility of nerves to these agents depends on size and myelination and usually follows the listed order: pain, warmth, touch, deep pressure, and motor function. The most common agents used include: lidocaine (60–120-minute duration of action) and bupivacaine (180–480-minute duration of action). Local anesthetics can be used topically (e.g. EMLA cream), as an epidural (usually combined with morphine PF), as an incisional line block, as a continuous infiltration along an incision via a catheter (common after thoracotomies), or in various peripheral nerve blocks (e.g. intercostal, intra-articular). Peripheral nerve blocks require good knowledge of the local anatomy in order to be performed correctly. Local anesthetics can be absorbed systemically and can lead to unwanted side effects, such as cardiac dysrhythmias (Gaynor and Muir, 2009).

If a catheter is going to be placed prior to anesthesia or if a blood sample needs to be drawn, a eutectic mixture of lidocaine and prilocaine (EMLA) cream can be applied topically to the ear or foreleg to facilitate placement and improve comfort in rabbits. For maximum efficacy, this cream needs to be applied to a shaved surface, approximately 60 minutes before venipuncture, and covered by an occlusive dressing for the 60-minute period (Flecknell et al., 1990).

Glucocorticoids

Glucocorticoids are mainly used post-operatively for their anti-inflammatory effects. These are achieved via inhibition of phospholipase A2 which is the precursor of arachidonic acid. This leads to decreased production of prostaglandins and leukotrienes, which prevents lowering of the nociceptive threshold (Gaynor and Muir, 2009).

Glucocorticoids can be used as a multi-modal approach to analgesia post-operatively but also can be used intra-operatively (e.g. intra-articular administration, epidural administration, wound irrigation).

Caution should be used when administering glucocorticoids as there are many potential adverse effects, including: delayed wound healing, gastric ulceration (do not combine with NSAIDs; rabbits are especially susceptible and if needed, a gastric protectant should also be administered), electrolyte and fluid imbalances, and immunosuppression.

N-Methyl-D-Aspartic Acid (NMDA) Antagonist

NMDA antagonists such as ketamine or tiletamine cause a catatonic or dissociative state characterized by analgesia, amnesia, immobility, and a detachment from surroundings. The animal's eyes may remain open and variable hypertonic contractions and gross muscle movement may occur independent of surgical stimulation. Pharyngeal reflexes are maintained as well as spontaneous breathing so animals are more prone to

laryngospasm upon intubation. This can be mitigated by use of another anesthetic agent or use of a topical anesthetic (e.g. lidocaine, cetacaine). Since ocular and pharyngeal reflexes are retained, or attenuated less, with ketamine than with other anesthetic agents, this can make traditional monitoring of anesthetic depth, using observation of physical signs, misleading.

Ketamine

Ketamine, is a water-soluble phencyclidine derivative available commercially as a racemic mixture. It possesses analgesia activity due to its ability to block pain impulses to cortical-thalamic regions of the brain. Ketamine is generally administered by intravenous, intraperitoneal, or intramuscular routes of injection. Due to its low pH, pain and irritation can be observed with intramuscular injection leading to self-mutilation (Beyers and Richardson, 1991). Ketamine undergoes hepatic metabolism to norketamine with renal and biliary excretion of metabolites.

Ketamine increases the sympathetic tone causing increases in heart rate, cardiac contractility, cardiac output, pulmonary arterial blood pressure, systemic vascular resistance, and myocardial oxygen demand. Ketamine should be avoided in conditions associated with high sympathetic tone and depleted catecholamine stores such as shock or with conditions such as hypertrophic cardiomyopathy due to its ability to cause diminished cardiac output and hypotension (Greene, 2002).

High doses of ketamine may be required for surgical anesthesia when ketamine is used alone (Green et al., 1981). Its use is associated with poor muscle relaxation and rough recoveries. Ketamine is generally used in combination with a benzodiazepine such as diazepam or midazolam or with an α_2 -agonist such as xylazine, medetomidine, or dexmedetomidine. In hamsters, intraperitoneal ketamine doses of 50–200 mg/kg combined with xylazine 10 mg/kg provided general anesthesia (Curl and Peters, 1983). The addition of an α_2 -agonist to ketamine augments analgesia and prolongs the duration of surgical anesthesia, yet is associated with dose-dependent bradycardia, respiratory depression, hypoxemia, and acidemia in rabbits (Hobbs et al., 1991; Lipman et al., 1990; Marini et al., 1992; Peeters et al., 1988; Popilskis et al., 1991; Sanford and Colby, 1980; Wyatt et al., 1989). In guinea pigs, ketamine–xylazine was associated with minimal respiratory depression, yet was associated with reductions in blood pressure and heart rate (Schwenke and Cragg, 2004). Supplemental oxygen is recommended. A study in rats by Olivar et al. (1999) demonstrated that ketamine was an effective analgesic for acute visceral nociceptive stimulation but was only effective on somatic pathways when injury or inflammation was present. A similar study in humans (Strigo et al., 2005) demonstrated analgesic efficacy for acute visceral pain. While

there was a decrease in the unpleasantness associated with acute cutaneous pain there was no change in the intensity noted by the subjects. In rabbits, the combination of xylazine–acepromazine–ketamine produced longer surgical anesthesia, yet was associated with severe hypothermia (Hobbs et al., 1991).

Additional side effects include atropine-responsive salivation, apneustic breathing (prolonged pause following inspiration), and emergence delirium characterized by ataxia, increased motor activity, hyper-reflexia, muscle rigidity, and agitation. Rough recoveries can be ameliorated with a benzodiazepine, phenothiazine tranquilizer, opioid, or α_2 -agonist. When used as an induction agent ketamine is commonly administered with diazepam.

Ketamine is a unique agent as it antagonizes the NMDA receptor, a glutamate excitatory receptor associated with dorsal horn neuronal sensitization, hyperalgesia, allodynia, and the temporal summation (wind-up) that is often associated with chronic pain. Ketamine and tiletamine–zolazepam are Schedule III controlled substances.

Tiletamine–Zolazepam

Tiletamine–zolazepam (Telazol®) is an NMDA antagonist–benzodiazepine tranquilizer combination of equal amounts with the dose based upon the combined weight of both drugs. Tiletamine is more potent and has a longer duration of action compared to ketamine. Tiletamine–zolazepam is used as an alternative to ketamine combinations (ketamine + opioid, benzodiazepine, or α_2 -agonist) to produce immobilization, sedation, or surgical anesthesia for minor to moderate procedures. Depending upon the dose and species, tiletamine–zolazepam may be associated with prolonged recoveries. Ocular, laryngeal, pharyngeal, and pedal reflexes remain intact. Side effects associated with tiletamine–zolazepam include dose-dependent decrease in cardiac output and hypotension.

In rabbits, tiletamine–zolazepam has been associated with renal toxicity when used at surgical anesthetic doses (Brammer et al., 1991). Renal toxicity was linked to the tiletamine component. When used at lower doses in combination with xylazine, tiletamine–zolazepam produced mean surgical anesthesia duration of 72 minutes versus 35 minutes for xylazine–ketamine with no nephrotoxicity observed (Doerning et al., 1992). In guinea pigs, tiletamine–zolazepam doses up to 50 mg/kg produced short-term immobilization, but inadequate surgical anesthesia (Radde et al., 1996). In hamsters, anesthesia but not analgesia was achieved at doses of 50–80 mg/kg (Silverman et al., 1983).

Propofol

Propofol is an intravenous sedative hypnotic agent solution used for the induction of general anesthesia,

endotracheal intubation, or maintenance of anesthesia. Propofol is not water-soluble and is commercially available as an oil-in-water emulsion. Due to the potential to support bacterial growth, propofol preparations should be immediately used within 6–12 hours of opening. This recommendation can vary by manufacturer so the package insert should always be consulted prior to reconstitution and use. Propofol has high lipid solubility and a rapid onset of action. Propofol is efficiently metabolized and has a clearance rate with minimal cumulative effects. As a result, propofol infusions are associated with rapid recovery and minimal residual central nervous system (CNS) effects.

Propofol use is best reserved for anesthesia induction or for maintaining unconsciousness for short procedures via intermittent boluses. A constant rate infusion via an infusion pump must be utilized for longer procedures or surgeries. Propofol does not possess analgesic properties and an analgesic should be used when performing procedures associated with intra-operative or post-operative pain. Premedication with a tranquilizer, an opioid, or an α_2 -agonist augments the sedative actions of propofol and reduces the dose or infusion rate needed to maintain surgical anesthesia (Hellebrekers et al., 1997; Ko et al., 1992; Luo et al., 1995). Propofol has been combined with opioids to provide total intravenous anesthesia (De Mulder et al., 1997).

Propofol is associated with rapid CNS depression and unconsciousness. Intraocular pressure, cerebral blood flow, and intracranial pressure are reduced with propofol. Dose-dependent respiratory depression is observed with the development of apnea during induction or after large doses. Additional side effects include hypotension secondary to vasodilation, decreased cardiac contractility, and diminished cardiac output. Hypotension is greater with large doses, rapid infusion, and pre-existing hypovolemia. Injection site pain, twitching, and myoclonus can occur, especially in non-pre-medicated patients. Due to the potential for respiratory depression and apnea, supplemental oxygen should be provided before and during a propofol infusion. Endotracheal intubation is recommended.

Because of the lack of easy venous access, propofol is reserved for larger species such as rabbits. However, propofol can be administered to smaller species such as guinea pigs or chinchillas via an intraosseous catheter (Lichtenberger and Ko, 2007). In rabbits, hypotension and hypoxemia were reported along with smooth inductions and rapid or slow recoveries depending upon the duration of anesthesia (Aeschbacher and Webb, 1993). However, propofol seems to be less effective in rabbits than other species (Flecknell, 1996). They require very high doses (>10 mg/kg IV) to even produce sedation but these doses provide little reflex depression or antinociception. Use of propofol as a continuous rate

infusion (CRI) in rabbits may lead to tolerance and eventually fatal propofol infusion syndrome after extended use (>26 hours) (Glen, 1980; Ypsilantis et al., 2006, 2007, 2009). There was no improvement in effect when propofol phosphate, a water-soluble propofol pro-drug was used. A dose of 64.5mg/kg IV only produced slight ataxia and a dose of 150mg/kg IV only produced only mild sedation prior to death at 158 minutes post-injection (Banaszczyk et al., 2002). Propofol should be used very cautiously in rabbits.

Initial studies involving intraperitoneal propofol in mice have shown variability in sedation and surgical anesthesia response. Propofol administered intraperitoneally either as a single agent or in combination with potent opioids did not maintain adequate sedation or anesthesia in mice (Alves et al., 2007). However, when combined with an opioid- α_2 -agonist combination, propofol produced short-term anesthesia with a fast recovery (Alves et al., 2009). It is unknown at this time whether similar effects would be observed in guinea pigs, hamsters, gerbils, and other small rodents administered propofol by the intraperitoneal route, though it seems appropriate that such use should be undertaken with caution.

Barbiturates

Pentobarbital is an oxybarbiturate analog of barbituric acid. Pentobarbital can be utilized as a sedative-hypnotic, anesthetic, and anticonvulsant. Pentobarbital's mechanism of action is similar to that of benzodiazepines and propofol in that GABA_A receptors are activated resulting in enhanced GABA binding and opening of transmembrane chloride channels leading to cellular hyperpolarization within the central nervous system. Administration of pentobarbital produces dose-dependent sedation, hypnosis, muscle relaxation, and depression of the sensory cortex and reticular activating system.

As with all GABA_A agonists, pentobarbital has little or no analgesic activity. Pentobarbital produces dose-dependent respiratory depression which may require respiratory assistance (Peeters et al., 1988). At higher doses, pentobarbital possesses anticonvulsant and hypotensive properties. Hypotension results from vasodilation, diminished myocardial contractility, and reduced cardiac output. These and other cardiovascular effects depend upon the route of administration and tend to be less pronounced with IP rather than IV administration. This is due to the fact that peak blood concentration is reached more slowly than with IV administration and the portion of drug absorbed into the portal system is subject to early destruction in the liver. Hypothermia is common and has been reported in gerbils (Weinandy et al., 2005). Hypothermia is most likely a result of decreased basal metabolism and vasodilation. Additive

sedation and respiratory depression can occur if pentobarbital is administered in conjunction with tranquilizers, benzodiazepines, opioids, α_2 -agonists, propofol, and inhalant anesthetic agents. Animals administered pentobarbital should be supplied supplemental oxygen or monitored with pulse oximetry. General anesthetic doses may require respiratory support or endotracheal intubation.

When pentobarbital is administered, a supplemental heat source should be applied to the animal during the surgical period and continued until full recovery to prevent hypothermia. Pentobarbital can be administered intraperitoneally or intravenously. When used as a general anesthetic, pentobarbital can be administered as a bolus (Borkowski et al., 1990) or CRI. Commercial solutions commonly contain propylene glycol, which can cause pain upon injection and thrombophlebitis. For this reason, the subcutaneous and intramuscular routes of injection are not recommended.

Inhalation Anesthetics

Isoflurane and sevoflurane are volatile inhalant anesthetics with low blood/gas solubility in blood and body tissues. Both agents are dose-dependent CNS depressants used for induction and maintenance of general anesthesia. Due to their intrinsic low solubility, rapid inductions, rapid changes in anesthetic depth, and fast recoveries are associated with both agents. Both isoflurane and sevoflurane are vasodilators and cause a dose-dependent decline in systemic vascular resistance and blood pressure. In addition, dose-dependent respiratory depression is observed with both. Cardiac output decreases when higher concentrations are used.

Sevoflurane and isoflurane showed similar effects upon intracranial pressure and cerebral blood flow in rabbits while the cranial cerebral metabolic rate for oxygen was reduced nearly 50% with both agents at an end-tidal 1.0 MAC (Scheller et al., 1988).

Isoflurane and sevoflurane require separate vaporizers since vaporizers for both agents must be calibrated to the specific vapor pressure of each anesthetic agent.

Isoflurane and sevoflurane undergo minimal hepatic metabolism and elimination is primarily through alveolar exchange. Direct renal-hepatic effects are not observed with clinically used concentrations of isoflurane or sevoflurane. In Wistar rats, sevoflurane was reported to react with soda lime to produce a nephrotoxic compound, (olefin-compound A) (Gonsowski et al., 1994a, 1994b). This has not been clinically observed in humans, dogs, and horses and is very unlikely to be a problem with the fresh gas flow rates used in non-rebreathing circuits (Gentz and Malan, 2001; Kharasch, 1998; Muir and Gadawski, 1998). Isoflurane and sevoflurane are not listed as controlled substances.

Inhalant agents are dosed to effect or by using their minimum alveolar concentration (MAC), the concentration that will inhibit gross purposeful movement in 50% of patients. MAC is a measure of potency, but not intrinsic efficacy. In general, agents with higher potencies have a lower MAC. A recent study in gerbils showed the isoflurane MAC was lower compared to the sevoflurane MAC ($1.55 \pm 0.08\%$ versus $2.90 \pm 0.12\%$), yet cardiac and respiratory parameters were not significantly different between the two agents (de Segura et al., 2009).

In humans, sevoflurane is reported to be non-pungent, less irritating, and more pleasant smelling than isoflurane, making it the preferred agent for mask inductions (Goa et al., 1999). However, this effect was not observed in rabbits who were reported to struggle and breath hold during inductions with sevoflurane and isoflurane (Flecknell et al., 1999). Mask or inhalant induction anesthetic techniques should not be used in cardiovascular-compromised or debilitated animals since the higher concentrations can severely affect cardiac output and respiratory function. Moreover, non-pre-medicated or non-sedated rabbits should not be induced via facemask due to the risk of injury secondary to struggling upon induction. For this reason, a pre-medication to enhance sedation and the use of an induction chamber is recommended if injectable agents will not be used to induce anesthesia.

Since induction times with inhalant anesthetics are generally slower than intravenous agents such as propofol and ketamine, struggling, breath holding, and excitement may occur. Due to their minimal effects upon the cardiovascular system, a benzodiazepine, opioid, or benzodiazepine–opioid combination can be used as a premedication to provide sedation and muscle relaxation prior to induction with an inhalant anesthetic, resulting in a shorter and smoother induction (Mutoh, 2007; Mutoh et al., 2001). In addition, lower inhalant concentrations should be used initially with facemasks or induction chambers and gradually increased as the animal loses its fighting reflex. Due to the higher concentrations used for facemask or chamber induction, a down-draft table or fume hood should be used with induction chambers to minimize personnel exposure to anesthetic vapors.

Disadvantages to the use of inhalant anesthetics include initial investment of equipment including a calibrated vaporizer, endotracheal tubes, breathing circuits, induction chambers, facemasks, and waste gas scavenging equipment. Waste gas exposure must be kept below the National Institute for Occupational Safety and Health (NIOSH) standard of 8 parts per million (ppm) per 8-hour time period. The advantages of using inhalant anesthesia far outweigh any disadvantages when considering moment-to-moment control

of anesthetic depth and faster recoveries. In the authors' opinion, with proper monitoring, complications leading to morbidity and mortality are reduced with inhalant anesthesia compared to injectable anesthesia. In addition, less recovery and post-operative monitoring time are required with inhalant anesthetic use allowing for allocation of resources and efficient workflow practices.

ANESTHESIA CIRCUITS

Induction Chambers

Induction chambers are widely used for the anesthetic induction of small and difficult-to-handle research animals. The major feature of induction chambers is very minimal or slight rebreathing of expired gases, therefore expired carbon dioxide is removed by dilution. The approximate fill time for the chamber can be estimated via the following equation: chamber volume/flow rate = time to fill (Flecknell, 1996). This time to fill (τ) value can be used to control and predict the concentration of anesthetic gas within the induction chamber. This rate of change is a function of the exponential wash-in and wash-out function (Meyer, 2008). Advantages of induction chambers include: low-cost equipment that facilitates sedation of animals without handling (Figure 2.1) and they have minimal resistance to breathing. Disadvantages of this type of induction system include: a large amount of the anesthetic gas is vaporized, the anesthetic gas may be irritating for the animal (it may be useful to apply eye lubricant prior to placement in the induction chamber), and it requires relatively high flow rates to prevent dilution with room air. There is usually no ventilation method with basic units, but new multiplex units now have vacuum removal of waste gases (Muir et al., 2007).



FIGURE 2.1 Commercial Plexiglass Small Mammal Induction Chambers (Smiths Medical PM, Inc., Waukesha, Wisconsin).

Breathing Circuits

The purpose of breathing circuits is to provide the patient with oxygen and inhalant while simultaneously removing carbon dioxide. Rebreathing systems are used for larger animals (>7kg) and will not be discussed in detail in this book chapter.

Non-rebreathing systems (e.g. Bain, Mapleson A thru F, Norman Elbow, Ayre's T-piece, Magill) are the most commonly used systems in biomedical research for small rodents and animals. This type of system relies on a relatively high, fresh gas flow rate to remove carbon dioxide, and the amount of rebreathing that occurs is largely dependent upon this flow rate (Meyer, 2008). The recommended flow rate to decrease rebreathing with this type of system is 2–3 times the minute volume (Thurmon et al., 1996).

Vaporizer Types and Machines

Anesthetic vaporizers volatilize liquid anesthetics and deliver clinically useful concentrations of anesthetic vapor to the common gas outlet and are considered out-of-circuit vaporizers (Muir et al., 2007). There are a wide range of anesthetic machines used for small research animals (Figure 2.2). A full description of the multiple rodent anesthetic machines and of anesthetic machines in general, is beyond the scope of this chapter.

It is possible to adequately induce and maintain anesthesia in rodents and other small animals using virtually any machine equipped with a non-rebreathing circuit. However, others have suggested that the term "rodent anesthesia machine" is usually understood to mean an anesthesia machine lacking all the components of a circle system, and intended for use with a Mapleson-E circuit, or functionally similar design,

without a reservoir bag or adjustable pressure-limiting (APL) valve. Today there are anesthetic units which have multiple breathing circuits and induction chambers to facilitate multiple rodent procedures in a short time frame (Vogler, 2008) (Figure 2.3).

Ventilators

One of the most crucial aspects of providing safe general anesthesia is the maintenance of normal respiratory gas homeostasis. This is defined as the maintenance of arterial carbon dioxide levels within normal limits. The arterial carbon dioxide level usually ranges between 35 and 45 mmHg. A value below 35 mmHg may indicate hyperventilation, while a value above 45 mmHg may indicate hypoventilation (Thurmon et al., 2006). Generally respiratory effort can be verified by observing the movements of the animal's chest and abdominal wall. These movements may be regular and give the appearance of satisfactory gas exchange; they do not ensure adequate movement of air in and out of the lungs (Muir et al., 2007).

Mechanical ventilators are used to free the anesthetist from manually assisting ventilation but require endotracheal intubation of the animal. These are most commonly used in rabbits and other small mammals during thoracotomies as entrance into the chest inhibits the animal's ability to breathe spontaneously. Consultation with a veterinarian as well as equipment manufacturers can be extremely helpful. Ventilators suitable for use with magnetic resonance imaging (MRI) equipment are available in units suitable for patients ranging in size from adult humans to mice.

Regulatory Monitoring Requirements

The Occupational Safety and Health Act requires employers to comply with hazard-specific safety and health standards and provide employees with a workplace free from recognized hazards likely to cause death or serious physical harm (OSHA, 2000). OSHA has guidelines regarding anesthetic gases and workplace exposures, but these guidelines are not regulations; they are intended to assist employers with providing a hazard-free work environment. The National Institute for Occupational Safety and Health (NIOSH) recommends that no one be exposed at ceiling concentrations >2 ppm to any halogenated anesthetic (sampling period not to exceed 1 hour). The American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value-time weighted averages (TLV-TWA) for halothane and enflurane for a normal 8-hour workday are 50 ppm and 75 ppm, respectively. Once again, it is important to note that these are guidelines and not standards or regulations.



FIGURE 2.2 Table Top Small Mammal Anesthetic Isoflurane Vaporizer (Smiths Medical PM, Inc., Waukesha, Wisconsin). Red arrow indicates area where a patient breathing circuit can be attached.

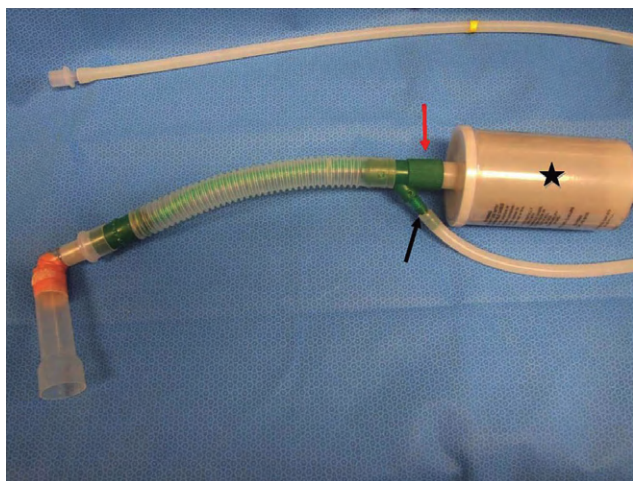


FIGURE 2.3 Mapleson-E Nonrebreathing System: The patient inspires fresh anesthetic gas from the corrugated reservoir tube. The patient expires air into the corrugated reservoir tube. Fresh gas is displaced into the expired gas out of the reservoir tube before the animals' next inspiration. Black arrow – where fresh anesthetic gas and oxygen enters the breathing circuit. Red arrow – where expired air exits the circuit. Black star – F/Air canister which contains charcoal and is a method of passive waste gas scavenging.

Anesthesia Waste Gas Scavenging

Two main types of gas scavenging are available, passive and active. Passive systems pipe directly to the atmosphere or to absorption devices (usually containing activated charcoal), while active systems include piped vacuum and active duct systems (Thurmon et al., 1996). Smith et al. (2003) compared the efficacy of three activated charcoal canisters for passive scavenging during rodent anesthesia with isoflurane and found there was great variability between brands and between individual canisters and that several canisters from one particular brand failed (emission of >100ppm isoflurane) prior to the end of their use life (gain of 50g). In another study by Smith et al. (2006), waste anesthetic gases were shown to be highest at the face/port interface when using a traditional rodent anesthesia unit and face mask. This level of exposure was mitigated when a tighter-fitting diaphragm was made for the face mask (better seal). Use of a flow hood during these types of procedures was also recommended (combining active and passive scavenging).

OSHA recommends that monitoring for anesthetic gases be performed every 6 months (OSHA, 2000). Personal, area, and source samples can be taken to evaluate worker exposure level, overall air containment, and leak detection/ineffective scavenger systems, respectively. Engineering controls (scavenging systems), safe work practices, employee training, and a comprehensive written hazard communication program are key in effectively managing anesthetic gas exposure. Specific recommendations for veterinary waste anesthetic gas

scavenging are available on the American College of Veterinary Anesthesiologists' website (<http://www.acva.org/professional/Position/waste.htm>). Proper monitoring and scavenging are extremely important as health consequences to exposed workers can be severe. Shirangi et al. (2008) demonstrated a 2.49-fold significant increase in the risk of spontaneous abortion in female veterinarians exposed to unscavenged anesthetic gases for one or more hours per week. Other possible health consequences include neurological dysfunction, hepatic and renal toxicity, and neoplasia.

ANESTHESIA MONITORING AND SUPPORT

Reflexes or response to surgical stimuli, body temperature, and the cardiopulmonary system should be thoroughly evaluated when monitoring animals under anesthesia. Redondo et al. (2007) evaluated the most common complications in 1281 dogs placed under general anesthesia and they are as follows: hypoventilation (63.4%), hypotension (37.9%), bradycardia (36.3%), hypoxia (16.4%), hypothermia (4.8%), and death (0.9%).

Anesthetic Depth

Pedal withdrawal, ocular and pinna reflexes can be used to assess anesthetic depth in rodents. Rabbit reflexes that can be used to evaluate depth of anesthesia include pinna, pedal withdrawal, corneal, and palpebral reflexes, with the pinna reflex considered to be the most accurate (Borkowski et al., 1990). It is important to note that although most animals lose palpebral responses early during anesthesia, rabbits may maintain this response even at deeper anesthetic planes (Smith and Danneman, 2008). Although the aforementioned reflexes can be used to assess anesthetic depth, response to surgical stimuli is probably the most reliable indicator (Whelan and Flecknell, 1992). While these reflexes, along with physiologic data such as heart rate and blood pressure, can be used, they are insufficient to fully determine anesthetic depth, especially whether or not an animal is unconscious (Antognini et al., 2005). Use of the electroencephalogram (EEG) is the only form of monitoring that has shown promise as a method of determining level of consciousness in both humans and animals (Antognini et al., 2005).

Respiratory Function

It is essential to assess the respiratory system to determine if anesthetized animals have adequate gas exchange. Respiratory rate can be determined in several

ways including: direct observation of the chest/abdominal wall, auscultation, and observation of reservoir bag movement. It may be difficult to visually assess respiratory rate in rodents (Smith and Danneman, 2008). Respiration depth and character must also be monitored for abnormalities such as bradypnea, tachypnea, or apnea.

Blood gas analysis is used to evaluate oxygenation, ventilation, and acid-base status. Arterial blood samples (femoral, metatarsal, auricular, etc.) are preferred for analysis. Once collected, samples should be analyzed immediately. If this is not possible, the samples should be stored on ice and analyzed within 2 hours. Depending on the type of analyzer used, the sample size can be very small (e.g. 95 μ l or less of whole blood for the VetScan i-STAT[®] 1 Handheld Analyzer). Normal pH, PaCO₂, and PaO₂ values are 7.35–7.45, 35–45 mmHg, and >90 mmHg, respectively. Corrective actions (e.g. endotracheal intubation, manual/mechanical ventilation, decreasing anesthesia, improving oxygenation/ventilation, and correcting acidotic/alkalotic states) should be performed as necessary.

Pulse oximetry reflects the oxygen saturation level of hemoglobin. Pulse oximeters also determine heart rate from the pulsatile flow of blood beneath the infrared (IR) diodes. Probes can be placed on the digits/paws, tongue, proximal tail, and the ear. The latter may give low readings in rabbits with use of some anesthetics that cause peripheral vasoconstriction, such as dexmedetomidine (Orr et al., 2005).

Capnography is used to measure CO₂ concentration in inspired and expired air. A capnograph is useful for detecting hypoventilation ($\uparrow$ ETCO₂), hyperventilation ($\downarrow$ ETCO₂), and malpositioned/malfunctioning endotracheal tubes. ETCO₂ has been evaluated in the rabbit and has been reported to reasonably reflect PaCO₂ (pulmonary tip) (Rich et al., 1990). However, this relationship is stronger in animals on controlled ventilation as the last gas exhaled more closely represents an end tidal sample.

Cardiovascular Function

The cardiovascular system must be monitored not only to determine if there is adequate circulation, but also because many anesthetics have cardio-depressive properties. Heart rate monitoring can be achieved by palpation, auscultation with a stethoscope, or use of an esophageal stethoscope. In order to determine the presence of bradycardia or tachycardia, the normal resting heart rate of each species being used should be known. Pulse rate and character can be determined by palpation of an accessible site (i.e. saphenous, carotid, femoral, lingual, auricular, or tail arteries) (Lipman et al., 2008; Smith and Danneman, 2008). Detection of digital

pulse may be difficult/impossible in rodents (Smith and Danneman, 2008). Capillary refill time (CRT) should be less than 2 seconds, and a prolonged CRT may be indicative of inadequate tissue perfusion. Heart rate, pulse rate/character, and CRT should be assessed collectively in order to adequately evaluate cardiac function.

Electrocardiographs (ECG) record the heart's electrical activity and allow heart rate/rhythm to be monitored. However, it is important to note that an ECG measures electrical activity but does not signify appropriate circulatory function. It is possible to have a normal ECG while cardiac output is actually zero (Flecknell, 1996). An ECG may be difficult to interpret in rabbits/rodents due to their rapid heart rate and low amplitude signals. However, ECG may still be a useful indicator of heart rate and presence of arrhythmias (Smith and Danneman, 2008). ECG patches can be used in larger animals, such as rabbits and chinchillas, if the fur in that area is removed but needle electrodes are usually necessary for smaller rodents (Flecknell, 1996). The electrodes can be placed on the left and right forelimb as well as the right hindlimb. Alternatively, there is a six-lead rodent ECG unit (ecgTUNNEL[®] from emka TECHNOLOGIES, Paris, France) that has demonstrated efficacy in conscious hamsters and rodents (Mongue-Din et al., 2007).

Esophageal stethoscopes may be used in rabbits to continuously monitor heart rate. Their use is impractical in small rodents and they may induce regurgitation in guinea pigs (Heard, 2003).

Arterial blood pressure is one type of measurement that can help determine tissue blood flow. For adequate perfusion of autoregulated tissues, the mean blood pressure should be greater than 60 mmHg (Haskins, 1987). Direct arterial monitoring is more ideal and more accurate than indirect monitoring if proper techniques are used. However, due to the invasiveness, difficulty, and monitor expense, the former technique is not frequently used. Indirect (oscillometric monitor and Doppler ultrasonic blood pressure monitor) measurements can be taken on limbs (both hind and fore), tails, and ears (rabbits). An appropriately sized cuff (~40% of limb circumference) is important for accuracy (Heard, 2003). There are a wide variety of rodent non-invasive blood pressure (NIBP) monitors available (e.g. Stoelting Plug-n-Play Non-Invasive Blood Pressure System, Wood Dale, IL) which can be used in mice, rats, guinea pigs, and hamsters.

Temperature

Small laboratory animals are prone to hypothermia due to their high surface area to body mass ratios. Shaving of fur, application of liquid disinfectants, contact with cold surfaces, and administration of some

pre-anesthetic and anesthetic agents (e.g. cold, dry inhalant anesthetics) further exacerbate heat loss. Reynolds et al. (2008) showed that hypothermia during anesthesia in humans predisposed them to post-operative complications such as: prolongation of action of anesthetics (inhaled and injectable), prolonged post-anesthetic recovery, increased blood loss during surgery, increased allogenic transfusion requirements, increased incidence of surgical wound infections, induction of hypoxia at peripheral wound tissues, impaired immune function, and increased risk of adverse post-operative myocardial episodes. Therefore, it is imperative to provide thermal support (e.g. table drapes, heating pads, forced air warming devices, circulating hot water blankets, and warm fluids) during the entire anesthetic period.

An appropriately sized thermometer should be used (when feasible) to measure body temperature. Core body temperature necessitates the use of an esophageal temperature probe. Alternatively, temperature probes are available on most pulse oximeters and can be inserted rectally or applied to a flat surface of the body. There are also feedback-controlled heating devices that utilize rectal temperature probes to maintain an animal's core body temperature within a preset range (e.g. Homeothermic Blanket System from Harvard Apparatus).

Fluid Therapy

Fluid therapy will depend on the patient (i.e. hydration and daily fluid requirements), presence of underlying diseases, and duration and invasiveness of the procedure. Crystalloid solutions (e.g. Lactated Ringer's, 0.9% sodium chloride, Normosol-R) are isotonic and can be used to replace fluid losses that occur during surgical procedures. Such fluid will quickly equilibrate between the intravascular and interstitial fluid compartments so that only 25% of the administered amount will remain in the intravascular space. Therefore, three times the volume needed must be administered in order to appropriately replace any intravascular losses (Thurmon et al., 1996). Colloid solutions (e.g. Hetastarch, Dextran, plasma, whole blood) are suspensions of large particles. The administration of these solutions leads to an increase in osmotic pressure within the vasculature which prevents fluid loss as well as draws water from the interstitial to the intravascular space. These types of solutions can be useful in cases of acute blood or protein loss during surgery. It should be noted that Hetastarch and Dextran are not replacements for blood products as they have no oxygen-carrying ability (Thurmon et al., 1996).

Rodent vessels are not usually amenable to intravenous fluid administration unless a vascular

cut-down procedure is performed, so warmed fluids are usually administered via intraperitoneal or subcutaneous routes. Rodents and rabbits require higher maintenance fluid rates than larger species (100ml/kg every 24 hours) (McKelvey and Hollingshead, 2003). The marginal auricular or cephalic veins are easily catheterized in rabbits, which allows administration of IV fluids. A rate of at least 10ml/kg/h is reported to be adequate for procedures of short to moderate duration (Lipman et al., 2008). Administration of fluids at this high of a rate (10ml/kg/h) for longer procedures (>2 hours) may lead to hemodilution and excessive accumulation of fluid within the interstitial space, so lower rates (5–8ml/kg/h) should be used if longer procedures are planned (Thurmon et al., 1996).

Intubation

Rabbit intubation is reported to be difficult due to the presence of a long/narrow oral cavity, large incisors, thick tongue, limited temporomandibular joint mobility (Lipman et al., 2008), and easily induced laryngospasm (Wixson, 1994). Endotracheal (ET) tubes ranging from 2.0–4.0mm (diameter), cuffed or uncuffed, are used for rabbit intubation. Topical anesthetic may be sprayed on the larynx to help reduce laryngospasm. Reported sequelae of ET intubation in rabbits include tracheal mucosal injury (Grint et al., 2006; Nordin et al., 1977; Phaneuf et al., 2006; Squire et al., 1990), and post-intubation tracheal strictures (Grint et al., 2006).

Guinea pigs are among the more difficult laboratory animals to safely anesthetize (Swindle et al., 2002). Some of the difficulties include: frequently food-filled cheeks, presence of palatal ostium (small opening which allows entry to the glottis) (Timm, 1987), and a small glottis relative to body size (Heard, 2004). Chinchillas also possess a palatal ostium. In addition, the pedal withdrawal reflex may not always reliably indicate anesthetic depth because guinea pigs make involuntary leg movements even under surgical anesthetic planes (Harkness and Wagner, 1995).

Medium- to large-sized rodents (e.g. guinea pigs, chinchillas, and hamsters) can also be intubated for anesthesia. Intravenous catheters (14G–16G) can be used for rodent endotracheal tubes. In guinea pigs, an 8 French modified urinary catheter or a 2.0–2.5-mm Cole or Murphy ET tube can be used for intubation (Johnson, 2010). In chinchillas, an 8 French modified urinary catheter can be used for intubation (Johnson, 2010).

Several methods for ET intubation have been described for rabbits, guinea pigs, chinchillas, and other rodents. The first is blind intubation. This requires proper positioning of the head and neck so the path from the oropharynx to the trachea is as

straight as possible so the ET tube can be directed into the trachea without visualization of the larynx. Proper placement can be determined by laryngeal palpation, patient response (e.g. cough, gag), listening for air flow through the ET tube, and the presence of condensation within the ET tube (Johnson, 2010). Direct visualization of the glottis is possible if an assistant hyperextends the head and neck and opens the mouth with a speculum or loops of gauze around the incisors while the operator uses an otoscope or small-bladed laryngoscope to raise the soft palate and depress the tongue. The ET tube can be placed once the vocal cords are visible (Johnson, 2010). Finally, indirect visualization of the glottis can be achieved by use of an endoscope or video laryngoscope. The endoscope is placed so the larynx can be seen and then the ET tube is passed parallel to it and into the trachea. In some instances, the ET tube can be placed over the endoscope and the entire unit passed directly into the trachea. Once in the trachea, the endoscope can be slid out of the ET tube and removed (Johnson, 2010; Tran et al., 2001). A recent report describes successful nasotracheal intubation in rabbits utilizing lubricated, clear silicon, uncuffed ET tubes (2.0–2.5 mm; 14.5 cm long) (DeValle, 2009).

An alternate method to use of ET intubation has been described for rabbits. Bateman et al. (2005) and Smith et al. (2004), describe the use of laryngeal mask airways (LMA) for inhalation anesthetics. Laryngeal mask airway is a tube which terminates in a cuffed, concave silicon mask which fits over the larynx (Vogler, 2008). Laryngeal mask airways are reported to be easier to place in comparison to ET tubes (Smith et al., 2004), but require a larger rabbit for appropriate fit and placement (>3 kg), emit more waste anesthetic gases (Smith et al., 2004), may lead to lingual cyanosis if the cuff is inflated (Bateman et al., 2005) although this has not been consistent in all studies (Cruz et al., 2000), and may lead to gastric tympany (Bateman et al., 2005). An LMA does allow for intermittent positive-pressure ventilation to be used and reduces the dead space and risk of hypoxia that is often associated with the use of a face mask (Bateman et al., 2005).

SURGICAL ANESTHESIA

Excellent reviews of anesthesia, anesthesia principles, and how they can be applied to small animals such as rabbits, gerbils, hamster, and small mammals are available (Fish et al., 2008; Flecknell, 1996, 2001; Lichtenberger and Ko, 2007).

Emphasis should be placed on using agents with analgesic, sedative, and muscle-relaxant properties

during the pre-surgical, intra-operative and post-operative periods. The unique pharmacologic profiles of each agent should be considered along with additive or synergistic effects when combined with other agents of a different pharmacologic class. By utilizing a multi-drug or multimodal approach one can enhance sedation, analgesia, and maintain general anesthesia with fewer side effects of the combined drugs than either drug when used alone. A general multimodal anesthetic regimen consists of administering an opioid to provide analgesia and sedation prior to surgery. Ketamine and a benzodiazepine can be used for induction to provide additional analgesia and NMDA receptor antagonism, thereby reducing central sensitization, and providing muscle relaxation. An inhalant anesthetic can be used to maintain anesthesia and an NSAID can be administered directly before recovery for post-operative analgesia and to reduce inflammation. In addition, local anesthetic blocks can be used at the surgical site prior to a surgical incision. Although this scenario is not practical for all cases, it demonstrates the utility of using a multiple drug approach focusing on the unique pharmacologic mechanisms of action of each drug to provide additive or synergistic anesthesia and analgesia. In addition, reversal agents are available for opioids, benzodiazepines, and α_2 -agonists that can be utilized in conditions or anesthetic scenarios of cardiac and respiratory depression or to hasten recovery. However, use of these agents will also reverse or attenuate the analgesic action of the anesthetic. A good example of using reversal agents to dramatically shorten recovery time was reported in chinchillas where fentanyl, medetomidine, and midazolam were reversed with naloxone, atipamezole, and flumazenil, respectively (Henke et al., 2004).

Administration of a tranquilizer, benzodiazepine, α_2 -agonist, or opioid prior to surgery provides sedation, enhances restraint, and allows for lower doses of injectable induction agents such as propofol or ketamine to be utilized. Use of premedication agents is summarized in Table 2.1. In addition, chamber or mask inductions with inhalant anesthetics are smoother and lower concentrations of inhalants can be utilized during the anesthesia maintenance period with fewer incidences of hypotension.

Although pre-medication with an analgesic-sedative agent or combination is recommended, short-term surgical anesthesia (generally 20–60 minutes) may be obtained with intraperitoneal administration of ketamine combined with an α_2 -agonist or a benzodiazepine in smaller species where intravenous access is not easily accessible. Alternatively, tiletamine-zolazepam can be used as a sole agent for inducing and maintaining anesthesia. Injectable anesthetic

TABLE 2.1 Premedication Agents

Drug	Rabbit (SQ, IM)	Guinea Pig (SQ, IM)	Hamster (SQ, IP)	Gerbil (SQ, IP)	Chinchilla (SQ, IM)
PHENOTHIAZINE					
Acepromazine	0.25–1	0.5–1.5	0.5–5	May increase seizure risk	0.5–1.5
Benzodiazepines					
Diazepam	0.5–5	1–5	3–5	3–5	1–5
Midazolam	0.25–2	1–2	1–2	1–2	1–2
ALPHA₂-AGONISTS					
Xylazine	1–5	5–10	5–10	2–10	5–10
Dexmedetomidine	0.05–0.25	0.15	0.05	0.05–0.1	0.025 (IM)
OPIOIDS*					
Buprenorphine	0.01–0.05	0.05	0.1		0.05
Morphine	2–5	2–5	2–5		2–5
Hydromorphone	0.05–0.1				
Butorphanol	0.1–0.8	1–2	1–5		1–2
ANTICHOLINERGICS					
Atropine	0.8–1	0.05	0.05	0.05	0.05
Glycopyrrolate	0.01–0.02	0.01–0.02	0.01–0.02	0.01–0.02	0.01–0.02

Adapted from Heard, D. (2004) *Anesthesia, Analgesia, and Sedation of Small Mammals*. In "Ferrets, Rabbits, and Rodents", 2nd ed. (K.E. Quesenberry and J.W. Carpenter, Eds.), pp. 356–369. Saunders, St. Louis.; Flecknell, P.A. (1996). "Laboratory Animal Anaesthesia: A Practical Introduction for Research Workers and Technicians," 2nd ed. Academic Press, London; Carpenter J.W. (2005). *Exotic Animal Formulary*, 3rd ed. St. Louis: Elsevier Saunders Publishing; and Gaynor, J.S. and W.W. Muir III (2009). *Handbook of Veterinary Pain Management*, 2nd ed. St. Louis: Mosby Elsevier Publishing.

All doses are mg/kg unless stated otherwise and administered 15–30 minutes prior to anesthesia or surgery.

*If also using the opioids for post-operative pain management, please follow the dosing intervals listed in Table 2.3.

combinations and constant rate infusions are summarized in Table 2.2. Due to the difficulty of maintaining moment-to-moment changes in the depth of anesthesia and prolonged recovery periods associated with injectable anesthetic agents, the authors recommend utilizing inhalant agents whenever possible.

POST-OPERATIVE MONITORING AND PAIN ASSESSMENT

Pain Assessment

Assessing pain in rabbits, guinea pigs, and other small mammals post-operatively can be extremely difficult as these are prey species and they have evolved behaviors to mask signs of pain or weakness. Any procedure performed on these animals that would be expected to cause pain in either humans or other animals, should be assumed to cause pain in the species covered by this book. The person assessing the level of post-operative pain should be familiar with the normal behaviors in these species and be as familiar as possible with the normal behaviors of the specific animals. Observing them for several days prior to the surgical procedure can be helpful. The presence of an observer

can often alter their normal behavior so the use of video equipment for post-operative monitoring can be helpful. However, the animals should be acclimated to the presence of this equipment prior to the observation period.

Behaviors to look for in rabbits that may indicate pain, include: anorexia, bruxism, decreased movement, sitting for long periods in a far corner of the cage, rapid fits of locomotion when handled, hunched abdomen, vocalization, decreased respiratory rate with a nasal flare and deep breathing pattern, decreased grooming (scruffy appearance), epiphora, and serous nasal discharge (Gaynor et al., 2009). Leach et al. (2009) documented inactive pain behaviors (twitch, wince, stagger, flinch, press, adjust slow and shuffle) and active pain behaviors (press, shuffle, quiver and arch) that were observed in rabbits after ovariohysterectomy.

Behaviors to look for in guinea pigs, hamsters, and other rodents that may indicate pain are: aggressive behavior, vocalization, decreased activity, decreased grooming (scruffy appearance), hiding in the cage, piloerection, separation from cagemates, and anorexia (Flecknell et al., 2000). Assessing behaviors that are indicative of pain are especially difficult in some species (e.g. hamster, gerbil) as they are nocturnal and therefore normally inactive when laboratory or veterinary staff

TABLE 2.2 Injectable Anesthetic Combination and Constant Rate Infusions

Drug or Combination	Rabbit	Guinea pig	Hamster	Gerbil	Chinchilla
Ketamine:xylazine*	10–40:3–5 (IM)	20–40:3–5 87:13 (IM)	50–150:5–10 (IP)	50–70:2–3 (IP)	20–40:3–5 (IM)
Ketamine:diazepam*	4–10:0.25–5 IV 20–40:1–5 IP, IM	20–50:3–5 (IP, IV)	50–150:2–5 (IP)	40–150:3–5 (IP)	20–50:3–5 (IM)
Ketamine:acepromazine*	25–50: 0.25–1 (IM)	20–50:0.5–1 (IP, IV)	50–100:2.5–5 (IP)		20–50:0.5–1 (IM)
Ketamine:dexmedetomidine*	15–25:0.25 (IM, SQ)	40:0.25 (IP)	100:0.125 (IP)	75:0.25 (IP)	
Tiletamine–zolazepam	5–25 (IM)	20–40 (IP, IV)	50–80 (IP)	50–80 (IP)	20–40 (IP, IV)
Pentobarbital	30–45 (IV)	37 (IP)	50–90 (IP)	60–80 (IP)	
Propofol bolus (induction)	3–10 (IV)**	10 (IV)	NA	NA	10 (IV)
CONSTANT RATE INFUSIONS (IV)					
Ketamine:fentanyl	5–20 µg/kg/h: 0.3–0.4 mg/kg/h				

Adapted from Heard, D. (2004) *Anesthesia, Analgesia, and Sedation of Small Mammals*. In "Ferrets, Rabbits, and Rodents", 2nd ed. (K.E. Quesenberry and J.W. Carpenter, Eds.), pp. 356–369. Saunders, St. Louis., Flecknell, P.A. (1996). "Laboratory Animal Anaesthesia: A Practical Introduction for Research Workers and Technicians," 2nd ed. Academic Press, London, Carpenter J.W. (2005). *Exotic Animal Formulary*, 3rd ed. St. Louis: Elsevier Saunders Publishing, and Gaynor, J.S. and W.W. Muir III (2009). *Handbook of Veterinary Pain Management*, 2nd ed. St. Louis: Mosby Elsevier Publishing.

All doses are mg/kg unless stated otherwise and administered immediately prior to anesthesia or surgery.

*Only re-dose during surgery with ketamine component at one-third the original dose.

**Propofol should be used with caution in rabbits as extended use may lead to propofol infusion syndrome.

would be most likely to be performing post-operative examinations (Flecknell et al., 2000).

There are numerous pain assessment scoring systems that have been developed for cats and dogs (Gaynor et al., 2009). It may be useful for the laboratory or investigator to develop a similar system for post-operative pain monitoring that is both specific to the species being used and the procedure being performed. This would allow consistency between observers and therefore less of a chance to miss pain behaviors in the animal.

Post-Operative Analgesia

Analgesia and pain-relief protocols should be based upon the species, the procedure to be performed, the pharmacology and pharmacokinetics of available agents, anesthetic–analgesic drug interactions, and adverse effect profiles of individual agents (Table 2.3).

During the post-operative period, combining agents with different mechanisms of analgesia (i.e. multimodal therapy) such as opioids and NSAIDs is useful to augment analgesia and reduce inflammation. Furthermore, the addition of an NSAID prior to recovery may reduce peripheral sensitization and improve the quality of recovery (Katz, 2001). If an opioid was administered as a premedication, there may be some residual analgesia remaining depending upon the length of the surgical

procedure and the duration of action of the drug. In this scenario an NSAID can be administered shortly before or during recovery with re-dosing of the opioid at a later time point. Many opioids have a shorter duration of action compared to daily dosing for some NSAIDs. By incorporating NSAIDs into an analgesic regimen, fewer analgesic doses need to be administered during the day, thus reducing handling and stress for the patient. For severe pain an opioid should be provided immediately after surgery along with an NSAID to allow onset of action time for the NSAID to provide analgesia.

Post-Operative Monitoring

Post-operative monitoring should be performed at least once daily, if not more frequently, for the first 7 days after surgery or until any skin sutures/staples, if present, are removed. Animals should be carefully assessed for the following parameters: appetite, water intake, body weight, urine output, fecal output, hydration status, appearance of the incision site, temperature and vital signs (if possible to obtain without stressing the animal), and evidence of any of the behaviors mentioned above that may indicate pain. This is especially important in rabbits, guinea pigs, and chinchillas because ileus is a common and serious post-operative complication in these species. If ileus does develop,

TABLE 2.3 Post-operative Analgesics

Drug	Rabbit	Guinea Pig	Hamster	Gerbil	Chinchilla
OPIOIDS					
Buprenorphine	0.01–0.05 (SQ, IM, IV) q6–12h	0.05 (SQ, IM) q6–12h	0.1 (SQ) q6–12h	0.1 (SQ) q6–12h	0.05 (SQ, IM) q6–12h
Morphine	2–5 (SQ) q4h; 0.1 epidurally (use morphine PF)	2–5 (SQ) q4h	2–5 (SQ) q4h	2–5 (SQ) q4h	2–5 (SQ) q4h
Hydromorphone	0.05–0.2 (IV, SQ, IM) q4–6h				
Butorphanol	0.1–0.8 (SQ) q4h	2 (SQ) q4h	1–5 (SQ) q4h	1–5 (SQ) q4h	2 (SQ) q4h
Oxymorphone	0.05–0.2 (SQ, IM) q8–12h	0.2–0.5 (SQ, IM) q6–12h	0.2–0.5 (SQ) q6–12h	0.2–0.5 (SQ) q6–12h	0.2–0.5 (SQ) q6–12h
NSAIDS					
Carprofen	4 (PO) q24h 2–4 (SQ) q24h	4 (SQ, IM) q24h	5 (SQ) q12h	5 (SQ) q12h	4 (SQ, IM) q24h
Meloxicam	0.1–0.5 (PO, SQ) q12–24h				
Ketoprofen	1–2 (SQ, PO) q24h	1 (SQ, IM) q12–24h	5 (SQ) q24h	5 (SQ) q24h	1 (SQ, IM) q12–24h
Flunixin meglumine	0.3–2 (PO, SQ, IM) q12–24h (no more than 3 days)	1–5 (SQ) q12–24h	2.5 (SQ) q12–24h	2.5 (SQ) q12–24h	1–3 (SQ) q12h
LOCAL ANESTHETICS					
Lidocaine	1–2 infiltrate; 0.4 epidurally (1.5%)	1–2 infiltrate	1–2 infiltrate	1–2 infiltrate	1–2 infiltrate
Bupivacaine	1–2 infiltrate	1–2 infiltrate	1–2 infiltrate	1–2 infiltrate	1–2 infiltrate
MISCELLANEOUS					
Tramadol	5–10 (PO) q12–24h	NA	NA	NA	NA
Dexamethasone	0.5–2 (IV, PO, SQ) then decreasing dose q12h × 3–14 days	0.5–2 (PO, SQ) then decreasing dose q12h × 3–14 days	0.5–2 (PO, SQ) then decreasing dose q12h × 3–14 days	0.5–2 (PO, SQ) then decreasing dose q12h × 3–14 days	0.5–2 (PO, SQ) then decreasing dose q12h × 3–14 days
Prednisone	0.5–2 (PO)	0.5–2.2 (PO, SQ, IM)	0.5–2.2 (PO, SQ)	0.5–2.2 (PO, SQ)	0.5–2.2 (PO, SQ, IM)

Adapted from Heard, D. (2004) *Anesthesia, Analgesia, and Sedation of Small Mammals*. In "Ferrets, Rabbits, and Rodents", 2nd ed. (K.E. Quesenberry and J.W. Carpenter, Eds.), pp. 356–369. Saunders, St. Louis., Flecknell, P.A. (1996). "Laboratory Animal Anaesthesia: A Practical Introduction for Research Workers and Technicians," 2nd ed. Academic Press, London, Carpenter J.W. (2005). *Exotic Animal Formulary*, 3rd ed. St. Louis: Elsevier Saunders Publishing, and Gaynor, J.S. and W.W. Muir III (2009). *Handbook of Veterinary Pain Management*, 2nd ed. St. Louis: Mosby Elsevier Publishing. All doses are mg/kg unless stated otherwise.

there are several treatment options available. Offering highly palatable treats (e.g. timothy hay, carrots, kale) and dietary supplements (e.g. Critical Care® from Oxbow) are a common first step. Any guinea pig that is inappetent or anorexic should be given parenteral vitamin C in order to prevent the development of scurvy as they cannot produce vitamin C and depend on dietary sources. If the animal appears dehydrated, providing

daily subcutaneous or intravenous crystalloid fluids is important. If none of these treatment options are effective and no gastrointestinal obstruction is present, use of a prokinetic, such as metoclopramide, can help stimulate gastric motility. Reassessing the post-operative analgesic plan is also important, as pain can be a large contributor to the development of ileus.

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Clinical Biochemistry and Hematology

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INTRODUCTION

This chapter reviews information on the clinical biochemistry and hematology of the rabbit (*Oryctolagus cuniculus*), guinea pig (*Cavia porcellus*), hamster (*Mesocricetus auratus*) and other rodents, including the gerbil (*Meriones unguiculatus*), chinchilla (*Chinchilla laniger*), degu (*Octodon degus*), deer mouse (*Peromyscus maniculatus*), dormouse (Gliridae family), kangaroo rat (*Dipodomys* spp.), cotton rat (*Sigmodon hispidus*), and sand rat (*Psammodmys obesus*).

The chapter begins with a review of sample collection and preparation, and a description of commonly measured parameters and analytical techniques. The reference values, sources of variation, and unique characteristics are then presented for each species, as available. We have attempted to include the most current literature on a backdrop of historical information for each of these topics, along with supporting references.

It is important to recognize that many variables affect the parameters of clinical biochemistry and hematology that are presented here, including methods of sample collection and preparation, equipment, reagents, and methods of analysis, as well as the age, sex, breed, and environment of the animals being sampled. Reference ranges are presented according to the most recent and reliable literature available. Some clinical biochemistry and hematology parameters for the less common species covered in this volume are not yet available in the literature.

CLINICAL BIOCHEMISTRY

Introduction

Abnormal clinical conditions are usually associated with specific biochemical changes in the blood and urine, and these changes are detected using specific analytical techniques. Values obtained from a clinical case are usually compared with reference values that are either produced in the same laboratory or in a similar group of animals and cited in the literature.

A joint committee formed by several scientific societies, the International Committee for Harmonization

of Clinical Pathology Testing (IHCPT), formulated a recommended core clinical chemistry panel for safety and toxicity studies in laboratory animals (Weingand et al., 1996). This core panel includes glucose, blood urea nitrogen, creatinine, total protein, albumin, globulin, calcium, sodium, potassium, chloride, and total cholesterol. Additionally recommended are at least two hepatocellular function tests, including alanine aminotransferase, aspartate aminotransferase, sorbitol dehydrogenase, glutamate dehydrogenase, or total bile acids, and two hepatobiliary function tests, including alkaline phosphatase, gamma glutamyltransferase, 5'-nucleotidase, total bilirubin, or bile acids. The U.S. EPA (2008) recommended the additional inclusion of assays for triglycerides, magnesium, protein electrophoresis, inorganic phosphate/cholinesterase, phosphorus, ornithine carbamoyl transferase, lactate dehydrogenase, and creatine (Evans, 2009).

Urinalysis tests recommended by the IHCPT and U.S. EPA include appearance (color and turbidity), volume, specific gravity, pH, microscopic sediment, protein, glucose, ketones, bilirubin, blood, nitrate/nitrite, and urobilinogen (Evans, 2009; U.S. EPA, 2008; Weingand et al., 1996).

Sample Collection

Restraint

Most small laboratory animals are prey species in the wild, and they are easily stressed by the handling and restraint needed to obtain a sample of blood or urine. Pre-conditioning has been shown to reduce plasma corticosterone levels in rats (Fluttert et al., 2000), but this practice is often impossible or impractical in many laboratory rodents, especially for the diagnosis of spontaneous disease. A restraint tube can be used to hold small rodents for obtaining samples if the tube is the appropriate size and is disinfected between animals.

Anesthesia may be needed to obtain samples from laboratory rabbits or rodents in certain situations, especially when larger volumes of blood, or sterile urine samples, are needed. However, anesthesia itself has been shown to produce changes in clinical biochemistry or hematology parameters, including decreased hematocrit, hemoglobin level, and red blood cell count

(Marini et al., 1994; Pilny, 2008). It is important to know what method of restraint was used to obtain samples when interpreting reference clinical biochemistry and hematology values.

Blood Collection

Optimal sites for blood collection vary between laboratory animals and are described in this chapter for each species for which information is available. Small animals typically lack superficial blood vessels, and deeper vessels are often obscured by fat. A variety of peripheral veins, such as the lateral saphenous vein and the cephalic vein, may be used for small samples of blood, as described for individual species. The jugular vein may be used in most species for larger blood samples, although appropriate positioning and restraint can be stressful and may require sedation or anesthesia (Thrall et al., 2004).

Blood collection from the smaller laboratory animals is challenging due to the limited blood volume available and because the restraint or sedation necessary to obtain a sample may alter the results of some assays (Pilny, 2008). A general guideline for the maximum volume of blood that can be safely withdrawn in a single sample is approximately 1% of the animal's body weight, or 10% of the circulating blood volume, in order to avoid hypovolemic shock and anemia. This volume will be replaced within 24 hours in most healthy animals, although a return to normal levels of all blood constituents may take up to 2 weeks (Thrall et al., 2004). An alternate standard states that the blood volume withdrawn should not exceed 7.5% of the total blood volume per week or 10% of the total blood volume every 2 weeks (McGuill and Rowan, 1989).

Clinical chemistry assays have become more sensitive with advances in technology, and successful analysis can now be achieved with smaller volumes of blood. Most clinical chemistry assays require a few milliliters of blood, and some assays now require only a few microliters of blood.

Serum is the preferable sample for most clinical chemistry assays to avoid interference by the actions of an anticoagulant. Some of the anticoagulants, such as ethylenediamine-tetraacetic acid (EDTA), and citrate, prevent clotting by chelating calcium ions, which are often cofactors in enzyme assays. Heparin is usually the preferred anticoagulant because it has minimal or no interference with the majority of clinical chemistry assays (Buetow et al., 1999). Heparin may, however, produce artificially elevated inorganic phosphorus values (Benjamin and McKelvie, 1978).

Serum is produced by collecting blood into tubes with no anticoagulant and allowing it to clot for 30–45 minutes at room temperature. The sample should not

stand for more than 45 minutes without refrigeration because many constituents may change concentration at room temperature, including glucose, phosphates, lipids, and chloride (Mitruka and Rawnsley, 1977). The tube is then centrifuged at approximately 1000g for 15 minutes to yield serum that can be analyzed for clinical chemistry parameters (Buetow et al., 1999).

Plasma is suitable for many clinical chemistry assays and is a practical necessity in many cases because of the small volume of blood available from laboratory rabbits and rodents. Blood samples may be treated with heparin and used for both hematology and clinical chemistry assays. Blood may be collected in a syringe containing 50U of heparin per ml, or placed in a heparinized tube, and centrifuged for 2 minutes at high speed, such as 12000g, to yield platelet-free plasma that can be used for clinical chemistry assays (Lindena and Trautschold, 1986). Alternatively, miniature blood collection tubes, such as those used in human pediatric medicine, can be used for small-volume blood samples. The advantage of using these tubes is that the anticoagulant is lyophilized and does not dilute the sample (Dyer and Cervasio, 2008).

Both serum and plasma samples may be used immediately, stored at 4°C for use in 24–48 hours, or stored frozen at –20°C for up to 90 days or at –70°C for 360 days or longer (Buetow et al., 1999; Cray et al., 2009). However, some enzymes, such as lactate dehydrogenase, are not stable if the sample is frozen (Mitruka and Rawnsley, 1981).

Certain factors, such as lipemia and hemolysis, may interfere with the results of clinical biochemistry assays that are performed by spectrophotometer. The increased turbidity associated with lipemia can produce artificially high values for certain enzymes. Lipemia also interferes with the assays for glucose, bilirubin, total protein, phosphorus, and urates (Saibaba et al., 1998). Hemolysis also produces errors in clinical biochemical values because most serum enzymes are present at high concentrations in red blood cells. Hemolysis causes the release of these enzymes into the bloodstream to yield artificially high serum values. Hemolysis may produce an artificial elevation of serum potassium and inorganic phosphorus levels (Owens et al., 2005; Stankovic and Smith, 2004), as well as bilirubin, transaminases, and lactate dehydrogenase (Gad, 2007).

The method and site of sample collection may also affect resulting values. For example, enzymes found in cardiac muscle, such as creatine kinase, aspartate aminotransferase, lactate dehydrogenase, and alanine aminotransferase, may be artificially elevated in the serum of some species when blood samples are obtained by cardiac puncture (Maxwell et al., 1985).

Urine Collection

Urine samples of satisfactory volume and quality are difficult to obtain from many small laboratory animals. Urine may be collected from most animals following spontaneous urination, manual bladder expression, cystocentesis, or catheterization. Cystocentesis should be used to obtain a sterile sample when a urine culture is needed, as for a bacterial culture and antibiotic sensitivity. A metabolism cage is useful when sequential urine samples are required or when a larger volume of urine needs to accumulate over a specified period of time. The volume of urine that is excreted per day and available for sampling depends on many factors, including food and water consumption, activity level, disease, and environmental temperature.

The simplest method of urine analysis is performed with a commercial dry reagent test strip, or dipstick, which requires only a few microliters of urine per test reagent square. This assay provides semiquantified measures of urine components, such as blood, glucose, ketones, and pH.

The animal's ability to concentrate urine is evaluated with a small drop of urine placed on a refractometer. Normal specific gravity falls in a range of approximately 1.003–1.050 and varies by species and environment. Increased specific gravity may indicate dehydration, heart failure, or shock, and decreased specific gravity may indicate renal failure, diabetes insipidus, or other conditions.

A small volume of 1–2 ml of urine can be centrifuged at approximately 10000g for 10 minutes. The sediment is then analyzed under the microscope for cells, bacteria, and crystals.

Reference values for urine characteristics of the laboratory animals covered in this volume are provided in Table 3.1.

Carbohydrate Metabolism

Glucose

Glucose is the parameter most commonly evaluated as a measure of carbohydrate metabolism. Glucose concentration is affected by many factors, including nutrition, hormones, hibernation, restraint, fasting, and anesthesia. Handling of the blood sample may also affect glucose values because glucose decreases in serum when erythrocytes are present. A decrease in glucose concentration by approximately 7% is reported in blood that sits at room temperature for 1 hour (Tietz, 1976). Therefore, serum should be separated and tested for glucose as soon as possible after a blood sample is taken. Alternatively, a preservative such as thymol or fluoride should be added to inhibit glycolysis, as described for the rat (Ringler and Dabich, 1979). Glucose is usually measured using glucose oxidase/peroxidase-linked chromogenic methods or by measuring hexokinase activity (Evans, 2009).

Reference ranges for serum glucose concentration in the laboratory animals covered in this volume are provided in Table 3.2.

Lipid Metabolism

The primary lipids contained in serum are cholesterol, triglycerides, fatty acids, and phospholipids. Of these, cholesterol and triglycerides are the most commonly measured in laboratory animals. There are significant interspecies differences in the normal serum concentrations of these lipid components. Reference ranges for cholesterol, triglycerides, and total lipids for the laboratory animals covered in this volume are provided in Table 3.2.

High levels of lipids cause lipemia, which can interfere with clinical biochemistry assays performed by photometric analysis. Solubilizers may be used on

TABLE 3.1 Urine Characteristics¹

	Volume (ml/kg/d)	pH	Specific Gravity	Normal Color	Turbidity	Protein (mg/wk)	Cells	Crystals	References
Rabbit	20–75	7.6–8.8	1.003–1.036	Yellow/ straw	Cloudy	Trace	Occas. RBC, WBC	TP, CaCO ₃	Kozma et al., 1974 Mitruka and Rawnsley, 1977 Mader, 2004
Guinea pig	N/A	8–9	N/A	Yellow/ amber	Opaque	N/A	N/A	Occas. CaCO ₃	Riggs, 2009
Hamster	7 ml/d	5.1–8.4	N/A	N/A	Cloudy	10	N/A	TP, CaCO ₃	Fiszer et al., 1979 Heatley and Harris, 2009
Chinchilla	N/A	8–9	N/A	Yellow/ amber	N/A	N/A	N/A	N/A	Riggs and Mitchell, 2009

N/A = values not available; RBC = red blood cell; WBC = white blood cell; TP = triple phosphate

¹Species for which no values are available are not listed.

TABLE 3.2 Carbohydrate and Lipid Metabolism¹

	Glucose (mg/dl)	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	Total Lipids (mg/dl)	References
Rabbit	75–155	10–80	15–160	150–400	Suckow and Douglas, 1996 Evans, 2009 Vennen and Mitchell, 2009
Guinea pig	60–130	20–80	10–70	N/A	Hrapkiewicz and Medina, 2007 Evans, 2009
Hamster	37–198	112–210	72–350	224–466	Cox and Gökçen, 1974 Heatley and Harris, 2009 Evans, 2009
Gerbil	50–135	90–150	N/A	N/A	Hrapkiewicz and Medina, 2007
Chinchilla	60–125	40–300	149–198	N/A	Hrapkiewicz and Medina, 2007 de Oliveira Silva et al., 2005
Degu	74–82	N/A	N/A	N/A	Opazo et al., 2004
Cotton rat	115–216	134–165	N/A	N/A	Dolyak and Leone, 1953
Sand rat	68–71	N/A	N/A	N/A	Frenkel and Kraicer, 1972

N/A = values not available.

¹Species for which no values are available are not listed.

lipemic samples to decrease this interference (Evans, 2009). Polyethylene glycol 6000 is a precipitating agent that is often used to remove lipoproteins from a serum sample (Thomson and Kunze, 1984).

Cholesterol

Cholesterol is an essential component of cell membranes and an important precursor for the synthesis of bile acids, steroid hormones, and some fat-soluble vitamins. Cholesterol is produced in the liver or obtained in the diet, and it circulates in free and esterified forms. Cholesterol is recycled through metabolism in the liver and excretion in bile into the digestive tract. Cholesterol levels typically peak after a meal, so fasting is required for an accurate assay.

Cholesterol has been the subject of many studies in laboratory animals because high levels of circulating cholesterol are associated with the development of atherosclerosis. There are several valuable laboratory animal models of cholesterolemia and atherosclerosis.

Cholesterol is usually measured using a cholesterol esterase followed by a cholesterol oxidase linked to a peroxidase-chromogen detection system (Richmond, 1992).

Lipoproteins are macromolecules that transport lipids and cholesterol within the bloodstream and can be classified as alpha or beta, or by a density value that varies inversely with size.

- *Low-density lipoproteins (LDL)* are also known as beta lipoproteins and have a diameter of 18–28 nm, density of 1.019–1.063 grams per ml, and carry cholesterol from the liver throughout the body.

- *High-density lipoproteins (HDL)* are also known as alpha lipoproteins and have a diameter of 5–15 nm, density of less than 1.063 grams per ml, and carry cholesterol away from the tissues of the body and back to the liver.

Different lipoprotein fractions can be separated and quantified using microaffinity chromatography columns (Tschantz and Sunahara, 1993), electrophoresis or other methods, including fast protein liquid chromatography, and high-performance liquid chromatography (Evans, 2009).

Triglycerides

Triglycerides are composed of a glycerol molecule bound to three fatty acids and are digested by pancreatic lipase. Triglycerides are a major component of very-low-density lipoprotein (VLDL) and serve as a source of energy. They are broken down in the intestine, absorbed by intestinal cells, and combined with cholesterol and proteins to form chylomicrons that are transported in lymph to the bloodstream.

Triglycerides are measured using enzymatic reagents, including lipase, glycerol kinase, and glycerol-3-phosphate oxidase linked to a peroxidase-chromogen detection system (Evans, 2009).

Enzymes

Enzymes are normally contained within the cells that synthesize them, and increased enzyme levels in serum or plasma are indicative of induction or cell damage. Several

factors affect the activity of enzymes in plasma or serum, including the concentration of enzyme in cells, the extent and duration of cell damage, and the accessibility and persistence of the enzyme in the bloodstream. Diet and nutritional deficiencies can also impact the levels of enzymes measured. The laboratory methods used to measure serum enzyme activity can affect the values obtained.

Enzyme activity is usually represented in International Units (IU), where one unit is the amount of enzyme that reacts with one picomole of substrate per minute. In theory, a standardized method of representing enzyme activities should allow better comparisons between laboratories. However, the choice of enzyme substrates and the experimental conditions used have important effects on the results of enzyme assays (Evans, 2009).

Reference values for several commonly assayed enzymes for laboratory animals covered in this volume are provided in Table 3.3.

Alkaline Phosphatase (ALP)

Alkaline phosphatase (ALP) represents a group of enzymes that remove phosphate groups from molecules such as nucleotides and proteins, and they work most effectively in an alkaline environment of pH 9–10 (Evans, 2009). The highest concentrations of ALP are found in liver and bone, and lower concentrations of ALP are present in kidney tubules, intestinal epithelium, lung, and placenta. Bone growth in young animals produces elevated ALP levels. Concentration of ALP varies according to species but in general is increased by digestion, cholestasis, or injury to intestinal or biliary epithelium. Levels of ALP may be decreased by fasting, hypothyroidism, or pernicious anemia (Fernandez and Kidney, 2007).

Alkaline phosphatase is typically measured using 4-nitrophenol phosphate as a substrate and 2-amino-2-methyl-1-propanol (AMP) or diethanolamine (DEA) as a buffer, although DEA yields higher ALP values. Isoenzymes of ALP can be distinguished using electrophoresis, HPLC, and other methods (Evans, 2009).

Alanine Aminotransferase (ALT)

Alanine aminotransferase (ALT) is a transaminase enzyme that was formerly known as serum glutamate pyruvate transaminase (SGPT). Alanine aminotransferase catalyzes the transfer of an amino group from alanine to alpha-ketoglutarate in the alanine cycle to form pyruvate and glutamate. The ALT enzyme is found in serum and organ tissues, especially liver, although significant concentrations are also found in kidney, skeletal muscle, and myocardium. Lower levels of ALT are present in pancreas, spleen, and lung. Alanine aminotransferase is elevated in serum under

conditions of significant cellular necrosis and is used as a measure of liver function. Levels of ALT may be elevated in cases of hepatitis, congestive heart failure, liver or biliary duct damage, or myopathy. Diet, restraint, and drug administration may also affect plasma ALT in rodents (Evans, 2009).

Concentration of ALT is quantified using the NADH oxidation method.

Aspartate Aminotransferase (AST)

Aspartate aminotransferase (AST) is a transaminase enzyme that catalyzes the conversion of aspartate and alpha-ketoglutarate to oxaloacetate and glutamate. The AST enzyme was formerly known as serum glutamate oxalate transaminase (SGOT) and is present in all tissues except bone, with highest levels in liver and skeletal muscle. Concentration of AST is elevated after bruising, trauma, necrosis, infection, or neoplasia of liver or muscle (Eugster et al., 1966). The AST enzyme is found in cerebrospinal fluid, exudates, and transudates in proportion to the amount of cellular damage. Low concentrations of AST are also found in urine but are not useful for diagnosis of renal damage (Evans, 2009).

AST is measured using the NADH oxidation method.

Lactate Dehydrogenase (LDH)

Lactate dehydrogenase (LDH) is an enzyme that catalyzes the conversion of pyruvate to lactate, along with NADH to NAD⁺, during glycolysis in conditions of hypoxia. The LDH enzyme is present in all cells, but it is concentrated in muscle, liver, and kidney. LDH exists as five isozymes, LDH-1 through LDH-5, each composed of four subunits. Differential LDH isozyme levels are used diagnostically (Markert et al., 1975). Isozyme LDH-1 (4H) has four heart subunits and is the major isozyme in heart. Isozyme LDH-2 (3H1M) has three heart and one muscle subunit and is the major isozyme in the macrophage-monocyte system and serum. The LDH-3 (2H2M) isozyme has two heart and two muscle subunits and is the major isozyme in lungs. Isozyme LDH-4 (1H3M) has one heart and three muscle subunits and is the primary isozyme in kidneys. The LDH-5 (4M) isozyme has four muscle subunits and is the major isozyme in liver and skeletal muscle. The concentration of LDH is elevated in serum as a result of organ infarction and significant cell death that results in loss of cytoplasm. For example, elevations of LDH result from conditions such as hepatitis, shock, hypoxia, extreme hypothermia, and meningitis, among others. The LDH enzyme has often been used in laboratory animals, along with troponin levels, to detect myocardial damage (Evans, 2009; Herman et al., 2006). However, LDH stability is highly susceptible to freezing, and values are affected by storage conditions (Mitruka and Rawnsley, 1981; Tietz, 1976).

TABLE 3.3 Enzymes¹

	ALP (IU/l)	ALT (IU/l)	AST (IU/l)	LDH (IU/l)	CK (IU/l)	SDH (IU/l)	Amylase (IU/l)	GDH (IU/l)	References
Rabbit	10–140	14–80	14–113	30–140	150–1000	170–177	200–500	6.39	Suckow and Douglas, 1997 Evans, 2009 Vennen and Mitchell, 2009
Guinea pig	80–350	10–90	10–90	20–120	50–150	N/A	995–1239	N/A	Waner et al., 1996
Hamster	50–186	20–128	20–150	100–300	23	N/A	154–196	N/A	Mitruka and Rawnsley, 1977 Evans, 2009 Heatley and Harris, 2009
Gerbil	12–37	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Hrapkiewicz and Medina, 2007
Chinchilla	10–70	10–35	15–100	N/A	0–300	N/A	N/A	N/A	Hrapkiewicz and Medina, 2007 Mitchell and Tully, 2009
Deer mouse	6–7	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Meagher, 1998
Cotton rat	10–14	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Dolyak and Leone, 1953

N/A = values not available.

¹Species for which no values are available are not listed.

Creatine Kinase (CK)

Creatine kinase (CK) is also known as creatine phosphokinase (CPK) and is an enzyme that catalyzes the phosphorylation of creatine. Creatine kinase is a dimer that exists as isoenzymes with greatest activity in muscle (CK-MM), heart (CK-MB), and brain (CK-BB) (Lang, 1981). An additional form of the CK enzyme exists in mitochondria (CK-Mt). The distribution of CK isoenzymes varies between tissues and species. Creatine kinase is found in serum, plasma, and urine and is elevated by muscle necrosis or disease.

Sorbitol Dehydrogenase (SDH)

Sorbitol dehydrogenase (SDH) is an enzyme that converts the alcohol form of glucose, sorbitol, into fructose, in the presence of NAD. Sorbitol dehydrogenase activity has been detected in liver, kidney, and seminal vesicles of many animals. Increased levels of SDH usually indicate hepatocellular damage (Dooley, 1984), and may be a more sensitive indicator of hepatic damage than ALT or AST, but changes in SDH levels may also be minimal (Evans, 2009).

Amylase

Amylase is a small enzyme that breaks down complex carbohydrates to ultimately form monosaccharides such as glucose. The pancreas produces large amounts of alpha-amylase, which requires calcium and acts as a major digestive enzyme at neutral pH. Amylase is also present in liver and salivary glands. Thus, increased plasma amylase is indicative of tissue damage in pancreas, liver, or salivary glands (Evans, 2009).

Chromogenic and immunologic methods are typically used to measure amylase for the diagnosis of pancreatic disease (Evans, 2009).

Glutamate Dehydrogenase (GDH)

Glutamate dehydrogenase (GDH) is a mitochondrial enzyme that is involved in the metabolism of glutamate to 2-oxoglutarate. The GDH enzyme is found primarily in liver, kidney, and cardiac muscle, with lower levels in brain, skeletal muscle, and leukocytes. The majority of GDH in the serum originates from hepatocytes in healthy as well as diseased animals. The distribution of GDH in the liver is mainly centrilobular, whereas ALT is periportal or centrilobular in rats, and may indicate the site of hepatocyte damage (Carakostas et al., 1986; Merrick et al., 2006). Levels of GDH are normally low in most species, and increased serum GDH is indicative of damaged or necrotic hepatocytes due to liver disease (Evans, 2009).

Hormones

There are many hormones that can be measured in the serum of laboratory animals, but a complete listing is beyond the scope of this chapter. This section will focus

on some of the more relevant hormones in laboratory animals, including adrenal, thyroid, and reproductive hormones. Reference values for hormones in laboratory animals covered in this volume are provided in Table 3.4.

Adrenal Hormones

Cortisol is a glucocorticoid produced by the zona fasciculata of the adrenal cortex and is involved in the response to stress. Cortisol acts to increase blood pressure and blood glucose and has an immunosuppressive action. Levels of cortisol are known to vary according to diurnal rhythm and other environmental influences.

Corticosterone is also produced by the adrenal cortex and is a significant glucocorticoid in some species, including rodents. Corticosterone has functions that are similar to those of cortisol.

Thyroid Hormones

Thyroxine (T4) is secreted by the follicle cells of the thyroid gland and transported in the bloodstream to control metabolic processes in the body. Thyroxine is a prohormone and is converted to the more potent form, *triiodothyronine (T3)* in the tissues of the body. Thyroid hormones are involved in growth and metabolism and act synergistically with other hormones, such as catecholamines and glucocorticoids. Circulating levels of thyroid hormones are affected by many internal and external factors to which an animal is exposed.

Reproductive Hormones

Luteinizing hormone (LH) is a glycoprotein hormone produced by the anterior pituitary gland. In the female, its sudden increase at the end of the follicular phase triggers ovulation and corpus luteum development. In the male, it is also called interstitial cell stimulating hormone, and it triggers testosterone production by the Leydig cells of the testis.

Follicle-stimulating hormone (FSH) is a glycoprotein hormone produced by the anterior pituitary gland. In both males and females, FSH stimulates germ cell maturation and helps regulate growth and sexual maturation.

Estradiol is a steroid hormone that is the predominant female sex hormone and the primary estrogenic hormone. In females, the majority of estradiol is produced by the ovaries, and smaller amounts are produced by the adrenal cortex. In males, small amounts of estradiol are produced by the testes and adrenal cortex.

Progesterone is a steroid hormone that is produced by the corpora lutea of the ovaries, the adrenal glands, and the placenta during pregnancy. Progesterone increases at the time of ovulation and helps support the maintenance of pregnancy.

Prolactin is a peptide hormone produced by the anterior pituitary gland and other organs and is primarily involved in lactation.

TABLE 3.4 Hormones¹

	Cortisol (µg/dl)	C'sterone (µg/dl)	T3 (ng/dl)	T4 (ug/dl)	LH (ng/ml)	FSH (ng/ml)	Estradiol (pg/ml)	Progest (ng/ml)	Prolactin (ng/ml)	Oxytocin (ng/ml)	Testost (pg/ml)	References
Rabbit	2.6–3.8	1.54	130–143	1.7–2.4	10–100	2–4.5	3	<1	10	10–20	3–5	DePaolo and Masoro, 1989 Suckow and Douglas, 1997
Guinea pig	5–30	N/A	22–56	2.3–3.5	5–55	100–200	30	1	N/A	<5	N/A	DePaolo and Masoro, 1989
Hamster	2.3–3.2	5.5–9.3	30–80	3–7	20–40	100–300	5–10	1	5–10	N/A	1.5–2	Cox and Gokcen, 1974 Neve et al., 1981 DePaolo and Masoro, 1989
Chinchilla	N/A	N/A	N/A	3.4–6.4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Martin et al., 2005
Degu	15	1.5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Gruss et al., 2006
Kangaroo rat	N/A	N/A	N/A	0.48–1.92	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Banta and Holcombe, 2002

N/A = values not available.

¹Species for which no values are available are not listed.TABLE 3.5 Liver Function¹

	Total Bilirubin (mg/dl)	Bile Acids (mMol/l)	Total Protein (g/dl)	Albumin (g/dl)	Globulins (g/dl)	References
Rabbit	0–0.8	0–40	5.4–8.3	2.4–4.6	1.5–2.8	Hrapkiewicz and Medina, 2007 Evans, 2009 Vennen and Mitchell, 2009
Guinea pig	0–1	N/A	4–7	2–5	2–4	Waner et al., 1996 Evans, 2009 Riggs, 2009
Hamster	0.1–0.9	N/A	5.2–7.0	3.5–4.9	2.7–4.2	Heatley and Harris, 2009
Gerbil	0.2–0.6	N/A	4.3–12.5	1.8–5.5	1.2–6.0	Heatley and Harris, 2009
Chinchilla	0.6–1.3	N/A	3.8–8.0	2.3–4.1	0.9–4.2	Hrapkiewicz and Medina, 2007 Riggs and Mitchell, 2009
Deer mouse	N/A	N/A	6.3–6.9	4.1–4.7	N/A	Meagher, 1998
Cotton rat	N/A	N/A	6.1–6.9	4.2–5.2	1.4–2.2	Dolyak and Leone, 1953

N/A = values not available.

¹Species for which no values are available are not listed.

Oxytocin is a peptide hormone produced in the hypothalamus and released from the posterior pituitary. Stimulation of the cervix during delivery causes release of oxytocin, which is involved in lactation and uterine contractions.

Testosterone is a steroid hormone that is the primary sex hormone in males and has anabolic effects. Testosterone is secreted primarily by the testes in males and the ovaries in females. Small amounts of testosterone are also secreted by the adrenal glands.

Most reproductive hormones are evaluated using commercial radioimmunoassay kits.

Liver Function

Enzymes

The aminotransferases, AST and ALT, are normally contained within liver cells and are released into the blood when liver cells are damaged. Increased levels of AST or ALT are therefore usually indicative of liver damage, as discussed above. Reference ranges for these enzymes in the laboratory animals covered in this volume are provided in Table 3.3.

Reference values for other indicators of liver function, including total bilirubin, bile acids, and serum proteins, in the laboratory animals covered in this volume are provided in Table 3.5.

Total Bilirubin

Bilirubin is produced in the macrophage-monocyte system by the breakdown of hemoglobin to biliverdin and then bilirubin. Bilirubin is carried in the plasma to the liver, where it is conjugated to form bilirubin diglucuronide and excreted in the bile. Bilirubin level thus serves as a measure of liver and bile tract function. Total bilirubin includes both the conjugated and unconjugated (free) forms and, if elevated, is usually indicative of liver damage or hemolysis.

Bilirubin oxidizes slowly when exposed to light, so specimens obtained for bilirubin levels should be protected from light exposure. Freezing also artificially alters bilirubin levels in plasma samples (Ringler and Dabich, 1979).

Elevated total bilirubin may be recognized by a visible yellow coloration of the plasma. A standard colorimetric assay for total bilirubin involves a diazotization reaction (Evans, 2009).

Bile Acids

Bile acids are synthesized from cholesterol and conjugated to amino acids in the liver, then stored in the gallbladder. Bile acids are released from the gallbladder during digestion to form micelles that assist in the processing of dietary fats. Bile acid levels are affected in most

species by diet, gastric and intestinal transit time, enteritis, and gallbladder contraction (Center et al., 1995). Bile acid concentration can be determined by gas or liquid chromatography-mass spectrometry (Perwaiz et al., 2001).

Serum Proteins

The liver is the source of many proteins, and liver function is often evaluated by measuring total serum protein, albumin, and globulin levels (Evans, 2009). Serum proteins include albumin and globulins, and total serum proteins represent the sum of these constituents. Protein electrophoresis is used to separate serum proteins, based on their mobility, into fractions of albumin, alpha-, beta-, and gamma-globulins and determine their concentrations (Thrall et al., 2004).

ALBUMIN

Albumin is the first fraction to appear by electrophoresis and is the predominant serum protein. Albumin is produced in the liver and helps maintain osmotic pressure within the intravascular compartment.

GLOBULINS

Alpha-1 and -2 globulins and beta globulins include a group of binding and carrier proteins in the blood. Alpha globulins are increased during acute infections and demonstrate the most rapid electrophoretic mobility of the globulin fractions. Beta globulins are binding proteins in plasma and thus should be measured in plasma rather than serum. Gamma globulins are primarily antibodies and are increased in response to infection.

TOTAL PROTEIN

Total protein levels can be affected by many factors including age, diet, pregnancy, disease, and dehydration. Technical issues such as assay method and sample handling can also impact the results of this assay.

Low total protein, or hypoproteinemia, is often due to chronic malnutrition, malabsorption, or protein loss due to kidney or liver disease. A specific decrease in the albumin fraction is suggestive of liver disease, although chronic malnutrition from poor diet or dental disease may also cause hypoalbuminemia. Reduced cecotrophy in some laboratory animals may also cause a decrease in total protein, especially albumin.

Increased total protein, or hyperproteinemia, is commonly caused by dehydration, but it may also indicate chronic infection, shock, or a metabolic disturbance.

The relative size of the albumin and globulin fractions may help determine the cause of an abnormal total protein value. The albumin to globulin ratio is often calculated as a measure of this relationship and is usually slightly greater than one. A low ratio could be the result of decreased albumin or increased globulins due to myeloma or autoimmune disease. A high ratio

TABLE 3.6 Kidney Function¹

	BUN (mg/dl)	Creatinine (mg/dl)	References
Rabbit	10–30	0.5–2.5	Evans, 2009 Vennen and Mitchell, 2009
Guinea pig	9–32	0.6–2.2	Hrapkiewicz and Medina, 2007 Riggs, 2009
Hamster	12–26	0.4–1.0	Heatley and Harris, 2009
Gerbil	17–27	0.6–1.4	Heatley and Harris, 2009 Hrapkiewicz and Medina, 2007
Chinchilla	10–40	0.4–2.3	Hrapkiewicz and Medina, 2007 Vennen and Mitchell, 2009
Kangaroo rat	30	N/A	Intress and Best, 1990
Cotton rat	13–14	0.9–1.5	Dolyak and Leone, 1953

N/A = values not available.

¹Species for which no values are available are not listed.

may be caused by low immunoglobulin levels as occurs in some leukemias.

There are several methods used to measure serum protein levels, and the specific method chosen has an effect on the values obtained. Total proteins can be measured with the biuret method, in which a cupric ion–peptide complex forms in an alkaline solution. The bromocresol green dye method produces higher total protein values than the method of cellulose acetate electrophoresis (Hall, 1992).

Kidney Function

Reference values for blood urea nitrogen and creatinine, which are typically used as indicators of kidney function, are provided in Table 3.6 for the laboratory animals covered in this volume.

Blood Urea Nitrogen (BUN)

Urea is produced by the breakdown of proteins and is excreted in the urine. Blood urea nitrogen (BUN) is often used to evaluate renal function in animals, including laboratory animals. Blood urea nitrogen level is affected by circadian rhythm, diet, liver function, hydration, and intestinal absorption (Melillo, 2007). The concentration of BUN is artificially increased by a high-protein diet or vigorous exercise, whereas BUN is decreased by low protein intake, liver failure, or treatment with anabolic steroids (McLaughlin and Fish, 1994).

Urea is measured using the urease enzyme followed by colorimetric detection methods (Evans, 2009).

Creatinine

Creatinine is a non-protein nitrogenous compound that is produced by the breakdown of creatine in muscle. Creatinine is found in serum, plasma, and urine and is excreted by glomerular filtration at a constant rate and in the same concentration as in plasma. Creatinine is a more reliable indicator of renal function than BUN because it is less influenced by other factors such as diet and hydration. Serum creatinine is increased by deficient glomerular filtration, although both BUN and creatinine remain in the reference range in most animals until 50–75% of renal function is lost (Finco, 1989). Creatinine is also increased by necrosis or atrophy of skeletal muscle, hyperthyroidism, infections, burns, or fractures.

When a renal disorder is suspected, the ratio of BUN to serum creatinine may be useful in diagnosing the location of the problem. A normal ratio is 10–20 mg/dl BUN to 1 mg/dl creatinine. A high (more than 20:1) BUN to creatinine ratio suggests pre-renal disease, whereas a low (less than 10:1) ratio is indicative of renal disease.

Creatinine is measured using an alkaline picrate colorimetric (Jaffé) method, by other enzymatic methods, or by HPLC (Evans, 2009). Endogenous chromogens such as bilirubin and ketones can artificially elevate creatinine values (Palm and Lundblad, 2005).

Electrolytes

Reference values for electrolytes in the laboratory animals covered in this volume are provided in Table 3.7.

Bicarbonate (HCO_3^-)

The bicarbonate anion (HCO_3^-) affects the acid–base balance in the body and is necessary for its buffering capacity in blood. The main influences on bicarbonate levels in the blood are respiratory production of carbon dioxide and renal excretion of bicarbonate after its conversion from carbonic acid (Evans, 2009). Increased levels of bicarbonate are indicative of respiratory acidosis and metabolic alkalosis.

Calcium (Ca)

Calcium is the most prevalent mineral in the body and is involved in bone metabolism and conduction of electrical impulses. Total calcium is the sum of bound and ionized (Ca^{2+}) forms. Approximately half of total calcium is bound to plasma proteins and the other half exists in the free cation form in serum. Ionized calcium is difficult to test but represents the more physiologically important value (Evans, 2009).

Total calcium is affected by diet, serum proteins, metabolism, and disease. Calcium is increased in animals with kidney or bone neoplasia. Calcium is decreased in animals with hypoparathyroidism, chronic renal failure, or magnesium deficiency.

TABLE 3.7 Electrolytes¹

	HCO ₃ ⁻ (mEq/l)	Ca (mg/dl)	Cl ⁻ (mEq/l)	Mg ²⁺ (mg/dl)	P (mg/dl)	K ⁺ (mEq/l)	Na ⁺ (mEq/l)	References
Rabbit	16.2–38.0	5.6–17	92–120	2.0–5.4	2.3–6.7	3.5–6.9	131–155	Melillo, 2007 Evans, 2009 Vennen and Mitchell, 2009
Guinea pig	N/A	5.3–12.0	90–115	3.5–4.1	3.0–12.0	4.0–8.0	120–150	Waner et al., 1996 Hrapkiewicz and Medina, 2007 Riggs, 2009
Hamster	N/A	5.3–12.0	93–110	N/A	3.0–9.9	3.9–6.0	128–150	Evans, 2009 Heatley and Harris, 2009
Gerbil	N/A	3.7–6.2	93–118	N/A	3.7–7.0	3.8–6.3	141–172	Hrapkiewicz and Medina, 2007 Heatley and Harris, 2009
Chinchilla	N/A	5.6–15.0	105–130	3.6–4.0	4–8	3–7	130–170	Hrapkiewicz and Medina, 2007 de Oliveira Silva et al., 2005 Riggs and Mitchell, 2009
Cotton rat	12.6–15.9	N/A	N/A	N/A	6.0–7.9	N/A	N/A	Dolyak and Leone, 1953

N/A = values not available.

¹Species for which no values are available are not listed.

Total calcium is typically measured using a colorimetric assay. Measurement of ionized calcium is not as common in animals because it requires the use of a selective ion electrode.

Chloride (Cl⁻)

Chloride (Cl⁻) is a major extracellular anion and, along with sodium, is important for the maintenance of osmolality and acid–base balance. Chloride ions are found in serum, plasma, urine, and cerebrospinal fluid and are reabsorbed in the kidney. Elevated chloride occurs in metabolic acidosis or respiratory alkalosis. Chloride is decreased in metabolic alkalosis and conditions of high fluid loss, such as diarrhea and dehydration (Evans, 2009).

Magnesium (Mg²⁺)

The magnesium (Mg²⁺) cation is involved in enzymatic reactions, conduction of electrical stimuli, and bone formation. Approximately half of total magnesium is ionized and the other half is bound to albumin or otherwise complexed (Evans, 2009).

Elevated magnesium may occur with dehydration, hyperthyroidism, tissue trauma, or adrenocortical insufficiency and can depress myocardial contractions. Low magnesium levels may be associated with myopathies.

Magnesium is typically measured using a colorimetric assay (Evans, 2009).

Phosphorus (P)

Phosphorus (P) is present within cells and is required for normal Krebs cycle function and formation of bone and teeth. Serum phosphorus exists mainly as the phosphate anion (PO₄⁻), which is the form normally assayed. Phosphorus is increased in metabolic bone disease, healing fractures, and during anesthetic recovery. Phosphorus is decreased in animals with renal acidosis, hypopituitarism with growth hormone deficiency, and Gram-negative septicemia.

The kidney is the main organ involved in phosphorus balance by regulating glomerular filtration and tubular reabsorption, so phosphate levels are indicative of renal function. Hyperphosphatemia usually indicates pre-renal, renal, or post-renal effects or soft tissue trauma.

Hypophosphatemia occurs but its clinical significance is not known.

Phosphorus is typically measured using a colorimetric assay (Evans, 2009).

Potassium (K⁺)

Potassium (K⁺) is primarily an intracellular cation and is important for maintenance of membrane potential. Intracellular and extracellular potassium levels are regulated by a complex ion exchange mechanism and affected by several compounds, including aldosterone, insulin, and catecholamines (Evans, 2009). Elevated plasma potassium levels increase aldosterone secretion

from the adrenal glands and may also cause arrhythmias and cardiac arrest. Hypokalemia results in decreased aldosterone secretion.

Potassium is usually measured with an ion-selective electrode (Evans, 2009).

Sodium (Na^+)

Sodium (Na^+) is a cation present in all tissue spaces, and its concentration is associated with osmotic homeostasis. Plasma sodium is normally in balance with renal excretion of sodium, with most reabsorption of sodium occurring in the distal tubules and collecting ducts of the kidney. Sodium is increased when there is high fluid loss that spares electrolytes, or with inadequate water intake. Sodium is decreased in conditions of fluid loss when water replacement includes no electrolytes.

Sodium, as with potassium, is typically measured using an ion-selective electrode (Evans, 2009).

RABBIT

Sample Collection

Restraint

Rabbits are relatively docile with repeated handling, but they are easily frightened by unusual noises and movements. If rabbits are not held correctly, the combination of strong hind legs and a fragile skeleton can result in a broken back. A rabbit should be picked up by the scruff of the neck with one hand and supported under the hind end with the other hand to prevent the rabbit from kicking (Brewer, 2006).

Rabbits may be placed in a restraint device, a cat bag, or wrapped in a towel for blood collection without anesthesia.

Blood

The best site for blood collection in a rabbit depends on the volume and number of samples needed and whether or not the rabbit will be anesthetized for blood collection.

The marginal ear vein is the most common site for collection of small blood samples in the unanesthetized rabbit and can be used for single or repeat samples of 1–5 ml. The rabbit should be restrained as described above and the ear warmed by gentle stroking or using a lamp. Acepromazine maleate (1–5 mg/kg IM) may be given to calm the rabbit and aid in vasodilation. Topical oil of wintergreen, xylene, and local anesthetics have all been used to aid in vasodilation (Hrapkiewicz and Medina, 2007). Xylene especially may be irritating if not thoroughly rinsed off the ear after blood withdrawal (Suckow and Douglas, 1997). The collection site should be shaved and then wiped with alcohol or an antiseptic

solution. Blood is first collected from the vein near the base of the ear, with successive samples taken from the alternate ear or by moving distally toward the tip of the ear. A 25G or 26G needle or butterfly catheter may be inserted into the vein while pressure is applied to the vein at the base of the ear. It is difficult to obtain sufficient blood volume using this method in dwarf breed rabbits due to the small size of their ears (Melillo, 2007), and catheterization of this vein in dwarf breeds may lead to vascular necrosis, thrombosis, or sloughing of the ear (Vennen and Mitchell, 2009).

Other peripheral veins that may be used in a rabbit for small blood samples without anesthesia include the lateral saphenous vein and the cephalic vein, both of which can be accessed using a 25G needle, with or without a 1 ml syringe. The lateral saphenous vein runs superficially and is easily visualized on an extended leg when held off at the stifle. Visualization of this vein is assisted by applying alcohol or clipping the overlying fur. Pressure should be placed on the vein after blood withdrawal to prevent hematoma formation (Mader, 2004). The cephalic vein may be used to collect blood samples or for intravenous catheter placement (Vennen and Mitchell, 2009).

The central ear artery can be used for collection of larger volumes of up to 30 ml of arterial blood in the unanesthetized rabbit. The rabbit should be restrained as described above and a 21G or 22G needle or butterfly catheter inserted into the artery near the distal tip of the ear. Blood may be obtained faster using a vacutainer tube or a vacuum ear bleeder (Fox and Laird, 1999). Subsequent samples can be taken from the alternate ear or by moving proximally toward the base of the ear. Several minutes of digital pressure should be applied to ensure hemostasis after blood withdrawal. Potential side effects of blood collection from the ear vessels of rabbits include thrombosis, hematoma, and vascular necrosis of the pinna.

The jugular vein is the preferred sampling site for venous blood volumes greater than 0.5 ml (Vennen and Mitchell, 2009). An unanesthetized rabbit can be positioned with its neck gently extended upward and its front feet held straight down over the edge of a table. Anesthesia is recommended for jugular venipuncture to avoid the stress of restraint. The anesthetized rabbit is placed in dorsal recumbency and the jugular vein occluded near the apex of the sternum. The vein is most easily found by clipping the overlying hair. The dewlap may partially obstruct access to the jugular vein in a large female rabbit (Mader, 2004).

Cardiocentesis (cardiac puncture) may be used as a terminal procedure to collect a large volume of blood from a rabbit under anesthesia. A large-bore (18G–21G) 1.5-inch needle can be used to enter the left ventricle through the chest wall, through the diaphragm,

or from the cranial or caudal end of the sternum, and blood should be withdrawn slowly. A blood volume of 60–200 ml may be obtained using this method, depending on the size of the rabbit.

Urine

The method used for urine collection in a rabbit depends on the need for an uncontaminated sample and whether or not the animal is anesthetized. Normal rabbit urine is dense and rich in minerals and should be centrifuged and filtered before it is analyzed. Urine samples should be assayed immediately or stored refrigerated until used.

If sample contamination is not a concern, urine may be collected from a pan placed beneath the wire grid floor of the cage in which the rabbit is housed. Alternatively, manual expression of the bladder may be performed on an awake or anesthetized rabbit and urine collected directly from the urethra or a collection pan. Manual expression must be performed carefully due to the thin wall of the rabbit bladder, which may rupture if the rabbit struggles or if there is a urethral obstruction.

Repeated urine samples may be collected over a period of time using a metabolism cage, which separates urine output from feces.

Relatively uncontaminated urine samples may be obtained directly from the urinary bladder of a rabbit by catheterization or cystocentesis under sedation or light anesthesia. A sterile lubricated 9 French flexible catheter may be inserted into the external urethral opening and directed into the urinary bladder. Urine is withdrawn by gentle aspiration with an attached syringe, or it flows out due to gentle manual pressure applied to the caudal abdomen. Cystocentesis may be performed on a rabbit placed in dorsal recumbency after aseptic preparation of the caudal ventral abdomen. The bladder is located by palpation cranial to the pelvis, and a 20G–22G needle is attached to a syringe and inserted through the skin and bladder wall at the ventral midline. Urine is withdrawn under gentle aspiration pressure (Vennen and Mitchell, 2009).

Reference Values and Sources of Variation

Blood

Total blood volume of the rabbit is discussed in the Hematology section of this chapter.

Rabbit blood clots fairly rapidly and may be separated by centrifugation at 1100 rpm after it stands for 30–45 minutes at room temperature. If inadequate time is allowed for clotting to occur, fibrin clots may form in the cell-free fraction (Fox and Laird, 1999).

Rabbit blood may require more anticoagulant than other species due to its higher calcium content (Bjoraker and Ketcham, 1981).

Urine

Characteristics and reference ranges of rabbit urine are presented in Table 3.1.

Rabbits have a wide reference range for urine volume, 20–75 ml per kg body weight, collected over 24 hours. Normal rabbit urine has an alkaline pH of 7.6–8.8 and is fairly dilute, with a specific gravity that ranges from 1.003–1.036. Rabbit urine is normally cloudy due to a high concentration of triple phosphate (ammonium magnesium phosphate), calcium carbonate monohydrate, and anhydrous calcium carbonate crystals. Crystals are identified by examining the urine sediment after centrifugation. Trace protein and a few red or white blood cells are commonly found in rabbit urine.

The color of normal rabbit urine can vary from light yellow or straw color to a reddish-brown. Reddish coloration may be due to dietary porphyrins but must be differentiated from the presence of blood. A dipstick may be used to detect hematuria, and a woods lamp will detect porphyrins due to their natural fluorescence. Clear urine suggests low excretion of calcium, which can be due to renal failure, or may be normal in a young rabbit or lactating doe. Excess dietary calcium may cause rabbit urine to be thick and white with a reddish-orange pigment (Harkness and Wagner, 1989).

Abnormalities in rabbit urine are usually identified by simple diagnostic tests using a few drops of urine. Rabbit urine with an acidic pH indicates acidosis due to anorexia, fever, pregnancy toxemia, or hepatic lipidosis.

Hematuria may be caused by several conditions in the rabbit, including uterine adenocarcinoma, uterine polyps, endometrial hyperplasia, abortion, urolithiasis, cystitis, renal infarcts, cystitis, and urinary tract infection.

Glucosuria may be due to stress hyperglycemia. True glucosuria is indicative of hepatic lipidosis or diabetes mellitus. Ketonuria is abnormal in rabbits and is indicative of anorexia, hepatic lipidosis, pregnancy toxemia, or diabetes.

Proteinuria with low specific gravity typically indicates renal disease. Ratio of urine protein to urine creatinine is normal if it is less than 0.6.

Spores of *E. cuniculi* are visualized using Gram or trichrome stain on urinary sediment obtained by centrifugation.

Carbohydrate Metabolism

GLUCOSE

The reference range for blood glucose in rabbits is 75–155 mg/dl (Table 3.2). Blood glucose remains fairly consistent due to the fact that rabbits eat throughout the day and use volatile fatty acids as their primary source of energy (Melillo, 2007). Normal glucose levels are maintained, even after a prolonged fast of up to 96 hours, probably due to coprophagy (Hunt

and Harrington, 1974; Kozma et al., 1974). However, some reports suggest that glucose levels in rabbits are affected by diurnal variation (Fox and Laird, 1970). There is some strain variation in glucose concentration, with higher levels in New Zealand White rabbits than in Dutch Belted or Polish rabbits (Kozma et al., 1974).

Glucose levels in neonatal rabbits increase to a peak level 9 hours after suckling, then decrease at 24 hours. Glycogen stores are depleted by 6 hours in newborns that do not nurse. Glycogen stores typically increase in neonatal rabbits after 9 days old (Mottaz et al., 1989). At 15 days old, rabbits that are fasted for 48 hours have no change in blood glucose, but a decline in concentration of ketone bodies.

Elevated blood glucose in rabbits is usually due to disease or environment. Hyperglycemia may occur in rabbits anesthetized with urethane, halothane, or isoflurane (Bito and Eakins, 1969; Gil et al., 2007). Rabbits with diabetes mellitus may have blood glucose concentrations higher than 500mg/dl, accompanied by the common diabetic symptoms of polyuria, polydipsia, and polyphagia. Hyperglycemia of approximately 350mg/dl may also be caused by acute intestinal blockage or mucoid enteropathy. Other causes of hyperglycemia in rabbits include traumatic or hypovolemic shock, cold stress, and hyperthermia (de la Fuente et al., 2007; Melillo, 2007).

Low blood glucose in rabbits may be the result of prolonged anorexia and metabolism of adipose tissue, which may lead to hepatic lipidosis. Hypoglycemia may also result from acute sepsis, or terminal mucoid enteropathy, liver failure, or other chronic disease (Melillo, 2007). Rabbits housed with unfamiliar rabbits were found to have decreased glucose concentrations (de la Fuente et al., 2007).

Low glucose values may also result from technical handling, which includes allowing blood samples to stand at room temperature for prolonged periods. Glycolytic enzymes are released from platelets and increase in serum with prolonged clotting time, which lowers glucose in the sample (Lindena and Trautschold, 1986).

Lipid Metabolism

The rabbit is recognized as a valuable model for human disturbances in lipid metabolism, such as the metabolic syndrome and hypercholesterolemia leading to atherosclerosis. The lipoprotein profile of rabbits is similar to that of humans, and rabbits have the same cholesteryl ester transfer protein as in humans, which is not present in rodents (Brousseau and Hoeg, 1999). However, the measurement of cholesterol and triglycerides in rabbits may be confounded by cecotrophy in this species.

CHOLESTEROL

The reference range for cholesterol levels in the rabbit is 10–80mg/dl (Table 3.2), with normal variation in cholesterol levels in rabbits depending on age, sex, and time

of day. Cholesterol levels in neonatal rabbits are similar to adult values and then increase to a peak at 25 days old and decline to adult values at 60–80 days old (Laird and Fox, 1970). Serum cholesterol is higher in female than in male New Zealand White rabbits, and cholesterol levels typically increase at the end of the day (Melillo, 2007).

High-cholesterol diets are particularly effective in the rabbit for producing hypercholesterolemia, and the rabbit is often used as a model to study cholesterol metabolism and atherogenesis (Fekete, 1993). Cholesterol levels are particularly elevated in certain strains of rabbit. For example, the Watanabe Heritable Hyperlipidemic (WHHL) rabbit has a single gene defect that produces 8–14 times the reference level of cholesterol in homozygotes and a slight elevation of cholesterol in heterozygotes. The WHHL strain serves as a model for human familial hypercholesterolemia (Kita et al., 1981).

A high-fat diet, obesity, or liver disease may also cause high cholesterol and triglycerides in rabbits. Hypercholesterolemia during anorexia suggests end-stage hepatic lipidosis and has a poor prognosis. High-density lipoproteins have been found to be reduced, and low-density lipoproteins increased, in pregnant rabbits and their cesarean-derived neonates (Bortolotti et al., 1989). Hypercholesterolemia may be seen in rabbits with disease conditions such as pancreatitis, diabetes mellitus, nephrotic syndrome, or chronic renal failure (Saunders and Davies, 2005). Decreased serum cholesterol may be found in rabbits with liver failure, malnutrition, or pregnancy (Matsuoka et al., 2009).

TRIGLYCERIDES

The reference range for triglyceride levels in the rabbit is 15–160mg/dl (Table 3.2). Recent models of hypertriglyceridemia have been produced by mating WHHL strain rabbits with normal Japanese White rabbits. One resulting strain, TGH, has naturally high cholesterol levels of 1000–1500mg/dl and triglycerides greater than 500mg/dl, with widespread atherosclerotic plaques and xanthomas. The other resulting strain, PHT, has postprandial cholesterol of 100–150mg/dl and triglycerides of 500–3000mg/dl, with no atherosclerosis (Mitsuguchi et al., 2008).

Decreased triglycerides have recently been reported in late-gestational pregnant rabbits on a restricted feeding regimen (Matsuoka et al., 2009).

TOTAL LIPIDS

The reference range for total lipid levels in the rabbit is 150–400mg/dl (Table 3.2).

Enzymes

ALKALINE PHOSPHATASE (ALP)

Reference levels of alkaline phosphatase (ALP) in the rabbit are 10–140IU/l (Table 3.3). Levels of ALP have

been found to vary by breed and strain of rabbit (Fox and Laird, 1970; Kozma et al., 1974) but not by sex or diurnal variation (Fox and Laird, 1970; Kozima et al., 1974). Levels of ALP are 2–4 times higher in young rabbits compared to adults (Kramer, 1989).

Rabbits produce three isoenzymes of alkaline phosphatase, including one intestinal form and two different isoenzymes in the liver and kidney. This is in contrast to most other animals, which produce only two ALP isoenzymes.

Elevated ALP in the rabbit is caused by conditions associated with bile stasis in the liver, including lipodosis, coccidiosis, liver lobe torsion, abscesses, and neoplasia (Melillo, 2007). Bone lesions are also associated with elevated ALP in the rabbit.

Alkaline phosphatase is decreased in pregnant rabbits (Viard-Drouet et al., 1984), in contrast to an increase in ALP during late gestation in many other species.

ALANINE AMINOTRANSFERASE (ALT)

Reference levels of alanine aminotransferase (ALT) in the rabbit are 14–80 IU/l (Table 3.3). The ALT enzyme is not specific to the liver in herbivores, including the rabbit. The ALT enzyme has a short half-life of approximately 5 hours in the rabbit and is therefore not a very useful indicator of liver damage in this species (Melillo, 2007). However, ALT is not increased by restraint in the rabbit, so an elevated ALT value is suggestive of tissue damage in this species (Melillo, 2007). The ALT enzyme is found at high concentrations in cardiac muscle in rabbits (Hoffmann et al., 1982).

The ALT enzyme can be mildly elevated in healthy rabbits after exposure to low levels of lead or mycotoxins (Harcourt-Brown, 2002) or halothane anesthesia (Gil et al., 2007). This enzyme may also be elevated in rabbits with advanced hepatic neoplasia (Reznik and Padberg, 1991).

Liver enzymes in rabbits are not liver-specific, since ALT is found in liver and cardiac muscle, and AST is found in liver, as well as muscle, kidney, and pancreas (Vennen and Mitchell, 2009).

High levels of liver enzymes have been described in cases of viral hemorrhagic disease in rabbits (Melillo, 2007).

ASPARTATE AMINOTRANSFERASE (AST)

Reference levels of aspartate aminotransferase (AST) in the rabbit are 14–113 IU/l (Table 3.3). The AST enzyme is found in many tissues in rabbits, including the heart and liver, and it has a relatively short half-life in the rabbit of approximately 5 hours (Melillo, 2007).

Levels of AST may be elevated due to disease, environment, or technical reasons. Increased AST is found in rabbits with liver damage and in rabbits anesthetized with halothane or isoflurane (Gil et al., 2007). Experimental infection with *Eimeria stiedae* has also been shown to produce elevated AST levels in rabbits

(Gomez-Bautista et al., 1987). Experimental heat stress induced by hyperthermia in rabbits causes a three-fold increase in AST (Marder et al., 1990). Decapitation, heart puncture, or aortic incision produces an artificial elevation of AST due to muscle cell damage (Lindena and Trautschold, 1986). The AST value may also be elevated by physical restraint of a rabbit or when the blood sample becomes hemolyzed (Melillo, 2007).

Liver enzymes in rabbits are not liver-specific, since ALT is found in liver and cardiac muscle, and AST is found in liver, as well as muscle, kidney, and pancreas (Vennen and Mitchell, 2009).

High levels of liver enzymes have been described in cases of viral hemorrhagic disease in rabbits (Melillo, 2007).

LACTATE DEHYDROGENASE (LDH)

Reference levels of lactate dehydrogenase (LDH) in the rabbit are 30–140 IU/l (Table 3.3). The LDH enzyme is not usually helpful in disease diagnosis because it is produced by both muscle and liver cells.

The LDH value may be increased by physical restraint of a rabbit or when the blood sample is hemolyzed because of its high concentration in erythrocytes (Melillo, 2007). Rabbits exposed to noise stress have elevated LDH levels (de la Fuente et al., 2007).

CREATINE KINASE (CK)

Reference levels of CK in the rabbit are 150–1000 IU/l (Table 3.3). Dutch rabbits are reported to have elevated levels of the MM isoform of CK compared to other rabbit breeds and other animals (Yi et al., 1991).

Creatine kinase is commonly increased in the serum by physical restraint or the process of blood collection (Melillo, 2007). The different CK isoforms may be elevated depending on the method of blood collection. For example, the CK-MM and CK-BB isoforms are predominant after blood collection from peripheral veins, and the CK-MB isoform is predominant after blood collection by cardiac puncture (Lindena and Trautschold, 1986). Rabbits exposed to stressors, such as cold, noise, and housing with unfamiliar rabbits, have elevated levels of CK (de la Fuente et al., 2007).

SORBITOL DEHYDROGENASE (SDH)

Reference levels of sorbitol dehydrogenase (SDH) in the rabbit are 170–177 IU/l (Table 3.3).

AMYLASE

Reference levels of amylase in the rabbit are 200–500 U/l (Table 3.3).

In the rabbit, amylase is produced almost exclusively in the pancreas, with little contribution from the salivary glands, intestines, or liver. Amylase levels are

lower in the serum of rabbits than in most other animals (Lindena and Trautschold, 1986).

Elevated amylase levels in rabbit serum may be caused by pancreatitis, pancreatic duct obstruction, peritonitis, or renal failure, or treatment with corticosteroids.

Amylase values may be lower in hemolyzed blood samples.

GLUTAMATE DEHYDROGENASE (GDH)

The reference level of glutamate dehydrogenase (GDH) in the rabbit is 6.39 IU/l (Table 3.3). There is little information on the diagnostic value of this enzyme in rabbits. Its concentration in the centrilobular area of the liver could potentially identify the primary site of hepatocyte damage in liver disease in this species, as in others. Rabbits, like guinea pigs, are different from most animals in that GGT is released into the serum as clotting occurs (Clifford and White, 1999).

OTHERS

Atropine esterase is present in approximately one-third of domestic rabbits. This enzyme hydrolyzes atropine and thus interferes with the pharmacologic action of atropine in many rabbits (Hrapkiewicz et al., 1998).

Hormones

ADRENAL HORMONES

Reference levels of adrenal hormones in the rabbit include 2.6–3.8 µg/dl cortisol and 1.54 µg/dl corticosterone (Table 3.4). Cortisol is the predominant steroid hormone found in rabbits (Fecteau et al., 2007).

The stress of handling and restraint in rabbits causes the release of steroids in an age- and strain-related manner (Redgate et al., 1981). Steroids can affect some biochemical assays (Lindena and Krautschold, 1986). Therefore, it is important to standardize handling methods, blood draw technique, and length of anesthesia in rabbits to reduce the impact of stress on assay results.

A study on the effect of inhalant anesthetics in New Zealand White rabbits found that halothane increases serum corticosterone levels, whereas isoflurane increases corticosterone and serotonin levels in this species (Gil et al., 2007). A recent study in New Zealand White rabbits showed that group housing produced lower white blood cell counts and higher plasma cortisol levels than in individually housed rabbits (Fuentes and Newgren, 2008). Heat stress has also been shown to produce increased cortisol levels in rabbits (de la Fuente et al., 2007). Changes in leukocytes correlated with changes in cortisol levels (Toth and Krueger, 1989).

Stress causes an increase in blood pressure due to increased release of adrenocorticotrophic hormone (ACTH) and adrenaline. This results in a decreased

marginal pool of neutrophils as they move to the central pool and are found at higher levels in the venous blood. Halothane anesthesia, which is now rarely used, has also been shown to increase ACTH levels in the rabbit (Gil et al., 2007).

THYROID HORMONES

Reference levels of thyroid hormones in the rabbit include 130–143 ng/dl triiodothyronine (T3), and 1.7–2.4 µg/dl thyroxine (T4) (Table 3.4).

REPRODUCTIVE HORMONES

Reference levels of reproductive hormones in the rabbit include 10–100 ng/ml luteinizing hormone (LH), 2–4.5 ng/ml follicle stimulating hormone (FSH), 3 pg/ml estradiol, <1 ng/ml progesterone, 10 ng/ml prolactin, 10–20 ng/ml oxytocin, and 3–5 pg/ml testosterone (Table 3.4). There was no difference found between adult male and female neutered rabbits in the serum concentrations of several reproductive hormones, including progesterone, 17-hydroxy-progesterone, androstenedione, and testosterone. Elevated prolactin levels and enlarged mammary glands have been described in a 4-year-old female New Zealand White rabbit with a prolactin-secreting pituitary adenoma (Sikoski et al., 2008).

Liver Function

TOTAL BILIRUBIN

Reference levels of total bilirubin in the rabbit are 0–0.8 mg/dl (Table 3.5). The rabbit produces a large amount of bile per body weight when compared with other animals. Biliverdin is the primary component of rabbit bile, comprising approximately 70% of excreted bile. There is no assay available for biliverdin and it is therefore not measured. Approximately one-third of biliverdin is converted to bilirubin, which is measured in the blood.

In the rabbit, bilirubin is secreted by the liver in the monoconjugated form, in contrast to the diconjugated form secreted in most species (McLaughlin and Fish, 1994). Bilirubin comprises approximately 30% of the excreted bile.

Elevated bilirubin concentration is typically a marker of liver dysfunction or hemolytic disease. In rabbits, hyperbilirubinemia is caused primarily by bile flow obstruction. This obstruction can be the result of hepatic coccidiosis in young rabbits or biliary neoplasia, hepatic lipidosis, or liver lobe torsion in older rabbits. Hepatic fibrosis and hyperbilirubinemia may also be a result of aflatoxicosis from ingesting moldy food (Melillo, 2007).

BILE ACIDS

Reference levels of bile acids in the rabbit are 0–40 mMol/l (Table 3.5), although this is not a standard

biochemical assay in this species. Serum bile acids are used as a measure of liver function in other animals by comparing pre- and postprandial levels, but cecotrophy makes it difficult to fast a rabbit for the pre-prandial sample.

Persistently elevated bile acid concentrations have been reported in rabbits with liver disease (Harcourt-Brown, 2002).

SERUM PROTEINS

The reference range for total serum protein levels in the rabbit is 5.4–8.3 g/dl (Table 3.5), which is higher than those in most other animals (Vennen and Mitchell, 2009). Total protein levels vary by rabbit strain, with descending reference values in Polish rabbits, Dutch Belted rabbits, and New Zealand White rabbits, respectively (Kozma et al., 1974). There is also an effect of age on total proteins in the rabbit, as these values are lowest at birth and increase with age to reach adult values by 12 weeks old (Jain, 1986).

The reference range for albumin levels in the rabbit is 2.4–4.6 g/dl, and the reference range for globulin levels is 1.5–2.8 g/dl (Table 3.5). The albumin fraction of the total protein value is higher in female New Zealand White rabbits than in males (Kozma et al., 1974), but lower in pregnant females than in other adults. Caesarian-derived neonates have low total protein levels, with low albumin and high alpha-globulin levels (Bortolotti et al., 1989).

Feed restriction has been shown to result in lower total protein levels, albumin, and triglycerides in late-gestation pregnant rabbits and to produce a high percentage of near-term abortions (Matsuoka et al., 2009).

Kidney and liver disease are usually associated with decreased albumin and total protein, whereas hyperthermia, dehydration, or shock cause elevated total proteins (Marder et al., 1990). Coronavirus infections have been shown to produce increased globulins in rabbits.

Hemolyzed serum samples can produce artificially increased total protein values (Melillo, 2007).

Cold stress causes an increase in total plasma proteins and beta globulins, and a decrease in serum albumin in the rabbit.

Kidney Function

BLOOD UREA NITROGEN (BUN)

The reference range for blood urea nitrogen (BUN) concentration in the rabbit is 10–30 mg/dl (Table 3.6), and these levels vary according to several factors. Rabbit urea levels increase in late afternoon and early evening and are higher in female than in male New Zealand White rabbits (Melillo, 2007). Polish rabbits are reported to have higher BUN levels than New Zealand White or Dutch Belted strains (Kozma et al., 1974).

Some causes of elevated BUN include urolithiasis and obstruction of urine flow, or kidney tumors or ureteral ligation (Adjarov et al., 1976). Low BUN may be due to hepatic insufficiency or loss of muscle mass due to dental disease (Melillo, 2007).

CREATININE

The reference range for creatinine levels in the rabbit is 0.5–2.5 mg/dl (Table 3.6) and can be increased by renal insufficiency or muscle injury. Creatinine, and often BUN, is elevated when there is reduced blood flow to the kidneys associated with 50–70% loss in renal function (Vennen and Mitchell, 2009). Dehydration can cause pre-renal azotemia in rabbits due to the limited ability of this species to concentrate urine. Blood urea nitrogen and creatinine levels are also increased after stroke, cardiac disease, or bleeding within the gastrointestinal tract (Melillo, 2007).

A study on the effect of inhalant anesthetics in New Zealand White rabbits found that halothane and isoflurane both cause increased levels of BUN and creatinine (Gil et al., 2007).

Renal failure in rabbits can be a result of renal disease due to neoplasia, glomerular nephritis, or pyelonephritis. In addition, renal failure can result from pre-renal disease, such as dehydration, or post-renal disease, such as urethral calculi (Vennen and Mitchell, 2009). The parasite *E. cuniculi* is the most common cause of renal failure in rabbits due to granulomas and fibrosis in the renal parenchyma (Melillo, 2007).

Electrolytes

BICARBONATE (HCO_3^-)

The reference range for the bicarbonate anion in the rabbit is 16.2–38.0 mEq/l (Table 3.7). Rabbit kidneys have minimal ability to correct acid–base imbalances, making this species fairly susceptible to electrolyte alterations. Anorexia in rabbits may also result in metabolic acidosis (Melillo, 2007).

CALCIUM (Ca)

Total serum calcium (Ca) concentration is higher in normal rabbits than in other mammals and has a wide range of reference values in this species, from 5.6–17 mg/dl (Table 3.7). Calcitrol is a metabolite of vitamin D and has a major effect on serum calcium levels in most animals by regulating calcium absorption from the gut. In contrast, gut absorption in the rabbit is independent of calcitrol, and serum calcium concentration depends directly on calcium levels in the diet (Brewer, 2006). Total serum calcium concentrations up to 15 mg/dl and higher have been reported in the rabbit (Hrapkiewicz and Medina, 2007; Melillo, 2007). Young rabbits and pregnant does have a higher calcium requirement than normal adult rabbits,

resulting in lower blood calcium levels in these conditions (Melillo, 2007). Ionized calcium (Ca^{2+}) concentration in rabbit serum is similar to that of other animals.

Excess calcium is excreted in the urine of rabbits, compared to bile excretion in other animals (Hrapkiewicz and Medina, 2007), and the Ca level in urine varies with serum calcium concentration. Urinary excretion rate of calcium is 45–60% in rabbits, compared to 2% in most other mammals (Melillo, 2007). This situation predisposes the rabbit to the formation of Ca-rich uroliths. Hypercalcemia can result when rabbits are unable to eliminate excess calcium and may be a sign of renal disease (Melillo, 2007).

Hypocalcemia is rare in rabbits but may be indicative of poor nutrition, hypoalbuminemia, diarrhea, chronic renal failure, or hyperparathyroidism (Vennen and Mitchell, 2009). Parathyroid hormone levels affect blood calcium levels, and calcium is readily moved from the blood to bones in rabbits (Melillo, 2007). Seizures resulting from hypocalcemia have been reported in late-pregnant and lactating does (Kerr, 1989).

CHLORIDE (Cl^-)

Reference levels of chloride (Cl^-) levels in rabbits are 92–120 mEq/l (Table 3.7) and remain fairly constant across different ages and sexes (Fox and Laird, 1999). Differences in chloride levels have been reported when compared among different strains of rabbits (Fox et al., 1970). Chloride levels are increased in rabbits with dehydration or increased dietary salt intake, and decreased in rabbits with diarrhea or low dietary salts (Vennen and Mitchell, 2009).

MAGNESIUM (Mg^{2+})

Magnesium (Mg^{2+}) levels in normal rabbits are 2.0–5.4 mg/dl (Table 3.7) and are not significantly affected by age, sex, or strain. Increased magnesium levels are seen in rabbits with dehydration, hyperthyroidism, tissue trauma, or adrenocortical insufficiency (Fox and Laird, 1999).

In the rabbit, Mg^{2+} ions are excreted mainly in the urine instead of in the bile.

PHOSPHORUS (P)

Phosphorus (P) levels in normal rabbits are 2.3–6.7 mg/dl (Table 3.7) and show significant differences between strains of rabbits (Fox and Laird, 1970).

Increased phosphorus concentration is indicative of renal disease in rabbits, whereas decreased phosphorus occurs with urinary Ca^{2+} excretion (Vennen and Mitchell, 2009).

Hemolysis of the blood sample may cause an artificially elevated phosphorus value.

POTASSIUM (K^+)

The reference range for potassium (K^+) in normal rabbits is 3.5–6.9 mEq/l (Table 3.7) and may fluctuate

physiologically in rabbits, as in other herbivores (Melillo, 2007).

Hyperkalemia in the rabbit is usually due to acute renal failure, obstructed urine flow, or metabolic acidosis. Hyperkalemia in this species can also be due to severe tissue damage and loss of potassium into the extracellular space, or by excess administration of potassium-rich fluids (Melillo, 2007). Artificially elevated potassium values may be obtained when a blood sample is hemolyzed for technical reasons.

Low potassium levels are seen in cases of acute renal failure, starvation, low-potassium diet, or after administration of excess potassium-poor fluids (Vennen and Mitchell, 2009). Hypokalemia can also result from a stress-induced increase in catecholamines. A condition referred to as “floppy rabbit syndrome” has been associated with low potassium levels in the blood (Harcourt-Brown, 2002). Potassium values may also be decreased for technical reasons, including the use of plastic tubes for blood collection (Caisey and Kling, 1980) and hemolysis of serum samples (Melillo, 2007).

SODIUM (Na^+)

The reference range for sodium (Na^+) in rabbits is 131–155 mEq/l (Table 3.7), and levels vary considerably among different strains of rabbits (Fox and Laird, 1970). Sodium level in rabbits is not very useful as a diagnostic indicator. The serum sodium value may be artificially low in rabbits with lipemia or hyperproteinemia. True hyponatremia in the rabbit may be associated with acute or chronic renal failure and polyuria (Melillo, 2007). Sodium depletion may also be the result of polydipsia in fasted rabbits that drink excessively (Cizek, 1961). Sodium is elevated in rabbits with dehydration or fluid loss due to diarrhea, peritonitis, burns, or myiasis (Melillo, 2007).

GUINEA PIG

Sample Collection

Restraint

Guinea pigs are generally docile, especially with frequent and gentle handling. However, guinea pigs are difficult to restrain for blood and urine samples because they resist being held securely, and their short legs and neck make it difficult to access peripheral vessels without anesthesia. Guinea pigs should be held under the trunk with one hand and supported under the hind end with the other hand, being careful not to constrict the thorax or abdomen (Hrapkiewicz and Medina, 2007).

Blood

Venipuncture in guinea pigs is difficult because the peripheral veins in this species are small and often covered by layers of fat. It is often necessary to shave

the skin and apply alcohol to help visualize peripheral veins in this species (Dyer and Cervasio, 2008).

Small volumes of blood can be obtained in unanesthetized guinea pigs from a variety of peripheral veins using a sterile lancet or a 23G–27G needle. A marginal ear vein can be nicked with a 25G needle and blood drops collected directly into a microhematocrit tube. The lateral saphenous vein runs across the tarsal joint and can be sampled with a 1-ml syringe on a 23G–27G needle after applying digital pressure on the thigh to hold off the vein. The cephalic vein and tarsal vein are also accessible on the guinea pig, although only small volumes can be obtained from these sites, and bruising and hemorrhage often result. Alternatively, a toenail can be cut short using a nail clipper to yield small volumes of blood (Clifford and White, 1999).

Larger volumes of blood can be obtained from anesthetized guinea pigs using large central veins, including the jugular vein, cranial vena cava, and femoral vein. The short neck of the guinea pig makes the jugular vein and cranial vena cava difficult to access, but shaving and proper positioning allow optimal exposure (Pilny, 2008). In the guinea pig, the right jugular vein is larger than the left and will yield up to 2.5 ml of blood. A 24G needle on a 1-ml syringe can be directed caudally to enter the jugular vein just cranial to the clavicle (Shomer et al., 1999). The cranial vena cava can be accessed with a 22G or 23G needle on a 3- or 6-ml syringe that is directed caudally into the sternal notch under the first rib. A ketamine–xylazine mixture has been described as the preferable method of anesthesia for this route of blood sampling (Dang et al., 2008). However, the cranial position of the heart makes cardiac hemorrhage a significant risk when blood is sampled from the cranial vena cava (Joslin, 2009). The femoral vein can be used to collect up to 3 ml of blood with the guinea pig under anesthesia, in dorsal recumbency, and with the rear leg abducted. A 23G needle on a 1–3-ml syringe can be directed at a 45–90° angle to the skin and inserted just ventral to the pulsing femoral artery within the femoral triangle (Joslin, 2009).

Alternative sites for blood collection in a guinea pig under anesthesia include an interdigital vein and the retro-orbital sinus. The interdigital vein technique in the guinea pig is adapted from that described in the rat (Snitily et al., 1991). The foot is disinfected with alcohol and stimulated by rubbing with a methyl salicylate soaked gauze. Approximately 0.4 ml of blood is collected into a capillary tube after puncturing the interdigital vein with a 20G needle. The sample is then transferred into a sodium citrate solution, and pressure is applied to the sampling site for hemostasis (Keino et al., 2002). The retro-orbital sinus will also yield small volumes of blood in an anesthetized guinea pig (Joslin, 2009).

Cardiocentesis can be performed as a terminal procedure in an anesthetized guinea pig. The animal is

placed in dorsal recumbency, and a 20G–25G needle is advanced toward the heart under the xiphoid cartilage to enter the left ventricle. Alternatively, up to 15 ml of blood can be obtained from the descending aorta or caudal vena cava following a laparotomy as a terminal procedure in an anesthetized guinea pig (Joslin, 2009). Decapitation, performed by a trained individual, may yield 10–15 ml of trunk blood (Clifford and White, 1999).

Urine

Urine may be readily collected from the guinea pig using a metabolic cage, free catch after manual pressure on the bladder, or placement on a cold surface (Hrapkiewicz and Medina, 2007). If a urine culture is needed, a cystocentesis should be performed using sedation and aseptic preparation, as described for the rabbit and other animals (Riggs, 2009).

Reference Values and Sources of Variation

Blood

Total blood volume and plasma volume of the guinea pig are discussed in the Hematology section of this chapter.

Clotting of guinea pig blood occurs more slowly when collected into plastic tubes (Clifford and White, 1999).

Urine

Characteristics and reference values of normal guinea pig urine are presented in Table 3.1.

Guinea pigs are herbivores and produce alkaline urine, with a pH of 8–9 (Riggs, 2009).

Normal guinea pig urine is yellow to amber in color, and is fairly opaque. Dietary factors may cause the urine to be darker and more orange in color. It is important to distinguish dietary pigmentation from hematuria using a dipstick to detect blood or Wood's lamp to detect porphyrins. Crystalluria is not uncommon in the guinea pig and should prompt a thorough examination for urinary calculi (Riggs, 2009). A study of urinary calculi in guinea pigs found that the vast majority are composed of 100% calcium carbonate (Hawkins et al., 2009).

Urinary tract infections may occur in older female guinea pigs, and uroliths are an occasional finding in this species (Hrapkiewicz and Medina, 2007).

Carbohydrate Metabolism

GLUCOSE

Reference range for blood glucose level in guinea pigs is 60–130 mg/dl (Table 3.2).

Glucose is elevated in guinea pigs with diabetes mellitus, as in other animals. Spontaneous diabetes mellitus has been reported in guinea pigs at 3–6 months old and is associated with hyperglycemia, glucosuria, and often ketonuria (Hrapkiewicz and Medina, 2007).

Differences in serum glucose have been reported in guinea pigs treated with different anesthetics. For example, serum glucose was higher in guinea pigs anesthetized with 30mg/kg ketamine and 2.5mg/kg xylazine IP, compared to 37mg/kg pentobarbital IP prior to blood draw from the cranial vena cava. This difference was thought to be due to xylazine's inhibition of insulin secretion from pancreatic beta cells in the guinea pig, as in other animals (Dang et al., 2008).

Pregnancy toxemia may occur in the guinea pig in late gestation and takes one of two forms, a fasting or metabolic form and a circulatory or toxic form. The fasting or metabolic form is referred to as ketosis and occurs especially in obese guinea pigs in the first or second pregnancy. This condition may be triggered by stress factors, such as a change in environment or feeding routine, and often results in death. Controlled food intake to prevent obesity can help prevent this condition. In the circulatory or toxic form of pregnancy toxemia, the gravid uterus compresses the descending aorta and causes ischemia of the uterus and placenta (Hrapkiewicz and Medina, 2007).

Obese old male guinea pigs may also become ketotic as a result of reduced carbohydrate diet, resulting in mobilization of fat for energy (Hrapkiewicz and Medina, 2007).

Lipid Metabolism

CHOLESTEROL

The reference range for cholesterol levels in guinea pigs is 20–80mg/dl (Table 3.2). These values are reported to increase gradually with age in male guinea pigs of the inbred Weiser-Maples strain (Kitagaki et al., 2005).

The lipoprotein fraction in guinea pigs contains primarily LDL, with little HDL present unless cholesterol is present in the diet. Changes in dietary fiber primarily affect LDL levels (Fernandez et al., 1997).

The intestine is the primary site of cholesterol synthesis in the guinea pig, as it is in humans, whereas the liver serves this function in most species (Clifford and White, 1999). The guinea pig is very sensitive to cholesterol in the diet, with biochemical changes and lesions produced by dietary cholesterol as low as 0.1%. High dietary cholesterol in the guinea pig causes hemolytic anemia, enlarged spleen, and hyperplastic bone marrow, and may result in death prior to the production of atherogenetic plaques (Yamanaka et al., 1967). Hypercholesterolemia in the guinea pig results in fatty infiltration of the liver and other tissues. The guinea pig serves as a good model for hypercholesterolemia in humans (Sullivan et al., 1993).

TRIGLYCERIDES

The reference range for triglyceride levels in the guinea pig is 10–70mg/dl (Table 3.2). Triglyceride

levels have been found to increase gradually with age in male and female guinea pigs of the inbred Weiser-Maples strain (Kitagaki et al., 2005).

Enzymes

ALKALINE PHOSPHATASE (ALP)

The reference range for alkaline phosphatase (ALP) levels in guinea pigs is 80–350IU/l (Table 3.3). The ALP enzyme was found to decrease with body weight, as in other species (Clifford and White, 1999). Levels of ALP are reported to decrease sharply after birth and then level off in male and female guinea pigs of the inbred Weiser-Maples strain (Kitagaki et al., 2005).

A zinc-deficient diet causes an increase in ALP activity (Clifford and White, 1999), whereas fasting and ascorbic acid deficiency cause decreased ALP levels in guinea pigs (Mahmoodian et al., 1996; Tsuchiya and Bates 1994). Hairless Dunkin-Hartley guinea pigs are reported to have lower ALP levels compared to normal Dunkin-Hartley guinea pigs, although the significance of this difference is not known (Waner et al., 1996).

ALANINE AMINOTRANSFERASE (ALT)

The reference range for alanine aminotransferase (ALT) levels in guinea pigs is 10–90IU/l (Table 3.3), and these values are generally lower in this species than in other animals (Clampitt and Hart, 1978).

Elevated ALT is not a specific or sensitive marker of hepatocellular injury in the guinea pig compared to other animals (Clifford and White, 1999).

Hairless Dunkin-Hartley guinea pigs are reported to have higher ALT concentration compared to normal Dunkin-Hartley guinea pigs (Waner et al., 1996).

ASPARTATE AMINOTRANSFERASE (AST)

The reference range for aspartate aminotransferase (AST) levels in guinea pigs is 10–90IU/l (Table 3.3).

As with ALT and ALP, AST levels are reported to be lower in hairless Dunkin-Hartley guinea pigs than in normal Dunkin-Hartley guinea pigs (Waner et al., 1996).

LACTATE DEHYDROGENASE (LDH)

The reference range for lactate dehydrogenase (LDH) levels in guinea pigs is 20–120IU/l (Table 3.3).

CREATINE KINASE (CK)

The reference range for creatine kinase (CK) levels in guinea pigs is 50–150IU/l (Table 3.3).

Hairless Dunkin-Hartley guinea pigs are reported to have higher CK levels than normal Dunkin-Hartley guinea pigs (Waner et al., 1996). The method of anesthesia has been shown to affect CK levels in guinea pigs. For example, guinea pigs anesthetized with 37mg/kg pentobarbital given intraperitoneally have higher CK levels than

those anesthetized with 30mg/kg ketamine and 2.5mg/kg xylazine given intramuscularly (Dang et al., 2008).

Elevated CK may be seen in guinea pigs with myopathy associated with a deficiency of vitamin E and selenium (Hrapkiewicz and Medina, 2007).

AMYLASE

The reference range for amylase levels in guinea pigs is 995–1239 IU/l (Table 3.3).

Hairless Dunkin-Hartley guinea pigs are reported to have higher amylase levels than normal Dunkin-Hartley guinea pigs (Waner et al., 1996).

Hormones

ADRENAL HORMONES

The reference range for cortisol levels in guinea pigs is 5–30 µg/dl (Table 3.4). Cortisol is the primary glucocorticoid produced by the guinea pig, instead of corticosterone, which predominates in the mouse and rat (Clifford and White, 1999). Cortisol and corticosterone are readily interconverted in the guinea pig (Manin et al., 1982). Plasma cortisol levels vary by age and sex in the guinea pig, probably due to differences in its binding to transcortin (El Hani et al., 1980). Transcortin in guinea pigs has a higher binding affinity for cortisol than for progesterone, whereas transcortin binds both hormones with similar affinity in humans (Clifford and White, 1999). Plasma cortisol levels in males are higher than in females at ages greater than 30 days, similar in males and females at 90–120 days, and higher in adult male guinea pigs (Clifford and White, 1999).

THYROID HORMONES

Reference ranges for thyroid hormone levels in guinea pigs are 22–56 ng/dl for triiodothyronine (T3) and 2.3–3.5 µg/dl for thyroxine (T4) (Table 3.4).

REPRODUCTIVE HORMONES

Reference levels of reproductive hormones in guinea pigs include 5–55 ng/ml luteinizing hormone (LH), 100–200 ng/ml follicle stimulating hormone (FSH), 30 pg/ml estradiol, 1 ng/ml progesterone, and <5 ng/ml oxytocin (Table 3.4).

Guinea pigs, like other hystricomorphs, have a progesterone-binding globulin that is present during pregnancy and binds progesterone but not cortisol (Wriston, 1984).

Liver Function

TOTAL BILIRUBIN

The reference range for total bilirubin levels in guinea pigs is 0–1 mg/dl (Table 3.5).

Blood samples exposed to sunlight demonstrate bilirubin decreases up to 50% in 1 hour (Benjamin and McKelvie, 1978). Freezing of serum samples may also

cause an artificial decrease in bilirubin levels (Bayard, 1974).

SERUM PROTEINS

The reference range for total protein levels in guinea pigs is 4–7 g/dl (Table 3.5). Total protein is reported to increase sharply and level off at 150–200 days old in male and female guinea pigs of the inbred Weiser-Maples strain (Kitagaki et al., 2005).

The reference range for albumin levels in guinea pigs is 2–5 g/dl (Table 3.5). Hairless Dunkin-Hartley guinea pigs are reported to have higher total protein and albumin levels compared to normal Dunkin-Hartley guinea pigs (Waner et al., 1996).

The reference range for globulin levels in guinea pigs is 2–4 g/dl (Table 3.5).

Kidney Function

BLOOD UREA NITROGEN (BUN)

The reference range for blood urea nitrogen (BUN) levels in guinea pigs is 9–32 mg/dl (Table 3.6). Levels of BUN were found to increase gradually with age in male and female guinea pigs of the inbred Weiser-Maples strain (Kitagaki et al., 2005).

The concentration of BUN was found to be higher in guinea pigs anesthetized with 37 mg/kg pentobarbital given intraperitoneally compared to 30 mg/kg ketamine and 2.5 mg/kg xylazine given intramuscularly. This difference was thought to be due to pentobarbital's depression of cardiac output, renal blood flow, and glomerular filtration rate (Dang et al., 2008).

Renal disease is a common chronic condition in older guinea pigs, whereas acute renal disease may occur in younger animals. For example, acute renal disease, with elevated BUN and creatinine, has been described in a 1-year-old guinea pig after ingestion of oxalate-containing plants (Holowaychuk, 2006).

CREATININE

The reference range for creatinine levels in guinea pigs is 0.6–2.2 mg/dl (Table 3.6). Creatinine levels were found to increase gradually with age in male and female guinea pigs of the inbred Weiser-Maples strain (Kitagaki et al., 2005).

Hairless Dunkin-Hartley guinea pigs are reported to have higher creatinine levels compared to normal Dunkin-Hartley guinea pigs (Waner et al., 1996).

Electrolytes

CALCIUM (Ca)

The reference range for calcium levels in guinea pigs is 5.3–12.0 mg/dl (Table 3.7).

Hypercalcemia is rare in guinea pigs and is usually associated with poor nutrition and low phosphorus levels (O'Rourke, 2004).

Hairless Dunkin-Hartley guinea pigs are reported to have higher levels of most electrolytes measured compared to normal Dunkin-Hartley guinea pigs (Waner et al., 1996).

CHLORIDE (Cl^-)

The reference range for chloride levels in guinea pigs is 90–115 mEq/l (Table 3.7). Chloride levels are reported to decrease gradually with age in male guinea pigs of the Weiser-Maples strain (Kitagaki et al., 2005).

MAGNESIUM (Mg^{2+})

The reference range for magnesium levels in guinea pigs is 3.5–4.1 (Table 3.7).

A dietary imbalance of minerals, such as low magnesium and high phosphorus, can cause soft tissue calcification in guinea pigs older than 1 year. Mineral deposits may be found in the elbows and ribs or more widespread, in lung, trachea, heart, aorta, liver, and kidney (Hrapkiewicz and Medina, 2007).

PHOSPHORUS (P)

The reference range for phosphorus levels in guinea pigs is 3.0–12.0 mg/dl (Table 3.7).

Phosphorus was found to be higher in guinea pigs anesthetized with 37 mg/kg pentobarbital given intraperitoneally compared to 30 mg/kg ketamine and 2.5 mg/kg xylazine given subcutaneously (Dang et al., 2008).

POTASSIUM (K^+)

The reference range for potassium levels in guinea pigs is 4.0–8.0 mEq/l (Table 3.7).

Serum potassium values may be lower when blood is allowed to clot in plastic rather than glass tubes, because of slower clotting and decreased release of potassium from platelets (Caisey and Kling, 1980).

SODIUM (Na^+)

The reference range for sodium levels in guinea pigs is 120–150 mEq/l (Table 3.7). Sodium levels are reported to increase gradually with age in female guinea pigs of the Weiser-Maples strain (Kitagaki et al., 2005).

In contrast to most electrolytes, sodium levels are reported to be lower in hairless Dunkin-Hartley guinea pigs compared to normal Dunkin-Hartley guinea pigs (Waner et al., 1996).

HAMSTER

Sample Collection

Restraint

Hamsters are fairly docile when handled frequently but are typically more aggressive than mice. Hamsters

can be restrained manually by gathering the loose scruff skin in one hand and supporting the lower body against the palm of the same hand. A hamster may also be placed in a restraint tube for blood collection without anesthesia. Such a tube should have airholes at the nose end and be cleaned frequently to reduce the risk of cross-infection or stress from pheromones (Hem et al., 1998).

Blood

Small blood samples can be obtained in an unanesthetized hamster from the lateral saphenous vein, which runs dorsally and laterally across the tarsal joint. The hamster may be restrained manually or in a restraint tube, as described above, and the vein is held off with gentle pressure above the knee joint. A 20G–25G needle can be inserted into the vein, and blood is collected from the needle hub (Hem et al., 1998). Blood collection occasionally results in bruising, hemorrhage, or temporary favoring of the sampled limb (Joslin, 2009). The lateral vein of the tarsus may also be used to collect small volumes of blood without anesthesia. A clipped toenail, or a small cut in the ear, tail, or footpad, have also been described for obtaining a small blood sample in the hamster (Buetow et al., 1999; Hrapkiewicz and Medina, 2007).

Anesthesia is needed for blood collection from the cephalic vein in a hamster. A tourniquet is placed around the proximal leg, a 25G needle is inserted in the vein, and blood is collected from the needle hub (Joslin, 2009). Other peripheral veins that can be sampled under anesthesia include the jugular vein, femoral vein, sublingual vein, and ear vein (Joslin, 2009).

The retro-orbital venous plexus is commonly used for blood sampling in the hamster and can be accessed by penetrating the conjunctiva midway along the superior border of the eye (Dyer and Cervasio, 2008). A lateral or medial approach to the retro-orbital venous plexus is also described (Buetow et al., 1999). This method may be used by experienced personnel in unanesthetized hamsters, but anesthesia is recommended to avoid stress and damage to the eye. Up to 3 ml of blood can be withdrawn from this site in an adult Syrian hamster (Buetow et al., 1999), but there is risk of ocular damage using this method (Joslin, 2009).

Hamsters can be anesthetized to obtain a 1-ml sample of blood from the cranial vena cava using a 23G–25G needle inserted lateral to the manubrium, cranial to the first rib, and directed caudally. Cardiac puncture has been used for obtaining 1–2 ml of blood in the anesthetized hamster with a 3/8-inch 25G needle (Mitruka and Rawnsley, 1981). However, this technique requires practice, and repeated sampling is associated with a higher risk of mortality (Buetow et al., 1999).

Terminal blood collection of up to 5ml in a Syrian hamster can be performed under anesthesia by cardiocentesis, laparotomy, and venipuncture of the caudal vena cava, or by translumbar vena cava collection (Joslin, 2009).

Urine

Hamsters often urinate when they are picked up, so small urine samples can be obtained by this method. A metabolic cage may be used to collect larger samples of urine from a hamster. This method is advantageous for separating urine from fecal pellets, which are typically mixed on the floor of a conventional hamster cage.

Reference Values and Sources of Variation

Blood

Total blood volume of the hamster is discussed in the Hematology section of this chapter.

Urine

Characteristics and reference values of hamster urine are presented in Table 3.1.

Hamsters produce up to 7ml of urine per day. Chinese hamsters with diabetes mellitus may have significantly increased urine excretion of up to 75ml per day (Fiszer et al., 1979). The pH of hamster urine is normally alkaline and varies from 5.1–8.4.

Hamster urine is cloudy due to the normal presence of crystals and protein. Protein excretion rate in hamster urine is almost 10mg per week, which is approximately ten times higher than that in humans (Tietz, 1976). Cholesterol is the main lipid present in hamster urine, with an excretion rate of 11.7mg per week (Cox and Gökçen, 1973). Hamster urine has variable levels of sodium and potassium, averaging 70mM sodium and 120mM potassium (Fiszer et al., 1979). The primary crystals present in hamster urine include calcium carbonate and triple phosphate (Schuchman, 1980).

Carbohydrate Metabolism

GLUCOSE

The reference range for blood glucose concentration in the hamster is 37–198mg/dl (Table 3.2). Glucose level is affected by many factors, including diet, restraint, anesthesia, hibernation, and diurnal variation, as well as certain disease states (Tomson and Wardrop, 1987). These conditions must be taken into account when evaluating blood glucose concentration.

Blood glucose levels in the hamster are affected by dietary factors, including feeding schedule, duration of fast, and the food content of the stomach (Feldman et al., 1982).

The stress of restraint is likely an important factor producing elevated blood glucose in hamsters. This is demonstrated by higher glucose concentrations in blood samples repeated after 15 minutes. In addition, hamsters sedated for blood sampling yield lower glucose values than those that are unsedated (Tomson and Wardrop, 1987).

Thiobarbiturate anesthesia has been found to increase blood glucose to 300mg/dl in adult male Syrian hamsters, compared to approximately 145mg/dl in unanesthetized controls. This hyperglycemia has been found to persist for several hours (Turner and Howards, 1977).

Elevated glucose levels are reported during hibernation in the hamster. The hamster also has a circadian rhythm of glucose concentrations, with the lowest level just before the start of the dark period and the highest level at the start of the light period (Cincotta and Meier, 1984). This effect may be mediated by melatonin secretion by the pineal gland (Ortega-Corona et al., 1991).

Insulin-induced hypoglycemia is also affected by diurnal factors, with no hypoglycemia at 4 hours after light onset and significant hypoglycemia at 8–20 hours after light onset.

The Chinese hamster is often used as a model of diabetes mellitus due to the high incidence of the disease in this strain. Glucose levels of up to 500mg/dl may be seen in diabetic Chinese hamsters, and this level can be decreased with lithium supplementation in the drinking water (Hu et al., 1997).

Lipid Metabolism

CHOLESTEROL

The reference range for cholesterol concentration in the hamster is 112–210mg/dl (Table 3.2), which represents the highest reference range of normal rodents and rabbits (Cox and Gökçen, 1973). Cholesterol levels in the hamster are affected by a number of factors, including diet, strain, environment, diurnal variation, and temperature, as well as disease.

Cholesterol levels in the hamster are significantly affected by diet. A high-cholesterol, high-triglyceride diet is associated with elevated plasma cholesterol levels in the hamster. These abnormal levels can usually be reduced by fasting (Sullivan et al., 1993). A high-fructose diet produces higher VLDL triglycerides, free cholesterol, and phospholipid levels in the hamster, but no change in apolipoprotein B levels. In addition, a high-fructose diet increases LDL triglycerides, decreases LDL cholesteryl ester, and increases HDL fractions (Wang et al., 2008).

Group housing causes increased cholesterol levels in hamsters fed an atherogenic diet when compared to singly housed controls (Yoganathan et al., 1998).

A decrease in photoperiod from 14 to 10 hours of light reduces cholesterol levels in hamsters.

Photoperiods of 10 hours or less cause a decrease in cholesterol with no effect on other plasma lipids (Gad, 2007). Low environmental temperature causes a decrease in plasma cholesterol but no change in plasma triglycerides (Vaughan et al., 1984), whereas hibernation causes an increase in serum lipids (Gad, 2007).

Disease conditions have an effect on cholesterol levels in the hamster. Chronic hepatitis and biliary disease cause hypercholesterolemia in the hamster, probably due to cholestasis (Brunnert and Altman, 1991). Diabetes in Chinese hamsters, alloxan treatment in Syrian hamsters, pertussis toxin, and amyloidosis are all associated with hypercholesterolemia (Garcia-Sainz et al., 1987; Gerritsen, 1982; Murphy et al., 1984).

TRIGLYCERIDES

The reference range for triglyceride concentration in the hamster is 72–350 mg/dl (Table 3.2). A high-carbohydrate diet increases plasma triglycerides in the hamster. Low environmental temperature is reported to produce no change in plasma triglycerides in the hamster (Vaughan et al., 1984).

TOTAL LIPIDS

The reference range for total circulating lipids in hamsters is 224–466 mg/dl (Table 3.2), which is higher than in other rodents (Cox and Gökçen, 1974; Mitruka and Rawnsley, 1977).

Enzymes

ALKALINE PHOSPHATASE (ALP)

The reference range for alkaline phosphatase (ALP) concentration in the hamster is 50–186 IU/l (Table 3.3). Levels of ALP are affected by the age, sex, and strain of hamster (Maxwell et al., 1985). Immature hamsters have higher ALP levels than adults because of its release from growing bone, as in other animals (Dent, 1977).

Hamster ALP includes isoenzymes from bone, liver, and intestine (Cox and Gökçen, 1973), and placenta (Manning et al., 1970). Bone provides the main isoenzyme in hamster, and intestinal ALP provides 10–15% of the total ALP activity in this species. The liver ALP isoenzyme is sensitive to heat and declines from weaning to undetectable levels in older hamsters (Tomson and Wardrop, 1987).

Increased ALP in the hamster is a sensitive indicator of liver damage resulting from viral-induced hepatitis, as well as chronic hepatitis with biliary disease due to intrahepatic cholestasis and increased ALP synthesis (Brunnert and Altman, 1991). Increased ALP in hamsters has been reported in association with leukemias, prostate gland tumors, and bone and liver cancers (Eugster et al., 1966).

ALANINE AMINOTRANSFERASE (ALT)

The reference range for alanine aminotransferase (ALT) levels in the hamster is 20–128 IU/l (Table 3.3) and may vary due to a variety of factors, including disease or technical issues.

The ALT enzyme is elevated in hamsters with hepatic necrosis caused by virus or acetaminophen toxicity (El-Hage et al., 1983; Rollinson and White, 1983). Elevated ALT is seen in Syrian hamsters with chronic hepatitis and is correlated with the number of affected hepatocytes. Chronic hepatitis can also result in decreased ALT due to decreased hepatocyte numbers (Duncan and Prasse, 1986). Hamsters experimentally infected with pirital virus (Arenaviridae) have increased ALT and AST 5–8 days post-infection (Sbrana et al., 2006).

The cardiac puncture method may result in higher serum levels of several enzymes, including ALT, AST, LDH, and CK, compared to blood sampled from other sites (Tomson and Wardrop, 1987).

ASPARTATE AMINOTRANSFERASE (AST)

The reference range for aspartate aminotransferase (AST) levels in the hamster is 20–150 IU/l (Table 3.3).

The AST enzyme is increased in hamsters with liver neoplasia and metabolic alterations (Eugster et al., 1966).

LACTATE DEHYDROGENASE (LDH)

The reference range for lactate dehydrogenase (LDH) levels in the hamster is 100–300 IU/l (Table 3.3). Levels of LDH are increased in hamsters with hereditary myopathy (Homburger et al., 1966) and after inoculation with some viruses (Eugster et al., 1966).

Freezing of hamster serum samples has been shown to decrease LDH activity (Tietz, 1976).

CREATINE KINASE (CK)

The reference value for creatine kinase (CK) level in the hamster is 23 IU/l (Table 3.3).

Creatine kinase has been found in hamster striated muscle and nervous tissue, and its level is used to diagnose myopathies, muscular dystrophies, and cardiac necrosis in this species. The Syrian hamster has a hereditary myopathy characterized by increased CK levels up to 730 IU/l compared to 23.2 IU/l in controls (Bajusz and Homburger, 1966).

AMYLASE

The reference range for amylase levels in the hamster is 154–196 IU/l (Table 3.3).

Amylase is present in the hamster pancreas, intestine, and liver, as in other animals (Takahashi et al., 1981).

Hormones

ADRENAL HORMONES

The reference range for cortisol levels in the hamster is 2.3–3.2 µg/dl (Table 3.4), which is lower than in many other rodent species. Male hamsters generally have larger adrenal glands and higher cortisol levels than female hamsters.

Pregnancy is associated with increased cortisol levels in hamsters, with elevations from 0.3–30 µg/dl as parturition approaches (Brinck-Johnsen et al., 1981).

Cortisol fecal metabolites have been extracted and measured by radioimmunoassay in hamsters undergoing surgery. Cortisol metabolites were found to increase immediately following surgery and decrease thereafter (Chelini et al., 2006). Plasma cortisol levels were also elevated after chronic stress in the hamster (Ottenweller et al., 1985).

The reference range for corticosterone levels in the hamster is 5.5–9.3 µg/dl (Table 3.4).

The hamster adrenal cortex contains distinct glomerulosa, fasciculata, and reticularis layers, as in other species, and ACTH administration increases adrenal weight (Alpert, 1950).

Adrenal gland activity is sensitive to diurnal variation, with maximal secretion approximately 3 hours before the onset of the dark period (Frenkel et al., 1965).

Cold temperatures do not stimulate ACTH release in the hamster (Frenkel et al., 1965).

THYROID HORMONES

The reference ranges for thyroid hormone levels in the hamster are 30–80 ng/dl for triiodothyronine (T3) and 3–7 µg/dl for thyroxine (T4) (Table 3.4). Several factors affect T3 and T4 levels in the hamster, including hibernation, age, strain, and temperature. Chronic exposure of hamsters to shortened photoperiod and lower temperature, as occurs during periods of hibernation, results in a decrease in levels of TSH, T3, and T4 (Hoffman et al., 1982; Vaughan et al., 1982). Increasing age in the hamster, from 3 to 20 months, is accompanied by decreasing levels of T3 and T4 (Neve et al., 1981). There is also a smaller response to TSH in older hamsters.

Hamsters have decreased protein-bound iodine during pregnancy, which may alter thyroid hormone metabolism during this condition (Galton and Galton, 1966).

REPRODUCTIVE HORMONES

The adult female hamster has a 4-day estrous cycle associated with predictable variations in the reproductive hormones, including follicle stimulating hormone (FSH), luteinizing hormone (LH), and progesterone (PG). The luteal phase includes day 1 (ovulation) and day 2, while the follicular phase, or proestrus, occurs

on day 4 of the cycle. Hormonal fluctuations occur daily in the immature female hamster and shift to the mature 4-day cycle in the adult female (Donham and Stetson, 1991). Reproductive hormone levels have been well characterized in the hamster and are described below.

Reproductive hormone levels for both male and female hamsters are sensitive to photoperiod, with short periods of light producing decreased testosterone levels in males (Furuta et al., 1994), and decreased FSH, LH, and prolactin levels in females (Chandrashekar and Bartke, 1989).

LUTEINIZING HORMONE (LH) The reference range for LH levels in the hamster is 20–40 ng/ml (Table 3.4).

Normal adult female hamsters have one LH peak on the afternoon of proestrus (day 4), approximately 10 hours before ovulation. LH levels are fairly stable during days 1–3 of the estrous cycle in the Syrian hamster (Buetow et al., 1999). A similar pattern for LH has been reported in the Djungarian hamster (Erb and Wynne-Edwards, 1994).

FOLLICLE-STIMULATING HORMONE (FSH) The reference range for FSH levels in the hamster is 100–300 ng/ml (Table 3.4).

In the adult female hamster, FSH normally peaks at the time of the LH peak on the afternoon of proestrus (day 4) and then again on the morning of estrus (day 1), stimulating follicular growth for the next cycle. Levels of FSH are fairly stable during days 2 and 3 of the estrous cycle in the Syrian hamster (Buetow et al., 1999). A similar pattern for FSH has been reported in the Djungarian hamster (Erb and Wynne-Edwards, 1994).

Female Turkish hamsters demonstrate daily FSH variations when in anestrus and at immature stages (Ogilvie et al., 1992).

ESTRADIOL The reference range for estradiol levels in the hamster is 5–10 pg/ml (Table 3.4).

Estradiol levels are low during the luteal phase of the reproductive cycle of the hamster, showing an inverse relationship to levels of progesterone.

PROGESTERONE The reference value for progesterone level in the hamster is 1 ng/ml (Table 3.4).

Progesterone is the predominant reproductive hormone during the luteal phase, or day 1 and 2 of the hamster reproductive cycle. Progesterone then decreases and increases again on day 4, or proestrus (Buetow et al., 1999).

PROLACTIN The reference range for prolactin levels in the hamster is 5–10 ng/ml (Table 3.4).

Prolactin levels in the female hamster typically correspond with the persistence of corpora lutea during pregnancy and the onset of maternal behaviors

at parturition. During the estrous cycle, prolactin peaks each afternoon, with highest concentrations in proestrus. This pattern continues into gestation, but peak concentrations decrease as pregnancy advances (Buetow et al., 1999). In Djungarian hamsters, prolactin levels decrease in midgestation (Edwards et al., 1994).

Prolactin levels in most hamsters respond to photoperiod, although this response is less apparent in the Djungarian hamster (Ebling, 1994).

Prolactin increases in the female hamster in response to ether anesthesia and to the stress of handling (Matt et al., 1983), although similar stresses decrease prolactin and testosterone levels in the male hamster (Huhman et al., 1995).

TESTOSTERONE The reference range for testosterone levels in the hamster is 1.5–2.0 pg/ml (Table 3.4).

Liver Function

TOTAL BILIRUBIN

The reference range for bilirubin levels in the hamster is 0.1–0.9 mg/dl (Table 3.5), which are lower values than bilirubin in humans (Mitruka and Rawnsley, 1981).

Hamsters with hepatic dysfunction do not usually have abnormal serum bilirubin levels (Wardrop and Van Hoosier, 1989).

SERUM PROTEINS

The reference range for total protein concentration in the hamster is 5.2–7.0 g/dl (Table 3.5) and is affected by age, disease, and other factors. There are significant levels of fibrinogen in hamster plasma, making it necessary to accurately measure liver proteins in serum samples. Fibrinogen is linked to alpha-globulin rather than beta-globulin in the hamster.

The reference ranges for albumin and globulin concentrations in the hamster are 3.5–4.9 g/dl and 2.7–4.2 g/dl, respectively (Table 3.5). Albumin concentration decreases during the first year of life, whereas alpha-2-globulin increases by 6 months old, beta-globulin decreases at 8 weeks old, and levels of gamma-globulin and fibrinogen are variable between 8 weeks and 1 year of age (House et al., 1961). The albumin-to-globulin ratio decreases with age in the hamster.

Pregnancy in hamsters alters serum proteins, with decreased albumin and increased alpha-globulins in near-term hamsters. Hibernating hamsters have increased beta-globulin, albumin, and total protein, and decreased levels of gamma-globulin (South and Jeffay, 1958).

Disease states can also produce changes in protein concentrations. Experimentally induced inflammation produces an elevation of gamma-globulins (Betts et al., 1964). Hamsters experimentally infected with pirital virus (Arenaviridae) have decreased albumin 5–8 days post-infection (Sbrana et al., 2006).

Diabetes mellitus is associated with increased alpha-2 globulin levels in Chinese hamsters (Green and Yerganian, 1963). Older hamsters commonly have amyloidosis, which produces decreased levels of albumin and increased levels of gamma-globulin (Gleiser et al., 1971; Murphy et al., 1984).

Kidney Function

BLOOD UREA NITROGEN (BUN)

The reference range for blood urea nitrogen (BUN) concentration in the hamster is 12–26 mg/dl (Table 3.6) and is affected by diet, sex, and disease. Blood urea nitrogen is affected by the quality and quantity of the diet, as well as duration of fasting in the hamster. A diet containing 18–24% protein has been shown to produce a higher BUN level in the hamster than one with 12% protein (Feldman et al., 1982).

Some studies have demonstrated higher BUN values in the female hamster (Feldman et al., 1982; Maxwell et al., 1985), although findings have been variable.

Renal disease is common in older hamsters and is associated with increased BUN (Feldman et al., 1982). *Leptospira pomona* infection in the hamster is associated with elevated BUN, as high as 424 mg/dl, along with renal lesions and renal failure (Abdu and Sleight, 1965). Hamsters experimentally infected with pirital virus (Arenaviridae) were found to have increased BUN, and other abnormalities, 5–8 days post-infection (Sbrana et al., 2006).

CREATININE

The reference range for creatinine concentration in the hamster is 0.4–1.0 mg/dl (Table 3.6) and is affected by sex and age (Murphy et al., 1984) as well as disease states.

Elevated serum creatinine has been found in hamsters with renal amyloidosis and the nephrotic syndrome (Murphy et al., 1984). However, hamsters with chronic hepatitis were found to have no change in serum creatinine (Brunnert and Altman, 1991). Similarly, there is no difference in creatinine levels in inbred hypertensive (CHF 148) hamster strains compared to controls (Thomas et al., 1997).

Electrolytes

In general, electrolyte values in hamsters are similar to those of other rodents (see Table 3.7).

CALCIUM (Ca)

The reference range for calcium concentration in the hamster is 5.3–12.0 mg/dl (Table 3.7).

CHLORIDE (Cl⁻)

The reference range for chloride concentration in the hamster is 93–110 mEq/l (Table 3.7).

PHOSPHORUS (P)

The reference range for phosphorus concentration in the hamster is 3.0–9.9 mg/dl (Table 3.7). These values are higher in younger hamsters than older hamsters (Dent, 1977). Phosphorus levels in blood can be affected by the age, sex, and strain of the hamster (Maxwell et al., 1985).

POTASSIUM (K)

The reference range for potassium concentration in the hamster is 3.9–6.0 mEq/l (Table 3.7).

Hemolysis of blood samples can result in artificially elevated serum potassium levels (Bannon and Friedell, 1966).

SODIUM (Na⁺)

The reference range for sodium concentration in the hamster is 128–150 mEq/l (Table 3.7).

OTHER RODENTS

Gerbil***Sample Collection*****RESTRAINT**

The gerbil may be restrained by grasping the base of the tail with one hand and the scruff of the neck with the other hand. Alternatively, the gerbil may be enclosed within one hand and held firmly in an upright position. Gerbils should not be picked up by the tip of tail due to the risk of degloving. Gerbils are not aggressive but will readily jump in an attempt to escape (Hrapkiewicz and Medina, 2007).

BLOOD

Blood samples can be collected more frequently in the gerbil than in other laboratory animals because of their rapid rate of erythrocyte turnover. The lateral tail vein can be used for blood collection in an unanesthetized gerbil, although caution should be taken because of the increased risk of tail degloving injury in this species (Joslin, 2009). Anesthesia should be used in the gerbil for blood collection from sites other than the tail vein. The saphenous vein or metatarsal vein can be distended using a tourniquet proximal to the stifle and a small blood sample is collected into the hub of a 22G needle. A 0.1–0.3-ml blood sample can be collected from the retro-orbital venous sinus of a gerbil by advancing a microhematocrit tube through the lateral canthus and holding off the external jugular vein behind the mandible (Joslin, 2009). The medial canthus is also a viable approach to the retro-orbital sinus in the gerbil (Dyer and Cervasio, 2008). Alternatively, small volumes of blood may be obtained from the lateral metatarsal vein, or from a clipped toenail. Large volumes of blood can

be obtained by cardiac puncture under anesthesia as a terminal procedure in the gerbil, as in other species (Hrapkiewicz and Medina, 2007).

URINE

The gerbil is a desert species and produces small quantities of concentrated urine. However, a sample can usually be obtained when a gerbil is picked up (Hrapkiewicz et al., 1998). Other methods of urine collection, such as urinary catheterization or cystocentesis, require sedation or anesthesia.

Reference Values and Sources of Variation**BLOOD**

Total blood volume of the gerbil is discussed in the Hematology section of this chapter.

CARBOHYDRATE METABOLISM

GLUCOSE The reference range for glucose concentration in the gerbil is 50–135 mg/dl (Table 3.2).

Gerbils are susceptible to obesity and may develop reduced glucose tolerance and diabetes mellitus.

LIPID METABOLISM

CHOLESTEROL The reference range for cholesterol concentration in the gerbil is 90–150 mg/dl (Table 3.2).

Cholesterol metabolism of the gerbil is similar to that of humans, so this species has been used to study the effects of dietary cholesterol and fats (Gordon and Cekleniak, 1961). Gerbils fed a diet with no cholesterol have higher alpha lipoprotein fractions than pre-beta or beta lipoprotein fractions (Forsythe, 1986). This finding has been confirmed with micro-affinity chromatography (Tschantz and Sunahara, 1993). Gerbils fed high-cholesterol diets often develop gallstones.

Gerbil serum is often lipemic, especially in older gerbils of either gender, although this condition is more common in males. Sunflower seeds in the diet may contribute to lipemic serum in gerbils (Heatley and Harris, 2009). Repeatedly bred male and female gerbils may develop hyperadrenocorticism with associated elevation of serum triglycerides and development of fatty livers (Percy and Barthold, 2007).

ENZYMES

ALKALINE PHOSPHATASE (ALP) The reference range for ALP concentration in the gerbil is 12–37 IU/l (Table 3.3).

HORMONES

ADRENAL HORMONES The gerbil adrenal gland is very large and secretes primarily cortisol.

Repeatedly bred male and female gerbils may develop hyperadrenocorticism with associated mineralization in the aorta and peripheral arteries, as well as

pathology of the pancreas, thymus, and adrenal glands. This condition is also associated with obesity and diabetes (Percy and Barthold, 2007).

LIVER FUNCTION

TOTAL BILIRUBIN The reference range for bilirubin concentration in the gerbil is 0.2–0.6 mg/dl (Table 3.5).

SERUM PROTEINS The reference range for total protein concentration in the gerbil is 4.3–12.5 g/dl (Table 3.5).

The reference ranges for albumin and globulin concentrations in the gerbil are 1.8–5.5 and 1.2–6.0 g/dl, respectively (Table 3.5).

KIDNEY FUNCTION

BLOOD UREA NITROGEN (BUN) The reference range for BUN concentration in the gerbil is 17–27 mg/dl (Table 3.6).

CREATININE The reference range for creatinine concentration in the gerbil is 0.6–1.4 mg/dl (Table 3.6).

ELECTROLYTES

CALCIUM (Ca) The reference range for calcium concentration in the gerbil is 3.7–6.2 mg/dl (Table 3.7).

CHLORIDE (Cl⁻) The reference range for chloride concentration in the gerbil is 93–118 mEq/l (Table 3.7).

PHOSPHORUS (P) The reference range for phosphorus concentration in the gerbil is 3.8–7.0 mg/dl (Table 3.7).

POTASSIUM (K⁺) The reference range for potassium concentration in the gerbil is 3.8–6.3 mEq/l (Table 3.7).

SODIUM (Na⁺) The reference range for sodium concentration in the gerbil is 141–172 mEq/l (Table 3.7).

Chinchilla

Sample Collection

RESTRAINT

Chinchillas are relatively easy to handle and restrain for collection of blood and urine samples, and they rarely bite unless agitated. Chinchillas should be restrained by grasping the base of the tail with one hand and placing the other hand over the shoulders and thorax. Fur slip, or loss of a patch of fur, can occur if a chinchilla is handled roughly (Hrapkiewicz and Medina, 2007).

BLOOD

Blood collection in the chinchilla is best performed using anesthesia to reduce stress, unless the animal is very accustomed to being handled. Peripheral blood

vessels are very small in the chinchilla and yield volumes of blood that are sufficient for assays such as blood glucose and packed cell volumes (Riggs and Mitchell, 2009). Small blood samples can be obtained from several peripheral veins, including the lateral saphenous vein, cephalic vein, lateral abdominal vein, and ventral tail vein (Joslin, 2009). A 23G–27G needle on a 1-ml syringe can be used at these sites. Best success is achieved using a tourniquet or holding off the vein proximally, as in other animals. A toenail clip or needle stick between the toes may also be used for a small blood sample in the chinchilla (Hrapkiewicz and Medina, 2007).

The retro-orbital sinus may be used for blood collection in the anesthetized chinchilla, as in other rodents. Alternatively, the transverse sinus in the chinchilla runs superficially around the auditory bulla and can also serve as a site for blood collection. A 25G butterfly catheter attached to a 1-ml syringe can be used to penetrate this sinus from the dorsal surface of the head near the ear (McClure, 1999).

Larger blood samples, sufficient for a complete blood count or clinical biochemistry panel, may be obtained from the femoral vein, jugular vein, or cranial vena cava in the anesthetized chinchilla, using methods described for other animals (Joslin, 2009). A 25G needle on a 1-ml or 3-ml syringe can be used to collect a blood sample from these sites (Riggs and Mitchell, 2009). The jugular veins are very superficial in the chinchilla, making them visible and particularly accessible for blood sampling in this species (Pilny, 2008).

Cardiocentesis may be used as a terminal procedure under anesthesia for collection of a large volume of blood in the chinchilla, as in other animals.

URINE

Urine for routine urinalysis can be collected from a chinchilla by clean-catch, from the cage floor, using a metabolic cage, or by manual pressure on the bladder. Urine to be cultured should be collected by cystocentesis, with or without anesthesia. For cystocentesis, the animal is placed in dorsal recumbency, the caudoventral abdomen disinfected, and urine collected percutaneously with a 25G needle attached to a 3-ml syringe (Riggs and Mitchell, 2009).

Reference Values and Sources of Variation

BLOOD

Total blood volume of the chinchilla is discussed in the Hematology section of this chapter.

URINE

Reference values and characteristics of chinchilla urine are listed in Table 3.1.

Normal chinchilla urine is yellow to amber, although dietary porphyrins can produce dark orange urine

that must be distinguished from hematuria. Chinchilla urine is normally alkaline, with a pH of 8–9 (Riggs and Mitchell, 2009).

An overweight female chinchilla with diabetes mellitus has been described with hyperglycemia and severe glucosuria and ketonuria (Marlow, 1995).

CARBOHYDRATE METABOLISM

GLUCOSE The reference range for glucose concentration in the chinchilla is 60–125 mg/dl (Table 3.2).

Serum glucose is lower in blood obtained from chinchillas anesthetized with ketamine and xylazine than in blood obtained by cardiac puncture post-mortem (de Oliveira Silva et al., 2005).

Diabetes mellitus has been described in an overweight female chinchilla that was accompanied by hyperglycemia of greater than 400 mg/dl (Marlow, 1995).

LIPID METABOLISM

CHOLESTEROL The reference range for cholesterol concentration in the chinchilla is 40–300 mg/dl (Table 3.2).

TRIGLYCERIDES The reference range for triglyceride concentration in the chinchilla is 149–198 mg/dl (Table 3.2).

Serum triglycerides are lower in blood obtained from chinchillas anesthetized with ketamine and xylazine than in blood obtained by cardiac puncture post-mortem (de Oliveira Silva et al., 2005).

ENZYMES

ALKALINE PHOSPHATASE (ALP) The reference range for ALP concentration in the chinchilla is 10–70 IU/l (Table 3.3).

ALANINE AMINOTRANSFERASE (ALT) The reference range for ALT concentration in the chinchilla is 10–35 IU/l (Table 3.3).

ASPARTATE AMINOTRANSFERASE (AST) The reference range for AST concentration in the chinchilla is 15–100 IU/l (Table 3.3).

CREATINE KINASE (CK) The reference range for CK concentration in the chinchilla is 0–300 IU/l (Table 3.3).

HORMONES

THYROID HORMONES The reference range for T4 in the chinchilla is 3.4–6.4 µg/dl.

Thyroparathyroidectomy performed on chinchillas to create an animal model of hypothyroidism resulted in 9–89% reduction in thyroxine (T4) levels in 80% of animals at 6–14 days after surgery. This was followed by rapid regrowth of the thyroid gland and a return of T4

to baseline levels in most animals studied (Martin et al., 2005). The average baseline T4 level was 5.25 µg/dl, with no difference between males and females. Sham-operated controls had a significant increase in T4 levels, suggesting a non-specific response to surgery (Martin et al., 2005).

LIVER FUNCTION

TOTAL BILIRUBIN The reference range for bilirubin concentration in the chinchilla is 0.6–1.3 mg/dl (Table 3.5).

SERUM PROTEINS The reference range for total protein concentration in the chinchilla is 3.8–8.0 g/dl (Table 3.5).

The reference ranges for albumin and globulin concentrations in the chinchilla are 2.3–4.1 g/dl and 0.9–4.2 g/dl, respectively (Table 3.5).

KIDNEY FUNCTION

BLOOD UREA NITROGEN (BUN) The reference range for BUN concentration in the chinchilla is 10–40 mg/dl (Table 3.6).

Serum urea is lower in blood obtained from chinchillas anesthetized with ketamine and xylazine than in blood obtained by cardiac puncture post-mortem (de Oliveira Silva et al., 2005).

CREATININE The reference range for creatinine concentration in the chinchilla is 0.4–2.3 mg/dl (Table 3.6).

ELECTROLYTES

CALCIUM (Ca) The reference range for calcium concentration in the chinchilla is 5.6–15 mg/dl (Table 3.7).

CHLORIDE (Cl⁻) The reference range for chloride concentration in the chinchilla is 105–130 mEq/l (Table 3.7).

MAGNESIUM (Mg²⁺) The reference range for magnesium concentration in the chinchilla is 3.6–4.0 mg/dl (Table 3.7).

PHOSPHORUS (P) The reference range for phosphorus concentration in the chinchilla is 4–8 mg/dl (Table 3.7).

POTASSIUM (K⁺) The reference range for potassium concentration in the chinchilla is 3–7 mEq/l (Table 3.7).

SODIUM (Na⁺) The reference range for sodium concentration in the chinchilla is 130–170 mEq/l (Table 3.7).

Degu

Sample Collection

There is no published information available on methods of restraint or sample collection in the degu. In addition, blood volume and urine characteristics have not been reported in the literature.

Reference Values and Sources of Variation

CARBOHYDRATE METABOLISM

GLUCOSE The reference range for glucose concentration in the degu is 74–82 mg/dl (Table 3.2).

The degu is very intolerant of sugar in the diet and is very susceptible to developing diabetes mellitus when exposed to increased dietary glucose (Opazo et al., 2004). Cases of spontaneous diabetes mellitus in the degu may also be exacerbated by a carbohydrate-rich diet (Najecki and Tate, 1999; Nishi and Steiner, 1990). The protein structure of degu insulin and glucagon is similar to that of the guinea pig but varies from that of most other laboratory rodents, suggesting a difference in carbohydrate metabolism in histricomorph rodents (Nishi and Steiner, 1990).

A case of transitional cell carcinoma in a degu was found to be associated with hyperglycemia, increased ALT, and decreased total protein and albumin levels (Lester et al., 2005).

HORMONES

ADRENAL HORMONES The reference value for cortisol concentration in the degu is 15 µg/dl and the reference value for corticosterone in the degu is 1.5 µg/dl (Table 3.4).

The degu has been referred to as semi-precocial and has a more advanced hypothalamic–pituitary–adrenal axis at birth compared to that of more altricial rodents such as rats and mice (Gruss et al., 2006). The adrenal glands of the degu secrete both cortisol and corticosterone, with cortisol showing the greatest increase in response to stress (Kenagy et al., 1999). Plasma corticosterone levels in the degu are approximately 10–20% of cortisol levels (Gruss et al., 2006). Both male and female degus have elevated cortisol and corticosterone levels at birth, low levels at days 3–21, and higher, adult-like levels at days 45 and later (Gruss et al., 2006). There are no gender-dependent differences in these levels at any age.

The stress of parental separation causes elevated cortisol and corticosterone in male and female degus between 3 and 21 days of age. The absence of measurable ACTH activity is thought to be due to lack of degu-specific antibodies (Gruss et al., 2006).

Diurnal cycle affects cortisol levels in male degus, with a peak at the beginning of the light cycle and a trough near the end of a 12-hour light cycle (Mohawk et al., 2005).

KIDNEY FUNCTION

A case of transitional cell carcinoma in a degu was found to be associated with increased BUN, creatinine, and phosphorus levels (Lester et al., 2005).

Deer Mouse

Sample Collection

RESTRAINT

Peromyscus species readily adapt to the laboratory environment and may be handled in a similar manner to *Mus musculus*.

BLOOD

Blood can be collected from tail veins of the deer mouse under light anesthesia (Sealand, 1961). Larger blood samples may be collected directly from the heart in animals under deep terminal anesthesia (Gough and Kilgore, 1964).

There is very little information available in the literature regarding clinical biochemistry parameters in the deer mouse.

Reference Values and Sources of Variation

ENZYMES

ALKALINE PHOSPHATASE (ALP) The reference range for ALP concentration in the deer mouse is 6–7 IU/l (Table 3.3).

Deer mice infected with *Capillaria hepatica* were found to have increased concentrations of alkaline phosphatase compared with uninfected controls (Meagher, 1998).

LIVER FUNCTION

SERUM PROTEINS Deer mice infected with *Capillaria hepatica* were found to have increased total proteins and decreased albumin levels compared with uninfected controls (Meagher, 1998).

Dormouse

Sample Collection

BLOOD

Little information is available on obtaining blood samples from the dormouse. Blood samples of 1 ml were obtained by cardiac puncture in the anesthetized dormouse (Jallageas and Assenmacher, 1986).

Very little information is available in the literature regarding clinical biochemical parameters in the dormouse.

Reference Values and Sources of Variation

CARBOHYDRATE METABOLISM

GLUCOSE Glucose-induced insulin release was studied in the perfused pancreas of the edible dormouse (*Glis glis*) and demonstrated seasonal variation in this species. Exposure of the dormouse to 302 mg/dl glucose caused highest insulin release in the fall and winter and decreased glucose sensitivity in the spring and summer (Castex and Sutter, 1981).

HORMONES

THYROID HORMONES Artificially controlled photoperiod pattern and constant warm temperatures were found to interfere with the normal annual cycle of thyroxine levels in the dormouse (Jallageas and Assenmacher, 1986).

REPRODUCTIVE HORMONES The annual cycle of plasma testosterone levels in the dormouse is parallel and correlated to thyroxine concentrations (Jallageas and Assenmacher, 1983). Testosterone levels are decreased compared to reference levels in the dormouse when the thyroid gland is removed in June, at the beginning of the breeding season (Jallageas et al., 1992). Constant pattern of photoperiod and warm temperatures were found to interfere with the normal annual cycle of testosterone levels in this species (Jallageas and Assenmacher, 1986).

Kangaroo Rat

Sample Collection

RESTRAINT

The kangaroo rat has a fragile tail that should not be used for restraint. The recommended method of restraining a kangaroo rat is by grasping the loose skin at the nape of the neck (Fine et al., 1986).

BLOOD

Blood withdrawal from the kangaroo rat is best accomplished using sedation or light anesthesia. Blood sampling from the infraorbital sinus has been described in this species under light anesthesia (Banta and Holcombe, 2002). Blood samples can be obtained by tail clipping in the unanesthetized kangaroo rat (Scelza and Knoll, 1982).

URINE

Kangaroo rats rarely drink water in the wild and use internal metabolically derived water and concentration of urine to survive in an arid or semiarid environment. Kangaroo rats will drink water in captivity, but it is difficult to obtain a sufficient urine sample from this species to run a urinalysis (Donnelly and Quimby, 2002). A metabolism cage has been used successfully to evaluate urine volume and concentration in the kangaroo rat in one study (Banta and Holcombe, 2002).

Reference Values and Sources of Variation

URINE

Urine produced by kangaroo rats is very concentrated, which represents an adaptation to their desert environment in nature (Tracy and Walsberg, 2002). One

species (*D. panamintinus*) reportedly excretes urine that is concentrated to 3.28M (Intress and Best, 1990).

HORMONES

THYROID HORMONES The reference range for T4 in the kangaroo rat is 0.48–1.92 µg/dl (Table 3.4). Levels of circulating T4 are lower in the kangaroo rat than predicted based on its size and may explain the low metabolic rate in this species (Hulbert et al., 1985; Yousef and Johnson, 1975).

Kangaroo rats implanted subcutaneously with slow-release thyroxine pellets producing 0 (placebo) to 111.1 µg per day showed a dose-dependent increase in plasma thyroxine and basal metabolic rate, with no increase in urinary water loss (Banta and Holcombe, 2002).

KIDNEY FUNCTION

BLOOD UREA NITROGEN (BUN) The reference value for BUN in the kangaroo rat is 30 mg/dl (Table 3.6).

The kangaroo rat produces uric acid as the final product of purine metabolism, and it is excreted in the feces. This process is metabolically complex but advantageous for a desert-dwelling rodent because it conserves water.

It is possible to produce a condition similar to diabetes insipidus in the kangaroo rat if it is allowed to drink water ad libitum (Eisenberg and Isaac, 1963).

Cotton Rat

Sample Collection

RESTRAINT

Cotton rats are challenging to restrain for sample collection because they move quickly, jump vertically, and may attempt to bite when picked up. The tail skin is susceptible to degloving, so the tail should not be used to pick up a cotton rat (Donnelly and Quimby, 2002). Restraint with a leather glove followed by anesthesia is recommended to attain sufficient control for sample collection (Prince, 1994).

BLOOD

Cotton rats have tail veins that are not accessible for blood sampling, although blood samples can be obtained from the cut tip of the tail without anesthesia (Dolyak and Leone, 1953). A blood sample of up to 500 µl (per 100-g animal) may be collected from the retro-orbital venous sinus of an anesthetized cotton rat (Prince, 1994; Webb et al., 2003). Blood flow may be increased by applying pressure to the ipsilateral jugular vein (Prince, 1994). Blood sampling directly from the

heart of anesthetized cotton rats has also been reported (Dolyak and Leone, 1953).

Reference Values and Sources of Variation

CARBOHYDRATE METABOLISM

CHOLESTEROL The reference range for glucose level in the non-fasted cotton rat is 115–216 mg/dl (Table 3.2), or 78–124 mg/dl after a 24-hour period of fasting (Dolyak and Leone, 1953).

LIPID METABOLISM

GLUCOSE The reference range for cholesterol in the cotton rat is 134–165 mg/dl (Table 3.2).

ENZYMES

ALKALINE PHOSPHATASE (ALP) The reference range for ALP in the cotton rat is 10–14 IU/l (Table 3.3).

LIVER FUNCTION

SERUM PROTEINS The reference range for total protein in the cotton rat is 6.1–6.9 g/dl (Table 3.5).

The reference ranges for albumin and globulins in the cotton rat are 4.2–5.2 g/dl and 1.4–2.2 g/dl, respectively (Table 3.5).

KIDNEY FUNCTION

BLOOD UREA NITROGEN (BUN) The reference range for BUN in the cotton rat is 13–14 mg/dl (Table 3.6).

CREATININE The reference range for creatinine in the cotton rat is 0.9–1.5 mg/dl (Table 3.6).

ELECTROLYTES

BICARBONATE (HCO_3^-) The reference range for bicarbonate anion concentration in the cotton rat is 12.6–15.9 mEq/l (Table 3.7).

PHOSPHORUS (P) The reference range for phosphorus concentration in the cotton rat is 6.0–7.9 mg/dl (Table 3.7).

Sand Rat

Sample Collection

BLOOD

Blood samples of 100 μ l or more may be collected from the sand rat by cutting the tip of the tail (Frenkel and Kraicer, 1972).

URINE

Urine samples from sand rats may be collected using metabolic cages (Frenkel and Kraicer, 1972).

Very little information is available on the clinical biochemistry parameters of the sand rat.

Reference Values and Sources of Variation

CARBOHYDRATE METABOLISM

GLUCOSE The reference range for glucose concentration in the sand rat is 68–71 mg/dl (Table 3.2). This value does not vary significantly during a 60-hour period of fasting (Frenkel and Kraicer, 1972).

HEMATOLOGY

Introduction

Hematology is the study of blood and blood-forming organs, including the diagnosis, treatment, and prevention of diseases of the blood, bone marrow, and immunologic, hemostatic, and vascular systems. Hematologic analysis is often used for the diagnosis and treatment of animal diseases. As analytical methods have become more sensitive, requiring smaller sample volumes, hematology has become more important for disease recognition and treatment in the smaller laboratory animals.

Several physiologic and physical factors can affect the results of hematologic assays and make it difficult to establish reference values. Physiologic factors include age, breed and strain, gender, season and time of day, nutrition, disease, stress, and trauma. Important physical factors include site of blood withdrawal, presence and type of anticoagulant, and sample handling and preparation (Dyer and Cervasio, 2008). Interpretation of results must take these factors into consideration.

A joint committee formed by several scientific societies formulated a recommended core panel of hematology tests for safety and toxicity studies in laboratory animals (Weingand et al., 1996). This core panel includes total leukocyte count, differential leukocyte count, erythrocyte count, erythrocyte morphology, platelet count, hemoglobin concentration, hematocrit or packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration. Blood smears can be used for reticulocyte counts, and bone marrow cytology should be collected and evaluated if indicated. Tests of hemostasis recommended by the committee include prothrombin time, activated partial thromboplastin time, and platelet count (Weingand et al., 1996).

Blood Collection and Sample Preparation

Blood Collection

RESTRAINT

General approach and methods of restraint for blood collection in laboratory animals are discussed above in the Clinical Chemistry section of this chapter.

SAMPLE VOLUME

The size of the animal determines the total volume of blood that can be safely collected in a single sample. The standard safe volume of a single sample is 1% of the animal's body weight every 14 days, although a smaller volume is recommended in older animals or those with suspected anemia or hypoproteinemia. Blood volume is usually restored within 24 hours, but the return of some blood components to normal levels can take up to 2 weeks after blood collection (Joslin, 2009).

COLLECTION SITES

Many of the same peripheral vessels used for blood collection in larger animals can be used in laboratory animals, although the unique anatomy of certain species determines the success of one site over another. For example, the tail vein is an appropriate site for blood sampling in the mouse and gerbil but is not useful in the hamster. Blood collection sites for the respective species are discussed in the Clinical Biochemistry section of this chapter.

Regardless of the site used for blood collection, the overlying skin should be clipped or shaved and cleaned with an aseptic solution such as chlorhexidine gluconate or povidone iodine. A topical anesthetic, such as lidocaine and prilocaine, may also be applied to the skin prior to blood collection (Joslin, 2009).

Sample Preparation

Hematology parameters are best evaluated on a blood sample that is freshly collected and not hemolyzed or clotted. The blood sample may be collected into a tube with or without an anticoagulant.

Routine hematology assays are usually performed using an anticoagulant such as ethylenediaminetetraacetic acid (EDTA) or lithium heparin. EDTA is preferable to heparin as an anticoagulant because of its minimal alteration of cell morphology and staining characteristics. Coagulation studies are typically performed using sodium citrate.

If no anticoagulant is used, the blood should be well mixed by manually inverting or electronically rotating the tube, to ensure thorough mixing of blood components before removing an aliquot for evaluation. Blood clotting occurs more slowly in plastic tubes than in glass tubes.

Erythrocyte and leukocyte morphology and staining characteristics may be altered by anticoagulants. For this reason, blood smears should be made prior to mixing blood samples with anticoagulant (Dyer and Cervasio, 2008). A small volume of blood can be collected in the hub of a needle and used for blood smear preparation without exposure to anticoagulants.

Blood Volume

Total Blood Volume

Published values for total blood volume in a species can vary up to 10%, depending on the method used for calculation. Blood volume is also affected by physiologic factors such as age and lean body mass. In general, as an animal increases in size, the percentage of blood volume decreases (Joslin, 2009).

Blood volume is measured by the T-1824 or Evans blue dye method due to the high affinity of this azo compound dye to serum albumin.

Plasma Volume

In most animals, plasma volume is approximately 50–60% of the total blood volume. The plasma volume can be estimated from the size of the clear layer above the packed red cells and buffy coat in a microhematocrit tube after centrifugation.

Plasma volume can be calculated using radiolabeled albumin (McGuill and Rowan, 1989).

Reference ranges for blood and plasma volumes in the laboratory animals covered in this volume are provided in Table 3.8.

Peripheral Blood Cells

Erythrocytes

Red blood cells are anucleate and have a biconcave disc shape, which minimizes the distance that hemoglobin must travel to the surface of the red blood cell for gas exchange. The biconcave disc shape also increases the flexibility of the cell for movement through vessels for oxygen delivery.

The number of erythrocytes may be represented by red blood cell count, red blood cell concentration, hematocrit, or packed cell volume.

RED BLOOD CELL COUNT

An automated hematology instrument typically provides the red blood cell count in an isotonic dilution of the blood sample and calculates the red blood cell concentration from this value and the sample volume. Hematocrit and packed cell volume values both indicate the percentage of red blood cells in whole blood and are essentially equivalent but are derived by different methods.

Hematocrit (Hct) is usually calculated in an automated hematology instrument from the red blood cell concentration and mean cell volume (MCV) of red blood cells. Hematocrit is calculated using the formula $Hct = (MCV \times \text{red blood cell concentration})/10$ and is represented as percent.

Packed cell volume (PCV) is usually determined using the microhematocrit method (Goldenfarb et al.,

TABLE 3.8 Blood Values¹

	Total Blood Volume		Single Sample Vol (ml/2 wks)	Exsanguination Vol (ml)	Plasma Volume (ml/kg BW)	Bone Marrow (M:E ratio)	References
	(ml)	(ml/kg BW)					
Rabbit	160–480	44–70	20–40	60–160	28–50	1.1:1	Suckow and Douglas, 1997 Hrapkiewicz and Medina, 2007
Guinea pig	40–80	67–92	4–12	15–30	39	1.5–1.9:1	Dineen and Adams, 1970 Harkness and Wagner, 1995 Longley et al., 2008
Hamster	6.8–12	65–80	0.5–1.3	3–5	N/A	0.9:1	Gad, 2007 Hrapkiewicz and Medina, 2007 Brown and Donnelly, 2004
Gerbil	4.4–8	67	0.4–1.3	2–4	N/A	0.75–2.35:1	Dineen and Adams, 1970 Brown and Donnelly, 2004 Pilney, 2008
Chinchilla	20–32	N/A	2–7	8–12	N/A	N/A	Hrapkiewicz and Medina, 2007 Riggs and Mitchell, 2009

N/A = values not available.

¹Species for which no values are available are not listed.

1971). A microhematocrit tube is filled from the hub of the sampling needle or from the tube of anticoagulant-treated blood. The microhematocrit tube is filled at one end with clay sealant and centrifuged at high speed for 2–3 minutes in a microhematocrit centrifuge. The top clear layer is plasma, the middle white band is the buffy coat representing predominantly leukocytes and platelets, and the bottom red layer is packed erythrocytes. Normal plasma is clear, whereas icteric plasma is yellow, lipemic plasma is white, and hemolytic plasma is red. The PCV value is measured on a microhematocrit card reader by placing the tube on the card so that the sealant boundary with erythrocytes is on the zero line and the top of the plasma column is on the 100 line. The top of the red cell column then represents the percentage of the sample that is composed of red blood cells.

Errors of up to 8% can occur due to improper force or duration of centrifugation (Mitruka and Rawnsley, 1977).

PCV is fairly constant among mammals, whereas total red blood cell count and mean cell size vary among species (Campbell and Ellis, 2007).

HEMOGLOBIN (Hgb)

Hemoglobin is the protein that transports oxygen and carbon dioxide in the blood stream. There are several methods used to determine hemoglobin levels (Mitruka and Rawnsley, 1977).

Hemoglobin level is fairly constant among mammals and is usually approximately one-third of the hematocrit value, or one-third of the packed cell volume (Campbell and Ellis, 2007).

The cyanmethemoglobin method is usually performed by an automated hematology instrument, which uses a lytic dilution of the blood sample to release hemoglobin. Potassium cyanide converts hemoglobin to the stable pigment cyanmethemoglobin. Light absorbed at a specific wavelength is measured by spectrophotometry in a hemoglobinometer. Light is absorbed in proportion to the hemoglobin concentration, using a known concentration as a reference. Hemoglobin concentration is expressed as g/dl.

ERYTHROCYTE LIFESPAN

The lifespan of circulating red blood cells varies somewhat between species but typically ranges from 50–70 days. A variety of methods have been used to estimate this duration, including radiolabeled compounds and alveolar carbon monoxide.

ERYTHROCYTE SIZE AND STAINING

Mean cell volume (MCV) is the average size of red blood cells counted electronically. It is expressed in femtoliters or μ^3 .

Mean cell hemoglobin (MCH) is calculated using the hemoglobin concentration and red blood cell

concentration and therefore provides no additional clinical information. MCH is expressed in pg per cell.

Mean cell hemoglobin concentration (MCHC) is calculated from the hemoglobin concentration and hematocrit and provides the index of Hgb volume in grams relative to PCV in g per dl or percent.

$MCHC (g/dl) = [Hgb (g/dl)/PCV (\%)] \times 100$. Since Hgb is typically one-third the hematocrit value, the MCHC value is usually approximately 30g/dl.

In an automated hematology instrument, hemoglobin and packed cell volume are determined using different aliquots of a blood sample that are diluted separately.

A hemolyzed sample causes a falsely increased MCHC value.

Regenerative anemia causes decreased MCHC if the reticulocyte fraction is >20%. Severe iron-deficiency anemia or microcytic anemia also decreases MCHC due to decreased Hgb content.

$MCHC < 28 g/dl$ is usually due to instrument error.

RETICULOCYTES

Reticulocytes provide an assessment of the bone marrow's response to anemia. The rate of reticulocyte release from the bone marrow indicates the erythroid component of the bone marrow. A reticulocyte stain measures aggregates of residual ribosomes and mitochondria that form clumped granular material called reticulum.

Reticulocytes appear as polychromatophilic cells seen on a Wright- or Wright-Giemsa-stained blood film.

New methylene blue or brilliant cresyl blue are mixed with several drops of blood and incubated for 10 minutes in a tube before making a blood film. Reticulocytes are counted out of 1000 RBCs in a blood film and expressed as percentage of the RBCs. In cases of anemia, the number will be altered, so reticulocyte number per μl is calculated as $RBCs/\mu l \times \% \text{ reticulocytes}$.

Reticulocytes are increased as the bone marrow responds to anemia. A reticulocyte number of less than 10 000 per μl is considered to represent no or minimal regenerative response; 10 000–60 000 per μl is a poor regenerative response; 60 000–200 000 per μl is considered a moderate response; and 200 000–500 000 reticulocytes per μl is a maximal regenerative response.

Reference ranges for erythrocyte parameters in the laboratory animals covered in this volume are provided in Table 3.9.

Leukocytes

TOTAL WHITE BLOOD CELL (WBC) COUNT

Total leukocyte concentration is measured using the Unopette dilution method, automated nucleated particle counter, or by expanded buffy coat analysis. The Unopette system is a commercial kit that dilutes a blood

sample 1:100 in acetic acid, resulting in membrane lysis and removal of erythrocytes and platelets. The remaining nucleated particles are leukocytes and these are quantified within a grid under a microscope. Electronic cell counters use a similar approach, except that red blood cells are lysed with a detergent solution before nucleated particles are quantified. A quantitative buffy coat analysis system (QBC, Becton Dickinson) expands the buffy coat layer in a specialized microhematocrit tube and quantifies the leukocytes by optical scanning. An automated hematology instrument uses lytic dilution of the blood sample to provide white blood cell numbers.

BLOOD SMEAR

The optimal blood smear is a single layer of dispersed cells that accurately represents the distribution of cells in whole blood. The most common method of blood smear preparation is the wedge technique, in which a drop of blood is placed near the end of one glass slide. A second slide is held at a 30–45° angle, backed into the drop, and quickly pushed forward to the end of the first slide. The best area for cell counting is the monolayer of cells (counting area) between the thick region and the feathered edge (Thrall et al., 2004).

BLOOD STAINING

Blood staining is needed to perform a differential leukocyte count and to identify morphologic abnormalities. Romanowsky stains, such as Wright's or Wright-Giemsa stains, are employed in diagnostic laboratories to stain blood smears. Rapid hematology staining kits that mimic the Wright stain are commonly used in clinical settings.

DIFFERENTIAL LEUKOCYTE COUNT

The concentration of individual white blood cell types is the most important indicator of disease, and this is determined in a well-prepared and stained blood smear. The stained counting area is examined under high power to perform a differential count of leukocytes, evaluate blood cell morphology, and determine platelet number. The differential count of leukocytes is performed by classifying 100 consecutively encountered white blood cells as neutrophil, band neutrophil, lymphocyte, monocyte, eosinophil, and basophil. Morphology of white and red blood cells should be noted, and number of platelets per high-powered field recorded.

Flow cytometry can be used to count cell populations based on phenotypic markers and measuring cell functions (Kania, 2008). Antibodies are used that bind specifically to cell surface markers called cluster of differentiation (CD) antigens. The markers may correlate with specific cell functions.

Cell counting is performed with instruments that use light-scattering technology. The degree and angle

TABLE 3.9 Erythrocyte Values¹

	RBC Count ($\times 10^6/\mu\text{l}$)	Hct/PCV (%)	Hgb conc (g/dl)	RBC Lifespan (days)	RBC diam (microns)	MCV (μ^3)	MCH (pg/cell)	MCHC (g/dl)	Reticulocytes (%)	References
Rabbit	4.9–7.8	31–50	10–17.4	57	5.0–7.8	57.5–75	17.1–23.9	28.2–37	1.7–6.3	Thrall et al., 2004 Brewer, 2006 Melillo, 2007 Vennen and Mitchell, 2009
Guinea pig	4–11	30–50	11–17	N/A	6.6–7.9	70–95	23–27	25–40	0–6.1	Thrall et al., 1974 Riggs, 2009
Hamster	2.7–12.3	30–59	10–19.2	50–78	5–7	64–78	20–26	28–37	N/A	Thrall et al., 2004 Heatley and Harris, 2009
Gerbil	7–10	35–52	10–17.9	10	N/A	46.6–60	16.1–19.4	30.6–33.3	N/A	Donnelly and Quimby, 2002 Heatley and Harris, 2009
Chinchilla	5–10	30–55	9–15	N/A	N/A	32.1–69.2	10.4–19.8	20–38.5	N/A	Donnelly and Quimby, 2002 Riggs and Mitchell, 2009
Deer mouse	10–12	38–52	12–16	N/A	N/A	36–46	11–15	30–35	N/A	Sealander, 1960, 1961 Gough and Kilgore, 1964 Meagher, 1998
Kangaroo rat	7–9	47–53	15–17	N/A	N/A	57.3–66.0	18.6–25	30–35	N/A	Scelza and Knoll, 1982 Intress and Best, 1990
Cotton rat	N/A	N/A	13–14	N/A	N/A	N/A	N/A	N/A	N/A	Dolyak and Leone, 1953

N/A = values not available.

¹Species for which no values are available are not listed.

of light scatter is used to differentiate different types of cells. Automated differential WBC counting is fairly accurate for normal blood but can be inaccurate if the blood sample is abnormal and can be verified with a manual differential count.

Coulter technology is more commonly used for cell counting. This method is used to measure the size of RBCs, distinguish RBCs from platelets, and differentiate types of WBCs. Suspended cells impede the medium's ability to conduct current. Current deflection is measured as cells and current pass through an opening, and the amount of the deflection is proportional to the size of the cell.

LYMPHOCYTES Lymphocytes are found in blood, bone marrow, lymph nodes, gut-associated lymphoid tissue (GALT), and are associated with immunologic response.

NEUTROPHILS Mammalian neutrophils are phagocytic and function in the destruction of microorganisms. Inflammation, especially that caused by microorganisms, results in an increase in circulating neutrophils.

EOSINOPHILS Eosinophils are involved in the inactivation of histamine in response to allergens and some chronic diseases of the skin, lungs, gastrointestinal tract, and uterus, organs with high concentrations of mast cells.

Eosinophilic granules contain alkaline proteins that stain intensely pink with eosin dye. The granules have a crystalline shape that varies between species.

Eosinophils have phagocytic activity, but this function is much less effective than neutrophils.

Eosinophilia is typically associated with infections with helminth larvae, allergic inflammation, and antigen-antibody complexes (Thrall et al., 2004). Eosinophils primarily act to destroy parasitic microorganisms with the alkaline proteins contained in their granules.

BASOPHILS Basophils are typically the least common of the granulocytes and contain large cytoplasmic granules that stain with basophilic dyes. Basophils function in inflammatory reactions associated with allergies. Basophils contain heparin and histamine and are often found at sites of ectoparasite infection.

MONOCYTES Monocytes have a large nucleus with two lobes. Monocytes function in the immune system to restore the macrophages and dendritic cells in the tissues and respond to inflammatory mediators by differentiating into macrophages and dendritic cells as part of an immune response.

Platelets (Thrombocytes)

Platelets are cytoplasmic fragments from megakaryocytes in the bone marrow and contain cytoplasmic organelles. Platelets form the initial hemostatic plug during clot formation and prevent hemorrhage after damage to small blood vessels. Platelets often appear as clumps in blood smears. Large platelets represent those released early from the bone marrow. Platelets are much smaller than erythrocytes but are typically counted simultaneously by hematology analyzers, according to particle size. An experienced technician can count platelets using a hemocytometer and Unopette kit.

Reference ranges for leukocyte and platelet numbers in the laboratory animals covered in this volume are provided in Table 3.10.

Coagulation

Coagulation of blood is a defense mechanism to prevent excessive blood loss after vascular injury. It involves the formation of a clot at the site of damaged endothelium in response to thrombin, which converts fibrinogen into the fibrin matrix of the clot. After the endothelium has been repaired, the clot dissolves by fibrinolysis.

Prothrombin is the circulating precursor of thrombin, and there are several pro-coagulant proteins that interact to enhance thrombin formation. Endothelial damage initiates a cascade of events. A variety of tests are used to determine the functioning of the coagulation system.

Bleeding Time (BT)

Bleeding time is the time it takes, in minutes, for bleeding to stop after a standardized cut is made in a hairless area of skin. Bleeding stops when a platelet plug forms and BT thus assesses platelet function.

Prothrombin Time (PT)

Prothrombin time is a measure of the function of the extrinsic coagulation pathway. Blood is collected in a tube containing the anticoagulant sodium citrate and centrifuged to yield plasma. Tissue factor (III) is added and the time to clot formation is measured in seconds.

Activated Partial Thromboplastin Time (APTT)

Activated partial thromboplastin time is a measure of the function of the intrinsic and common coagulation pathways. A blood sample is collected into a tube containing citrate, which stops coagulation by binding calcium, and then centrifuged. A phospholipid, activator, and calcium are added to the plasma sample and time to clot formation is measured in seconds.

TABLE 3.10 Leukocytes¹

	Total WBC ($\times 10^3$ cells/ μ l)	Lymphocytes (%)	Neutrophils (%)	Eosinophils (%)	Basophils (%)	Monocytes (%)	Platelets ($\times 10^6/\mu$ l)	References
Rabbit	5.2–12.5	30–85	20–75	0–5	0–10	0–10	200–1000	Hrapkiewicz and Medina, 2007 Vennen and Mitchell, 2009
Guinea pig	6–17	30–80	20–60	0–7	0–3	1–12	250–850	Hrapkiewicz and Medina, 2007 Riggs, 2009
Hamster	3–15	50–96	17–35	0–5	0–5	0–5	200–590	Heatley and Harris, 2009
Gerbil	4.3–21.6	32–97	2–41	0–4	0–2	0–9	400–830	Hrapkiewicz and Medina, 2007 Heatley and Harris, 2009
Chinchilla	4–25	19–98	9–78	0–9	0–11	0–6	300–600	Donnelly and Quimby, 2002 Riggs and Mitchell, 2009
Deer mouse	1–4	N/A	N/A	N/A	N/A	N/A	N/A	Gough and Kilgore, 1964
Kangaroo rat	3–9	N/A	N/A	N/A	N/A	N/A	N/A	Scelza and Knoll, 1982 Intress and Best, 1990
Cotton rat	11–13	50–54	36–40	5–6	0–0.2	4–5	N/A	Webb et al., 2003

N/A = values not available.

¹Species for which no values are available are not listed.

TABLE 3.11 Coagulation Tests¹

	Bleeding Time (min)	Prothrombin Time (s)	APTT (s)	Thrombin Time (s)	Fibrinogen (g/dl)	References
Rabbit	1.1–2.7	7.2–7.8	35	7.8–12.0	0.17–0.31	Lee and Clement, 1990 Lewis, 1996
Hamster	1.5–2.4	10.3–10.7	N/A	N/A	188–316	Gad, 2007 Dodds et al., 1977 Tomson and Wardrop, 1987

N/A = values not available.

¹Species for which no values are available are not listed.

Thrombin Time (TT)

Thrombin time is also known as thrombin clotting time (TCT) and is a test that measures the time for a clot to form in plasma in a blood sample placed in anticoagulant to which an excess of thrombin is added. This test evaluates the conversion of soluble fibrinogen to insoluble fibrin. Failure to form a clot within 10–15 seconds is indicative of a possible fibrinogen deficiency.

Fibrinogen Concentration

Fibrinogen, or Factor I, originates in the liver and is converted to fibrin by the enzyme thrombin during coagulation. The fibrinogen test evaluates fibrinogen levels when there are suspected abnormalities in this protein that contribute to clotting disorders.

Reference ranges for coagulation tests in the laboratory animals covered in this volume are provided in Table 3.11.

Bone Marrow

Pluripotent stem cells in the bone marrow produce progenitor cells that differentiate into the erythrocyte, granulocyte, megakaryocyte, and monocyte/lymphocyte cell lines. These cells mature and are released into the circulation (Marshall, 2008).

Evaluation of the bone marrow is indicated for hematologic conditions that cannot be completely described by examination of peripheral blood elements. Conditions that may require bone marrow evaluation include non-regenerative anemias, thrombocytopenia, gammopathy, leukemias, and staging of lymphomas and mast cell tumors (Pilny, 2008).

General anesthesia is required for bone marrow aspiration in laboratory animals, and topical anesthetic should also be infiltrated at the sampling site. The proximal femur is the most common sampling site, but the proximal tibia, proximal humerus, and the ilium can also be used in larger animals (Pilny, 2008). Surgical exposure and resection of a bone to recover bone marrow may also be used, but this procedure is more invasive and has a greater risk of complications.

For bone marrow aspiration, the animal is placed in lateral recumbency, and the site to be sampled is shaved and aseptically prepared. A small incision is made in the overlying skin, and then a spinal needle is advanced into the medullary cavity and the stylet removed. A syringe is attached to the needle, and bone marrow is withdrawn. A smear can be prepared from a drop of the sample, and the remainder is usually stored in EDTA to prevent clotting (Pilny, 2008).

A bone marrow smear is prepared by placing a drop of marrow sample on one slide and covering it briefly with a second slide to spread the drop. The second slide is removed and the smear is air dried and stained with Wright-Giemsa or a rapid hematology stain. The smear is examined for normal cell morphology and myeloid vs. erythroid cell types. The myeloid to erythroid (M:E) ratio is calculated as an indication of bone marrow activity, and this ratio varies between 0.5:1 and 3:1 depending on the species. The maturation index, or ratio of proliferating to non-proliferating cells, is also calculated as an assessment of hematopoiesis (Pilny, 2008).

Bone marrow hypoplasia can result from infectious disease, estrogen or chemical toxicity, myelofibrosis, or immunosuppressive therapy or disease. Bone marrow aplasia indicates lack of cells from all lines (Pilny, 2008).

Bone marrow hyperplasia may represent a regenerative response to loss of myeloid or erythroid cells in the peripheral circulation, or it may be due to a lymphoproliferative or myeloproliferative neoplastic disease (Pilny, 2008).

Reference ranges for the myeloid to erythroid (M:E) ratio in the laboratory animals covered in this volume are provided in Table 3.8.

RABBIT

Sample Collection

Blood

Methods of restraint and acceptable sites and volumes of blood samples in the rabbit are described in the Clinical Biochemistry section of this chapter.

Bone Marrow

Bone marrow aspirates should be performed if there is a suspicion of anemia or other abnormalities of blood cell production. Collection of bone marrow should be performed with the rabbit under anesthesia and the sampling site prepared aseptically. Percutaneous aspiration may be used to sample bone marrow from the humerus or the femur with a Rosenthal pediatric needle (Horan et al., 1980) or from the proximal end of the tibia with a 15G needle (Sundberg and Hodgson, 1949). The proximal femur is generally the preferred site in most species, including the rabbit. A spinal needle with stylet is typically used and inserted in the trochanteric ridge of the femur (Vennen and Mitchell, 2009). The femur may also be surgically resected for direct access to the bone marrow (Powsner and Fly, 1962), but this approach is not recommended due to the importance of the rear limb for weight-bearing in the rabbit (Suckow and Douglas, 1997).

Reference Values and Unique Characteristics

Several factors can affect the results of hematology assays in the rabbit, including physiologic factors, such as age, breed or strain, sex, or pregnancy (Murray, 2000), as well as physical factors, such as sample handling and analytical techniques.

Blood Volume

Reference ranges for blood and plasma volumes in the rabbit are provided in Table 3.8.

TOTAL BLOOD VOLUME

The total blood volume of a normal adult New Zealand White rabbit is approximately 160–480ml, or 44–70ml per kg body weight. Rabbits have a higher blood volume during pregnancy (Prince, 1982). Blood volume per body weight in the young rabbit decreases significantly between birth and 4 months old.

Using the general guidelines for blood withdrawal based on body weight, approximately 20–40ml may be safely removed every 2 weeks.

The use of radio-iodinated albumin is preferable for estimating blood volume in a rabbit, instead of the dye T-1824 method often used in dogs (Brewer, 2006).

PLASMA VOLUME

Plasma volume in the rabbit is approximately 28–50ml per kg body weight and decreases significantly between birth and 4 months old.

Peripheral Blood Cells

Peripheral blood cells and elements of hematopoiesis in the rabbit are nicely illustrated in color photomicrographs in a recent publication (Marshall, 2008).

ERYTHROCYTES

There are no known blood groups in rabbits, and blood transfusions are well tolerated in this species (Marshall, 2008).

Reference values for erythrocyte parameters in the rabbit are provided in Table 3.9.

RED BLOOD CELL COUNT The reference range for red blood cell count in rabbits is $4.9\text{--}7.8 \times 10^6$ cells per μl (Table 3.9) and may be affected by several factors. For example, lower erythrocyte counts are reported in young rabbits of less than 12 weeks old (Melillo, 2007). Interestingly, the red blood cell count in a 28-day fetus is similar to that of the doe (Kriesten et al., 1987). The sex of the rabbit has been reported to have little or no effect on hematologic values (Jain, 1986).

An increase in red blood cell number has been reported in response to cold stress (de la Fuente et al., 2007), whereas hemolysis of the blood sample can result in a decreased red blood cell count (Melillo, 2007).

HEMATOCRIT (Hct) OR PACKED CELL VOLUME (PCV) The reference range for hematocrit in rabbits is 31–50% (Table 3.9) and may vary by age and strain. Hematocrit is reported to be high at birth and then falls by 20 days old, followed by a gradual increase to adult levels by 90 days old (Abelson and Simon, 1991). However, other studies report a PCV in neonatal rabbits that is similar to adult rabbits (Bortolotti et al., 1989). Differences exist in hematocrit values between different breeds and strains of rabbits but probably have little clinical significance (Kozma et al., 1974). There also appears to be little or no effect of sex on hematocrit values in the rabbit (Jain, 1986).

A PCV of less than 30% is indicative of anemia. A non-regenerative anemia may suggest the presence of a chronic disease. A regenerative anemia is associated with reticulocyte production and may occur in cases of blood loss due to external or internal hemorrhage, intravascular hemolysis, autoimmune hemolytic anemia, or lead toxicity (Melillo, 2007).

Hematocrit values of 13–31% were described in New Zealand White rabbits with hemolytic anemia resulting from systemic infection with *Achromobacter xylosoxidans* in contaminated intravenous fluids (Allison et al., 2007).

A PCV of more than 45% may indicate dehydration. Elevated PCV has also been reported as a consequence of cold stress and heat stress (de la Fuente et al., 2007).

HEMOGLOBIN (Hgb) The reference range for hemoglobin levels in rabbits is 10–17.4g/dl (Table 3.9). Similar to hematocrit levels, hemoglobin concentrations are high at birth and then fall by 20 days old, followed by a gradual increase to adult levels by 90 days old (Abelson, 1991). As with hematocrit,

hemoglobin differences between breeds and strains of rabbits exist but probably have little clinical significance (Kozma et al., 1974). Gender appears to have little or no effect on hemoglobin values in the rabbit (Jain, 1986).

Hemoglobin levels demonstrate diurnal variation in the rabbit, with hemoglobin levels highest in the morning and lowest in the late afternoon and early evening (Fox and Laird, 1970).

ERYTHROCYTE LIFESPAN Rabbit red blood cells have a short lifespan of 57 days (Table 3.9) and a high turnover rate. This results in polychromasia, which is not clinically significant in the rabbit. A few nucleated red blood cells (1–2%) and occasional Howell-Jolly bodies, which are basophilic nuclear remnants, are normal in the rabbit due to the release of young red blood cells into the circulation (Melillo, 2007).

ERYTHROCYTE SIZE AND STAINING Rabbit erythrocytes have an average diameter of 6.8 microns, although there is significant anisocytosis, with a diameter range of 5.0–7.8 microns (Table 3.9) (Melillo, 2007). Erythrocyte diameter decreases with age from a diameter of approximately 9 microns at birth to adult size by 20–120 days old (Kozma et al., 1974).

It is reported that red blood cells are not as deformable in the rabbit as in humans (Amin and Sirs, 1985). This is thought to be due to higher concentrations of oxygen-free radicals and plasma fibrinogen in the rabbit (Hirayama et al., 1986).

Mean Cell Volume (MCV) The reference range for mean cell volume (MCV) in rabbits is $57.5\text{--}75\mu^3$ (Table 3.9).

This value is reported to be higher in pregnant rabbits (Prince, 1982).

The MCV and erythrocyte diameter both decrease between neonatal and adult ages (Kozma et al., 1974).

Mean Cell Hemoglobin (MCH) The reference range for mean cell hemoglobin (MCH) in rabbits is 17.1–23.9 pg per cell (Table 3.9), and this value is reportedly higher in neonatal rabbits than in adult rabbits (Bortolotti et al., 1989).

Mean Cell Hemoglobin Concentration (MCHC) The reference range for mean cell hemoglobin concentration (MCHC) in rabbits is 28.2–37 g/dl (Table 3.9), and this value is reportedly similar in neonatal and adult rabbits (Bortolotti et al 1989).

RETICULOCYTES The reference range for reticulocyte numbers in the rabbit is 1.7–6.3% (Table 3.9). Reticulocytes are present at 2–4% in young rabbits (Jain,

1986) and after repeated bleedings or hemolytic anemia in adult rabbits (Brunner et al., 1983).

Reticulocytes are distinguishable from mature red blood cells by their larger size and more intense basophilic staining (Marshall, 2008). Reticulocyte count is elevated in neonates and declines with age to stable adult levels at 1 year old (Jain, 1986).

Nucleated red blood cells are found during endothelial changes and regenerative responses (Vennen and Mitchell, 2009).

LEUKOCYTES

Reference ranges for leukocyte parameters in the rabbit are provided in Table 3.10.

TOTAL WHITE BLOOD CELL (WBC) COUNT The reference range for total white blood cell count in rabbits is $5.2\text{--}12.5 \times 10^3$ cells per μl (Table 3.10) and varies with factors such as age, diurnal rhythm, and stress. Leukocyte counts are low in rabbits less than 12 weeks old and then gradually increase to maximum values by 3–6 months old (Jain, 1986; Melillo, 2007). Interestingly, the total white blood cell count in the 28-day rabbit fetus is also much lower than that of adult rabbits (Kriesten et al., 1987). A bimodal increase in leukocytes has been reported in rabbits, with the first peak at 3 months old, representing primarily lymphocytes, and the final peak at over 1 year old, due to an increase in neutrophils (Jain, 1986).

Total leukocyte numbers demonstrate diurnal variation, with lowest counts in the late afternoon and evening (Melillo, 2007).

Stress has been found to produce variable effects on total white blood cell count in rabbits. Some reports state that stress causes leukopenia in the rabbit (Brewer, 2006; Melillo, 2007). Application of electrical current in rabbits was found to produce a 15–30% decrease in the white blood cell count (Kaplan and Timmons, 1979). A recent study found lower white blood cell counts and higher plasma cortisol levels in group-housed compared with individually housed New Zealand White rabbits used for antibody production (Fuentes and Newgren, 2008).

Most studies, however, report that stress induces leukocytosis (Melillo 2007), and changes in leukocyte numbers have been found to correlate with changes in cortisol levels (Toth and Krueger, 1989). The stress associated with routine sample collection causes a physiological leukocytosis in rabbits (Harkness, 1987). It is reported that increased leukocyte numbers following stress consist primarily of mature neutrophils and lymphocytes and decreased eosinophils (Jain, 1986).

Increased white blood cell count is not a common response to infection in the rabbit. Instead, acute infection in rabbits has been shown to result in a

leukopenia of less than 5000 cells per ml and a normal differential count, or a normal white blood cell count with increased heterophils and decreased thrombocytes (Vennen and Mitchell, 2009).

LYMPHOCYTES The reference range for lymphocyte numbers in the rabbit is 30–85% (Table 3.10) and varies with several factors, including diurnal rhythm, stress, and disease. The lymphocyte nucleus is round or oval, acentrically located, and stains deep blue to purple. The cytoplasm of the lymphocyte stains a medium to deep blue, and there is typically a perinuclear halo of non-staining cytoplasm (Jain, 1986).

Lymphocytes are the predominant leukocyte in healthy rabbits, and both small and large lymphocytes are found in circulation. Small lymphocytes are 7–10 microns in diameter, and large lymphocytes are 10–15 microns in diameter. In addition to their presence in the bloodstream, lymphocytes are also found in the spleen, bone marrow, lymph nodes, and the lymphatic tissue of the gastrointestinal tract.

Lymphocyte numbers are typically highest in the early morning and lowest in late afternoon and evening (Fox and Laird, 1970; Melillo, 2007).

Acute stress and the associated increase in adrenaline may cause lymphocytosis, whereas prolonged stress and increased cortisol may induce lymphopenia (Melillo, 2007). However, other reports suggest that the epinephrine release associated with acute stress causes a mature heterophilia and relative lymphopenia (Marshall, 2008).

The stress induced by loud noises can cause an increase in the neutrophil to lymphocyte (NP:LC) ratio in rabbits that persists for 24–48 hours (Melillo, 2007).

Infection in the rabbit may also be accompanied by an increased NP:LC ratio, which is typically 1:1 in a normal adult rabbit (Melillo, 2007).

Increased lymphocyte numbers may indicate an immunologic response, such as occurs after a viral infection. Lymphocytosis may also be caused by lymphoma and lead toxicity (Melillo, 2007). A reversal in the NP:LC ratio due to elevated lymphocyte number was found to occur after experimental inoculation with several bacteria, as well as in spontaneously ill animals.

NEUTROPHILS (HETEROPHILS) The reference range for neutrophil numbers in the rabbit is 20–75% (Table 3.10) and is affected by several factors, including diurnal rhythm, stress, and disease.

Rabbit neutrophils have a diameter of 9–15 microns and have acidophilic cytoplasmic granules that stain with eosin and give them the appearance of eosinophils. For this reason, rabbit neutrophils are often referred to as pseudoeosinophils or, more commonly, heterophils. The neutrophil nucleus has a

polymorphous shape and stains light purple to light blue. The neutrophil cytoplasm stains pink because of numerous small granules, with a reddish hue due to several larger dark-staining granules (Campbell and Ellis, 2007). By electron microscopy, these granules are identified as primary or azurophilic granules and secondary or specific granules, respectively (Jain, 1986). Primary and secondary granules are believed to arise from two different sites on the Golgi apparatus (Marshall, 2008). The small pink granules comprise 80–90% of the neutrophil granules and contain peroxidase, alkaline phosphatase, lipase, and antibacterial activity (Benson and Paul-Murphy, 1999).

Neutrophils of female rabbits and other species may contain a drumstick configuration, which is referred to as the Pelger-Huet anomaly and is likely due to abnormal maturation of these cells (Kozma et al., 1974). The homozygotic form of this disease in rabbits is usually lethal (Jain, 1993).

Neutrophil numbers in the rabbit are lowest in the early morning and increase to peak numbers in late afternoon and evening (Fox and Laird, 1970; Melillo, 2007).

Prolonged stress induces an increase in neutrophils and a concomitant decrease in lymphocytes and eosinophils (Melillo, 2007). This may result in an overall leukopenia in the rabbit (Brewer, 2006). Stress due to loud noises can also cause an increase in the NP:LC ratio in rabbits that persists for 24–48 hours (Melillo, 2007). Acute stress is associated with catecholamine release that results in a mature neutrophilia and relative lymphopenia (Marshall, 2008).

Acute bacteria-induced inflammation causes an increase in neutrophils, which migrate to the site of infection and engulf pathogens. Lysosomal granules in the neutrophil cytoplasm fuse with phagosomes to kill and degrade infectious organisms (Campbell and Ellis, 2007).

Infection in the rabbit is usually associated with an increased NP:LC ratio, although the presence of band neutrophils and a left shift is uncommon. A reversal in the NP:LC ratio has also been reported after experimental inoculation with several bacteria, as well as in spontaneously ill animals. A neutrophil count of 60–80% can occur as a result of inflammation, although there is often a concomitant decrease in lymphocytes, with little or no change in total white blood cells. Alternatively, acute infection in the rabbit has been reported to result in increased neutrophils and decreased thrombocytes (Vennen and Mitchell, 2009).

EOSINOPHILS The reference range for eosinophil numbers in rabbits is 0–5% (Table 3.10) and is affected by several factors, including diurnal rhythm, stress, and disease. A low or absent eosinophil count is common in healthy rabbits.

The rabbit eosinophil has a diameter of 12–16 microns and is larger than the neutrophil. The eosinophil contains large acidophilic granules that fill the cytoplasm and are 3–4 times the size of neutrophil granules. These granules give the cytoplasm of the eosinophil a pink-orange appearance (Benson and Paul-Murphy, 1999). The eosinophil nucleus is bi-lobed or horseshoe-shaped (Vennen and Mitchell, 2009).

Eosinophil numbers are lowest in the early morning and increase to highest numbers in the afternoon or evening (Fox and Laird, 1970; Melillo, 2007).

Stress has been reported to induce a mature eosinophilia in rabbits. However, other reports suggest that glucocorticoid release is associated with eosinopenia.

Diseases of the skin, lungs, gastrointestinal tract, or uterus can result in eosinophilia in rabbits. For example, eosinophilia may be associated with abscesses or healing wounds in the rabbit or in cases of atopy and pyoderma (Melillo, 2007). Elevated eosinophil numbers may be caused by a parasitic disease such as *Encephalitozoon cuniculi* in the rabbit, although eosinophilia is reportedly an uncommon response to parasites or hypersensitivity in this species (Melillo, 2007).

BASOPHILS The reference range for basophil numbers in normal rabbits is 0–10% (Table 3.10), but can be up to 30% of the white blood cell differential count in healthy rabbits (Benson and Paul-Murphy, 1999; Vennen and Mitchell, 2009). The rabbit has higher basophil numbers than most species and may be the only laboratory animal with a significant number of basophils in circulation (Benson and Paul-Murphy, 1999). Circulating basophils are usually inversely proportional to the number of tissue mast cells. Rabbit basophils are 8–12 μm in diameter, which is similar in size to the neutrophil. The nucleus is light purple and is less segmented than the nuclei of eosinophils or neutrophils. The rabbit basophil has many purple-black cytoplasmic granules (Campbell and Ellis, 2007).

The function of the basophil is not well understood. Basophils demonstrate diurnal variation, with lowest numbers in the early morning and highest numbers in late afternoon (Fox and Laird, 1970). Cases of atopy and pyoderma in the rabbit are associated with elevated numbers of basophils and eosinophils (Melillo, 2007).

MONOCYTES The reference range for monocyte numbers in normal rabbits is 1–10% (Table 3.10) and varies according to diurnal rhythm and disease.

The monocyte is the largest white blood cell in the rabbit peripheral circulation. The monocyte nucleus has a horseshoe or bean shape and is lightly stained due to the diffuse nuclear chromatin. The monocyte cytoplasm is blue, with few vacuoles or granules in the healthy rabbit (Jain, 1986). In conditions of non-specific toxicity,

large dark-red granules may be seen in the cytoplasm of the rabbit monocyte (Benson and Paul-Murphy, 1999).

There is diurnal variation of monocytes in the rabbit, with lowest numbers in the early morning and highest numbers in late afternoon (Fox and Laird, 1970).

Elevated monocyte numbers in the rabbit may be caused by chronic inflammation from an abscess, mastitis, or tympanic bulla empyema, although monocyte counts are not always increased in these conditions (Melillo, 2007).

PLATELETS (THROMBOCYTES)

The reference range for platelet numbers in the rabbit is $200\text{--}1000 \times 10^6$ per μl (Table 3.10) and varies according to age, stress, and disease. Thrombocytes are present singly and in groups and appear as oblong or oval bodies measuring 1–3 microns in diameter. Thrombocytes have a pale periphery and a dark purple center when stained with Wright's stain (Kozma et al., 1974). Rabbit platelets have very high serotonin content (Fudge, 2000).

The platelet count in a 28-day rabbit fetus is significantly lower than that of the doe (Kriesten et al., 1987). Cold stress may cause an increase in platelets in rabbits (de la Fuente et al., 2007).

Decreased thrombocyte numbers may be a consequence of acute infection in rabbits (Vennen and Mitchell, 2009).

Coagulation

Rabbit blood clots rapidly at room temperature and must be treated with anticoagulants to evaluate complete blood count and plasma parameters.

Rabbits have high levels of intrinsic clotting factors, including V, VIII, IX, X, XI, and XIII, and low levels of factor I (fibrinogen), II (prothrombin), and XII, compared to other laboratory animals (Fudge, 2000).

Reference ranges for coagulation tests in the rabbit are provided in Table 3.11.

BLEEDING TIME (BT)

Bleeding time (BT) for rabbits is 1.1–2.7 minutes (Table 3.11).

PROTHROMBIN TIME (PT)

Prothrombin time (PT) varies with method but is 7.2–7.8 seconds in the rabbit (Table 3.11). Rabbits have approximately 89% prothrombin levels compared to dog (Kozma et al., 1974). Cold stress causes an increase in clotting time and a decrease in prothrombin time in rabbits (de la Fuente et al., 2007).

ACTIVATED PARTIAL THROMBOPLASTIN TIME (APTT)

Activated partial thromboplastin time (APTT) for the rabbit is approximately 35 seconds (Table 3.11).

THROMBIN TIME (TT)

Thrombin time (TT) for rabbits is 7.8–12.0 seconds (Table 3.11).

FIBRINOGEN CONCENTRATION

Fibrinogen concentration for rabbits is 0.17–0.31 g/dl (Table 3.11).

In the rabbit, fibrinogen degradation products do not stimulate the production of plasma fibrinogen, as occurs in humans (Kessler and Bell, 1980).

Bone Marrow

The maturation sequence and cell types in the rabbit bone marrow are similar to those seen in other species. Color photomicrographs of rabbit bone marrow are available (Sanderson and Phillips, 1981). The myeloid to erythroid ratio in normal adult rabbit bone marrow is approximately 1.1:1 (Sanderson and Phillips, 1981) (Table 3.8). This ratio is approximately 0.9–1 in 1-week-old rabbit kits and increases to adult levels by 4 weeks old (Sabin et al., 1936). Extramedullary hematopoiesis in the rabbit occurs in the liver and spleen.

GUINEA PIG

Sample Collection**Blood**

A discussion of blood sample methods and collection sites in the guinea pig is presented above in the Clinical Biochemistry section of this chapter.

Bone Marrow

Bone marrow in guinea pigs is usually obtained from the proximal end of the femur, as in other species (Feldman et al., 1982).

Reference Values and Unique Characteristics**Blood Volume**

Reference ranges for blood and plasma volumes in the guinea pig are provided in Table 3.8.

TOTAL BLOOD VOLUME

The reference range for total blood volume of the guinea pig is 40–80 ml, or 67–92 ml per kg body weight, (Table 3.8) and varies with age. The highest blood volume is reported at birth, followed by a steady decline in blood volume until the animals weigh approximately 900 grams (Sisk, 1976). The volume of a single sample of blood is 4–12 ml, and exsanguination yields 15–30 ml (Harkness and Wagner, 1995).

PLASMA VOLUME

The reference value for plasma volume of the guinea pig is 39 ml per kg body weight (Table 3.8) and varies with age (Sisk, 1976).

Peripheral Blood Cells

Color photomicrographs of representative peripheral blood cells of the guinea pig are presented in a recent publication (Thrall et al., 2004).

ERYTHROCYTES

Reference values for erythrocyte parameters in the guinea pig are provided in Table 3.9.

RED BLOOD CELL COUNT The reference range for total red blood cell count of guinea pigs is $4\text{--}11 \times 10^6$ per μl (Table 3.9), which is typically lower than in other rodents (Pilny, 2008). In general, most red blood cell indices are lower in the guinea pig than in other laboratory animals (Feldman et al., 1982).

Hematocrit (Hct) or Packed Cell Volume (PCV) The reference range for hematocrit of guinea pigs is 30–50% (Table 3.9).

Hairless Dunkin-Hartley guinea pigs were found to have higher PCV, hemoglobin concentration, MCV, MCH, and MCHC compared to normal Dunkin-Hartley guinea pigs (Waner et al., 1996).

A low hematocrit, or anemia, in the guinea pig is characterized by polychromasia and macrocytosis (Pilny, 2008). Anemia may be associated with cavian leukemia (Hrapkiewitz and Medina, 2007).

HEMOGLOBIN (Hgb) The reference range for hemoglobin level of guinea pigs is 11–17 g/dl (Table 3.9).

ERYTHROCYTE SIZE AND STAINING The reference range for diameter of guinea pig erythrocytes is 6.6–7.9 microns (Table 3.9), with normal anisocytosis and occasional microcytes of 3.5 microns in diameter.

Polychromasia varies with age, with approximately 25% in neonates, 4.5% in juveniles, and 1.5% in adults.

Mean Cell Volume (MCV) The reference range for MCV value in guinea pigs is 70–95 μ^3 (Table 3.9).

Mean Cell Hemoglobin (MCH) The reference range for MCH value in guinea pigs is 23–27 pg/cell (Table 3.9).

Mean Cell Hemoglobin Concentration (MCHC) The reference range for MCHC value in guinea pigs is 25–40 g/dl (Table 3.9).

RETICULOCYTES The reference range for reticulocyte number in guinea pigs is 0–6.1% (Table 3.9).

Reticulocytes were found to decrease in young male Weiser-Maples guinea pigs, then level off between 200 and 300 days old. There was no age-related change in female reticulocytes (Kitagaki et al., 2005).

LEUKOCYTES

Reference values for guinea pig leukocyte differential counts are provided in Table 3.10.

TOTAL WHITE BLOOD CELL (WBC) COUNT The reference range for white blood cell count in guinea pigs is $6\text{--}17 \times 10^3$ cells per μl (Table 3.10) and varies with age, strain, stress, and disease. Several studies have reported an age-related increase in total white blood cells (Kitagaki et al., 2005). Hairless Dunkin-Hartley guinea pigs were found to have a lower total leukocyte number compared to normal Dunkin-Hartley guinea pigs (Waner et al., 1996).

Stress can produce leukocytosis in the guinea pig. In addition, cavian leukemia is usually accompanied by a severe leukocytosis of 25000–500000 cells per mm^3 and is associated with high mortality and poor response to treatment (Pilny, 2008).

LYMPHOCYTES The reference range for lymphocyte number in guinea pigs is 30–80% (Table 3.10), making the lymphocyte the predominant circulating white blood cell in normal guinea pigs. There are small and large lymphocytes in the guinea pig, and the appearance of these cells is similar to lymphocytes in other species. The small well-differentiated lymphocytes predominate and are only slightly larger than red blood cells. These lymphocytes have a round dark nucleus surrounded by a narrow band of cytoplasm. The less common larger lymphocytes are approximately twice the size of the small lymphocytes, have a less densely stained more oval-shaped nucleus, and usually have small to large azurophilic granules.

Lymphocytes were found to decrease in number with increasing age in both male and female guinea pigs of the inbred Weiser-Maples strain (Kitagaki et al., 2005).

Hairless Dunkin-Hartley guinea pigs were found to have a lower number of lymphocytes, compared to normal Dunkin-Hartley guinea pigs (Waner et al., 1996).

NEUTROPHILS (HETEROPHILS) The reference range for neutrophil number in guinea pigs is 20–60% (Table 3.10) and varies with age and disease. The guinea pig neutrophil is the predominant circulating granulocyte in this species and has the same function as the neutrophil in other animals. As in several other laboratory animals, neutrophils are also known as heterophils or pseudoeosinophils in guinea pigs because of their staining characteristics. Guinea pig neutrophils are 10–12 microns in diameter and have more visible cytoplasm than eosinophils that contains small acidophilic granules. The neutrophil nucleus contains dense chromatin, is usually purple, and can have five or more segments (Feldman et al., 1984). In female guinea pigs, some neutrophils have a drumstick-shaped sex chromatin lobe.

Guinea pig neutrophils have moderate alkaline phosphatase activity (Tamburlin and Glomski, 1988).

Neutrophils lack myeloperoxidase, which is responsible for liquid purulent exudates, so abscess content in guinea pigs is caseous rather than liquid (Vennen and Mitchell, 2009).

The total neutrophil number was found to increase with increasing age in both male and female Weiser-Maples guinea pigs (Kitagaki et al., 2005).

The inflammatory response in the guinea pig is typically characterized by an increase in number of neutrophils while total leukocytes may remain constant (Riggs, 2009). Experimental infection with the skin mite, *Trixacarus caviae*, was found to cause an increase in neutrophils and other leukocytes (Rothwell et al., 1991).

EOSINOPHILS The reference range for eosinophil number in guinea pigs is 0–7% (Table 3.10). Guinea pig eosinophils are 10–15 microns in diameter, which is slightly larger than the diameter of neutrophils. The eosinophil nucleus is indented but less segmented than that of the neutrophil. The cytoplasm of eosinophils is completely full of large brightly eosinophilic granules. Cytoplasmic granules of eosinophils are larger than those of neutrophils and are round, rod-shaped, or trapezoidal and almost completely fill the cytoplasm (Jain, 1984).

Experimental infection with the skin mite, *Trixacarus caviae*, caused increased eosinophils and other white blood cells (Rothwell et al., 1991). Similarly, infestation with the tick, *Amblyomma americanum*, caused increased eosinophils in guinea pigs (Brown and Askenase, 1982). Syphilis infections induced with *Treponema pallidum* has been associated with eosinophilia in guinea pigs (Wicher et al., 1998).

BASOPHILS Basophils are rarely found in guinea pig differential counts, where the reference range for basophil number is 0–3% (Table 3.10). The basophil is approximately the same size as the neutrophil and has a purple-stained lobulated nucleus and round purple cytoplasmic granules of varying size.

Basophils were found to decrease sharply by 1 year of age in female Weiser-Maples guinea pigs using automated hematology analysis (Kitagaki et al., 2005). Other reports show no change in basophils due to age or sex using manual counts (Feldman et al., 2000).

Experimental infection with the skin mite, *Trixacarus caviae*, caused an increased number of basophils and other leukocytes (Rothwell et al., 1991). Tick infestation with either *Amblyomma americanum* or *Rhipicephalus sanguineus* produced basophilia in guinea pigs (Brown and Askenase, 1982; Szabo et al., 2003).

MONOCYTES The reference range for monocyte number in guinea pigs is 1–12% (Table 3.10). The

monocyte is the largest white blood cell in guinea pig circulation. The monocyte nucleus is oval and has less condensed chromatin and a variable shape, surrounded by blue-gray cytoplasm that is darker than the cytoplasm of lymphocytes (Jain, 1974).

Monocyte numbers were found to increase gradually with increasing age in male Weiser-Maples guinea pigs older than 300 days of age (Kitagaki et al., 2005).

Experimental infection with the skin mite, *Trixacarus caviae*, caused increased monocytes and other white blood cells (Rothwell et al., 1991).

FOA-KURLOFF CELLS Foa-Kurloff cells are a type of specialized mononuclear cell that are unique to guinea pigs and make up 3–4% of the differential leukocyte count in this species (Moore, 2000). These cells are approximately the same size as large lymphocytes and have a large granular intracytoplasmic inclusion called a Kurloff body. The Kurloff body is composed of sulfated proteoglycan that is rich in mucopolysaccharide and appears homogeneous, finely granular, or slightly vacuolated (Jain, 2004). The Kurloff body stains with toluidine blue and is PAS-positive. The nucleus of Foa-Kurloff cells is large and round and is usually eccentrically placed.

The exact origin and function of Foa-Kurloff cells are not known, but they lack lysosomes and phagocytic activity and may serve as natural killer (NK) cells in circulation (Pouliot et al., 1996). Foa-Kurloff cells are rarely seen in young animals or in males, and their presence in females is correlated with estrogen levels (Percy and Barthold, 2007). These cells are common in pregnant guinea pigs and may be induced by estrogen to shift from the lungs and red pulp of spleen to the thymus and placenta (Moore, 2000). It is thought that Foa-Kurloff cells may help form a barrier between mother and fetus during pregnancy by protecting fetal antigens in the placenta (Pilny, 2008).

PLATELETS (THROMBOCYTES)

The reference range for platelet number in guinea pigs is $250\text{--}850 \times 10^6$ per μl (Table 3.10), although the values are variable in the literature (Feldman et al., 1982). Guinea pig platelets have an irregular oval shape of approximately 2–3 microns in length, a pale periphery, and a darkly staining center.

Platelet numbers in guinea pigs are affected by factors such as age, strain, and disease. Platelet numbers were found to decrease between 100 and 200 days of age, followed by an increase, in male Weiser-Maples guinea pigs (Kitagaki et al., 2005). The hairless strain of Dunkin-Hartley guinea pigs were found to have higher platelet numbers compared to normal Dunkin-Hartley guinea pigs (Waner et al., 1996).

Inflammation in the guinea pig is associated with an increase in platelets, which can exceed one million per microliter without an increase in total leukocyte numbers (Riggs, 2009).

Bone Marrow

The reference range for myeloid to erythroid ratio in normal guinea pigs is 1.5:1 to 1.9:1 (Table 3.8).

HAMSTER

Sample Collection

Blood

Blood collection in the hamster is described in Clinical Biochemistry section above.

Reference Values and Unique Characteristics

Blood Volume

Reference values for blood and plasma volumes in the hamster are provided in Table 3.8.

TOTAL BLOOD VOLUME

The reference range for blood volume of the Syrian hamster is 6.8–12 ml or 65–80 ml per kg body weight (Table 3.8).

Due to the hamster's ability to conserve water, 72 hours of water deprivation in this species causes decreased whole blood volume and plasma volumes with little or no change in hematocrit (Kutscher, 1968). Hibernation in the hamster causes an increase in blood volume and other hematologic parameters (Hrapkiewicz and Medina, 2007). However, the site of blood collection reportedly has little effect on hematologic parameters in the hamster.

The volume of a single blood sample in the hamster is 0.5–1.3 ml per 2 weeks (Table 3.8).

The total exsanguination volume in the hamster is 3–5 ml (Table 3.8).

PLASMA VOLUME

Water deprivation for 72 hours was found to decrease plasma volume in the hamster (Kutscher, 1968).

Peripheral Blood Cells

ERYTHROCYTES

Reference values for erythrocyte parameters in the hamster are provided in Table 3.9.

RED BLOOD CELL COUNT The reference range for total number of red blood cells in the hamster is $2.7\text{--}12.3 \times 10^6$ cells per μl (Table 3.9).

Hibernation in the hamster causes an increase in red blood cell number (Heatley and Harris, 2009; Hrapkiewicz et al., 1998). Castration decreases circulating RBCs by 25–30% in male hamsters due to decreased testosterone levels (Heatley and Harris, 2009). There have been no red blood cell antigen differences identified in hamsters.

Hematocrit (Hct) or Packed Cell Volume (PCV) The reference range for hematocrit value in the hamster is 30–59% (Table 3.9) and is affected by several factors (Burns and De Lannoy, 1966). For example, hematocrit increases with age in hamsters. The neonatal hamster is relatively anemic, with a hematocrit of 34–39%. At this age red cells are characterized by hypochromia and macrocytosis, with 10–30% normoblasts (Gad, 2007). Ten to 30% of circulating red blood cells are nucleated in neonatal hamsters, compared to less than 2% in adults (Jain, 1974).

Hibernation in the hamster causes an increase in PCV and other hematologic parameters, accompanied by decreased red blood cell destruction (Hrapkiewicz and Medina, 2007).

HEMOGLOBIN (Hgb) The reference range for hemoglobin level in the hamster is 10–19.2 g/dl (Table 3.9) and is affected by factors such as age and hibernation state. Hemoglobin increases to a maximum concentration in hamsters at 8–9 weeks of age (Gad, 2007), although some studies report little change in hemoglobin levels between 1 and 27 weeks old (Rennie et al., 1981).

Hibernation causes increased Hgb concentration (Heatley and Harris, 2009), and hypothermia causes more complete oxygen saturation of hemoglobin than normothermia (Volkert and Musacchia, 1970).

ERYTHROCYTE LIFESPAN The reference range for erythrocyte lifespan in the hamster is 50–78 days (Table 3.9).

Hibernation causes increased life span of erythrocytes in the hamster (Mitchell and Tully, 2009).

ERYTHROCYTE SIZE AND STAINING The reference range for erythrocyte diameter in the hamster is 5–7 microns (Table 3.9).

Normal young hamsters (4–8 weeks old) have microcytic normochromic red blood cells with some variation in size and shape, as well as occasional spherocytes and target cells. Polychromasia and Howell-Jolly bodies are found normally in healthy hamsters (Pilny, 2008). Polychromasia is seen more often in hamsters than in humans (Gad, 2007). Hamster red blood cells are nucleated 2% of the time.

Mean Cell Volume (MCV)

The reference range for MCV in the hamster is 64–78 μ^3 (Table 3.9).

Most hamster erythrocyte values are similar to those of the rat, although MCV is slightly higher in the hamster (Dent, 1977).

Mean Cell Hemoglobin (MCH)

The reference range for MCH in the hamster is 20–26 pg per cell (Table 3.9).

Mean Cell Hemoglobin Concentration (MCHC)

The reference range for MCHC in the hamster is 28–37 g/dl (Table 3.9).

RETICULOCYTES The end of hibernation in the hamster is associated with an increase in reticulocyte numbers (Hrapkiewicz and Medina, 2007).

LEUKOCYTES

Reference values for leukocyte and platelet numbers in the hamster are provided in Table 3.10.

TOTAL WHITE BLOOD CELL (WBC) COUNT The reference range for total leukocyte count in the hamster is $3\text{--}15 \times 10^3$ cells per μl (Table 3.10) and varies with several factors including age, sex, strain, diurnal rhythm, hibernation, and disease.

Neonatal Syrian hamsters have a total leukocyte count of approximately 2000 cells per microliter. White blood cells increase with age in European hamsters, from 3500 per microliter at 13 days to 8200 per microliter at 65 days and are higher in males than females (Emminger et al., 1975).

Total white blood cell count in Chinese hamsters is higher in the middle of the light cycle than in the middle of the dark cycle (Lappenbusch, 1972).

Hibernation causes a decrease in total white blood cell count in Syrian hamsters from 5000–10000 per microliter to 2500 per microliter (Heatley and Harris, 2009; Mitruka and Rawnsley, 1977), although the NP:LC ratio remained at 1:1 (Hrapkiewicz and Medina, 2007).

In European hamsters, hibernation is also associated with a lower total white blood cell count and platelet count, with unchanged differential distribution (Reznik et al., 1975). White cell numbers fall to a low of 100 per microliter during hibernation in this strain (Heatley and Harris, 2009). After hibernation, a leukocytosis characterized by neutrophilia occurs, with 70–90% neutrophils and a total leukocyte count of 10000–20000 cells per microliter (Heatley and Harris, 2009; Pilny, 2008).

Hamsters experimentally infected with pirital virus, a novel arenavirus isolated from the cotton rat (*Sigmodon astoni*) in Venezuela, demonstrate

leukocytosis, including neutrophilia, eosinophilia, and basophilia on days 5–6 post-infection (Sbrana et al., 2006).

LYMPHOCYTES The reference range for total lymphocyte count in the hamster is 50–96% (Table 3.10). Lymphocytes are the predominant circulating leukocyte in hamsters, comprising 60–80% of the differential leukocyte count (Heatley and Harris, 2009). Most hamster lymphocytes are small round cells with a dark-blue nucleus that occupies the majority of the cell, surrounded by a rim of lighter-blue cytoplasm. Hibernation in the hamster causes decreased WBC numbers, with a 1:1 NP:LC ratio (Hrapkiewicz and Medina, 2007).

NEUTROPHILS (HETEROPHILS) The reference range for total neutrophil count in the hamster is 17–35% (Table 3.10). As in many other rodents, hamster neutrophils are also called heterophils because they resemble eosinophils, with a lobular nucleus and dense pink cytoplasmic granules. Hamster neutrophils have a diameter of approximately 10–12 microns. Hamster neutrophils have moderate alkaline phosphatase activity (Tamburlin and Glomski, 1988) but lack lysozyme activity (Thrall et al., 2004).

Hibernation in the hamster causes decreased WBC numbers, with a 1:1 NP:LC ratio (Hrapkiewicz and Medina, 2007). At the end of hibernation, neutrophilic leukocytosis occurs, with 70–90% neutrophils and total leukocyte count of 10000–20000 cells per microliter (Heatley and Harris, 2009).

EOSINOPHILS The reference range for total eosinophil count in the hamster is 0–5% (Table 3.10). Hamster eosinophils are similar in appearance to those of other rodent species.

BASOPHILS The reference range for total basophil count in the hamster is 0–5% (Table 3.10). Hamster basophils are similar in appearance to those of other rodent species.

MONOCYTES The reference range for total monocyte count in the hamster is 0–5% (Table 3.10). Hamster monocytes are similar in appearance to those of other rodent species.

PLATELETS (THROMBOCYTES)

The reference range for total platelet count in the hamster is $200\text{--}590 \times 10^6$ per μl (Table 3.10) and varies with hibernation and disease. Hibernation causes decreased platelet counts in European hamsters (Reznik et al., 1975) and Syrian hamsters (Heatley and Harris, 2009).

Hamsters experimentally infected with pirital virus had increased platelet numbers on days 5 and 6 post-infection (Sbrana et al., 2006).

Coagulation

Reference values for coagulation tests in the hamster are provided in Table 3.11.

BLEEDING TIME (BT)

The reference range for bleeding time in the hamster is 1.5–2.4 minutes (Table 3.11).

PROTHROMBIN TIME (PT)

The reference range for prothrombin time in the hamster is 10.3–10.7 seconds (Table 3.11).

Female hamsters have shorter prothrombin times than males (Dodds et al., 1977).

Hamsters experimentally infected with pirital virus had increased PT by 8 days post-infection (Sbrana et al., 2006).

FIBRINOGEN CONCENTRATION

The reference range for fibrinogen concentration in the hamster is 188–316 g/dl (Table 3.11). Female hamsters have higher concentrations of fibrinogen and plasminogen than males (Dodds et al., 1977).

Bone Marrow

The adult hamster has a highly cellular bone marrow, with a myeloid to erythroid ratio of approximately 0.9:1 (Table 3.8) (Gad, 2007). The largest cells in the hamster bone marrow are megakaryocytes. There are also significant numbers of eosinophils, plasma cells, histiocytes, and a few mast cells (Gad, 2007). The neonatal hamster bone marrow is primarily erythroblastic. The spleen does not play a major role in erythropoiesis in the hamster and becomes erythropoietically active only when stimulated by T3 (Boussios et al., 1982).

OTHER RODENTS

Gerbil

Sample Collection

BLOOD

Gerbil restraint techniques and blood collection are described in the Clinical Biochemistry section of this chapter.

Reference Values and Unique Characteristics

BLOOD VOLUME

Reference values for blood volume in the gerbil are provided in Table 3.8.

TOTAL BLOOD VOLUME The reference range for blood volume in the gerbil is 4.4–8 ml, or 67 ml per kg body weight (Table 3.8).

A single blood sample volume in the gerbil is 0.4–1.3 ml per 2 weeks (Table 3.8).

The exsanguination volume of blood in the gerbil is 2–4 ml (Table 3.8).

PERIPHERAL BLOOD CELLS

ERYTHROCYTES Reference values for erythrocyte parameters in the gerbil are provided in Table 3.9.

Red Blood Cell (RBC) Count The reference range for red blood cell count in the gerbil is $7\text{--}10 \times 10^6$ cells per μl (Table 3.9).

Neonatal gerbils have erythrocyte counts that are approximately half the adult values but increase to adult values by about 8 weeks of age (Pilny, 2008).

Hematocrit (Hct) or Packed Cell Volume (PCV) The reference range for hematocrit in the gerbil is 35–52% (Table 3.9).

Male gerbils have higher hematocrit values than females (Heatley and Harris, 2009).

Hemoglobin (Hgb) The reference range for hemoglobin concentration for the gerbil is 10–17.9 g/dl (Table 3.9).

Erythrocyte Lifespan The erythrocyte lifespan in the gerbil averages 10 days (Table 3.9), which represents a much faster turnover rate than seen in other laboratory animals (Joslin, 2009).

Gerbils up to 20 weeks old have a large number of circulating reticulocytes and erythrocytes with basophilic stippling and polychromasia. These cells are also abundant in older gerbils and are probably associated with the short erythrocyte lifespan (Heatley and Harris, 2009; Pilny, 2008).

Erythrocyte Size and Staining Neonatal gerbils have macrocytic erythrocytes, but this condition resolves by approximately 8 weeks of age (Pilny, 2008).

Mean Cell Volume (MCV) The reference range for MCV in the gerbil is $46.6\text{--}60 \mu^3$ (Table 3.9) and is elevated in neonatal gerbils until approximately 8 weeks old (Pilny, 2008).

Male gerbils have higher MCV values than females (Heatley and Harris, 2009).

Mean Cell Hemoglobin (MCH) The reference range for MCH in the gerbil is 16.1–19.4 pg per cell (Table 3.9).

Mean Cell Hemoglobin Concentration (MCHC) The reference range for MCHC for the gerbil is 30.6–33.3 g/dl (Table 3.9).

MCHC values are higher in male gerbils than in female gerbils (Heatley and Harris, 2009).

Reticulocytes Gerbils up to 20 weeks old have a large number of circulating reticulocytes and erythrocytes with basophilic stippling and polychromasia. These cells are also abundant in older gerbils and are probably associated with the short erythrocyte lifespan (Heatley and Harris, 2009; Pilny, 2008).

LEUKOCYTES Reference values for leukocyte and platelet numbers in the gerbil are provided in Table 3.10.

Total White Blood Cell (WBC) Count The reference range for white blood cell count in the gerbil is $4.3\text{--}21.6 \times 10^3$ cells per μl (Table 3.10).

Neonatal gerbils have panleukocytosis that resolves by approximately 8 weeks old (Pilny, 2008). Male gerbils have higher total leukocyte counts than females (Heatley and Harris, 2009).

Lymphocytes The reference range for lymphocyte count in the gerbil is 32–97% (Table 3.10).

The predominant leukocyte in gerbils is the lymphocyte, which is much more abundant than granulocytes in this species (Dillon and Glomski, 1975).

Male gerbils have higher total leukocyte count due to a higher number of lymphocytes. The lymphocyte-to-heterophil ratio in the male is 6:1 compared to 3:1 in the female (Heatley and Harris, 2009).

Neutrophils (Heterophils) The reference range for neutrophil count in the gerbil is 2–41% (Table 3.10).

Gerbil neutrophils have moderate alkaline phosphatase activity (Tamburlin and Glomski, 1988).

Eosinophils The reference range for eosinophil count in the gerbil is 0–4% (Table 3.10).

Basophils The reference range for basophil count in the gerbil is 0–2% (Table 3.10).

Monocytes The reference range for monocyte count in the gerbil is 0–9% (Table 3.10).

PLATELETS (THROMBOCYTES) The reference range for platelet number in the gerbil is $400\text{--}830 \times 10^6$ per μl (Table 3.10).

BONE MARROW

The reference range for myeloid to erythroid ratio in the gerbil is 0.75:1 to 2.35:1 (Table 3.8).

Chinchilla

Sample Collection

BLOOD

Blood collection in the chinchilla is described above in the section on Clinical Biochemistry.

Reference Values and Unique Characteristics

BLOOD VOLUME

Reference values for blood volume in the chinchilla are provided in Table 3.8.

TOTAL BLOOD VOLUME The reference range for blood volume in the chinchilla is 20–32 ml (Table 3.8).

Volume of a single blood sample in the chinchilla is 2–7 ml per 2 weeks (Table 3.8).

Exsanguination volume in the chinchilla is 8–12 ml (Table 3.8).

PERIPHERAL BLOOD CELLS

ERYTHROCYTES Reference values for erythrocyte parameters in the chinchilla are provided in Table 3.9.

Red Blood Cell Count The reference range for red blood cell count in the chinchilla is $5\text{--}10 \times 10^6$ cells per μl (Table 3.9).

Hematocrit (Hct) or Packed Cell Volume (PCV) The reference range for hematocrit in the chinchilla is 30–55% (Table 3.9).

The packed cell volume, hemoglobin concentration, and MCHC values are higher in blood obtained from chinchillas anesthetized with ketamine and xylazine than in blood obtained by cardiac puncture post-mortem (de Oliveira Silva et al., 2005).

Hemoglobin (Hgb) The reference range for hemoglobin concentration for the chinchilla is 9–15 g/dl (Table 3.9).

Erythrocyte Size and Staining

Mean Cell Volume (MCV) The reference range for MCV in the chinchilla is $32.1\text{--}69.2 \mu^3$ (Table 3.9).

Mean Cell Hemoglobin (MCH) The reference range for MCH in the chinchilla is 10.4–19.8 pg/cell (Table 3.9).

Mean Cell Hemoglobin Concentration (MCHC) The reference range for MCHC in the chinchilla is 20–38.5 g/dl (Table 3.9).

LEUKOCYTES Reference values for leukocyte and platelet numbers in the chinchilla are provided in Table 3.10.

Total White Blood Cell (WBC) Count The reference range for leukocyte number in the chinchilla is $4\text{--}25 \times 10^3$ cells per μl (Table 3.10). Total leukocyte number is higher in blood obtained from male chinchillas anesthetized with ketamine and xylazine compared to blood obtained by cardiac puncture post-mortem (de Oliveira Silva et al., 2005).

Lymphocytes The reference range for lymphocyte number in the chinchilla is 19–98% (Table 3.10).

The lymphocyte is the most common circulating leukocyte in the chinchilla. The chinchilla responds to inflammation with an early increase in neutrophils and decrease in lymphocytes, resulting in little change in the total leukocyte count (Riggs and Mitchell, 2009).

Neutrophils The reference range for neutrophil number in the chinchilla is 9–78% (Table 3.10). The neutrophil is the second most common circulating leukocyte in the chinchilla (Riggs and Mitchell, 2009). The chinchilla neutrophil has a nucleus that may be hyposegmented, similar to the Pelger-Huet anomaly in the rabbit. Heterophils lack myeloperoxidase, the enzyme that liquefies purulent material, causing chinchilla pus to be thick and caseous (Riggs and Mitchell, 2009).

Eosinophils The reference range for eosinophil number in the chinchilla is 0–9% (Table 3.10). These cells are uncommon in the circulation of normal chinchillas (Riggs and Mitchell, 2009).

Basophils The reference range for basophil number in the chinchilla is 0–11% (Table 3.10). These cells are uncommon in the circulation of normal chinchillas (Riggs and Mitchell, 2009).

Basophil and monocyte numbers were lower in blood obtained from male chinchillas anesthetized with ketamine and xylazine compared to blood obtained by cardiac puncture post-mortem (de Oliveira Silva et al., 2005).

Monocytes The reference range for monocyte number in the chinchilla is 0–6% (Table 3.10). These cells are uncommon in the circulation of normal chinchillas (Riggs and Mitchell, 2009).

PLATELETS (THROMBOCYTES) The reference range for platelet number in the chinchilla is $300\text{--}600 \times 10^6$ per μl (Table 3.10). A dramatic increase in platelets, of more than 1000 per ml, may occur in response to inflammation in the chinchilla (Riggs and Mitchell, 2009).

Degu

There is little specific information available on the hematologic characteristics of the degu.

The degu is a diurnal rodent and has been the subject of many studies of the effect of circadian rhythm on physiology, behavior, and disease states.

Hematologic and serum protein values for degus of both sexes and all ages studied are reported to be

similar to those of other laboratory rodents (Murphy et al., 1978).

Deer Mouse

Sample Collection

BLOOD

Blood collection in the deer mouse is described above in the section on Clinical Biochemistry.

There is limited information available in the literature regarding hematology parameters in the deer mouse.

Reference Values and Unique Characteristics

PERIPHERAL BLOOD CELLS

ERYTHROCYTES Reference values for erythrocytes in the deer mouse are provided in Table 3.9.

Red Blood Cell Count The reference range for red blood cell count in the deer mouse is $10\text{--}12 \times 10^6$ cells per μl (Table 3.9).

Hematocrit (Hct) or Packed Cell Volume (PCV) The reference range for hematocrit in the deer mouse is 38–52% (Table 3.9).

Deer mice infected with *Cuterebra* sp. were found to have lower packed cell volumes than uninfected deer mice (Sealander, 1961). By contrast, deer mice infected with *Capillaria hepatica* had elevated hematocrits compared with uninfected controls (Meagher, 1998).

Hemoglobin (Hgb) The reference range for hemoglobin concentration in the deer mouse is 12–16 g/dl (Table 3.9).

Deer mice infected with *Cuterebra* sp. were found to have a lower hemoglobin concentration than uninfected deer mice trapped during the same period (Sealander, 1961).

Erythrocyte Size and Staining

Mean Cell Volume (MCV) The reference range for MCV in the deer mouse is $36\text{--}46 \mu^3$ (Table 3.9).

Mean Cell Hemoglobin (MCH) The reference range for MCH in the deer mouse is 11–15 pg per cell (Table 3.9).

Mean Cell Hemoglobin Concentration (MCHC) The reference range for MCHC in the deer mouse is 30–35 g/dl (Table 3.9).

LEUKOCYTES Reference values for leukocyte numbers in the deer mouse are provided in Table 3.10.

Total White Blood Cell (WBC) Count The reference range for total leukocyte number in the deer mouse is $1\text{--}4 \times 10^3$ cells per μl (Table 3.10).

Dormouse

There is no information available in the literature regarding hematology values in the dormouse.

Kangaroo Rat

Sample Collection

BLOOD

A discussion of blood sample methods and collection sites in the kangaroo rat is presented above in the Clinical Biochemistry section of this chapter.

Reference Values and Unique Characteristics

PERIPHERAL BLOOD CELLS

ERYTHROCYTES Reference values for erythrocyte characteristics in the kangaroo rat are provided in Table 3.9.

Red Blood Cell Count The reference range for red blood cell count in the kangaroo rat is $7\text{--}9 \times 10^6$ per μl (Table 3.9). Values are lowest in the winter and highest in the summer (Intress and Best, 1990).

Hematocrit (Hct) or Packed Cell Volume (PCV) The reference range for hematocrit in the kangaroo rat is 47–53% (Table 3.9). Values are lowest in the summer and highest in the winter (Scelza and Knoll, 1982).

Hemoglobin (Hgb) The reference range for hemoglobin concentration in the kangaroo rat is 15–17 g/dl (Table 3.9). Values are fairly consistent throughout the year (Scelza and Knoll, 1982).

Erythrocyte Size and Staining

Mean Cell Volume (MCV) The reference range for MCV in the kangaroo rat is $57.3\text{--}66^3$ (Table 3.9).

Mean Cell Hemoglobin (MCH) The reference range for MCH in the kangaroo rat is 18.6–25 pg per cell (Table 3.9). Values are lowest in the summer and variably higher during the other seasons (Intress and Best, 1990).

Mean Cell Hemoglobin Concentration (MCHC) The reference range for MCHC in the kangaroo rat is 30–35 g/dl (Table 3.9). Values are fairly consistent throughout the year (Scelza and Knoll, 1982).

LEUKOCYTES Reference values for leukocyte numbers in the kangaroo rat are provided in Table 3.10.

Total White Blood Cell (WBC) Count The reference range for total leukocyte number in the kangaroo rat is $3-9 \times 10^3$ per μl (Table 3.10). Values are highest in the spring and variable during the other seasons (Intress and Best, 1990; Scelza and Knoll, 1982).

Cotton Rat

Sample Collection

BLOOD

Blood collection in the cotton rat is described in the Clinical Biochemistry section of this chapter.

Reference Values and Unique Characteristics

PERIPHERAL BLOOD CELLS

ERYTHROCYTES Reference values for erythrocyte characteristics in the cotton rat are provided in Table 3.9.

Cotton rats exposed to 1000ppm lead in drinking water showed decreased erythrocyte indices in a dose-dependent manner (McMurry et al., 1995).

Hemoglobin (Hgb) The reference range for hemoglobin concentration in the cotton rat is 13–14g/dl (Table 3.9).

LEUKOCYTES Reference values for leukocyte numbers in the cotton rat are provided in Table 3.10.

Total White Blood Cell (WBC) Count The reference range for total leukocyte number in the cotton rat is $11-13 \times 10^3$ cells per μl (Table 3.10).

Several studies have examined the effect of diet and environmental conditions on hematology and immune function of the cotton rat.

Immunocompetence has been suggested as an important influence on survival in herbivore populations (Robel et al., 1996). Supplementation of food quantity and quality (enhanced methionine) did not have a consistent effect on hematology or immune function in adult male cotton rats, although total leukocyte number was increased (Webb et al., 2003).

Cotton rats exposed to 1000ppm lead in drinking water showed decreased total leukocytes in a dose-dependent manner (McMurry et al., 1995).

Lymphocytes The reference range for lymphocyte number in the cotton rat is 50–54% (Table 3.10).

Neutrophils (Heterophils) The reference range for neutrophil number in the cotton rat is 36–40% (Table 3.10).

Eosinophils The reference range for eosinophil number in the cotton rat is 5–6% (Table 3.10).

Basophils The reference range for basophil number in the cotton rat is 0–0.2% (Table 3.10).

Monocytes The reference range for monocyte number in the cotton rat is 4–5% (Table 3.10).

PLATELETS (THROMBOCYTES) Supplementation of food quantity and quality (enhanced methionine) increased platelet number in the cotton rat (Webb et al., 2003).

Sand Rat

Sample Collection

BLOOD

Blood collection in the sand rat is described above in the section on Clinical Biochemistry.

Reference Values and Unique Characteristics

There is very little information available on hematology parameters in the sand rat.

A study in sand rats found that glucose concentration within red blood cells is higher than in plasma of this species, as it is in other mammals. This phenomenon was explained by loose binding of glucose to macromolecules within the erythrocyte (Halperin and Adler, 1985).

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Euthanasia and Necropsy

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INTRODUCTION

This chapter is designed to give a general overview of euthanasia and necropsy techniques that may be used for laboratory rabbits, guinea pigs, rats, mice, and other rodents. The euthanasia techniques described are those approved by the American Veterinary Medical Association at the time of publication (AVMA, 2007).

EUTHANASIA

Introduction and Background

“Euthanasia” is a noun from Greek origins: *eu* (meaning “good”) and *thanatos* (meaning “death”). In

other words, euthanasia is death that is induced with minimal pain and distress. Euthanasia of an animal should be performed in a humane manner. From a biological standpoint, the techniques used should cause rapid unconsciousness, respiratory or cardiac arrest, and loss of brain function (AVMA, 2007).

Research and animal care staff should handle animals with respect prior to and during the euthanasia procedure. Personnel should be properly trained to ensure that the animal is handled in such a way as to minimize pain and distress to the animal and ensure personnel safety. Euthanasia can be distressing to personnel, especially when performed repeatedly (Reilly and Rose, 2001). To reduce human distress during the euthanasia process, personnel may be provided with counseling, education, and training.

Pain is not experienced if the cerebral cortex and subcortical structures are non-functional (AVMA, 2007). These structures can be made non-functional by induction of hypoxia, the use of various anesthetics and analgesics, or other physical means.

Distress occurs when an animal's response to stimuli interferes with its well-being and comfort. Distress can occur even during a basic activity, such as routine animal husbandry (Sharp et al., 2002, 2003). The amount of distress during handling can vary with the methods used, and factors such as single versus group housing can be very important. Factors such as species, breed, age, size, disease state, previous handling experience, and other variables can heighten or even decrease distress associated with euthanasia. Animals undergoing euthanasia may release pheromones that can cause conspecifics to experience undue distress; thus, it is recommended that animals not be euthanized in the presence of others not being euthanized at that time.

The euthanasia method selected should be irreversible. Death should be verified immediately by confirming cessation of respiration; lack of pulse; lack of heartbeat; loss of color of the mucous membranes; absence of corneal, palpebral, and pedal reflexes; and glazing of the eyes. Experimental endpoints which define the moribund state in the context of the work being performed should be clearly defined to reduce terminal suffering (NRC, 2008; Toth, 1997), which should also help to reduce distress.

Approved methods for euthanasia are typically chemical or physical, and the choice of which method is optimal in a particular situation varies based on age, use, and species differences. For example, inhalant methods of euthanasia may take longer to produce death in neonates because they are resistant to hypoxia (AVMA, 2007; Klaunberg et al., 2004; NIHARAC, 2004; Singer, 1999). Some injectable anesthetic methods of euthanasia involve use of controlled substances, and require appropriate registration with federal authorities and documentation of use. In larger species, it may be appropriate to utilize captive bolt, while cervical dislocation would typically be used only in smaller animals (AVMA, 2007).

The method of euthanasia selected should be based on compatibility with postmortem data collection needs. Agents used for euthanasia may produce changes in research data and should therefore be carefully selected (Artwohl et al., 2006; Reilly and Rose, 2001). Additional criteria to be considered when selecting the method of euthanasia should include safety of personnel. Some physical methods of euthanasia may pose safety issues for personnel; for example, the guillotine, if improperly used, can cause injury to the user; and human exposure to inhalant anesthetics is associated with liver and kidney damage, cancer, and complications during pregnancy (Buring et al., 1985).

Occasionally, specific needs of the research may require the method of euthanasia to deviate from the recommendations of the American Veterinary Medical Association. Such deviations must be scientifically justified and approved by the Institutional Animal Care and Use Committee (IACUC) or a similar body and reviewed by a veterinarian familiar with the species and methods of euthanasia. An example would be the use of cervical dislocation without anesthesia, use of which might be justified when tissues or biological specimens of interest are altered by anesthetics.

Chemical Methods

Inhalants

CARBON DIOXIDE

Carbon dioxide (CO₂) is an inexpensive, non-flammable, non-explosive, nearly odorless gas that is commonly used to euthanize rodents in the laboratory environment. It is naturally present in room air, which is composed of 79% nitrogen, 20.9% oxygen, and <0.1% carbon dioxide, water vapor, and other inert gases (Gill et al., 2002). Carbon dioxide may be obtained as a compressed gas or as dry ice, though use of dry ice as a source of carbon dioxide is unacceptable due to the risk of thermal injury to animals and personnel, as well as the inability to precisely control the concentration of CO₂ within the euthanasia chamber. As dry ice melts, it undergoes sublimation (conversion of a solid directly to a gas), thus producing carbon dioxide. Currently, acceptable delivery of carbon dioxide for euthanasia requires compressed gas because the inflow rate of carbon dioxide gas into the euthanasia chamber (Figure 4.1) can be regulated and the rapid decrease of temperature associated with dry ice can be avoided (Artwohl et al., 1999; AVMA, 2007). The same system may be used to euthanize a single animal or a group of animals. It is recommended that mice and rats have an appropriate dwell time (length of exposure) to the carbon dioxide gas to ensure that death is achieved. It has been the authors' experience that if rodents are removed prior to death and exposed to room air, they recover. Because of the number of cases in which this has occurred, it has been suggested that a second, physical form of euthanasia, such as thoracotomy, be performed to ensure that the animals have been appropriately euthanized (National Institutes of Health, 2002). Unconsciousness and death typically occur when the concentration of carbon dioxide reaches 10% (Gill et al., 2002). The authors recommend verifying that death has occurred with any inhalant method used and perhaps augmenting with a secondary physical method. Commercial systems are available that ensure the dwell time is appropriate by preventing removal of the animals from the euthanasia chamber and providing removal



FIGURE 4.1 Example of CO₂ chambers and CO₂ tank with regulator valve.

of waste gases prior to allowing access to the chamber. Such equipment eliminates the possibility of animals not being euthanized and ensures safety of personnel; the limit for human exposure to carbon dioxide is 0.5% for an 8-hour time-weighted average based on the National Institute of Occupational Safety and Health's permissible exposure limit (PEL) of 5000 parts per million (NIOSH, 2005).

There are other considerations when using carbon dioxide for euthanasia of rodents. Individuals should be properly trained on euthanasia techniques. Euthanasia chambers should not be overcrowded, and it is best not to remove animals from their housing cage or mix animals that have not been previously housed together in the same container because it may be distressing (Artwohl et al., 2006; National Institutes of Health, 2002). It has often been stated that using carbon dioxide for euthanasia of the dam in transgenic mouse colonies may affect the yield of healthy embryos. A controlled study did not reveal a difference between the use of cervical dislocation and carbon dioxide in this regard (Howell et al., 2003).

It is best to use carbon dioxide delivered using a two-stage regulator, with the second stage capable of adjustable fixed flow rates (Artwohl et al., 2006). The recommended flow rate of carbon dioxide is to displace 20% of the chamber volume per minute (AVMA, 2007).

The use of carbon dioxide for euthanasia of rodents and other species has been controversial. For example, disagreement exists with regard to the need to pre-fill the euthanasia chamber with CO₂ prior to introduction of the animals. The Newcastle Consensus Meeting on Carbon Dioxide Euthanasia of Laboratory Animals concluded that concentrations of carbon dioxide >50% caused 10–15 seconds of pain prior to loss of consciousness. This group also concluded that a flow rate of 20% per minute using 100% carbon dioxide can cause loss of

consciousness without evidence of pain (Hawkins et al., 2006). Increasing concentrations of carbon dioxide were judged by human volunteers to be unpleasant (50% CO₂) to painful (100% CO₂) (Danneman et al., 1997). The pain was due to the mixing of carbon dioxide with the moisture in the nares and the subsequent formation of carbonic acid (AVMA, 2007). Although the time to loss of consciousness is minimized in prefilled chambers, adding oxygen to the carbon dioxide may not make the procedure less distressful in rodents (Blackshaw et al., 1988; Coenen et al., 1995; Hewett et al., 1993; Sharp et al., 2006; Smith and Harrap, 1997). Rats and mice showed aversion to flow rates as low as 15% and 20% carbon dioxide compared with their response to room air (Leach et al., 2002; Niel and Weary, 2007; Niel et al., 2008). Even though there are disagreements, based on current data, we recommend that best practices would be to utilize a fill rate of 20% of the chamber volume per minute to achieve euthanasia of rodents.

All methods of euthanasia are associated with specific histological changes in various tissues (Feldman and Gupta, 1976). Although carbon dioxide gas euthanasia may cause perivascular edema and extravasation of blood in the lung (Fawell et al., 1972), it is still considered a suitable method of euthanasia for preventing postmortem histological changes in abdominal and pulmonary viscera (Feldman and Gupta, 1976).

CARBON MONOXIDE

Carbon monoxide (CO) is a colorless, odorless gas that is non-flammable. The use of carbon monoxide is associated with significant safety issues because it can be explosive (at concentrations that exceed 10%) and is toxic to personnel, thus appropriate ventilation is imperative. Carbon monoxide euthanasia has been banned by several states (AVMA, 2009). Death occurs due to fatal hypoxemia; carbon monoxide combines with hemoglobin to form carboxyhemoglobin, thus blocking the uptake of O₂ by red blood cells. Guinea pigs exposed to 8% carbon monoxide collapsed in <2 minutes, and death occurred in 6 minutes (AVMA, 2007). Carbon monoxide is delivered using compressed gas.

INHALANT ANESTHETICS

Various inhalant anesthetics, including halothane, isoflurane, and enflurane, may be used for euthanasia. Threshold levels of these halogenated anesthetics have been established at two parts per million (AVMA, 2007). Enflurane and sevoflurane have been documented to cause seizures in cats (Kurata et al., 1994; Osawa et al., 1994). Among halothane, enflurane, and isoflurane, halothane was the least aversive in rats and mice (Leach et al., 2002). These agents vary in the induction time (time to unconsciousness) and the solubility and amount of odor. Odors may cause the animal to hold its breath,

thus increasing the time until unconsciousness. The induction and recovery times are based on solubility as defined by the relative affinity between gas, blood, and tissue. Halothane is the least soluble and has the longest induction time, followed by enflurane, isoflurane, and sevoflurane, in that order (Preckel and Bolten, 2005).

INERT GASES

Nitrogen (N₂) and argon (Ar) are inert gases that are colorless, odorless, non-flammable, and non-explosive. Use of argon and nitrogen gas as agents for euthanasia is conditionally acceptable in rabbits and rats; animals should be heavily sedated or anesthetized prior to being placed in a prefilled chamber (AVMA, 2007). Convulsions, vocalizations, and gagging occurred in pigs exposed to 90% argon in air or a mixture of 30% carbon dioxide and 60% argon in air (Raj, 1999). Heavy sedation or anesthesia may delay euthanasia. Argon and nitrogen are delivered using compressed gas, are inexpensive, and can be easily administered. Argon is recommended to be used at a concentration of 90%, but rats have shown an aversion to its use when given the opportunity to enter a cage with argon and a food reward. Rats had a median dwell time of 3 seconds, and no food reward was eaten (Niel and Weary, 2007). Argon and nitrogen gases at 100% concentration caused unconsciousness in 54 seconds and >2 minutes, respectively, in rats (Sharp et al., 2006). It is interesting to note that one outcome of the Newcastle Consensus Meeting on Carbon Dioxide Euthanasia of Laboratory Animals was to not recommend argon or nitrogen for euthanasia in rodents (Hawkins et al., 2006) which is in disagreement with the American Veterinary Medical Association Guidelines on Euthanasia (AVMA, 2007).

Injectable Agents

BARBITURATES

Barbiturates act by depressing the nervous system. This depression leads to unconsciousness, respiratory depression, and eventually to cardiac arrest. Barbiturates are given intravenously, intraperitoneally, or intracardially. The intracardiac route should only be used when the animal is heavily sedated, unconscious, or anesthetized (AVMA, 2007). The barbiturate pentobarbital is commonly used for euthanasia in combination with other agents. Commercial euthanasia formulations of pentobarbital, including those combined with other agents such as phenytoin, are listed as schedule III drugs by the United States Drug Enforcement Agency, and appropriate registration and documentation are necessary to obtain and use them in the United States. Pentobarbital should not be combined with a neuromuscular blocking agent for euthanasia (AVMA, 2007). Pentobarbital has been shown to be useful as a euthanasia agent when histopathological

studies of the abdominal viscera or the pulmonary tract are to be performed (Feldman and Gupta, 1976). One commercially available euthanasia compound that incorporated pentobarbital, reduced sperm motility in rats (Stutler et al., 2007). Guarniere et al. (1999) compared the ingredients of three commercially available formulations of pentobarbital and determined that 40% propylene glycol, sterile water, and 40% ethanol contributed to hemolysis when injected intravenously into rabbits. Use of sodium pentobarbital dissolved into 10% ethanol and sterile water for a concentration of 50 mg/ml prevented hemolysis during euthanasia.

POTASSIUM CHLORIDE

A metal halide salt, potassium chloride (KCl), must be used in conjunction with a surgical plane of anesthesia. KCl can be given either intravenously or intracardially (AVMA, 2007; Cartner et al., 2007). Administration of KCl leads to cardiac arrest and death due to depolarization of the cardiac cells.

UNACCEPTABLE INJECTABLE AGENTS

A number of compounds should be avoided for use in the euthanasia of animals due to animal distress associated with their use and/or risk to personnel (AVMA, 2007). These agents include strychnine, nicotine, caffeine, magnesium sulfate, potassium chloride, cleaning agents, solvents, disinfectants, and other toxins or salts, and all neuromuscular blocking agents (AVMA, 2007).

Physical Methods

Physical methods of euthanasia are best used when the animal is anesthetized. If the animal cannot be anesthetized due to research protocol or other justifications, physical methods should be used with care. It is important that personnel are well trained in the method, and that the equipment used is fully operational and well maintained.

Cervical Dislocation

Cervical dislocation is the manual, rapid separation of the cervical vertebrae with accompanying lethal trauma to the spinal cord. Cervical dislocation when performed without prior anesthesia should be scientifically justified and approved by the IACUC and personnel should demonstrate proficiency with the technique (AVMA, 2007). When performed correctly, cervical dislocation appears to be a humane method of euthanasia. This technique may be used in smaller species, typically those weighing less than 200 g. The technique involves immobilization of the head and rapid disarticulation of cervical vertebrae, preferably by use of specialized equipment such as a cervical dislocator, though this may be performed manually by quick and firm traction

and pulling of the body in a direction away from the head (Donovan and Brown, 2006). A study reported in 2007 indicated that cortical function was disrupted in mice within 10 seconds after cervical dislocation was performed (Cartner et al., 2007).

Captive Bolt

Penetrating captive bolts, pointed rods that are propelled into the animal's brain, have been used for euthanasia of several, mostly larger, species and has occasionally been used for euthanasia of rabbits. Adequate restraint is imperative when using the captive bolt, which is powered by either gunpowder or compressed air. The penetrating captive bolt causes concussion and damage to the cerebral hemisphere and brain stem. In contrast, the non-penetrating captive bolt achieves unconsciousness through percussion by a strong blow to the skull, and the brain is not penetrated. The non-penetrating captive bolt should not be used as the sole means of euthanasia and neither the penetrating nor the non-penetrating captive bolt should be used on an unanesthetized animal unless strongly justified (AVMA, 2007). The operator should be well trained to ensure accuracy of stunning. Equipment must be cleaned and serviced regularly according to manufacturer's instructions to decrease the chances of poor stunning.

Decapitation

Decapitation involves direct loss of brain function due to ischemia. Decapitation is a physical means of euthanasia that does not chemically contaminate tissues or alter tissue architecture. The guillotine (Figure 4.2A), which is used to decapitate adult rodents and rabbits, should be properly maintained to ensure that it is sharp and functional, and personnel should be properly trained in its use. Personnel should be cognizant of the potential for injury when utilizing a guillotine. Personnel may prefer to restrain the rodent in a disposable restrainer, such as the DecapiCone® (Figure 4.2B) to ensure proper placement in the guillotine. Exsanguination contributes to the effectiveness of decapitation as a method for euthanasia (AVMA, 2007). Measurements of electroencephalogram (EEG) signals after decapitation in various species ranged from 3 seconds to 40 seconds depending on whether anesthesia was previously used (Holson, 1992). In a study by Sharp et al. (2002), group-housed male Sprague-Dawley rats did not experience significant elevations of their heart rates or mean arterial pressure while in the presence of other rats during decapitation procedures. Nevertheless, because of potential pheromone production or auditory stimuli that could cause undue distress, it is recommended that performing euthanasia by decapitation or other methods in the presence of other animals should be avoided unless approval has been obtained from the IACUC and scientific justification provided.

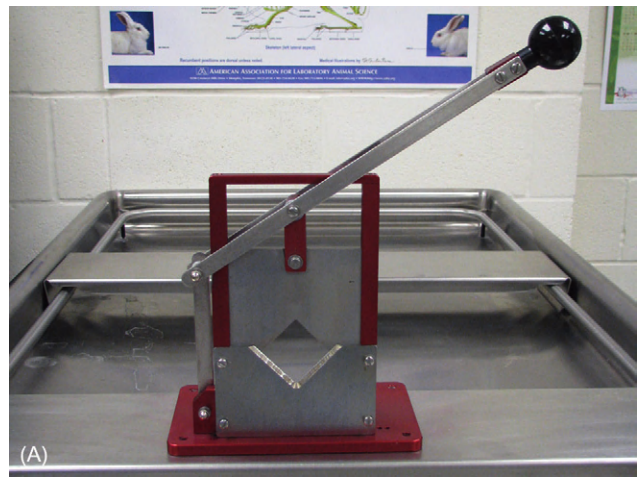


FIGURE 4.2A Guillotine.



FIGURE 4.2B A DecapiCone disposable plastic sleeve, often used to restrain mice and rats for decapitation using the guillotine. Photo courtesy of Oscar Contreras at The University of Texas M. D. Anderson Cancer Center.

Microwave Irradiation

Focused high-intensity microwave irradiation has been used for euthanasia by neurobiologists for measurement of highly labile substances or metabolites without loss of the anatomic integrity of the brain (Delaney and Geiger, 1996; Ikarashi et al., 1984, 1985; Mayne et al., 1999). It should not be used as a routine method of euthanasia and should be scientifically justified and approved by the Institutional Animal Care and Use Committee. Specialized equipment that is designated for this purpose should be used (Figure 4.3), not appliances that are available for general public use. Euthanasia is accomplished in <3 seconds using this method by heating of the brain tissue. Brains of animals euthanized using microwave irradiation may exhibit some protein denaturation because of the elevated



FIGURE 4.3 Microwave instrument used for euthanasia. Photo courtesy of Dr. Jonathan Geiger at The University of North Dakota, School of Medicine and Health Sciences.

temperature (Ikarashi et al., 1986). Personnel should be trained in this procedure, and the animal should be properly positioned to ensure that the animal's brain is accurately exposed to the field (Close et al., 1997; Ikarashi et al., 1986).

Euthanasia of the Rabbit

The agents and methods for euthanasia that are widely considered to be acceptable in rabbits include the use of barbiturates, inhalant anesthetics, carbon dioxide, carbon monoxide, and potassium chloride combined with general anesthesia. Other agents and methods that would be generally acceptable with justification include nitrogen, argon, cervical dislocation, decapitation, and penetrating captive bolt. Cervical dislocation may be used on rabbits that are <1 kg (AVMA, 2007).

A physiologic mechanism allows burrowing and diving animals to have a high threshold for hypercapnia. Since rabbits are burrowing animals, they should be

monitored closely to ensure the irreversibility of hypoxemia when induced utilizing gases such as carbon dioxide. Because of this physiologic mechanism, rabbits may become immobile during the euthanasia process, and the individual performing the procedure may prematurely withdraw the gas, leading to distress for the rabbit and the operator while the rabbit struggles to regain consciousness. As for all animals undergoing euthanasia, death should be verified.

Euthanasia of the Guinea Pig

Agents and methods generally acceptable for euthanasia of guinea pig adults and neonates include barbiturates, inhalant anesthetics, carbon dioxide, and microwave irradiation. Potassium chloride may be used to euthanize guinea pigs only after induction of general anesthesia.

Guinea pig fetuses less than 34 days old may be euthanized by euthanasia of the dam. Guinea pigs fetuses 35 days old or greater should be euthanized using one of the common methods of euthanasia, including decapitation or injection of chemical anesthetics. Rapid freezing or placing fetuses in a chemical fixative without previous anesthesia is not acceptable (Artwohl et al., 2006; NIHARAC, 2004).

Euthanasia of the Hamster

Agents and methods generally acceptable for euthanasia of adult hamsters include carbon dioxide, carbon monoxide, inhalant anesthetics, barbiturates, microwave irradiation, and potassium chloride while under general anesthesia. Fetal hamsters less than 15 days old may be euthanized by euthanasia of the dam. Fetuses greater than 15 days old can be euthanized using injectable anesthetics; and decapitation or cervical dislocation, if required for the needs of the research. Carbon dioxide may be used for neonatal and fetal hamsters greater than 15 days old, keeping in mind that they are resistant to hypoxia, so prolonged exposure time may be needed (Klaunberg et al., 2004; Ronca and Alberts, 1995; Singer, 1999).

Euthanasia of Mice, Rats, and Other Rodents

Because many rodents are similar in size and physiology to common laboratory mice and rats, euthanasia of these other rodents can be based upon what is known for the latter. In rat and mouse fetuses that are less than 15 days into gestation, it is believed that neural development is minimal and that they are non-viable once the blood supply is eliminated; therefore, euthanasia of the dam is adequate to ensure appropriate euthanasia of the fetuses (Committee on Guidelines for the Use

of Animals in Neuroscience and Behavioral Research, 2003; NIHARAC, 2004; Smotherman and Robinson, 1985). Mouse and rat fetuses may be euthanized using injectable anesthetics, carbon dioxide, decapitation, or cervical dislocation. For studies requiring rapid freezing in liquid nitrogen or whole-body fixation using a chemical fixative or perfusion, fetuses should first be anesthetized. Although fetuses are generally resistant to hypoxia, they may be euthanized by extended exposure to carbon dioxide or other inhalant anesthetics (Artwohl et al., 2006; Klaunberg et al., 2004; NIHARAC, 2004; Ronca and Alberts, 1995). Rats and mice which are 0–10 days post-birth may be euthanized using injectable anesthetics, carbon dioxide, decapitation, or cervical dislocation. Anesthesia should be used prior to immersion in liquid nitrogen or chemical fixatives (Artwohl et al., 2006). Hypothermia may be used in rats and mice less than 6 days old. Inhalant or injectable anesthesia may also be used prior to other forms of euthanasia. In a study in which rats 0–10 days old were exposed to 100% carbon dioxide, it was determined that the time required to euthanize rats increased by 3 minutes for every day of increase in age. The time required for death by carbon dioxide increased inversely based on the age of the rat (Pritchett-Corning, 2009). Inbred mice in this age range were less susceptible to carbon dioxide than outbred mice, especially between 0 and 8 days of age. It was determined that carbon dioxide exposure time could vary from 5 minutes to 60 minutes for mice 0–10 days old (Artwohl et al., 2006; Pritchett et al., 2005). European Union guidelines recommend decapitation, concussion, and hypothermia (Close et al., 1997), but not carbon dioxide, for euthanasia of neonates.

Mice and rats greater than 10 days old may be euthanized using barbiturates, inhalant anesthetics, carbon dioxide, carbon monoxide, and microwave irradiation; cervical dislocation or decapitation may be used, preferably on anesthetized animals unless otherwise justified by the scientific needs of the study. Potassium chloride may be used following induction of general anesthesia. Rats should be <200g if cervical dislocation is used as the sole means of euthanasia (AVMA, 2007).

NECROPSY

General Considerations

Necropsy is an important procedure for diagnostic investigations of laboratory animals and for obtaining valuable data in biomedical research. The origin of the term “necropsy” is from the Greek words *nekros* (meaning “corpse”) and *opsis* (meaning “to view”), and together they mean “to look at the dead body with naked eyes”; that is, macroscopic examination of a dead

body. Although “autopsy” and “necropsy” are synonymous, “necropsy” is the conventional term for postmortem examination of non-human species, and “autopsy” is used only for postmortem examination of humans. Because there is only one opportunity to perform the necropsy of an animal, it is important to conduct a thorough macroscopic examination with identification and detailed description of all lesions. The necropsy procedure includes not only the dissection of the dead animal and macroscopic examination of all organs but also collection of appropriate tissue samples and measurements of the carcass, internal organs, and body fluids (i.e. weight, size, length, volume). The gross lesions observed at necropsy should be documented promptly in a descriptive written report and by taking good-quality photographs (Relyea et al., 2000; Sundberg and Miller, 2000).

Necropsy methods differ between laboratories, pathologists, and experiments. These differences are generated by the objectives of the study, established protocols, and experience of persons performing the necropsy. In planning and determining the appropriate necropsy procedure, several important factors need to be considered in concert with the objectives of the study (Table 4.1). Preparing for and conducting the necropsy, as well as identifying and interpreting the lesions, is a complex process that requires a veterinary pathologist’s expertise. The pathologist should be consulted at the beginning of the experiment for assistance in designing the study, as well as for performing postmortem examinations and evaluation of observed lesions.

Due to postmortem autolytic changes that begin quickly after death of the animal, the necropsy should be performed immediately after euthanasia. The central nervous system, eyes, adrenal medulla, gastrointestinal mucosa, pancreas, liver, and kidney are the first organs affected by postmortem autolysis. Fibrous tissues, muscles, bones, and skin are more resistant to postmortem changes. If necropsy cannot be performed immediately after death of the animal, the carcass should be preserved in a refrigerator until necropsy. Refrigeration slows the autolytic processes of the dead animal. Carcasses should not be frozen before necropsy because the freezing and thawing processes cause severe morphologic artifacts of tissues that obscure the gross and histological lesions.

Tissue artifacts caused by manipulation, dissection, and examination during necropsy and by fixation, trimming, and processing of tissues into histological sections should be minimal or non-existent. Gentle manipulation of tissues during dissection, examination, and sampling will avoid distortion of tissue architecture. Proper fixation of tissues is accomplished by immersing the tissue samples in an adequate amount and type of fixative, quickly after the animal’s death. Trimming of fixed tissue samples and processing of histological sections should be

TABLE 4.1 Issues to Consider in Logistical Planning of Necropsy

- What is the method of euthanasia, and is it compatible with experimental needs?
- What are expected “target organs,” and are any special procedures warranted such as ancillary clinical pathology studies?
- What special prelabeling and identification requirements are needed?
- Do fixatives and/or clinical preservatives need to be prepared fresh?
- Are there randomization issues and/or special timing issues required between experimental groups?
- Are target organs to be weighed, and is exsanguination required?
- Do terminal clinical, clinical chemistry, urinalysis, or hematology specimens need to be collected and, if so, by what method? Is food restriction required before this collection?
- Are microbiology samples to be collected, and is aseptic technique required?
- Are there critical steps that require a “single individual” to collect target tissues for consistency purposes?
- Is a special tissue collection order required for target organ study owing to autolysis issues or biochemistry needs?
- Are special identification methods to be used for multiple or paired tissues?
- Are study protocols, standard operating procedures, and clinical records available to the prosectors?
- Are frozen specimens to be collected and, if so, by what method?
- Are morphometric techniques to be used, and does this require special tissue handling such as organ perfusion?
- Are there special postfixation needs for molecular, ultrastructural, histochemical, or immunohistochemical studies?
- What are the microbial, radiological, and chemical hazards posed by the necropsy procedures, and what precautions are to be used?

From Black, 1986, and Everitt and Gross, 2006.

performed in histology laboratories by skilled histotechnologists. Tissue artifacts and autolytic changes observed at necropsy or microscopic examination should be differentiated from lesions by the pathologist.

Preparation for Necropsy

It is advisable to establish a detailed necropsy protocol before euthanasia and necropsy, preferably when designing the animal experiment. The preparation for necropsy is dictated by the clinical history of the animal, objectives of the experiment, types and methods of treatments/experiments, and tissue samples that need to be collected. All equipment and supplies listed in [Table 4.2](#) should be prepared and instruments and specimen containers prelabeled before euthanasia and necropsy occur. The necropsy should be performed only in designated and adequately equipped necropsy rooms. The person performing the necropsy should wear protective equipment that includes a laboratory coat, protective gloves, and safety eyewear. Additional biosafety measures should be implemented when the animals have been exposed to, or experiments conducted with, biohazardous materials. Aseptic instruments and

TABLE 4.2 Equipment, Instruments, and Materials for Necropsy of Rabbits, Guinea Pigs, Hamsters, Rats, Mice, and Other Rodents

- Laboratory coats, surgical gowns, and safety gloves and glasses
- Biological safety cabinets with equipment for handling biological hazards and animals exposed to biohazardous materials
- Anesthesia and euthanasia chamber with CO₂ tank and regulator valve or other euthanasia equipment
- Vented necropsy table
- Corkboards, Styrofoam boards, and disposable sheets for dissection of animals
- Calibrated balance or scale and weigh trays for measuring body and organ weights
- 1-ml syringe with 20- or 22-gauge needle, tubes with EDTA or heparin, and serum separator tubes for collection of blood samples for hematology and serology tests
- Sharp and blunt-tip hypodermic needles (16- to 25-gauge) and a 5- to 20-ml syringe filled with fixative for infusion of lungs, urinary bladder, intestines, and stomach
- 1-, 5-, 10-, and 20-ml syringes for collection of biological fluids
- Serrated and rat-toothed tissue forceps
- Straight and curved (iris) tissue forceps of different sizes
- Scalpel blades (numbers 11, 20, and 22) with handles or finger scalpels (numbers 10 and 22)
- Large and small scissors, straight and curved, with blunt and sharp points
- Bone-cutting forceps, nail-cutting forceps, bone rongeurs, and a small spatula
- Prelabeled containers, plastic bags, and Eppendorf tubes for specimen collection
- Prelabeled containers with fixative (10% neutral buffered formalin for routine histologic analysis)
- Bacteriological swabs and sterile petri dishes for specimen collection for microbiology tests
- Microscope glass slides for blood smears and cytological preparations
- Clear tape for taking anal and perianal samples, which are then placed on a glass slide for microscopic detection of parasites
- Embedding bags or biopsy bags and tissue cassettes (small and regular size)
- Cryomolds, optimal cutting temperature (OCT) compound, and liquid nitrogen for preparation of frozen tissue samples
- Beaker or squeeze bottle with physiologic saline (0.9% sodium chloride) for rinsing the tissues
- Cotton balls soaked in 70% alcohol or a squeeze bottle of 70% alcohol
- Paper towels, gauze sponges, and silk suture material (3/0, 1/0)
- Push pins, index cards or cardboard, marker, pencil, and pen
- Calipers and metric rulers for measuring masses, cysts, and other lesions
- Dyes for labeling tissue lesions
- Magnifying glass for identifying smaller organs or lesions
- Camera for taking photographs of lesions
- Plastic disposal bags

containers should be available at necropsy for collecting samples for microbiological analyses that are necessary for investigating the etiology of disease or the health status of sentinel animals in the animal colony. Photographic equipment and a dissection microscope or stereomicroscope should be available in the necropsy

room for taking photographs of the lesions during the necropsy procedure. Other imaging techniques, such as radiographs of the entire animal or only target organs, shortly after euthanasia or during the necropsy, may need to be performed in certain experiments. Having readily available instruments, supplies, materials, and prelabeled containers during the necropsy enables the prosector to accomplish quick sampling and immediate fixation of tissues after the animal's death.

Necropsy Procedure

It must be reiterated that there is no single best method of necropsy that will fit all circumstances and desires of the pathologist or investigator performing the necropsy. Any necropsy technique is suitable as long as a thorough examination of all organs is performed, all gross findings are recorded properly, appropriate tissue samples are collected, and the objectives of the experiment and diagnostic investigations are addressed. Therefore, the appropriate method of necropsy should be determined based on the objective of the experiment, clinical history of the animal, planned use of tissue samples, and planned diagnostic tests. It is essential to develop and perform a routine standard necropsy procedure with examination and sampling of all organs in a consistent, systematic sequence. Having a routine will prevent missing lesions or sample collections and will improve considerably the efficiency and speed of performing the necropsy. The necropsy of animals in preclinical drug studies and phenotyping of genetically engineered mice are commonly conducted in a consistent manner in all animals, according to an established protocol or standard operating procedures.

A number of sources provide detailed information regarding the necropsy of rodents and rabbits (Bono, 1994; Bono et al., 2000; Everitt and Gross, 2006; Feinstein, 1994; Feldman and Seely, 1988; Hayward et al., 2007; King et al., 2005; Registry of Industrial Toxicology Animal Data, 2010; Relyea, et al., 2000; Seymour et al., 2004; Sharp and LaRegina, 1998; Silverman, 1987). Further, a detailed anatomy atlas will aid the prosector in dissecting and recognizing the organs (Greene, 1963; Hayakawa and Iwaki, 2008; McLaughlin, 1970; Olds and Olds, 1979; Popesko et al., 1990a, 1990b; Wingerd, 1985).

Any necropsy should begin with identification of the animal and investigation of the clinical history, research protocol, applied experimental procedures, and reason for submission. Subsequently, external examination, body weight, and collection of blood and other tissue samples should be performed. Because the amount of blood sampled can vary greatly from one animal to another, the animal's body weight should be recorded before the collection of blood samples. The sequence of dissection, gross examination, and tissue sampling

of a complete systemic necropsy in laboratory rodents is described below. This necropsy technique has been applied successfully in most types of research and pre-clinical drug studies in our laboratory. Although the mouse is used as an example of necropsy here, a similar necropsy technique can be performed in rabbits, guinea pigs, hamsters, rats, and other rodents. The differences between these species in necropsy procedures will be addressed as appropriate.

Collection of Blood and Urine Samples

Blood samples for diagnostic tests are often collected at necropsy from the anesthetized animal just before euthanasia or immediately after euthanasia and death of animal. In mice, rats, guinea pigs, hamsters, and other rodents, the blood samples can be collected from the following sites: heart, retro-orbital sinus, abdominal aorta, femoral artery, caudal vena cava, and medial saphenous vein. In mice and rats, the tail vein or tail artery can also be used to sample a small volume of blood (Adams, 2002; Everitt and Gross, 2006; Fukuta, 2004; Relyea et al., 2000). For hematological testing, the blood must be collected quickly with minimal tissue trauma and mixed promptly with anticoagulant in plastic lavender-top tubes (Figure 4.4) that contain ethylenediaminetetraacetic acid (EDTA) to prevent clotting of blood (Adams, 2002; Everitt and Gross, 2006; Fukuta, 2004; Relyea et al., 2000). For blood chemistry analysis and other serologic tests, the blood is collected in yellow-top tubes (Figure 4.4). In our experience, collection of blood from the heart by cardiac puncture with a 1–2 ml syringe and 20–25 gauge needle has proven to be a consistently successful technique in mice and rats (Figure 4.4). In rabbits, the blood samples can be collected from the heart, auricular (marginal ear) vein, auricular (central ear) artery, jugular vein, or medial saphenous vein (Bivin, 1994; Hrapkiewicz et al., 2007; Suckow and Douglas, 1997). Blood smears should always be prepared and examined along with hematological testing. The sampling site of blood collection should be consistent in animals from the same study because the values of total and differential white blood cell count, red blood cell count, platelet count, and other hematological parameters are significantly different from one site of sampling to another (Doeing et al., 2003; Nemzek et al., 2001; Schnell et al., 2002). Collection of blood samples by cardiac puncture is most recommended because this technique avoids variations in hematological values caused by various biological factors, manipulation, and other events (Schnell et al., 2002).

When urinalysis is required at the time of euthanasia or necropsy, the urine can be collected either before the euthanasia and collection of blood samples, or during the necropsy directly from the urinary bladder with a sharp needle and syringe. Obtaining a sufficient amount of urine at necropsy is often challenging in mice due to

the small volume of urine that can be found in the urinary bladder after euthanasia. In mice, we recommend collecting the urine from live animals before the anesthesia and euthanasia, because the urinary bladder of euthanized mice is commonly empty of urine at necropsy. The urine can be collected before the anesthesia and euthanasia using the clear-plastic-wrap method (Kurien and Scofield, 1999; Kurien et al., 2004); housing the mouse in a metabolic cage; or by collection of urine in a plastic tube held at the external urethra while gripping the mouse (Fukuta, 2004; Relyea et al., 2000). In our laboratory, we have successfully collected 50–250 µl of fresh non-contaminated urine excreted by an individual mouse on clear plastic wrap. Because rabbits, guinea pigs, hamsters, and rats have a higher volume of urine in the urinary bladder, their urine samples can be collected directly from the urinary bladder during necropsy shortly after euthanasia, with a syringe and sharp needle. In rabbits, the urine can also be collected by catheterization or cystocentesis (Adams, 2002; Bivin, 1994; Hrapkiewicz et al., 2007; Suckow and Douglas, 1997).

Exsanguination and Euthanasia

Once the blood samples are collected, the anesthetized animals can be euthanized by exsanguination and/or other methods of euthanasia. Due to the small total blood volume in mice, the collection of blood samples of approximately 400 µl or more of blood can be used to euthanize an anesthetized animal by exsanguination (Figure 4.4). Exsanguination of rabbits, hamsters, guinea pigs, rats, and other rodents can be accomplished by either cardiac puncture, transection of the abdominal aorta, transection of the vena cava, transection of the axillary and femoral arteries, or decapitation (Everitt and Gross, 2006). Complete exsanguination of animals is recommended for obtaining consistent organ weights, generating better-quality histological sections for chemical and immunohistochemical analysis, and eliminating pooled blood in tissues during dissection (Everitt and Gross, 2006; Sullivan, 1985).

External Examination

The necropsy begins with a thorough external examination. This includes verification of the animal's identification information (animal identification number, sex, date of birth, strain, genotype, and pedigree) and visual inspection and palpation of the animal's fur, skin, oral cavity, nasal mucosa, conjunctiva, eyes, anus, and external genitalia (Figure 4.5). Examination of the skin should include evaluation of the skin for abnormal color, presence of ulcerations, traumatic wounds, ectoparasites, cutaneous or subcutaneous tumors, fur condition and soiling, and any other external abnormalities. Presence of nasal, ocular, or aural discharge should be assessed. The anus and perineal region are examined for diarrhea and traumatic lesions.

The external genital organs and urethra are examined for blood or purulent discharge and for presence of tumors or other abnormalities. Photographs of the entire animal and external lesions should be taken at this time, particularly in research and studies involving the phenotyping of genetically modified mice.

Oral, anal, and skin swabs, skin scrapes, and an anal clear-tape test should be performed immediately after euthanasia and before the immersion of the mouse in disinfectant solution. Similarly, skin samples for bacteriologic or other diagnostic tests should also be collected before the immersion of the mouse in disinfectant. Hair samples should be collected by manual plucking using the thumb and forefinger from an area of skin that is not sampled for histological examination (Relyea et al., 2000). In genetically modified mice, a 1-cm-long section from the tip of the tail is usually sampled in an Eppendorf tube for genetic testing. To prevent contamination, the samples must be collected aseptically using sterile instruments and petri dishes or other sampling containers (Hayward et al., 2007).

Dissection, Gross Examination, and Collection of Tissue Samples

After completing the external examination and collection of appropriate skin samples, the euthanized animal is immersed in a solution of germicidal detergent or 70% alcohol. Alternatively, one can moisten the ventral surface of the body with 70% alcohol or another disinfectant (Figure 4.6). This measure will prevent contamination of tissues with hair. The animal is then positioned in dorsal recumbency on a corkboard, and the legs are pinned down by placing a pushpin through each paw and into the corkboard (Figure 4.6). Immobilization of the carcass increases the precision and speed of dissection and helps to prevent the contamination of instruments and organs during necropsy.

SECTIONING OF SKIN AND EXAMINATION OF SUBCUTANEOUS TISSUES

With scissors, a ventral midline incision of skin is made beginning immediately cranial to the external genitalia and ending at the mandibular symphysis. The skin is then sectioned from the midline inguinal region to the distal region of the tibia on the medial aspect of both hind legs. After that, the skin should be reflected laterally on both sides of the midline incision by applying gentle lateral traction of the skin with forceps and carefully excising the subcutaneous loose connective tissue (Figure 4.7). For better exposure of subcutaneous tissues and to avoid the contamination of internal organs with hair, we recommend pinning down the reflected skin as demonstrated in Figure 4.7. This measure allows good exposure of the salivary glands, mammary glands, preputial or clitoral glands, and subcutaneous lymph nodes (Figures 4.7–4.9). The mammary glands are within the

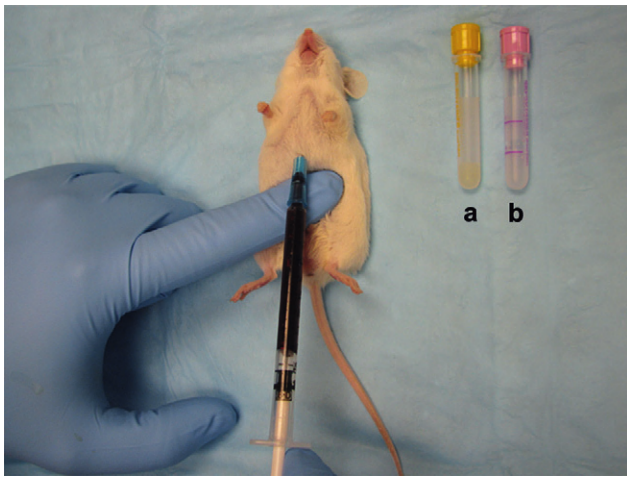


FIGURE 4.4 Collection of blood samples by cardiac puncture. The yellow-top tube (a) is used for collecting blood for serological tests. The lavender-top tube (b) contains EDTA and is used for hematological tests.

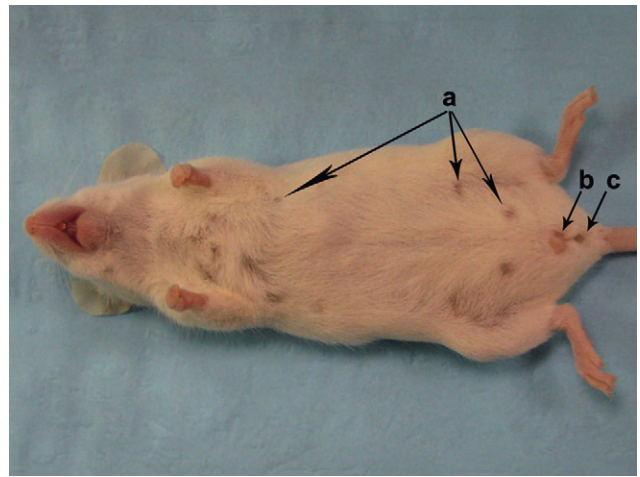


FIGURE 4.5 External examination of a euthanized female mouse. Shown are the nipples of mammary glands (a), vulva (b), and anus (c).



FIGURE 4.6 Immobilization of a mouse on corkboard. The fur on the ventral aspect of the mouse is moistened with 70% alcohol.

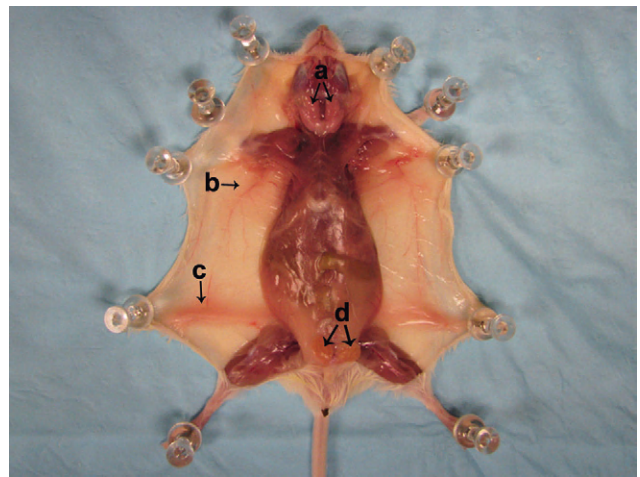


FIGURE 4.7 Sectioning and lateral retroflexion of skin with exposure of subcutaneous tissues in a female mouse. The salivary glands (a), thoracic mammary glands (b), inguinoabdominal mammary glands (c), and clitoral glands (d) should be examined and sampled.

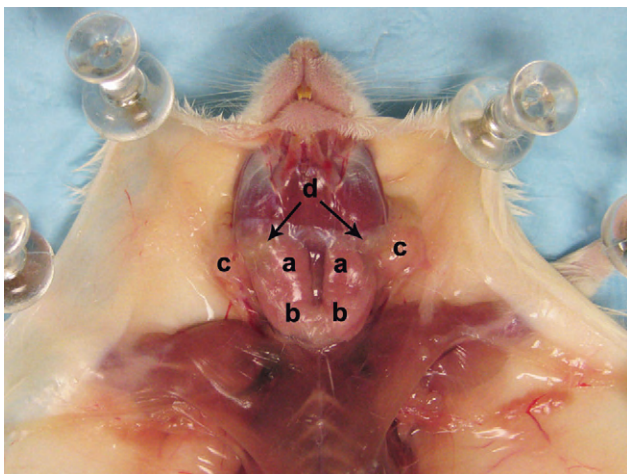


FIGURE 4.8 The salivary glands. Shown are the sublingual glands (a), submandibular glands (b), parotid glands (c), and submandibular lymph nodes (d).

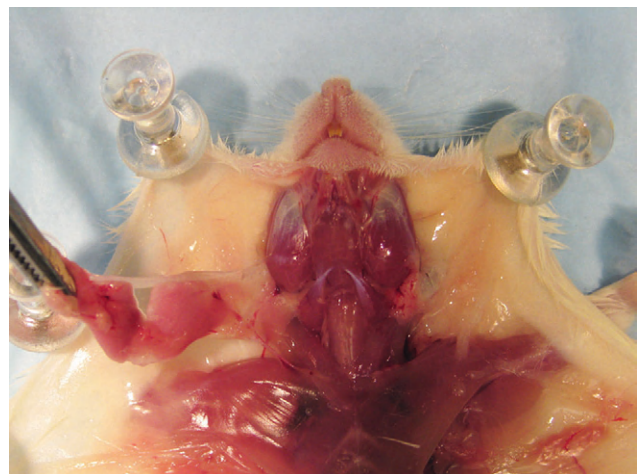


FIGURE 4.9 Sampling of the salivary glands as a single unit.

subcutaneous fat pad attached to the skin (Figure 4.7) and are usually sampled for histological examination together with the adjacent skin (see Figure 4.29). In studies that require detailed histological investigations of the entire mammary tissue, the inguinoabdominal and cervicothoracic mammary glands, including the mammary lymph nodes, are separated from the skin with a scalpel blade together with subcutaneous adipose tissue (Figure 4.10) and attached to a glass slide (Figure 4.11) or paper cardboard. Maintaining the mammary tissue on a glass slide or cardboard for 1–2 minutes before immersion in fixative allows the mammary tissue to stick to the glass slide or cardboard during fixation. This measure will preserve the anatomically flat orientation of the mammary glands during fixation and will facilitate obtaining of histological sections through the entire mammary tissue. Whole-mount preparation of mammary glands is performed in certain investigations. The salivary glands (parotid, submandibular, and sublingual) and submandibular or superficial cervical lymph nodes are collected together in fixative. The connective tissue surrounding the parotid glands near the ear canal is grabbed with forceps, and the parotid, submandibular, and sublingual salivary glands including the submandibular lymph nodes are separated as a single unit by sectioning the connective tissue attachments all the way to the other ear canal (Figure 4.9). The other subcutaneous lymph nodes, the preputial glands in males, and the clitoral glands in females (Figure 4.7) should be collected at this time and placed in fixative.

OPENING THE ABDOMINAL CAVITY

The abdominal cavity is opened by performing a medial incision of the abdominal wall from the pubic symphysis to the xiphoid, through the linea alba. Immediately after opening the abdominal cavity, the presence of abnormal fluid in the abdominal cavity should be noted and collected, uncontaminated, with a syringe for evaluation of volume, color, and consistency, and possible microscopic or microbiologic investigations. Subsequently, the abdominal wall is incised bilaterally from the medial incision toward the spinal column along the rib cage and similarly in the inguinal region through the lateral abdominal wall. This incision will allow adequate exposure of the abdominal viscera (Figures 4.12, 4.13 and 4.15). At this time, before any manipulation and disturbance of organs, it is important to perform *in situ* examination of the abdominal organs to evaluate the anatomical position of the viscera and to collect uncontaminated peritoneal swabs or other tissue samples as needed (Hayward et al., 2007).

UROGENITAL ORGANS

The testicles and epididymides should be collected and immersed in formalin as a single unit, without

sectioning the testicles, in order to preserve the normal histological orientation of the seminiferous tubules. The testicles and epididymides are easily exposed from the scrotum by grasping the epididymal fat with forceps and applying gentle traction. In rabbits, rats, and other rodents, the epididymis can be separated from the testis before fixation, but the testicle should be immersed intact in fixative for 6–12 hours (depending on the type of fixative used and the size of the testicle) before sectioning for faster penetration of fixative. This preliminary fixation will prevent histological distortion of the seminiferous tubules when cutting the testicles.

In order to collect the male reproductive tract, the pelvic cavity should be opened first by sectioning the pubic symphysis with scissors (mice) or bone-cutting forceps (rabbits, rats, and other rodents). The penis, urethra, accessory sex glands, and urinary bladder are collected as one unit by grasping the tip of the penis with forceps and using a scalpel blade or scissors to separate the penis, urethra, accessory sex glands, and urinary bladder from the anus, rectum, colon, and surrounding tissues (Figure 4.14). For preserving the anatomical orientation of reproductive organs during fixation, it is advisable to attach the entire male reproductive tract to a dry glass slide or index card before immersion of the tissues in fixative.

For female animals, the pelvic cavity is opened first as described for males. Then, the vulva, vagina, uterus, ovaries, and urinary bladder should also be collected as one unit by grasping the vulva with forceps and by severing the attachments to surrounding tissues with scissors (see Figure 4.16). Similar to the procedure for males, the entire female urogenital tract should be placed on a dry glass slide or a piece of index card before immersion in fixative in order to preserve the anatomical orientation during fixation. For adequate fixation of the urinary bladder urothelium, fixative should be injected into the urinary bladder with a syringe. This can be accomplished either by inserting a blunt-tip needle through the urethra, followed by ligation of the urethra after infusion of fixative, or by direct injection of fixative with a sharp needle through the urinary bladder wall.

SPLEEN, MESENTERIC LYMPH NODES, PANCREAS, AND DIGESTIVE TRACT

The spleen is collected in a small tray and weighed before being placed in fixative. Frozen-tissue samples and tissue samples for microbiology tests or other molecular investigations must be collected from the spleen and other organs before introducing them into fixative solution. Before removing the digestive tract, the patency of the common bile duct can be demonstrated by opening the proximal duodenum with a longitudinal incision, and subsequently applying pressure on the gallbladder

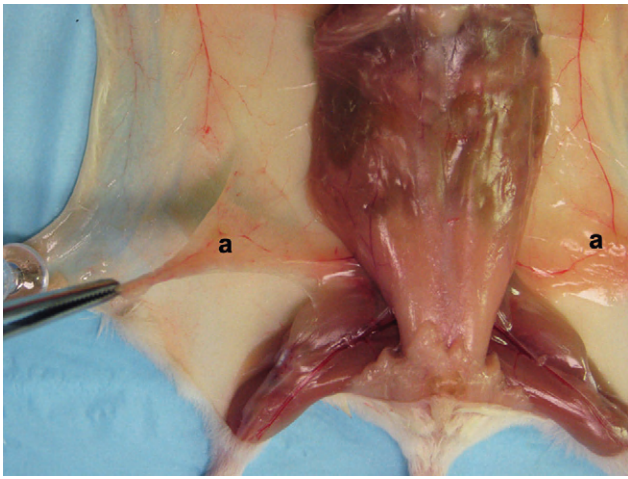


FIGURE 4.10 Sampling of the inguinoabdominal mammary glands by dissecting the subcutaneous adipose tissue (a).

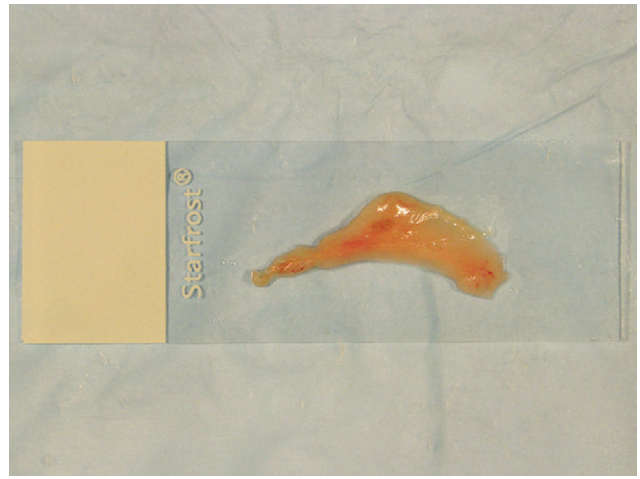


FIGURE 4.11 The mammary gland attached to a glass slide before immersion in fixative solution.

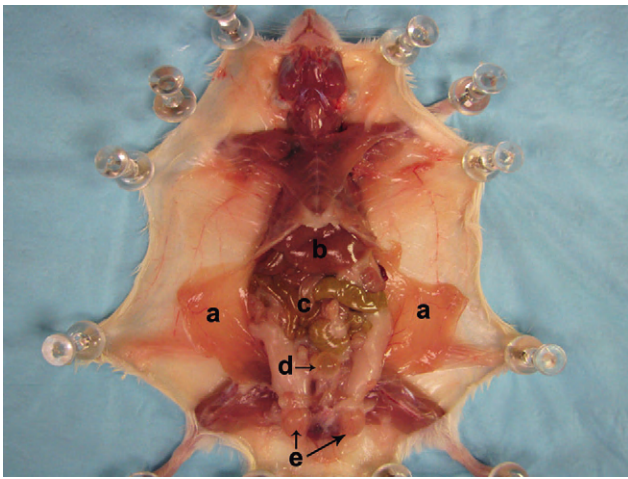


FIGURE 4.12 Opening of the abdominal cavity by medial incision and bilateral retroflexion of the abdominal wall (a). In situ examination of abdominal organs in a male mouse: liver (b), intestines (c), urinary bladder (d), and testicles (e).

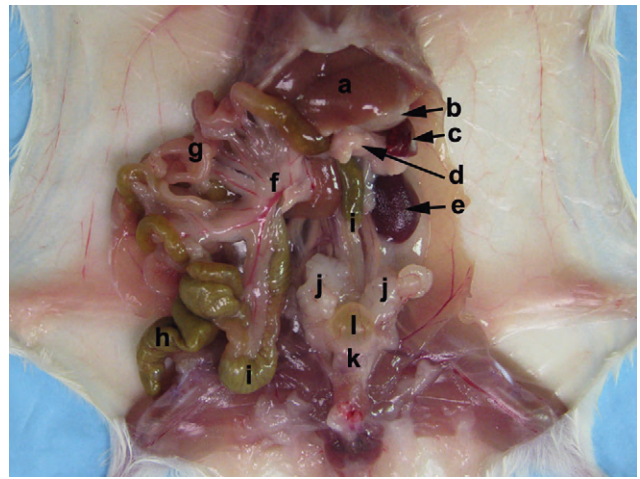


FIGURE 4.13 Examination of abdominal organs: liver (a), stomach (b), spleen (c), pancreas (d), kidney (e), mesenteric lymph nodes (f), small intestine (g), cecum (h), colon (i), seminal vesicles (j), prostate glands (k), and urinary bladder (l).

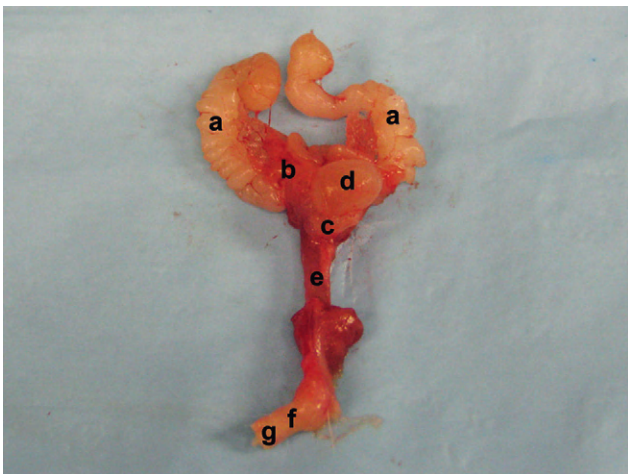


FIGURE 4.14 After splitting the pubic symphysis, the male urogenital tract is removed as a single unit: seminal vesicles (a), anterior prostate gland or coagulating gland (b), ventral prostate gland (c), urinary bladder (d), urethra (e), penis (f), and preputium (g).

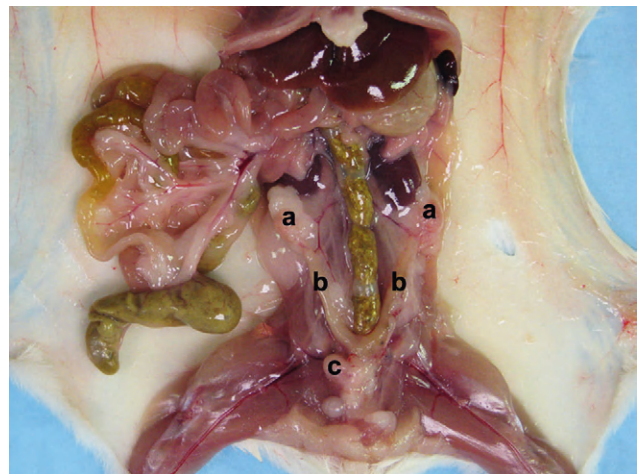


FIGURE 4.15 In situ examination of the female urogenital tract: ovaries (a), uterine horns (b), urinary bladder (c).

(Seymour et al., 2004). If the common bile duct is patent, the bile should be expressed in the duodenum at the bile duct orifice. Among laboratory rodents, rats lack a gallbladder. The mesenteric lymph nodes and the pancreas can be easily located within the mesentery and should be separated from the intestine before the intestinal tract is removed (Figure 4.13).

The stomach and entire gastrointestinal tract are removed as a single unit by first cutting the esophagus caudal to the diaphragm, without sectioning the diaphragm. Then, grasping the stomach with forceps, the intestinal tract is pulled away from the carcass and all mesenteric and other tissue attachments are cut from stomach to anus. The stomach and the entire intestinal tract (duodenum, jejunum, ileum, cecum, colon, rectum, and anus), including the feces, should be examined in more detail at this time (Figure 4.17), and samples for bacteriological, parasitological, or other diagnostic tests should be collected as required. Due to rapid postmortem autolysis of gastrointestinal mucosa, we recommend that fixative be injected into the intestinal lumen in multiple sites using a 5–10ml syringe with a 20–22 gauge needle. Other investigators prefer to ligate one end of the intestine and use the other end for infusing the fixative; that end is subsequently ligated as well in order to maintain the fixative within the intestinal lumen for better fixation of the mucosa. The stomach can be infused with fixative and fixed without cutting the stomach wall, or the stomach can be opened along the greater curvature to examine the mucosa and ingesta and then placed in fixative. The stomachs of rabbits contain a larger amount of ingesta and should always be opened along the greater curvature before immersion in fixative. In mice, rats, guinea pigs, hamsters, and other rodents, the entire intestinal tract is immersed in fixative after intraluminal infusion of fixative. In rabbits, multiple 1–2 cm long segments of intestine from all regions (duodenum, jejunum, ileum, cecum, colon, rectum, and anus) are usually collected in fixative. When the entire length of intestine needs to be examined microscopically in mice, “Swiss rolls” of the small intestine or colon should be prepared in histology cassettes before immersion of the tissue in fixative. When preparing the Swiss roll, the intestine should be infused with fixative first, and then, while the proximal end of the intestine is held in the center of the cassette, the distal end is twisted around in a spiral-like shape (Figure 4.18). It is recommended to make the rolls smaller than the cassettes to avoid draining of fixative while rolling the intestine. It is also recommended to not overinflate the intestine with fixative (Relyea et al., 2000). This technique is practiced in certain types of research and when sentinel mice are examined for the presence of pinworms or other parasites in monitoring the health of the mouse colony. The colon of mice, rats, and other rodents can be fixed in formalin either as Swiss roll preparations or as multiple segments of 1–2 cm.

LIVER, GALLBLADDER, ADRENAL GLANDS, AND KIDNEY

In mice, the liver and gallbladder are collected together and fixed as a single unit. Using forceps, the liver is grasped from the connective tissue under the medial lobe, and with scissors or a scalpel blade, the blood vessels and ligaments are severed without sectioning the diaphragm. The liver with the attached gallbladder should be weighed, and the required samples should be collected before the immersion of liver in the fixative solution. In rats, guinea pigs, hamsters, and other rodents, the liver is removed in a similar manner, but before immersing the liver in fixative, sections of liver at 3–5 mm intervals are recommended for better fixation of tissue. From rabbits, pieces of liver of $1 \times 1 \times 0.5$ cm from each hepatic lobe, including the gallbladder and observed gross lesions, should be collected in fixative.

After removal of the digestive tract and liver, there should be good exposure of the adrenal glands, kidneys, and ureters (Figure 4.19). Before removing the kidneys, the adrenal glands within the adipose tissue at the cranial pole of each kidney should be sampled intact (Figure 4.19). Both adrenal glands are excised without adipose tissue, weighed if the study protocol requires, and placed in a small cassette (Figure 4.20) prior to immersion in fixative. Similarly the kidneys should be collected without the surrounding adipose tissue and weighed before fixation. After examining the surface of the renal capsules, the kidneys should be examined on the cut surface by sectioning the right kidney in a transverse plane and the left kidney in a medial longitudinal plane, through the renal cortex, medulla and pelvis.

THORACIC CAVITY

Cutting the diaphragm and the ribs on both sides of the sternum, dorsal to the chondrocostal junction and from the last rib to the first rib, should open the thoracic cavity (Figure 4.21). The entire sternum with attached ribs is collected in fixative (Figure 4.21, inset). The organs within the thoracic cavity are examined in situ, the presence of abnormal fluids in the thoracic and pericardial cavities is evaluated, and samples for microbiology testing are collected before further manipulation and contamination of tissues (Hayward et al., 2007). The entire respiratory tract, heart, aorta, tongue, larynx, esophagus, thyroid and parathyroid glands, thymus, and mediastinal lymph nodes are collected and fixed as a single unit (Figure 4.22, inset), also known as “the pluck”. To accomplish this, the soft tissues on the medial aspect of the mandible are cut until the oral cavity can be accessed, and then the mandibular symphysis is split. The tongue is grasped with forceps, and while the tongue is gently pulled caudally and away from the carcass, the soft palate and connective tissues around the larynx, trachea, esophagus, and the mediastinum are cut in order to separate the pluck from the carcass

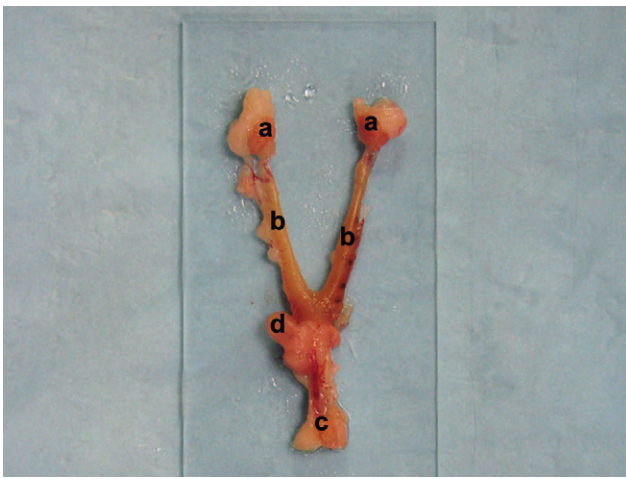


FIGURE 4.16 After the pubic symphysis is split, the female urogenital tract is collected as a single unit and is attached to a glass slide or index card before immersion in fixative. Ovaries (a), uterine horns (b), vagina (c), and urinary bladder (d).



FIGURE 4.17 Collection and examination of the digestive tract: stomach (a), duodenum (b), jejunum (c), ileum (d), cecum (e), proximal colon (f), distal colon (g), and rectum (h).

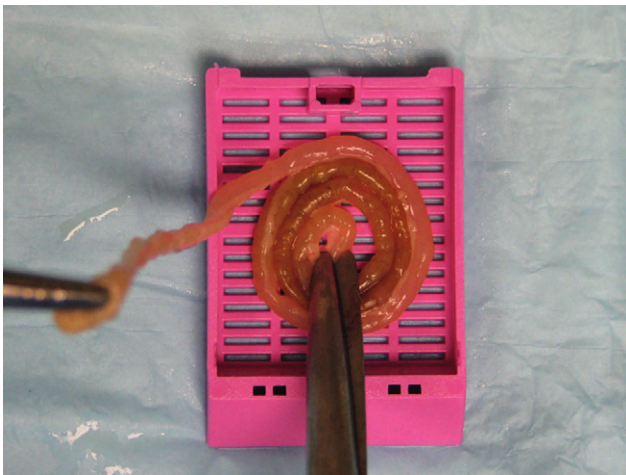


FIGURE 4.18 Preparation of a "Swiss roll" after fixative has been infused into the lumen of the intestine.

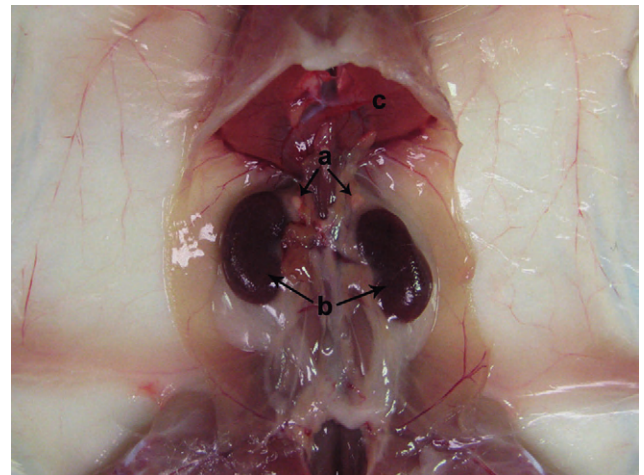


FIGURE 4.19 The adrenal glands (a) found at the cranial pole of the kidneys (b) should be examined and sampled before removing the kidneys. The diaphragm is intact (c).

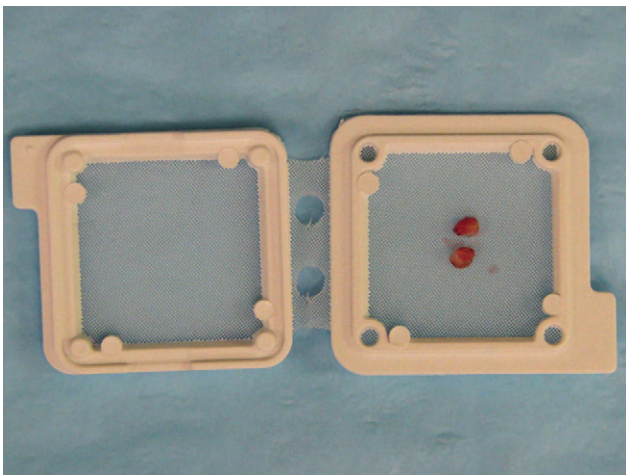


FIGURE 4.20 The adrenal glands are collected in small cassettes before immersion in fixative.

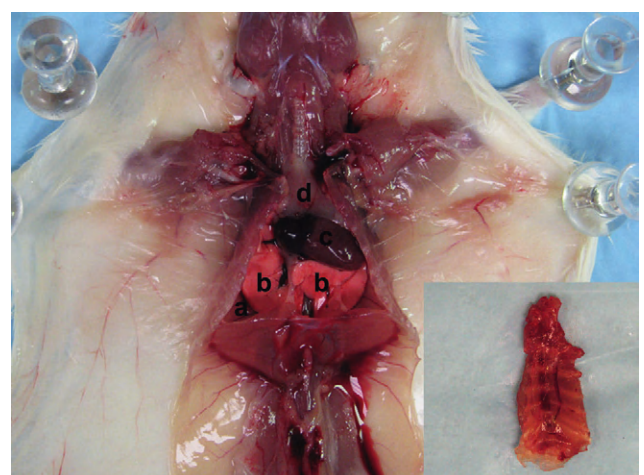


FIGURE 4.21 Opening of the thoracic cavity and in situ examination of the pleura and thoracic cavity (a), lungs (b), heart (c), and thymus (d). The sternum is collected and placed in fixative (inset).

(Figure 4.22). Due to its small size the heart of the mouse is usually fixed without sectioning. In rabbits, rats, guinea pigs, hamsters, and other rodents, the heart is opened by a section in a median plane through the right and left ventricles and atria, or by other methods of dissection. This sectioning will allow not only exposure and examination of all chambers and walls of the heart, but also a better fixation of the cardiac tissues. After external examination and collection of samples for microbiology testing as necessary, the lungs of rabbits, guinea pigs, hamsters, rats, mice, and other rodents should be infused with fixative to prevent histological artifacts caused by alveolar collapse (Renne et al., 2001). Using a blunt-tip hypodermic needle and a 5–20ml syringe filled with fixative, the needle is introduced into the lumen of trachea through the pharynx, and then the trachea with the inserted needle is clamped down with forceps to prevent backflow of fixative solution (Figure 4.23). Applying gentle pressure, the fixative should be infused into the lungs until the lungs attain the size of the thoracic cavity, and then the entire pluck should be dipped into the fixative. Overinflation of the lungs with fixative will cause artifacts from rupture of the alveolar walls.

SCIATIC NERVE, SKELETAL MUSCLE, BONES, AND JOINTS

The sciatic nerve can be easily located between the muscles of the thigh, close to the femur, as shown in Figure 4.24, and should be placed in a small cassette (Figure 4.24, inset) before immersion in fixative. In mice, rats, guinea pigs, hamsters, and other rodents, the entire front and hind limbs are usually sampled in fixative (Figure 4.25) for the evaluation of skeletal muscle, joints, bone marrow, and bone tissue. Additional samples of skeletal muscle can be collected from muscles along the vertebral column.

EYES, BRAIN, AND SPINAL CORD

The entire spinal column and the head should be separated from the skin and remaining carcass as one unit by starting from the coccygeal region of the spinal column and continuing cranially to the nostrils (Figure 4.26A). In mice and similarly sized rodents, the eyes can be fixed in situ and processed in histological sections together with the cranial bones, or can be extracted from the orbits and fixed individually. In rabbits, rats, guinea pigs, hamsters, and any study that involves ocular histology, it is recommended to extract the eyes from the orbital cavity and fix them individually in Davidson's, Zenker's, or Bouin's fixative to obtain better fixation and high-quality histological sections. For a better fixation, larger ocular globes may need to be infused with fixative into the vitreous chamber before immersion in fixative. The eye globe is usually collected with attached optic nerve and Harderian gland. This can be achieved by grasping the conjunctiva with

forceps and pulling the eye globe out of the orbit, while cutting into the orbital cavity around the eye globe with a small scalpel blade or scissors and severing the optic nerve at the end. To prevent autolytic changes of eye tissues, particularly when the eyes are the target organs of the investigation, they are preferably collected and placed in fixative at the beginning of necropsy.

The head is separated from the spinal column by disarticulating the atlanto-occipital joint as shown in Figure 4.26. The brain is collected by removing first the calvaria, with scissors (mice) or bone-cutting forceps (rats, hamsters, guinea pigs, and rabbits), without damaging the brain tissue. The calvaria is removed by cutting bilaterally the occipital, parietal, and frontal bones beginning at the foramen magnum dorsal to the occipital condyle, and ending at the frontal bones between the orbits (Figure 4.27). With the brain cavity opened and the skull turned upside down to allow gravitational falling of brain, the brain should be extracted intact from the cranial cavity (Figure 4.28). This is accomplished by gently pulling away the brain from the ventral aspect with a small spatula or a scalpel blade, beginning at the foramen magnum and advancing toward the olfactory lobes, and concurrent sectioning of the nerve roots. For all species, the extracted brain should be examined and introduced intact in the fixative (Figure 4.28) unless the protocol of the study requires otherwise. Sectioning the brain before fixation causes severe distortion of the histological architecture of the brain tissue. Once examined, the remaining bones and soft tissues of the head, including the nasal cavity and maxillary and mandibular bones, should be introduced into fixative. Perfusing the nasal cavity with fixative is recommended for proper fixation of nasal tissues (Everitt and Gross, 2006).

After trimming away the ribs and muscles tissues from the spinal column, the cervical, thoracic, lumbar, and sacral regions of the spinal column are separated by transverse sections of the vertebral column (Figure 4.26B). This sectioning enables faster penetration of fixative and better fixation of the spinal cord.

SKIN

Recommended routine samples of the skin should include skin from the dorsal, lateral, and ventral aspects of the body, and from the head, neck, and tail regions (Relyea et al., 2000). These samples, which may also include the mammary glands, are collected from the lumbar and inguinoabdominal regions as shown in Figure 4.29. To avoid undesirable folding of skin during fixation, the subcutaneous surface of the collected skin sample should be attached to a piece of index card before it is immersed in fixative. The skin of the head and neck, including the ear pinna, should be sampled routinely in fixative. In addition to the described routine tissue sampling of all organs, any lesion observed upon gross

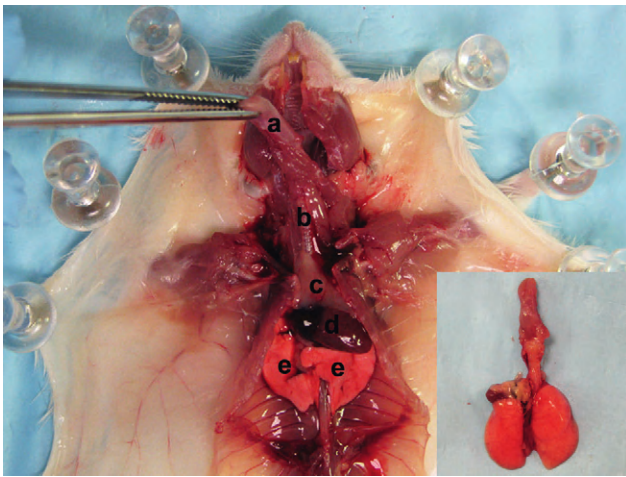


FIGURE 4.22 After the mandibular symphysis is split, the respiratory system and adjacent tissues are removed as a single unit (the pluck): tongue (a), trachea (b), thymus (c), heart (d), lungs (e), and mediastinum with mediastinal lymph nodes. The dorsal view of the removed pluck (inset).

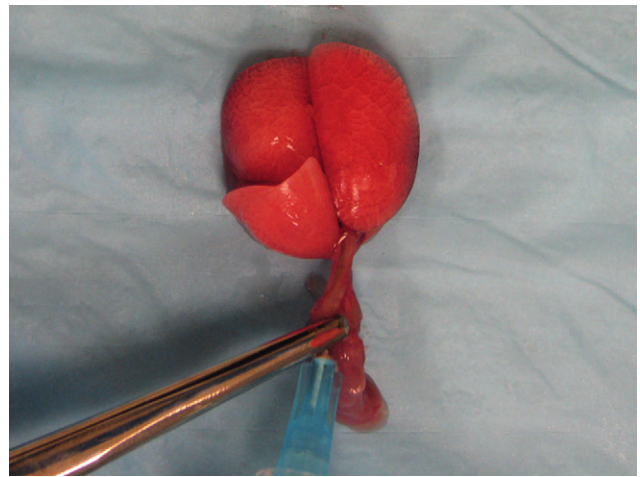


FIGURE 4.23 Infusion of the lungs with fixative before immersion in fixative solution.

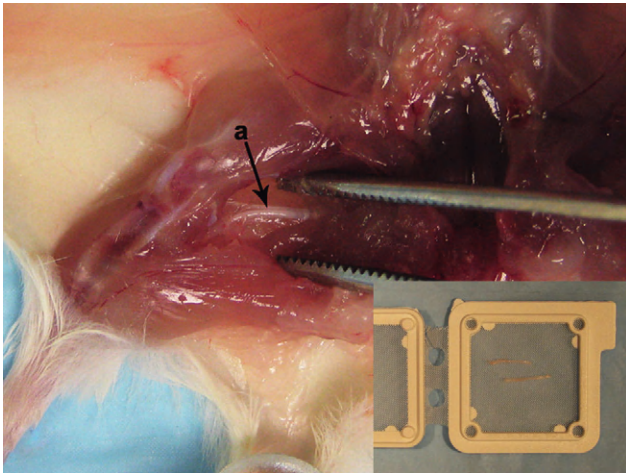


FIGURE 4.24 Sampling of the sciatic nerve (a) in a small cassette (inset) before immersion in fixative.

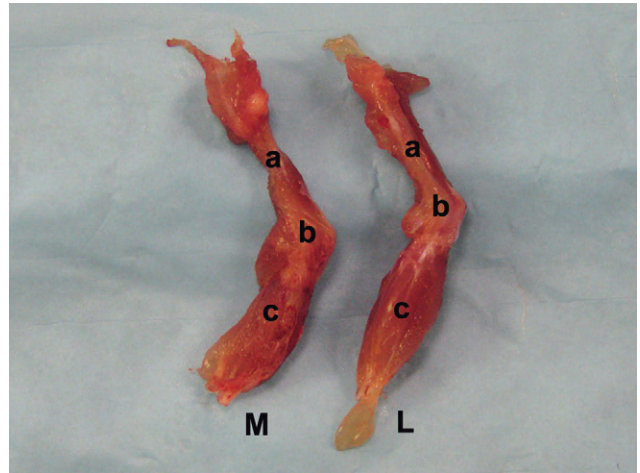


FIGURE 4.25 Sampling of the hind limbs containing the femur (a), stifle joint (b), and tibia (c) with attached skeletal muscles. Medial view (d) and lateral view (e).

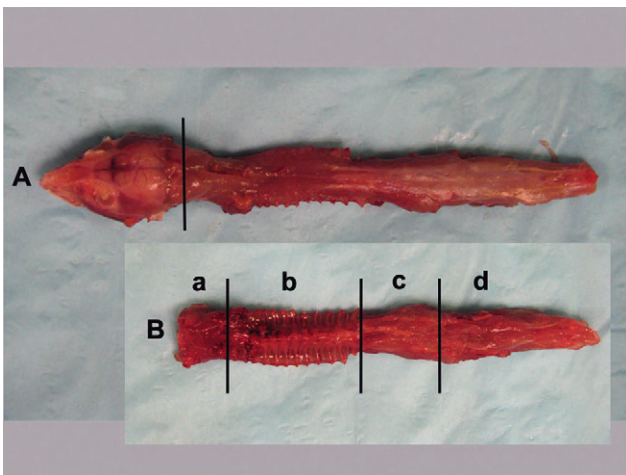


FIGURE 4.26 The spinal column and the head are separated from the remaining of the carcass (A, dorsal view), and then the head is separated from the spinal column (B, ventral view). The vertebral column (B) is sectioned transversally, separating the cervical (a), thoracic (b), lumbar (c), and sacral (d) regions before immersion in fixative.



FIGURE 4.27 The calvaria is removed and the cranial cavity is opened for sampling the brain.



FIGURE 4.28 The brain is removed and immersed in fixative intact.

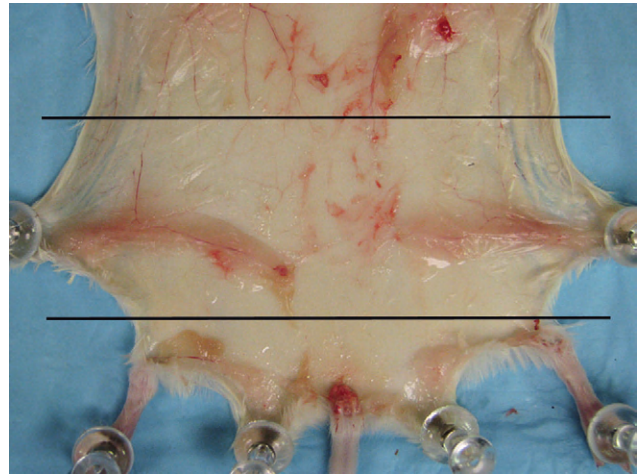


FIGURE 4.29 Sampling of skin from the dorsal, lateral, and ventral abdominal and inguinal regions, with attached mammary glands.

examination should be collected in fixative for histological examination.

PATHOLOGY CONSIDERATIONS

Descriptive Pathology

During necropsy, the pathologist or technician must record in the written report the animal's identification information, organ weights, and all lesions observed, using precise measurements and descriptive common terminology but without interpretation of the lesions (i.e. "dark-red area", not "hemorrhage"; "tissue mass", not "neoplasia"). In describing the lesions, it should first be recorded what organs are affected, the distribution or pattern of the lesions, and the percentage of the organ affected or the extent of the lesion. The following characteristics of the lesions should be described using precise terminology: size, number, color, shape, consistency or texture, weight, location, severity, odor, and volume (Bono et al., 2000; Everitt and Gross, 2006; Feldman and Seely, 1988; King et al., 2005). Tissue artifacts caused by handling or dissection during the necropsy or due to postmortem autolysis should be differentiated from pathologic lesions. Following the written report, a veterinary pathologist should interpret the observed gross lesions, give the morphological diagnosis of the lesions found, and elaborate a definitive, differential, or suspected diagnosis. The severity of gross and histological lesions can be graded using a score from 1 to 4 (1 = minimal, 2 = mild, 3 = moderate, 4 = marked). A grade of zero or no grade is used when no lesion is present. Consistency among pathologists in using similar diagnostic terminology and grading scores for histological lesions is particularly important in preclinical drug

studies (Crissman et al., 2004; Shackelford et al., 2002). Accompanying the pathology report, photographs of gross and histological lesions should be included.

In biomedical science, images are an important tool for documenting, analyzing, teaching, learning, and publishing medical knowledge and research data. Therefore, photographs of necropsy specimens should be accurate and of high quality. High quality can be achieved by using adequate photography equipment, preparing gross specimens properly, and learning basic photography skills. Most necropsy laboratories are equipped with a copy stand that has arms with lights and camera support. Digital cameras with autofocus are more commonly used today because they are more practical for routine work, but they are not recommended for taking pictures in macro-focus range. For the latter, because organs and lesions are commonly small in mice and other laboratory rodents, a digital single-lens reflex (SLR) camera with telephoto or close-up lens (50- or 100-mm macro lens) is recommended. The necropsy specimen, as well as the background, should be clean of blood, contaminants, and other materials. A suitable background color with good contrast, an adequate light source, absence of shadows, and good focus on the specimen are needed for obtaining high-quality images. Pictures of the well-exposed organ and/or the lesions should be taken before removing them from the animal, in order to capture their anatomical orientation and relationship with surrounding organs (Sundber and Miller, 2000). Additional photographs with more details of the organ and lesions can be taken after removing the organ. Including a control normal organ in the same picture is recommended when comparison is important. Dissection microscopes or scanning electron microscopes are used for taking photomicrographs of smaller lesions.

Tissues Sampling, Fixation, and Trimming

Tissue samples must be collected with sharp instruments and gentle manipulation to avoid distortion of the cellular and histological architecture of tissues. Histological sections of tissues can be obtained from frozen or fixed tissue samples.

Frozen tissue samples are usually prepared when molecular analysis of tissue or specific immunohistochemical, histochemical, or immunofluorescence stains of histological sections are required. High-quality frozen tissue samples can be obtained by embedding the fresh tissue sample in a cryomold with optimal cutting temperature (OCT) compound, followed by immediate immersion into liquid nitrogen or dry ice. Subsequently, these samples should be preserved in a freezer at -80°C . Freezing of tissue samples is not recommended for routine histological examination because the freezing artifacts damage cellular morphology and tissue architecture, and therefore may obscure or disable recognition of lesions. Routine microscopic examination of tissues is performed on hematoxylin and eosin (H&E) stained sections from tissue samples fixed in 10% neutral buffered formalin.

For obtaining good-quality histological sections, autolysis after the collection of tissues must be prevented by performing appropriate fixation (Eltoum et al., 2001; Grizzle, 2009; Jones, 2001). Proper fixation of tissues can be accomplished by sampling and immersing the tissues in fixative within 15 minutes after euthanasia, by collecting tissue samples no thicker than 5 mm, and by maintaining the tissue samples in an adequate amount of fixative for a minimum of 24–48 hours. An experienced prosector can usually finish a rodent necropsy in 10–15 minutes, which allows rapid collection of tissues in fixative. Considering that the fixative solution penetrates through the surface of the tissue gradually, approximately 1 mm per hour, collecting small pieces of tissue will prevent autolysis within the center of the tissue sample. Most organs in mice can be sampled and fixed without sectioning because of their small size. Rabbits, guinea pigs, rats, and hamsters have larger organs, and therefore tissue samples no thicker than 5–6 mm have to be sectioned from these organs to accomplish adequate fixation. Regardless of size, it is recommended that the brain and testicles be fixed intact for a few hours before sectioning these organs in order to prevent distortion of the histological architecture by cutting. After 6–24 hours of preliminary fixation, depending upon the type of fixative and the size of the organ, the brain and testicles should be sectioned in 3–5 mm thick slices and returned to fixative for another 12–24 hours to complete the fixation. This sequence will enhance the penetration of fixative into the tissue and avoid autolysis within the center of the tissue sample, while preserving the morphology of tissue.

The volume of fixative in the container should be 10–20 times more than the volume of tissues sampled (Everitt and Gross, 2006; Feldman and Seely, 1988; Seymour et al., 2004). Maintaining the container with tissues and fixative on a shaker device during fixation will enhance the exposure of all surfaces of tissues to the fixative and will improve the quality of fixation.

Several chemical solutions are used for fixation of tissues, and all have different advantages and disadvantages, which determines their application in research and diagnostic investigations. Ten percent neutral-buffered formalin solution is the most commonly used fixative for preparation of tissues for histological examination and provides the best preservation of tissue morphology, at a convenient price and with less severe toxic effects than are seen with other fixatives. A major disadvantage of formalin fixation, however, is the continuous formation of cross-linking of amino groups in the tissue sample, resulting in conformational changes in the tertiary and quaternary structures of proteins and thus modifying the epitopes recognized by antibodies (Arnold et al., 1996; Ramos-Vara, 2005; Ramos-Vara and Saetelee, 2007; Ramos-Vara et al., 2008). Therefore, prolonged formalin fixation has a detrimental effect on immunohistochemical staining and RNA preservation of tissues (Leong and Gilham, 1989; van der Loos, 2007; van Maldegem et al., 2008; Werner et al., 2000), although many antibodies can detect antigens following up to 7 weeks of fixation of tissues in formalin (Webster et al., 2009). On the other hand, incomplete fixation of tissue may have a more detrimental effect on immunohistochemical results than overfixation (Webster et al., 2009; Werner et al., 2000; Yaziji and Barry, 2006). Most studies concluded that tissue samples should be immersed in formalin within 30 minutes of the animal's death or collection of a biopsy, and the tissues should be fixed in formalin for 24–48 hours (Werner et al., 2000). After 24–48 hours of fixation, the tissue samples should be extracted from the formalin and immediately trimmed for tissue processing and paraffin embedding. If trimming and tissue processing cannot be performed after the 48-hour fixation period, the tissue samples should be transferred to and maintained in 70% ethanol, which will prevent further cross-linking of peptides by the formalin. However, unlike ethanol, formalin can preserve indefinitely the morphological quality of tissue samples.

In addition to commonly used 10% neutral-buffered formalin and 70% ethanol, there are other fixative solutions that can be used for the fixation of tissues: Davidson's fixative, Bouin's fixative, Tellyesniczky's or Fekete's fixative, B5 fixative, Carnoy's fixative, Clarke's fixative, 4% paraformaldehyde fixative, glutaraldehyde fixative, Karnovsky's fixative, and JB4 fixative. Every fixative has different properties and different applications. The selection of proper fixative and fixation protocol is determined by the objectives of the

experiment, the methods of tissue examination, and the staining techniques required for the diagnostic or research investigation (Relyea et al., 2000).

A special fixation procedure is the perfusion method, which combines euthanasia with fixation (Relyea et al., 2000; Rostgaard et al., 1993). First, the animal is anesthetized; then, the thoracic cavity is opened and the blood samples collected. Next, the walls of the right atrium or the jugular veins are cut, and physiologic saline is injected into the left ventricular chamber of the heart for washing the blood out of the system. Subsequently, while the heart is still contracting, the fixative is injected into the left ventricular chamber of the heart through the same needle until the entire animal becomes stiffened and the animal is euthanized. After this procedure, the necropsy and tissue collection can be performed as usual. This fixation technique is performed when instant fixation of the brain, liver, kidney, or other tissue is necessary.

Once tissues are trimmed into small pieces no thicker than the depth of histology cassettes (2–3 mm) to permit adequate penetration of solvents and paraffin, trimmed pieces of tissue are placed in the desired orientation into labeled histology cassettes for processing and embedding in paraffin blocks. Trimming must be performed with sharp blades and gentle manipulation using toothless forceps in order to avoid damage of tissues and formation of artifacts. When trimming the tissue samples, it is important to capture in the selected section the lesion that was observed grossly and an area of peripheral, less-affected or normal tissue. Standard trimming techniques with specific sections of each organ are presented in previous publications (Bahnmann et al., 1995; Bono et al., 2000; Kittel et al., 2004; Morawietz et al., 2004; Relyea et al., 2000; Ruehl-Fehlert et al., 2003). Each histology cassette can contain one or multiple pieces of tissues. Related tissues of similar size and density or texture may be placed together in the same cassette.

Before trimming and tissue processing, formalin fixed bone tissues have to be decalcified properly with a decalcifying solution such as 5% or 10% formic acid or hydrochloric acid solution. However, excessive decalcification for too long (more than 24–48 hours) will damage the tinctorial properties of tissues and compromise the quality of H&E staining. Decalcified bones must be rinsed in running water for 3–4 hours before processing to avoid inadequate staining of tissues. Histology cassettes containing trimmed tissues that were only fixed or decalcified and rinsed should be placed in a container with 10% formalin or 70% ethanol solution and submitted to the histology laboratory for processing and paraffin embedding (Seymour et al., 2004). The cassettes containing decalcified but not previously rinsed tissues should be placed in water and rinsed thoroughly before introducing them into 70% ethanol. For histologic examination, paraffin-embedded tissues

should be cut in 3–5 µm thick sections mounted on a glass slide, and stained with H&E. Special histochemical or immunohistochemical stains of these tissues may be requested after the histopathological examination of H&E-stained sections.

Standard techniques of tissue sampling, fixation, trimming, embedding, processing, and staining must be applied uniformly to all tissues from the same study to avoid differences in staining quality and from one tissue to another, which could cause misinterpretation of tissue changes. This uniformity is particularly important in studies that require complex digital image analysis or morphometric measurements of tissues and lesions using sophisticated image analysis software. If the histologic sections are used in molecular biology investigations such as in situ hybridization, in situ polymerase chain reaction, and other RNA-based studies, specific protocols of tissue collection and processing must be employed to preserve the biological targets. All details presented here must be considered when performing the necropsy and processing the tissues for microscopic examination.

Acknowledgments

We thank Maurice J. Dufilho IV at The University of Texas MD Anderson Cancer Center for technical support with necropsy.

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Zoonoses and Occupational Health

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INTRODUCTION

This chapter will review zoonoses that can be acquired from handling hamsters, guinea pigs, rabbits, and other miscellaneous rodent species. Although the risk of acquiring a disease from many types of laboratory animals has decreased since most laboratory animals are now produced with defined microbiologic profiles, there are still instances when wild animals or field-collected animals are required for research. The use of wild rodents or field-collected rodents may expose the research staff to diseases that have been excluded, if not eliminated, from research colonies. Allergic responses to animals, particularly rodents, can also be a concern.

In general, some of these diseases require vectors for the transmission to humans while others can be directly transmitted. The control of vectors such as biting insects can effectively limit the spread of some vector-transmitted diseases. For some parasitic diseases, humans can serve as an intermediate host; for others humans are the final host of the parasite. In the latter case, the elimination of the intermediate host should prevent the complete lifecycle and protect the laboratory animal worker. Knowledge about the transmission of the disease will play a part in effectively reducing the risk of the pathogen.

The focus here is to review diseases that might be present in research animals and might be transmitted to immunocompetent research staff during experiments. Diseases that might be transmitted specifically to immune-compromised individuals are not covered. Finally, allergic reactions to laboratory animals are discussed because exposure to animal allergens represents a significant risk to personnel who handle animals.

VIRAL DISEASES

Hantavirus

Reservoir and Incidence

Hantavirus is a member of the Bunyaviridae family. At least 20 different viruses are recognized in this genus (Diglisic et al., 1999). Wild rodents are the reservoirs for these viruses (Fox et al., 2002). The incidence of serologic evidence for Hantavirus infection in both the human population and the resident rodent population has been reported by Diglisic et al. (1999). Norway rats in Baltimore, Maryland were reported to have a prevalence rate of 44% although the human rate is 0.74% demonstrating that although the incidence of the infection in the rodent population is prevalent, the human infection rate still remains low (Diglisic, 1999).

In contrast, nine workers at a laboratory animal facility were reported to have become infected with Hantavirus while working with known infected rats (Lee and Johnson, 1982). One person visited the animal housing rooms only twice, before acquiring the infection and being hospitalized for 2 weeks.

Mode of Transmission

Horizontal transmission via aerosolization of urine, feces, and saliva is the most common mode of transmission (Fox, 2002). Transmission can also occur via a rodent bite (Fox et al., 2002). Numerous cases of Hantavirus infection have occurred among laboratory animal facility personnel through exposure to infected rats with outbreaks reported in Korea, Japan, Belgium, France, and England (LeDuc, 1987). Person-to-person transmission has also been reported (Enríe et al., 1995).

Clinical Signs

In humans clinical signs are related to the strain of virus involved. Clinical signs can include fever, back pain, headache, myalgia, anemia, hematuria, gastrointestinal bleeding, and coughing (Committee on Occupational Safety and Health in Research Animal Facilities, 1997).

Diagnosis and Prevention

Colonies of rodents should be tested for Hantavirus. Animal Biosafety Level 2 is recommended for work conducted with experimentally infected rodent species known not to excrete the virus. All work involving inoculation of the virus into *P. maniculatus* or other permissive species should be conducted at Animal Biosafety Level 4 (Centers for Disease Control, 1994).

Lassa Virus

Reservoir and Incidence

Lassa virus, the causative agent of Lassa fever, is a member of the family Arenaviridae. The multimammate mouse (*Mastomys natalensis*) is the only known non-human host for Lassa virus (Monath et al., 1974). Lassa antibody and antigen were found in 96 (11%) and 46 (5%), respectively, of 884 tested *Mastomys* (Demby et al., 2001). The proportion of Lassa virus-infected *Mastomys* per region in Guinea ranged from 0 to 9% and was highest in the savannah and forest zones. The proportion of infected animals per village varied considerably, even between villages in close proximity. Infected animals tended to cluster in relatively few areas, suggesting the existence of focal "hot spots" of Lassa virus-infected *Mastomys*, possibly accounting for the observed heterogeneous distribution of Lassa fever (Demby et al., 2001).

Mode of Transmission

Infection is believed to be transmitted through contact with the feces and urine of infected rodents. In the multimammate mouse, antibody and antigen were essentially mutually exclusive and showed profiles consistent with vertical transmission of both Lassa virus and antibody (Demby et al., 2001).

Clinical Signs

Lassa virus has a predilection for person-to-person spread within the hospital environment, with a high risk of infection in medical personnel. The hazard to laboratory investigators is also great (Monath et al., 1974). Case-fatality rates vary and have been reported as 18% by Bausch et al. (2001), and 43% among hospitalized patients by Monath et al. (1974).

Diagnosis and Prevention

The infection is not thought to be typically present within well-monitored populations of laboratory animal species. An ELISA antibody detection system exists and has been used to determine the prevalence of the virus in wild rodent populations (Demby et al., 2001).

Lymphocytic Choriomeningitis Virus

Reservoir and Incidence

Mice and hamsters are known to be reservoirs for lymphocytic choriomeningitis virus (LCMV). LCMV is a member of the family, Arenaviridae (Fox et al., 2002).

Mode of Transmission

LCMV can be transmitted among mice horizontally through secretions (urine, saliva, and feces). Offspring develop persistent infections and shed the virus during most of their lives (Jacoby et al., 2002). Infections in humans can occur after inhalation, ingestion, and direct contact with LCMV-contaminated urine, feces, and saliva of infected mice, hamsters, and guinea pigs (Committee on Occupational Safety and Health in Research Animal Facilities, 1997).

There are many case reports of zoonotic disease related to LCMV in the literature. One specific case report documented several cases of LCMV transmission to humans from a pet hamster with a propensity for biting its owners (Amman et al., 2007; Gregg, 1975; Hirsch et al., 1974).

Several case reports have been published that traced the source of contamination to hamster tumor lines. One outbreak of 48 human cases occurred between 1971 and 1973. Most cases occurred in personnel visiting or using a photocopier in the basement of the radiotherapy department of a large hospital in upper New York State. The photocopier was located in the same room as about 200

Syrian hamsters. Clinical signs in humans were characterized mainly by sudden onset of fever (102–104°F), severe prostration, headache, and myalgia. Convalescence was protracted, but there was little evidence of meningitis. No deaths were reported with this outbreak (Hotchin et al., 1974). This case report demonstrated that limited contact with persistently infected hamsters is sufficient to transmit the disease to humans. The outbreak was traced to a supplier of hamster tumor lines that were found to be positive for LCMV (Gregg, 1975).

The following year, a nationwide epidemic in the United States in 1973 and 1974 involving 181 human cases was associated with one pet hamster breeder in Birmingham, AL. This breeder was also employed by a biologics firm that sold hamster tumor lines that were implicated in the New York outbreak (Gregg, 1975).

In early April 2005, four recipients of solid-organ transplants in three hospitals in Rhode Island and Massachusetts became gravely ill shortly after transplantation; three subsequently died. All four recipients shared a common donor. LCMV was identified as the etiologic agent. Viral sequences from the organ recipients were identical to those from a pet hamster acquired by the donor's household 17 days before organ donation (Amman et al., 2007). The source of the hamster was found to be a breeding facility in Arkansas. Further investigation by the United States Centers for Disease Control and Prevention (CDC) found that the animals from this breeding colony were distributed to 22 states. The CDC and pet industry are now working toward ways of educating consumers to the risks of owning pet rodents and also developing guidelines to prevent additional infections (Nolen, 2006).

Clinical Signs

Approximately half of the hamsters infected congenitally or as newborns develop a chronic progressive fatal disease characterized by inactivity and weight loss. Other animals infected in vivo or as newborns and young adults develop subclinical infections (Hankenson and Van Hoosier, 2002).

Approximately one-third of human cases of lymphocytic choriomeningitis have been asymptomatic. Humans with the infection manifest symptoms of aseptic meningitis, meningoencephalitis, transverse myelitis, Guillain-Barré syndrome, and permanent or transient hydrocephalus (Barton and Hyndman, 2000). Other clinical signs in humans include: fever, myalgia, headache, photophobia, nausea, and variable involvement of the testes and salivary gland. The disease is rarely fatal. Intrauterine infections of fetuses have been well documented and can result in hydrocephalus, microencephaly, macroencephaly, intracranial calcification, mental retardation, seizures, and fetal death (Barthold and Smith, 2007).

Diagnosis and Prevention

Regular testing of hamster colonies as well as mice and guinea pigs for evidence of LCMV infection should be conducted. If an infection with LCMV is diagnosed, all animals in the colony should be euthanized. If a cell line or transplantable tumor is determined to be the source of the infection, it also should be destroyed.

Rabies Virus

Reservoir and Incidence

Although wild animals were shown to be the reservoir for rabies virus in one study, lagomorphs were not incriminated (Childs et al., 1997). In contrast, another review of cases in New York between 1992 and 2001 documented seven rabid rabbits, and one rabid guinea pig was also described (Eidson et al., 2005). Reports of rabid guinea pigs and wild rodents are extremely rare (Fishbein et al., 1986).

Mode of Transmission

The mode of transmission is through the bite of a rabid animal or the contamination of a wound by saliva from an infected animal.

Clinical Signs

In one review of seven cases of rabies in rabbits, most rabbits presented with neurologic symptoms, although lethargy and inappetance were also noted in one case (Eidson et al., 2005). The one rabid guinea pig reported in the literature became aggressive approximately 21 days after being bitten by a rabid raccoon and bit the owner (Eidson et al., 2005).

Diagnosis and Prevention

Histological examination of the brain is required to confirm a diagnosis of rabies.

The capture of wild rabbits or rodents poses a relatively minor risk of rabies exposure for laboratory personnel. The time from exposure to clinical signs for guinea pigs and rabbits appears to be less than 4 weeks (Eidson et al., 2005). Rabies should be suspected in feral animals with neurologic abnormalities. This disease poses few concerns when using laboratory-raised animals, as it is extremely unlikely for well-maintained laboratory-raised animals to be infected with the rabies virus.

RICKETTSIAL DISEASES

Coxiella burnetii

Reservoir and Incidence

Rickettsial organisms are obligate intracellular, Gram-negative bacteria. *Coxiella burnetii* is the etiologic

agent of Q fever. In national disease surveillance reports for 1948–1986, 1396 human cases were reported, from almost every state (McQuiston and Childs, 2002). Among published individual case reports and outbreak investigations, occupational exposures (i.e., at research facilities, farm environments, and slaughterhouses) were commonly reported, and sheep were most frequently implicated as a possible source of infection. *Coxiella burnetii* was also reported among various wild-animal species, including coyotes, foxes, rodents, skunks, raccoons, rabbits, deer, and birds (McQuiston and Childs, 2002). One human case report documented four patients with atypical pneumonia and a history of exposure to wild rabbits (Thomas et al., 1986).

Mode of Transmission

Inhalation of the organism is the most common mode of transmission. Infection via aerosol may occur directly from parturient fluids of infected animals, and may be associated with newborn animals, placental tissue, or wool. *Coxiella burnetii* is very resistant to inactivation in nature and may survive for several weeks in areas where rodents and rabbits have been present. The organism may also be spread by the wind (Maurin and Raoult 1999).

Clinical Signs

Animals infected with *Coxiella burnetii* do not usually experience symptomatic infections. In the acute phase, the presence of *C. burnetii* may be demonstrated in the blood, lungs, spleen, and liver. Most animals remain totally asymptomatic, including a lack of fever. *C. burnetii* infection often becomes chronic, with persistent shedding of the pathogen in feces and urine (Maurin and Raoult, 1999).

Chronic endocarditis, myocarditis, pericarditis, skin rash, and meningoencephalitis are some of the many clinical signs observed in humans. Infections during pregnancy have been reported in the literature many times, and Maurin and Raoult (1999) summarized 27 of these cases. Premature birth was reported in eight cases; spontaneous abortions were reported in six cases; and death in utero was reported in two cases. Only five healthy infants were born at term in these 27 cases.

Diagnosis and Prevention

Serology is an important tool for diagnosis of Q fever in humans and animals (Thomas et al., 1986). Colonies of animals should be screened for serum antibodies to *C. burnetii* and infected animals eliminated from the colony. Sources of sheep, goats, and cattle should be monitored to assure freedom from infection, and any research involving placental tissues from these species should be considered an additional risk and appropriate precautions taken.

This disease poses few concerns when using laboratory-raised rodents or rabbits, but the use of wild rabbits in research may expose laboratory workers to *C. burnetii*.

Rickettsia akari

Reservoir and Incidence

Rickettsial pox is caused by infection with *Rickettsia akari*. It was originally described along the east coast of the United States, but has recently been reported in the Ukraine and Croatia. The organism has also been isolated from voles in Korea, but the human disease has not been reported in Korea (Krusell et al., 2002). There are also reports of the disease in Russia, Egypt, and South Africa (Fox et al., 2002). Wild mice and voles are reservoirs of the bacteria (Fox et al., 2002; Krusell et al., 2002).

Mode of Transmission

The pathogen is transmitted by the mite *Liponyssoides sanguineus*. Data suggest that silent sylvan cycles of *R. akari* infection exist and that the organism is more widely distributed than generally appreciated (Krusell et al., 2002).

Clinical Signs

There are no clinical signs associated with infection in animals. In man, the rash of rickettsial pox commences as a discrete maculopapular rash which then becomes vesicular. About 90% of affected persons develop an eschar, with a shallow ulcer covered by a brown scab (Fox et al., 2002). The palms of the hands and soles of the feet are not affected.

Diagnosis and Prevention

Ectoparasite control in wild rodent populations is essential, particularly the elimination of free-living mites capable of transmitting the agent (Committee on Occupational Safety and Health in Research Animal Facilities, 1997).

Rickettsia rickettsii

Reservoir and Incidence

In the western hemisphere, wild rodents and rabbits are reservoirs for *Rickettsia rickettsii*, the causative agent for Rocky Mountain spotted fever (Elliot et al., 1985). In humans, illness is usually seen during the months of high tick activity, however only 60% of the patients have a history of observed tick exposure (Elliot et al., 1985).

Mode of Transmission

Zoonotic transmission of this disease in the laboratory has involved aerosols, accidental parenteral

inoculation, and bites by natural ectoparasites (Committee on Occupational Safety and Health in Research Animal Facilities, 1997).

Clinical Signs

The disease is asymptomatic in animals. The most common findings in humans are fever (97%); headache (87%); truncal rash (85%); and rash on the palms and soles (62%) (Elliot et al., 1985).

Diagnosis and Prevention

Ectoparasite control in wild rodent populations is essential, particularly the *Rhipicephalus sanguineus* tick which is known to transmit the pathogen (Fox et al., 2002). Often, the control of ectoparasites involves control of wild rodent populations.

CHLAMYDIAL DISEASES

Chlamydophila caviae

Reservoir and Incidence

Guinea pigs can serve as a reservoir for *Chlamydophila caviae*. Rabbits can also be infected with *Chlamydophila caviae* (Lutz-Wohlgroth et al., 2006).

Mode of Transmission

The mode of transmission in the one case reported was never determined (Lutz-Wohlgroth et al., 2006).

Clinical Signs

Only one case of human infection exists in the literature. This report described a single case of seromucous ocular discharge and mild conjunctivitis in a single individual. This person was the owner of approximately 200 guinea pigs. Approximately 25% of these guinea pigs exhibited clinical signs that include ocular discharge, conjunctivitis, chemosis, entropion, keratitis, corneal edema, corneal erosion, nasal discharge, sneezing, stridor, dyspnea, pneumonia, vaginal discharge, and abortion (Lutz-Wohlgroth et al., 2006).

Diagnosis and Prevention

PCR amplification was used to diagnose all cases of the infection (Lutz-Wohlgroth et al., 2006). Due to the paucity of reported cases, it is believed that this disease poses few concerns when using laboratory-raised animals.

Chlamydophila psittaci

Reservoir and Incidence

Chlamydial agents are widely distributed among birds but can also occur in laboratory animals including

mice, guinea pigs, hamsters, rabbits, ruminants, swine, cats, ferrets, muskrats, and frogs (Fox et al., 2002).

Mode of Transmission

The organism is spread to humans from infectious material in exudates, secretions, or desiccated fecal material via direct contact or the aerosol route (Committee on Occupational Safety and Health in Research Animal Facilities, 1997).

Clinical Signs

Clinical signs in animals can include conjunctivitis, pneumonitis, myocarditis, hepatitis, enteritis, arthritis, meningoencephalitis, endometritis, and abortion (Fox et al., 2002). In humans, clinical signs can include those associated with upper or lower respiratory disease, conjunctivitis, thrombophlebitis, myocarditis, hepatitis, and encephalitis (Committee on Occupational Safety and Health in Research Animal Facilities, 1997).

Diagnosis and Prevention

Diagnosis can be made by demonstrating serum antibodies to *C. psittaci*, or by using PCR to amplify the bacterial DNA (Hankenson et al., 2003).

BACTERIAL DISEASES

Bordetella bronchiseptica

Reservoir and Incidence

A single case report implicated rabbits as the reservoir for the infection (Gueirard et al., 1995).

Mode of Transmission

The mode of transmission was never determined but assumed to be by nasal inoculation of aerosolized bacteria.

Clinical Signs

It was reported that a 79-year-old woman developed acute febrile bronchopneumonia (Gueirard et al., 1995). She lived on a farm and had significant exposure to a cat and to rabbits, and the cat and the rabbits had symptoms compatible with bordetellosis (cough associated with rhinorrhea, conjunctivitis, and diarrhea). Nasal and pharyngeal swabs were performed on the cat, but *B. bronchiseptica* was not isolated. During a subsequent infection, the cat had no sign of bordetellosis, but the rabbits did. One rabbit was sacrificed, and though cultures from the upper respiratory tract failed to isolate bacteria, *B. bronchiseptica* was isolated in pure culture from the hilus and bronchi (Gueirard et al., 1995).

Diagnosis and Prevention

Bacterial culture of respiratory secretions or tissues of the nasopharynx and respiratory tract is typically used to isolate *B. bronchiseptica* from animals suspected of being infected. This disease poses few concerns when using well-maintained laboratory-raised animals due to low prevalence of infection in such animals.

***Borrelia burgdorferi* (Lyme's Disease)**

Reservoir and Incidence

Sylvilagus floridanus (cottontail rabbit) can serve as a host for the cycle of *Borrelia burgdorferi* (Telford and Spielman, 1989). The disease in humans is called Lyme disease and is endemic in the eastern United States, Europe, and Asia (Steere, 2001).

Mode of Transmission

Ixodes dammini (the deer tick) is the vector which transmits *B. burgdorferi* to susceptible animals. In one study, 90% of cottontail rabbits sampled had serum antibodies against *B. burgdorferi* (Telford and Spielman, 1989). Control of the ticks or tick larvae should limit the exposure to *B. burgdorferi*. Rabbits are not considered to be infectious since an infection cannot be directly transmitted from the rabbit (Telford and Spielman, 1989).

Clinical Signs

Clinical signs related to infection with *B. burgdorferi* have not been reported in rabbits. At least 80% of the infected patients in the United States exhibit a slowly expanding skin lesion, erythema migrans, which occurs at the site of the tick bite. This is frequently accompanied by influenza-like symptoms including malaise, fatigue, headache, arthralgias, myalgias, fever, and regional lymphadenopathy (Steere, 2001). Advanced stages of the disease can be characterized by meningitis, polyneuropathy, encephalopathy, arthritis, and atrio-ventricular block (Steere, 2001).

Diagnosis and Prevention

Serologic analysis to determine if antibodies against *Borrelia burgdorferi* are present is a typical diagnostic approach (Telford and Spielman, 1989). This disease poses few concerns when using laboratory-raised animals.

***Francisella tularensis* (Tularemia)**

Reservoir and Incidence

Rabbits, ticks from rabbits, and deerflies feeding on rabbits are the main reservoirs for *F. tularensis* (Hoff et al., 1975; Klock et al., 1973). In the United States, wild

rabbits of the genus *Sylvilagus* (cottontails, marsh rabbits, and swamp rabbits) present the greatest hazard. Jackrabbits and snowshoe hares are susceptible to tularemia but have rarely been implicated as direct sources of human infection. The domestic rabbit (*Oryctolagus cuniculus*) has not been documented as a source of human tularemia (Centers for Disease Control, 1986).

Mode of Transmission

Exposure of the skin or conjunctiva to blood and other infectious tissue while skinning and dressing rabbits account for most cases. In some instances, pulmonary tularemia has resulted from breathing aerosols generated while handling unopened rabbit carcasses or merely by direct contact with a dead rabbit (Centers for Disease Control, 1986). In 1970, 18 people living in Hungary were diagnosed with tularemia. The source of the infection was wild-caught hamsters and all people involved were hamster trappers (Szekelyfoldi, 1970).

Clinical Signs

In humans the clinical signs depend on the inoculation route. The most common presentation is the ulceroglandular form, due to inoculation of the extremities or the conjunctiva. Ingestion of contaminated food or water or hand-to-mouth contact after handling an infected animal can cause oropharyngeal tularemia. Inhalation of infected material produces pneumonic disease characterized by severe dyspnea (Elliot et al., 1985).

Diagnosis and Prevention

Serology and a history of exposure to wild rabbits or hamsters are important for diagnosis. This disease poses few concerns when using laboratory-raised animals. The maintenance and use of wild rabbits or rodents may pose a minor risk of exposure for laboratory personnel.

***Leptospira* spp.**

Reservoir and Incidence

Leptospira spp. has a worldwide distribution in domestic and wild animals. It can also be isolated from dogs, pigs, sheep, goats, cats, gerbils, and hamsters (Hankenson et al., 2003).

Mode of Transmission

Leptospiral agents are shed in the urine of reservoir animals. Transmission can be by oral ingestion; exposure to contaminated urine, placenta, fetal tissues; and by inhalation. Organisms can also infect hosts through abrasions in the skin or musosal surfaces (Hankenson et al., 2003).

Clinical Signs

Animals are asymptomatic and carry the organism in their renal tubules for years. Mice are believed to harbor the organism for life ([Committee on Occupational Safety and Health in Research Animal Facilities, 1997](#)).

Diagnosis and Prevention

Serology or isolation of the organism is typically used for diagnosis. PCR amplification of *Leptospira* DNA from wild rodent kidney tissue has been reported as a means of diagnosis ([Pezzella et al., 2004](#)). The capture of wild rodents may pose a risk of exposure for laboratory personnel; however this disease poses few concerns when using laboratory-raised animals.

***Salmonella* spp.**

Reservoir and Incidence

Many mammals, as well as non-mammal vertebrates, and wild mice can serve as reservoirs for *Salmonella* spp. ([Meerburg et al., 2006](#); [Pocock et al., 2001](#)). In this regard, wild-caught rodents may pose some risk to personnel, though most rodents available from high-quality commercial vendors are free of *Salmonella*. Hamsters from pet stores or from pet distributors have been demonstrated to be a potential source of infection for humans. In 2004, a pet distributor reported to the Minnesota Department of Health that two hamsters died from salmonellosis. The bacterial isolate involved in this outbreak was determined to be multidrug-resistant *Salmonella typhimurium* ([Swanson et al., 2007](#)). Further epidemiology identified 13 human cases of *Salmonella* from patients who had contact with rodents purchased at retail pet stores ([Centers for Disease Control, 2005](#); [Swanson et al., 2007](#)). Eventually, the strain involved in the outbreak was also found in rodent cages and transport containers from another pet distributor ([Peota, 2007](#); [Swanson et al., 2007](#)).

Mode of Transmission

Ingestion of water or food that has been contaminated by the feces of infected animals is the primary mode of transmission.

Clinical Signs

Enteritis was the clinical presentation in one outbreak in hamsters ([Swanson et al., 2007](#)). This is unusual since enteritis is not usually a clinical feature of *Salmonella* infection in the hamster. The disease is more often characterized by multifocal necrosis of the liver ([Hankenson and Van Hoosier, 2002](#)). Previously, the disease was reported to present in hamsters as septic pulmonary phlebothrombosis ([Innes, 1953](#)).

Diagnosis and Prevention

The use of *Salmonella*-free hamsters bred specifically for research has greatly diminished the concern for this disease in the research laboratory. The use of hamsters from pet store sources may pose a risk for laboratory humans.

Streptobacillus moniliformis

Reservoir and Incidence

Wild or laboratory rats, mice, *Motomys alexis* (Spinifex hopping mice), gerbils, squirrels, guinea pigs, dogs, cats, ferrets, weasels, calves, pigs, turkey, koala, and non-human primates can serve as the reservoir for this bacteria ([Gaastra et al., 2009](#)). When the disease is transmitted via a bite or scratch the disease is called rat bite fever. If humans become orally infected, the disease is called Haverhill fever ([Gaastra et al., 2009](#)).

Mode of Transmission

The bacteria can be transmitted by urine and the secretions of the mouth, nares, or conjunctival sac. The disease can also be transmitted through indirect inoculation via contaminated fomites or through trauma to unprotected skin ([Hankenson et al., 2003](#)). The disease can also occur in individuals who have no history of rat bites, but who reside or work in rat-infested areas ([Fox et al., 2002](#)).

Clinical Signs

The disease is asymptomatic in rodents. Humans can develop chills, fever, headache, myalgia, weakness, regional lymphadenopathy, and joint pain.

Diagnosis and Prevention

Diagnosis is made after the isolation of the agent from the oral cavity, nares, and conjunctival sacs on appropriate culture media. PCR has been successfully used to diagnose the disease ([Gaastra et al., 2009](#)). The exclusion of infected rodents from production colonies by commercial supplies has been largely successful in preventing the disease in laboratory personnel ([Committee on Occupational Safety and Health in Research Animal Facilities, 1997](#)).

Spirillum minus

Reservoir and Incidence

Spirillum minus has been isolated from the oropharynx, blood, and exudates of up to 25% of wild rats. Nasopharyngeal carriage rates of 50–100% in wild rats have also been reported ([Gaastra et al., 2009](#)).

Mode of Transmission

The transmission is the same as for *S. moniliformis*. This disease occurs less frequently than rat bite fever and occurs mainly in Asia. In Japan, the disease is known as sodoku (Gaastra et al., 2009).

Clinical Signs

The disease is asymptomatic in rodents. Humans develop a distinctive rash of red to purple-colored plaques. Compared to *S. moniliformis*, arthritis rarely develops due to infection with this organism (Committee on Occupational Safety and Health in Research Animal Facilities, 1997).

Diagnosis and Prevention

The agent does not grow well in culture. Diagnosis can be made after animal inoculation of infected tissue or fluid and identification of the organism by dark field microscopy (Hankenson et al., 2003). The exclusion of infected rodents from production colonies of commercial suppliers has been largely successful in eliminating the disease in the laboratory (Committee on Occupational Safety and Health in Research Animal Facilities, 1997).

Yersinia pestis

Reservoir and Incidence

Rabbits and other wild rodents are known to be reservoirs for *Yersinia pestis* (von Reyn et al., 1976). The incidence of this disease has been well documented in areas where people encounter a nidus of sylvatic plague (Kartman, 1970).

Mode of Transmission

The disease is transmitted by the bite of an infected flea, but infections can also occur via cuts or abrasions in the skin or exposure of mucous membranes to infected aerosols (Fox et al., 2002).

Clinical Signs

There are no clinical signs in rabbits infected with *Y. pestis*. The disease in humans presents in three distinct forms: bubonic or suppurative lymphadenopathy; pneumonic; and septicemic plague. All forms of the disease include fever, headache, and weakness as clinical signs.

Diagnosis and Prevention

A presumptive diagnosis of *Y. pestis* is made after identification of Gram-negative bipolar staining rods in sputum or lymph node biopsy samples (Fox et al.,

2002). Infections with *Y. pestis* are rare in laboratory-raised animals. Feral or random source animals acquired from plague-endemic areas should be quarantined and treated with appropriate insecticides to kill fleas (Fox et al., 2002).

FUNGAL DISEASES

Dermatophytosis

Reservoir and Incidence

The most common genera of fungi responsible for disease in animals and humans are the dermatophytes: *Microsporum*, *Epidermophyton*, and *Trichophyton* (Fox et al., 2002). In New Zealand, *Trichophyton mentagrophytes* was found to be widespread among a variety of animals including: mice, rats, hedgehogs, opossums, guinea pigs, cats, dogs, horses, and cows (Smith et al., 1969). In Italy, the eastern cottontail (*Sylvilagus floridanus*) has been shown to be a carrier of several zoonotic dermatophytes (Gallo et al., 2005). Other case reports of fungal infections in guinea pigs (Hironaga et al., 1981), and rabbits (Aho, 1980) are found in the literature. *Microsporum canis* has also been reported in laboratory-housed New Zealand White rabbits (Vogtsberger et al., 1986).

Mode of Transmission

Transmission to humans occurs from direct or indirect contact with symptomatic or carrier animals; contaminated bedding, caging, or other equipment; or fungal contamination of the environment (Fox et al., 2002).

Clinical Signs

The skin lesions on laboratory animals are often localized to the head or trunk. The disease in rabbits is characterized by focal irritation and inflammation of the skin. Crusting lesions, scabs, and hair loss are also common (Chomel, 1992).

Diagnosis and Prevention

Diagnosis is made by identification of hyphae in skin scrapings or by isolation of the dermatophyte on fungal culture media (Chomel, 1992). The use of the ultraviolet Wood's lamp is generally non-diagnostic, since most pathogenic dermatophytes of small mammals do not fluoresce (Pollock, 2003). This disease poses relatively limited concern when using laboratory-raised animals; however, feral or random source animals may pose a greater risk of dermatophytosis to personnel handling such animals.

CESTODE INFECTIONS

Rodentolepis nana

Reservoir and Incidence

This tapeworm of hamsters was formerly known as *Hymenolepis nana*. It can be found in the small intestinal tract of hamsters, mice, rats, non-human primates, and humans (Hankenson and Van Hoosier, 2007).

Mode of Transmission

R. nana can have either a direct or indirect lifecycle. Flour beetles and fleas can serve as the intermediate host. The direct lifecycle is 14–16 days and autoinfection can occur (Hankenson and Van Hoosier, 2002).

Clinical Signs

The condition is often benign, but depends on the number of tapeworms present and the degree of intestinal occlusion (Hankenson and Van Hoosier, 2002). Catarrhal enteritis, chronic enteritis, abscesses of mesenteric lymph nodes, and hyperplasia of Peyer's patches have been observed in infected hamsters (Wagner, 1987). Symptoms in humans include nausea, vomiting, abdominal pain, diarrhea, irritability, restless sleep, and allergic symptoms. One-third of infected humans develop peripheral eosinophilia of greater than 5% (Feldman and Easton, 2006).

Diagnosis and Prevention

Diagnosis can be made by the identification of cestode eggs in the feces or finding adult worms in the gastrointestinal tract. The use of hamsters from colonies free of the infection is recommended. The use of wild rodents may increase the risk for husbandry workers and other individuals handling such animals.

ARTHROPOD INFECTIONS

Cheyletiellosis

Reservoir and Incidence

Cheyletiella mites are non-burrowing mites that have a 36-day life cycle, with two nymphal stages and one larval stage. The rabbit, cat, and dog serve as reservoirs for the mite. All stages of the lifecycle occur on the animal (Parish and Schwartzman, 1993).

Mode of Transmission

This mite can be transmitted to humans through direct contact with infested animals. The mite can also be transmitted by flies, fleas, and lice (Parish and Schwartzman, 1993).

Clinical Signs

The *Cheyletiella* mite produces a non-specific cutaneous scaling with or without pruritus. The lesions on humans vary from vesicles to papules, most often on the trunk and in particular at locations where the infested animal is held against the person's body (Parish and Schwartzman, 1993).

Diagnosis and Prevention

This disease poses few concerns when using laboratory-raised animals. The handling of wild rabbits poses a minor risk of exposure to laboratory personnel.

ALLERGIES

Allergic reaction to animals is common and represents an important occupational health concern. One study of 5641 laboratory workers in Japan found that 23.1% had one or more allergic symptoms related to laboratory animals (Aoyama et al., 1992). Much work on this issue has been done using rat, mouse, and rabbit antigens. Fewer case reports exist that describe the development of symptoms for people working with guinea pigs and hamsters. Lutsky et al. (1983) reviewed the literature and reported that a large number of research animal facilities documented workplace hypersensitivity to rats (72.2%) and rabbits (67.7%), while only a few observed occupational hypersensitivity to hamsters (4.5%).

The most common symptom associated with allergy to animals is rhinitis. In contrast, another study found that allergic reaction to animal dander is estimated to occur in only 6% of the general population (Elliot et al., 1985). Secondary exposure to animal allergens is also well documented. One study performed in Poland described that children of parents who work with laboratory animals presented significantly more positive skin prick tests against allergens from the hair of laboratory animals compared to children of non-exposed parents (Krakowiak et al., 1999). It was thought that the children were secondarily exposed due to poor hygienic conditions at their parents' workplace.

Cross reaction between allergic responses to allergens of different laboratory animal species may occur. The presence of allergy to mice in some laboratory animal technicians who had worked with only rats, but not mice, suggested that there is some cross sensitization between allergens present in rat and mouse urine (Agrup et al., 1986). Cross sensitivity also seems to exist between guinea pig and hamster allergens. One study reported that nine people who were allergic to guinea pigs also reacted to hamsters, although only one had had occupational contact with hamsters (Agrup et al., 1986).

The incidence of laboratory animal allergies increases as a factor of time spent exposed to the animal antigens, but only for the first several years. One report indicated that it is rare for laboratory animal allergies to develop later than 3 years after exposure (Bland et al., 1987); and most workers were found to have developed laboratory animal allergies between 1 and 4 years from the time of first exposure (Lutsky et al., 1983).

Exposure to the environment in which rabbits are housed has been closely studied as a factor in the allergic response to rabbit allergens. Ooms et al. (2008) found that airborne contaminants in rabbit rooms include particulate material, endotoxin, ammonia, carbon dioxide, and rabbit salivary secretions which contain high levels of rabbit salivary protein allergen. Other airborne contaminants in rabbit rooms include fur, epidermal cells, feces, urine, saliva, feed, and bedding. It was shown that the level of rabbit salivary protein in the room increased over the 2-week sampling period but not significantly, indicating that replacement of dirty litter pans did little to decrease environmental contamination with that allergen (Ooms et al., 2008). In contrast to the Ooms' study, Edwards et al. (1983) determined that increasing the humidity from 54% to 77% caused a significant reduction in laboratory animal allergen levels. These two studies strongly implicate the animal room environment, and not just the animal itself, as an important factor in animal-related allergies.

PREVENTION OF ALLERGY AND ZOONOTIC DISEASE

The prevention of laboratory animal allergy has been addressed in several ways. Pre-employment screening of susceptible individuals using a family history of allergy and positive skin prick reactions against environmental allergens has been suggested as one means to mitigate allergy risk (Sjostedt and Willers, 1989). Consideration should be given to protecting workers from environments or objects contaminated by animal allergens rather than just the animal itself (Lutsky, 1987). Engineering controls, workplace protection systems, and personal protective equipment should have a potential role in controlling allergen exposure and the severity of laboratory animal allergies.

Engineering controls are a combination of safety equipment and physical features of the facility that help minimize hazardous exposures of personnel and the surrounding environment. Safety equipment provides a barrier between the worker and the hazard. Physical features can prevent or reduce the exposure to the hazardous agent (Committee on Occupational Safety and Health in Research Animal Facilities, 1997). The principle of dilution and room air turnover rates have not

been shown to be effective in the removal or dilution of submicron aeroallergens except at energy-intensive fresh air exchange rates in excess of 100 air changes per hour (Swanson et al. 1990). However, other engineering controls can be important protective measures. Common engineering controls used to reduce the exposure of workers to animal allergens and zoonoses include: use of isolation cages, air locks, temperature and humidity controls as well as facility ventilation systems that prevent airborne contaminants from leaving the animal facility.

Work practices are another means to reduce the exposure of workers to laboratory animal allergens and zoonoses. Employees should understand the hazard associated with the procedures that they are performing, recognize the route through which they can be exposed and select work practices that minimize exposure (Committee on Occupational Safety and Health in Research Animal Facilities, 1997). One of the easiest ways to prevent exposure is to restrict access to animal areas. Additionally, exposure can be reduced by washing and decontamination of areas and equipment.

The use of personal protective equipment is an important measure for controlling exposure to allergens as well as other potentially hazardous agents. Personal protective equipment provides a physical barrier to hazardous material that might otherwise come into contact with employees' skin, eyes, mucous membranes, and clothing. Proper consultation with occupational safety professionals should occur as part of establishing standards for use of personal protective equipment.

The prevention of pathogen transmission from animals to personnel should be a major concern when working with such agents or animals thought to carry pathogens. The use of pathogen-free animals is not always feasible. When pathogens cannot be eliminated, or when pathogen status of an animal is unknown, the use of protective clothing, personal hygiene, sanitation measures, engineering controls, and workplace protection systems are essential for diminishing exposure risk. When known agents are used, the use of vaccines can also assist in protecting personnel.

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The Domestic Rabbit, *Oryctolagus Cuniculus*: Origins and History

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INTRODUCTION AND BACKGROUND

All varieties of domestic rabbits used in modern biomedical research were developed from the European rabbit, *Oryctolagus cuniculus*. The European rabbit possesses a mixture of features that make it an appealing model for in vivo research: an intermediate body size, tractable disposition, ability to breed readily in captivity, and a short generation time. Given its obvious utility as a research subject, it seems equally obvious that the European rabbit would be richly represented in the scientific literature from the Age of Enlightenment onward. However, the rabbit as a research model rates barely a mention until the mid-19th century, when the Viennese physician Schroff noted that some rabbits were able to thrive on a diet of belladonna, an observation that led to the discovery of atropine esterase and is credited with being the first documented discovery of a heritable response to a pharmacological agent (Liebenberg and Linn, 1980; Stormont and Suzuki, 1970). As breed standards improved and production facilities moved from the backyard to commercial rabbitries, acceptance of rabbits as research models increased. During the early to mid-20th century, the European rabbit became an indispensable tool of

biomedical research, contributing to pivotal discoveries across multiple disciplines including immunology, vaccine development, genetics, and infectious disease research. The suitability of the European rabbit as a research animal was greatly enhanced by the efforts of the Rabbit Experimental Station in Fontana, California. Established by the United States Department of Agriculture in 1928, the Fontana Station operated continuously until 1965 and was dedicated to the improvement of breeding and husbandry of rabbits raised for meat and fur. The techniques developed at the Fontana Station laid the foundation for best practices used in modern large-scale production facilities that now supply healthy laboratory rabbits to academia and industry.

The publication of Russell and Burch's *Principles of Humane Experimental Technique* in 1959 ushered in the postmodern era of in vivo research. The scientific community's adoption of the principles of reduction, replacement, and refinement, combined with the burgeoning of genetic engineering technologies in the last quarter of the 20th century, put the laboratory mouse at the forefront of biomedical research, at least in terms of numbers used. Analyzing international trends in animal use, particularly at the species level, is problematic due to differing reporting requirements and counting

methods (Omoe, 2006); however, the number of rabbits used in research over the last two decades appears to have declined. In the United States, Great Britain, and Canada, where accounting for numbers of rabbits used in research is government-mandated, the numbers reported have leveled off or decreased over the last several years (Gauthier, 2004; Reports of Research Facilities, 1998–2004; Statistics on the use of animals in Great Britain, 2002–2008). In Japan, where animal use data are collected by survey methods and reporting is voluntary, the numbers of all animals used in research, excluding rats and mice, are believed to have decreased since 1991 (Matsuda, 2004). However, it would be incorrect to infer that the European rabbit's usefulness as an animal model has waned. A keyword search of the Ovid MEDLINE database using the terms "experimental model, animal" and "rabbit" resulted in 6329 citations for the period 2000–2010. Clearly, the European rabbit continues to play an essential role in biomedical progress, particularly in safety testing, preclinical studies, and translational research.

At various times throughout history, man has regarded the European rabbit as a game species, agricultural commodity, research model, invasive pest, endangered species, and companion animal. Yet despite the European rabbit's familiarity, economic and ecological significance, and scientific importance, its evolutionary history is still debated, and the story of its domestication is largely a matter of myth and conjecture.

PHYLOGENY AND TAXONOMY

Historically, naturalists have hypothesized a close relationship between rodents and lagomorphs based on their shared characteristic of hypsodontic incisors adapted for gnawing (Meng and Wyss, 2005; Rose, 2006). Linnaeus initially proposed the clade Glires as a way of grouping the rodents, rabbits, and closely related taxa based upon morphological similarity (Douzery and Huchon, 2004; Rose, 2006). Despite its intuitive appeal and the convergence in the last 50 years of the molecular, paleontological, and morphological evidence, the hypothesis that these animals have a monophyletic origin has been debated ever since. Craniodental morphological analyses mainly support the Glires hypothesis (Asher et al., 2005; Frahner, 1999; Mess, 1999), as do most phylogenetic studies based on analysis of mitochondrial and nuclear DNA sequences (Douzery and Huchon, 2004; Huchon et al., 2002; Lin et al., 2002; Meng and Wyss, 2005). Even so, the question "What, if anything, is a rabbit?" (Wood, 1957) lives on into the 21st century. Two recent studies appear to refute the monophyly of Glires altogether. Phylogenetic trees based on probability

analysis of orthologous proteins (Graur et al., 1996) and nuclear genes (Misawa and Janke, 2003) demonstrate that lagomorphs may be more closely related to humans than to rodents, suggesting that at least some of the morphological similarities between lagomorphs and rodents may have evolved independently.

The taxonomic history of the European rabbit is equally convoluted. Originally named *Lepus cuniculus* by Linnaeus in 1758, the European rabbit initially shared this genus with hares and some cottontails. The European rabbit was renamed *Oryctolagus cuniculus* by Lilljeborg in 1873–1874 (Angermann et al., 1990; Hoffman, 1993) but was still considered a subgenus of *Lepus* (Corbet, 1994). The obvious physical and behavioral differences between European rabbits and other leporids ultimately led to the recognition of *Oryctolagus* as a separate genus, with *O. cuniculus* as the only member species. Despite the increasingly complex classification schemes at the genus and species levels, taxonomists continued to classify rabbits, hares, and pikas as rodents until 1912, when they were recognized as distinct and reclassified to the order Lagomorpha (Chapman and Flux, 1990; Gidley, 1912). Today, most taxonomists continue to group rabbits and rodents, with a few exceptions, in the Glires superorder (McKenna and Bell, 1997).

Table 6.1 shows the current classification scheme for the European rabbit from the ordinal to genus level. Within the order Lagomorpha, there are two families and 13 genera. The family Leporidae contains

TABLE 6.1 Classification of Lagomorphs

Order: Lagomorpha
Family: Ochotonidae—Pikas (2 genera and 22 species)
Genus: Ochotona—Pikas (21 species)
Genus: Prolagus—Sardinian Pika (extinct)
Family: Leporidae—Hares and Rabbits (11 genera and 47 species)
Genus: Pentalagus—Ryukyu Rabbit
Genus: Bunolagus—Bushman Rabbit
Genus: Pronolagus—Red Rabbits (3 species)
Genus: Nesolagus—Sumatra Short-eared Rabbit
Genus: Romerolagus—Volcano Rabbit
Genus: Brachylagus—Pygmy Rabbit
Genus: Poelagus—Central African Rabbit
Genus: Caprolagus—Bristly Rabbit
Genus: Lepus—Hares (23 species)
Genus: Sylvilagus—Cottontails (13 species)
Genus: Oryctolagus—European Rabbit



FIGURE 6.1 A Tibetan Woolly Hare, *Lepus oiostolus*. Photo credit: Andrew Smith, Lagomorph Specialist Group, IUCN.



FIGURE 6.2 A cottontail rabbit of the genus *Sylvilagus*. Photo credit: Rachel Rommel, Houston Zoo.

11 genera, including *Lepus* (jackrabbits and hares; Figure 6.1), *Sylvilagus* (cottontails; Figure 6.2), and *Oryctolagus* (European rabbits; Figure 6.3). The family Ochotonidae contains a single surviving genus, *Ochotona* (pikas; Figure 6.4). There are more than 80 recognized species of lagomorphs. In 1999, a new cottontail species, *Sylvilagus varynaensis*, was identified in the Venezuelan lowlands (Durant and Guevara, 2001).

GEOGRAPHIC ORIGINS AND DIVERSITY

An exact determination of the antiquity, evolution, and geographic origins of the European rabbit is complicated by its physical attributes, its behavior, and the history of human-mediated dispersal. All lagomorphs, including *Oryctolagus*, are distinguished by a highly conserved body plan adapted for hopping, and the fossil records are themselves often incomplete, making



FIGURE 6.3 The European rabbit, *Oryctolagus cuniculus*. Photo credit: Jamie Greaver, The University of Texas Health Science Center at Houston.



FIGURE 6.4 A pika, genus *Ochotona*. Photo credit: Rachel Rommel, Houston Zoo.

fine morphological distinctions between genera and species difficult (Chapman and Flux, 1990; Corbet, 1994; Fox, 1994). *Oryctolagus* species have undergone population contractions and expansions congruent with climatic fluctuations, with corresponding increases and decreases in species diversity. European rabbits create and inhabit complex burrows up to 3 meters deep, crossing geological strata and confounding interpretation of archeological records (Corbet, 1994; Thompson, 1955). Finally, *O. cuniculus* was not domesticated until the Middle Ages, and the zooanthropological record does little to clarify the paleontological or molecular evidence.

Until recently, lagomorphs were thought to have diverged in Asia during the Early to Middle Eocene, with diversification into the two representative families, Leporidae (rabbits and hares) and Ochotonidae (pikas), occurring during the late Eocene (Asher et al., 2005). However, a recent molecular study (Springer et al., 2003) and even more recent fossil discoveries in west-central India (Rose et al., 2008) have suggested a much earlier emergence of Lagomorpha during the early Eocene, millions of years earlier than previously believed. In addition, the Indian fossils have tarsal adaptations consistent with those of modern leporids, suggesting that Leporidae and perhaps *Oryctolagus* may have emerged significantly earlier than previously supposed (Rose et al., 2008).

The oldest recorded *Oryctolagus* species, *O. larynsis*, existed at least 3.5 million years ago (Mya) on the Iberian Peninsula and possibly in southeast France (Lopez-Martinez, 2008). Fossil records of *O. cuniculus* from the Iberian Peninsula date to about 0.6Mya (Lopez-Martinez, 2008). Several species of *Oryctolagus* coexisted during the mid-Pleistocene, but by the late Pleistocene, all except *O. cuniculus* were extinct. Competing theories of an African (Ferrand, 2008) or Chinese (Long et al., 2003) origin for *O. cuniculus* have been ruled out by analysis of mitochondrial DNA (mtDNA) and microsatellite markers.

mtDNA analysis (Branco et al., 2000; Hardy et al., 1995) and protein polymorphism (Ferrand and Branco, 2007) of wild European rabbits indicate that two subspecies of *O. cuniculus* presently inhabit the Iberian Peninsula; the date of divergence is estimated to be 1.8Mya (Carneiro et al., 2009). The subspecies *O. c. algirus* is restricted to southern Spain, and *O. c. cuniculus* is found in northern Spain and southern France, with a zone of mixing transecting the center of the Iberian Peninsula running northwest to southeast (Branco et al., 2000, 2002). Molecular markers have confirmed that domestic breeds as well as feral populations in Europe, Britain, and Australia are nearly genetically identical to the *O. c. cuniculus* of southern France (Ferrand, 2008; Queney et al., 2002). The subspecies *O. c. algirus* exhibits a higher degree of polymorphism, despite its restricted home range. The existence of allopatric subspecies may provide new avenues in the development of research models.

DOMESTICATION AND BREED DEVELOPMENT

Long before the fossil record lent support to the hypothesis of a European origin of *O. cuniculus*, man considered the Iberian Peninsula the European rabbit's ancestral home. Credit for discovery of rabbits

generally goes to Phoenician sailors, c. 1000BC, who reported numerous creatures resembling today's rabbits inhabiting the coast of the Iberian Peninsula. Regardless of who receives credit for the discovery, it was the rabbit's prolific breeding capacity under captive conditions that ultimately led to its exploitation for food. This ability of *Oryctolagus* to reproduce easily in captivity set it apart from *Sylvilagus* species and hares, and placed the European rabbit forever in the category of agricultural commodity. Seafaring civilizations from the time of the Phoenicians to the 18th century have left caches of rabbits on islands along shipping routes, where they served as a sustainable game supply for sailors; as a result, feral rabbit populations exist today on more than 800 oceanic islands (Ferrand, 2008; Flux and Fullagar, 1992; Gibb, 1990).

The earliest record of rabbits kept in captivity comes from the Romans, who imported them to Italy beginning around the third century BC (Flux, 1994). The Romans were fond of rabbit meat and considered the newborn kits and unborn fetuses, called laurices, a delicacy. They kept rabbits penned in walled gardens called leporaria for sport hunting (Fox, 1994), setting a precedent that was followed in France, Germany, and Britain into the Middle Ages (Flux, 1994). There is evidence that the Romans captured rabbits and kept them in hutches for fattening, though there are no documented attempts at true domestication (Clutton-Brock, 1999). The Romans, like many societies that followed, quickly discovered that rabbits not only bred astonishingly well in captivity, but were escape artists that rapidly invaded the countryside and became a serious pest problem (Clutton-Brock, 1999; Fox, 1994). Human transport brought rabbits to Western Europe and eventually to the British Isles, where they were introduced during the Norman Conquest, around 1100AD.

Compared with dogs, cats, and ferrets, rabbits are the most recently domesticated small animal species. Perhaps because of their perceived untrainability, lack of natural weapons, and ability to breed in captivity without human assistance or intervention, little effort was made to domesticate rabbits until the 6th century AD. French Catholic monks cultivated rabbits as a sustainable food source and, following the example of the Romans, kept them in walled, paved gardens. In 600AD Pope Gregory I declared that laurices were aquatic and therefore could be classified as fish, thus permitting the monks to enjoy newborn and fetal rabbit as an alternative to meat at Lent. Selection for size may have begun around this time, and by the mid-1500s, variations in size and coat color were well established. In 1595 the Italian monk Agricola described the relatively large rabbits found in his native Verona as piebald, black, and white (Bixby and Whitman, 2005). With the selection of traits for domestication, breed development inevitably

followed. The oldest breed on record is the Champagne d'Argents, developed more than 400 years ago from the "silver-plated" rabbits of France. Himalayan rabbits were first described in 1858, and later in the 19th century the Dutch rabbit was developed. The New Zealand White was developed in the early 20th century by W. S. Preshaw (Verhoef-Verhallen, 1998). A large (maximum weight 12 pounds) albino rabbit originally intended for meat production, the New Zealand White became the model of choice for the acute irritancy test developed by J. H. Draize in 1944. The New Zealand White has since become the breed most commonly used in biomedical research; the Dutch breed is also used, particularly where pigmented or smaller animals are desired. Both the New Zealand White and the Dutch are available from commercial vendors as conventional or specific-pathogen-free stocks.

Currently, 47 breeds of rabbits are recognized by the [American Rabbit Breeders Association](#). Unfortunately, as preferences for meat and fur have changed, some breeds have dwindled in popularity and are now threatened with extinction, leading the [American Livestock Breeds Conservancy](#) to include these historic breeds in its conservation mission. Table 6.2 is a list of currently recognized breeds and their conservation status.

ECOLOGY AND CONSERVATION

Although wild European rabbits exist only in their native habitat of the Iberian Peninsula, *O. cuniculus* today is inarguably one of the most ubiquitous animals on earth. Their ubiquity is primarily the unintended consequence of human introduction of domestic animals followed by accidental or deliberate release into non-native habitats. The rabbits' legendary reproductive capacity, burrowing behavior, and adaptability to a diverse range of climates and herbivorous diets more than compensate for its small size, appeal to predators, and lack of natural defenses, resulting in their colonization, with varying degrees of success, of every major continent except Antarctica and, as mentioned above, more than 800 oceanic islands (Flux and Fullagar, 1992). These free-living populations of European rabbits, descended from domestic animals, are more properly classified as feral (Fox, 1994). In locales where predation, trapping, and hunting keep feral rabbit populations in check, numbers may never rise above nuisance level. This has been the case on the North American mainland (Flux et al., 1990); the only established feral rabbit population in North America resides on the San Juan Islands off the coast of Washington (Couch, 1929; Hall, 1977). However, when rabbits are introduced to an ecosystem where few natural competitors, geological barriers, or predators exist, populations can quickly

TABLE 6.2 Currently Recognized Breeds and their Conservation Status

American ^a	Harlequin
American Chinchilla ^a	Havana
American Fuzzy Lop	Himalayan
American Sable	Holland Lop
Belgian Hare ^{b,*}	Jersey Wooly
Beveren ^c	Lilac ^c
Blanc de Hotot ^b	Mini Lop
Britannia Petite	Mini Rex
Californian	Mini Satin
Champagne d'Argent	Netherland Dwarf
Checkered Giant	New Zealand
Cinnamon	Palomino
Crema d'Argent ^c	Polish
Dutch ^{**}	Rex
Dwarf Hotot	Rhineland ^c
English Angora	Satin
English Lop	Satin Angora
English Spot	Silver ^b
Flemish Giant	Silver Fox ^a
Florida White	Silver Marten
French Angora	Standard Chinchilla
French Lop	Tan
Giant Angora	Thrianta
Giant Chinchilla ^c	

Sources: American Rabbit Breeders Association (list of breeds), American Livestock Breeds Conservancy (endangered breeds).

*The Belgian Hare was developed from selective breeding of *Oryctolagus cuniculus*.

Animals classified in the genus *Lepus* are the only true hares.

**"Dutch" is the breed name recognized by the American Rabbit Breeders Association; this breed is also known as the Dutch Belted.

^{a, b, c} Endangered breeds. a – critical; b – threatened; c – watch.

get out of hand, with devastating ecological, economic, and public human and animal health consequences. The most stunning example is Australia, where 24 rabbits were deliberately released by an English immigrant in 1859. Within 10 years, the feral population topped 2 million, and by 1881 farmers in the state of Victoria were abandoning their farms (Myers, 1986). While the Australian example is extreme, similar scenarios have occurred in Great Britain, New Zealand, and Argentina (Flux et al., 1990; Novillo and Ojeda, 2008). A non-indigenous species everywhere except the Iberian Peninsula, the European rabbit's destructive impact on non-native habitats is primarily attributable to its voracious appetite. Rabbits overgraze native vegetation,

altering ecosystems by contributing to soil erosion and displacing native species, leaving indigenous prey species vulnerable to overpredation due to loss of protective ground cover (Danoff-Burg, 1993; Lees and Bell, 2008; Novillo and Ojeda, 2008). Their impact on agriculture is equally devastating. In addition to destroying crops outright, rabbit grazing contributes to weediness and insect penetration (Crawley, 1989; Myers et al., 1994) and reduces the carrying capacity of grazing areas, resulting in declines in meat and wool production (Myers et al., 1994; Thompson, 1955).

Ironically, the numbers of native wild rabbits on the Iberian Peninsula have dwindled since the 1950s as a result of habitat loss, overhunting, myxomatosis, and rabbit calicivirus (Delibes-Mateos et al., 2009). The decline of this keystone species has placed survival of other species, including the Iberian lynx (*Lynx pardinus*) and the Iberian imperial eagle (*Aquila adalberti*), in jeopardy (Lees and Bell, 2008; Ward, 2005). Recovery efforts aimed at providing species-friendly habitats, controlling hunting, and removing generalist predators have not succeeded, and rabbit populations remain at only 5% of pre-1950 levels. In 2008, the International Union for Conservation of Nature classified wild *O. cuniculus* as “Near Threatened with extinction” throughout its native range.

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Rabbit Genetics and Transgenic Models

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RABBIT GENETICS

Introduction

Genetic studies using laboratory rabbits will receive an essential boost with the publication of the complete

European rabbit genome, *Oryctolagus cuniculus*. All domestic rabbits are derived from the European rabbit, which originated in the Iberian Peninsula, and was domesticated in the 6th century. The Broad Institute has compiled a 7× coverage of the rabbit genome

(<http://www.broadinstitute.org/science/projects/mammals-models/rabbit/rabbit-genome-data-links>), National Center for Biotechnology Information (NCBI) accession # AAGW00000000 (published in 2009) from the inbred Thorbecke strain that provides an important data base for rabbit researchers. However, there remain significant gaps in this sequence database. A deeper 7× (seven times) coverage was started in 2007. Today, more than 100 purebred rabbit breeds exist worldwide, and these breeds have provided commercial sources of meat, fur, pets, research animals, and biopharmaceuticals (via transgenics). Researchers using rabbit models to study diverse projects such as infectious diseases, atherosclerosis, anti-viral treatments, toxicology, diabetes, transplantation, eye diseases (both inherited and infectious), milk production, breed sizes, quantitative traits, immunology, and transgenics will all benefit from the upcoming newly compiled *Oryctolagus cuniculus* genomic sequence.

European Rabbit: Genetic Origins of Domestic Rabbit Breeds

Breeding stocks of domestic rabbits are derived from the European rabbit *Oryctolagus cuniculus*, and wild populations of these rabbits are now widely distributed throughout the world via human transportation (Queney et al., 2002). The geographical origins of the European rabbit are confined to the Iberian Peninsula (Hardy et al., 1995; van der Ferrand et al., 1991). Two highly divergent clades of the European rabbit (clades A and B) are found in this region of southern France and Spain (Branco et al., 2000, 2002) and the clades are represented as two subspecies, *O. cuniculus cuniculus* (Clade A) in the south western Iberian region, and *O. cuniculus algirus* (Clade B) in the northeastern region of the Iberian peninsula (Geraldès et al., 2005; Loreille et al., 1997). There are a number of published comparative studies on these wild rabbits that define their genetic diversity, polymorphisms and origins of genetic stocks for domestic rabbit breeds (Branco et al., 2000, 2002; Geraldès et al., 2005, 2006; Hardy et al., 1994, 1995; Payseur, 2009; Terrance et al., 2007; Vieira and Ferrand, 1995; Webb et al., 1995). Ancient mitochondrial DNA (mtDNA) extracted from European rabbit bones has been sequenced to assess genetic diversity in populations from Europe (Hardy et al., 1994, 1995). The studies included rabbit bones that spanned the time period from 11000 years ago to the present day, and examined nucleotide sequence diversity in a fragment of the mitochondrial cytochrome b gene. Unique findings included data that supported a period of genetic stability in rabbit populations until the Middle Ages when a novel type of mtDNA appeared in most wild rabbit populations and which corresponds to the sequence present in modern domestic rabbit breeds (Hardy et al., 1995). The introduction of this unique genetic marker into wild rabbit populations

provides a timeline (2000 to 1000 years ago), to support the documented establishment of rabbit warrens after the tenth century A.D. in Europe, and the widespread development into all regions of France of animals that carried this genetic marker (Hardy et al., 1995). Parallel genetic analyses of mitochondrial DNA in domestic rabbit breeds identify their primary origins from Clade B European rabbits (Geraldès et al., 2005; Loreille et al., 1997).

Domestic Rabbit Breeds

More than 100 breeds of domestic rabbits have been identified (Table 7.1) and vary in body size, coat color, fur characteristics, ear size, and eye color. The American Rabbit Breeders Association (ARBA) additionally provides a resource for authentication of new and existing breeds. Recent genetic tools such as mitochondrial DNA markers have been used to define the matrilineage of some breeds that arise due to interbreed crosses and the genetic management of stocked rabbit breeding populations (Terrance et al., 2007). Earlier work with breeding studies described extensive reports and data on the genetic inheritance of body weight and size, litter size, morphology, blood groups, and mutations (summarized in Fox, 1994).

Breeds and Traits

Coat Color

Substantial breeding studies have been conducted to determine the inheritance of breed coat color and hair characteristics. A number of reviews cover these findings in significant detail and thus need not be duplicated in this chapter (reviewed in Fox, 1994). Rabbit coat color is primarily determined by five major color genes A, B, C, D, and E. The A or Agouti gene, the B or Black/chocolate gene, the C or Color gene, the D or Dense/dilute gene, and the E or Extension gene. The A gene has three forms that include: A for Agouti (wild-type) rabbits; at for otters, tans and martens; and a, which is responsible for “self” colored rabbits. Agouti rabbits include chestnut, opal, chinchilla and ermine, and the at gene produces black otters, sable point martins and blue otters. The B color gene is either dominant BB or recessive bb and black family rabbits are either BB or Bb, whereas chocolate (or brown) family rabbits are bb. The C or color gene controls where and how much color will be expressed in the coat, and has several major mutations. These mutants include C (full color), C_{ch3} (chinchilla-dark), C_{ch2} (chinchilla-medium), C_{ch1} (sable or chinchilla-light), C_H (Himalayan), cc (recessive or albino). The D gene is either DD, Dd (dense color rabbits), or dd (Dilute color rabbits), and controls the amount of coat color. The E/e gene controls whether the basic hair color is extended throughout the hair shaft. Rabbits with the EE and Ee genes are the same color all over, and ee rabbits look shaded in color

TABLE 7.1 Breeds (Fox, 1994; Fontanesi, et al., 2006; and various websites, see below table)

(American Rabbit Breeders Association) ARBA Breeds	Adult Size	Genotype (if known)	ARBA Breeds	Adult Size	Genotype (if known)
American	12lbs		Himalayan	2.5–4.5lbs	
American Chinchilla	9–12lbs	c ^{ch3} c ^{ch3}	Black		aa c ^H c ^H
American Fuzzy Lop	4lbs		Blue		aa dd c ^H c ^H
American Sable	10lbs	aa c ^{ch1} c ^{ch1}	Chocolate		aa bb c ^H c ^H
Belgian Hare	9.5lbs	A- E-	Lilac		aa bb dd c ^H c ^H
Beveren	8–12lbs		Holland Lop	2.5–4lbs	
Black		aa c ^H c ^H	Jersey Wooly (Agouti, Broken, Self, Tan pattern)	2lbs	
Blue		aa dd	Lilac rabbit		
White		vv	Lionhead	8lbs	aa bb dd
Blanc de Hotot	11 lbs	aa E-	Mini Lop	2.5–4lbs	
Britannia Petite	2.5lbs		Mini Rex	3.5lbs	
Black		aa	Mini Satin	3–4.5lbs	
Black Otter			Netherland Dwarf	3–4lbs	
Chestnut			New Zealand rabbit	1–2.5lbs	
Sable Martin			White	9–12lbs	
Ruby-Eyed White			Red		cc
Californian	8–10.5lbs	aa c ^H c ^H	Black		ee
Champagne d'Argent	9–12lbs	aa sisi E-	Palomino rabbit		aa
Checkered Giant	11 + lbs		Golden	9–10lbs	
Black		aa E ^D e	Lynx		
Blue		aa dd E ^D e	Polish		bb dd
Cinnamon	8.5–11 lbs	ee sisi	Black	3.5lbs	
Crème d'Argent	8–11 lbs		Blue-eyed white		aa
Dutch	3.5–5.5lbs	du ^d du ^w aa	Broken		
Black		du ^d du ^w aa dd	Chocolate		
Blue		du ^d du ^w aa bb	Ruby Eyed White		aa bb
Chocolate		du ^d du ^w aa E ^D e	Rex (also Amber, Broken, Californian)		cc
Steel		du ^d du ^w	Black	8–10.5lbs	
Gray		du ^d du ^w aa ee	Blue		
Tortoise			Castor		rr aa
Dwarf Hotot	3lbs		Chinchilla		rr aa dd
Black			Chocolate		rr
Chocolate		ll cc (white)	Himalayan		rr c ^{ch3} c ^{ch3}
English Angora	7.5lbs		Lilac		rr aa bb
English Lop	9–11 lbs		Lynx		rr c ^H c ^H
English Spot	5–8lbs	Enen aa	Opal		rr aa bb dd
Black		Enen aa dd	Red		rr bb dd

(Continued)

TABLE 7.1 (Continued)

(American Rabbit Breeders Association) ARBA Breeds	Adult Size	Genotype (if known)	ARBA Breeds	Adult Size	Genotype (if known)
Blue		Enen aa bb	Sable point		rr dd
Chocolate			Tortoise		rr ee
Gold		Enen	White		rr aa c ^{ch2} c ^{ch2}
Gray		Enen aa bb dd	Otter		rr aa ee
Lilac		Enen aa ee	Rhineland rabbit		rr cc
Tortoise			Satin (11 varieties)		rr a ^t a ^t
Flemish Giant	14–18lbs	aa	Satin Angora	7–10lbs	
Black		aa dd	Siberian rabbit	8–11lbs	sasa
Blue		ee	Silver rabbit	9.5lbs	
Fawn			Silver Fox	5–7lbs	
Light Grey		ww	Black	4–7lbs	sisi
Sandy		E ^D e	Blue	9–12lbs	
Steel Grey		cc	Silver Martin		sisi aa
White		cc	Standard Chinchilla		sisi aa dd
Florida White	6lbs	ll cc (white)	Tan	6–9lbs	a ^t a ^t
French Angora	10.5lbs	Enen	Black	5–7.5lbs	c ^{ch3} c ^{ch3}
French Lop	10–12lbs		Blue	4–6lbs	a ^t a ^t
Giant Angora	10–11lbs		Chocolate		a ^t a ^t
Giant Chinchilla	12–16lbs	c ^{ch3} c ^{ch3}	Lilac		a ^t a ^t dd
Harlequin	9.5lbs	e ^e e ^j	Thrianta		a ^t a ^t bb
Havana	4.5–6.5lbs				a ^t a ^t bb dd
Black		aa		4–6lbs	
Blue		aa dd			
Chocolate		aa bb			
Broken					
Additional British Rabbit Council (BRC) breeds	Adult Size	Genotype (if known)	Other Breeds	Adult Size	Genotype (if known)
Alaska	7–9lbs	aa E-	Belgian Silver	8–9lbs	
Blanc de Bouscat	12–14lbs		Blue of Sint-Niklaas	10–12lbs	
Blanc de Termonde	10–12lbs		Blue of Hem	10–12lbs	
British Giant	12.5lbs		Brazilian	7–10lbs	
Chinc-Gi	12lbs		Elfin	5lb	
Conti	15lbs		Enderby Island rabbit	4.5lbs	
Deilenaar	7–9lbs		German Grey	23lbs	
Giant Papillon	11–13lbs		Spanish Giant	12lbs	
Perflee	5–7lbs				
Pointed Beveren	7lbs				
Martin Sable	5–7lbs				
Siamese Sable	5–7lbs				

TABLE 7.1 (Continued)

(American Rabbit Breeders Association) ARBA Breeds	Adult Size	Genotype (if known)	ARBA Breeds	Adult Size	Genotype (if known)
Sallander	6–9lbs				
Smoke Pearl	5–7lbs				
Sussex	7–8lbs				
Swiss Fox	5–7lbs				
Thuringer	9lbs				
Tri-Dutch	4–5lbs				
Vienna	8–12lbs				
Hulstandler	5–6lbs				
Fauve de Bourgogne	9–10lbs				
Tri-colored English	6–8lbs				

Various rabbitry websites (including the ARBA and BRC). <http://www.arba.net/Breeds.htm#>; <http://www.thebrc.org/stds.htm>; <http://www.youpet.com>; http://en.wikipedia.org/wiki/List_of_rabbit_breeds; <http://www.rabbits-online.co.uk/>; <http://www.thenaturetrail.com/Rabbit-Coat-Color-Chart.htm>.

due to the short hairs only having full color. Additional color genes include the *v* or Vienna white gene which produces white, blue-eyed rabbits. Other color genes (such as *En*, or English spotted) control whether the coat color is solid, broken, or spotted. Numerous color combinations are possible as suggested by listings in commercial rabbitry web sources (Table 7.1), and review publications (Fontanesi et al., 2006; Fox, 1994).

More recent studies have examined polymorphisms in the melanocortin 1 receptor (MC1R) gene that contributes to coat color (Fontanesi et al., 2006). In mammals, mutations of the MC1R gene causing a constitutively active receptor are dominant and produce black coat color, whereas inactivating mutations are recessive and result in red/yellow pigmentation (reviewed in Cone et al., 1996; Klungland and Vage, 2003). Four MC1R alleles were identified in rabbits in which two wild-type alleles were defined by synonymous single nucleotide polymorphisms (SNPs) (Fontanesi et al., 2006). A third allele with a 30-bp deletion was found to represent the recessive *e* allele at the Extension locus (Fontanesi et al., 2006) as identified through earlier genetic inheritance studies in rabbits (Fox, 1994). Both Agouti and Extension are two genetic loci known to control the amounts of eumelanin (brown-black) and pheomelanin (yellow-red) pigments, and the antagonist/activation interactions of agouti and alpha-melanocyte-stimulating hormone with the MC1R provide molecular pathways for the understanding of the genetics of inherited coat color (Cone et al., 1996).

Hair Characteristics

Additional breeding studies have looked at hair characteristics as an important component of the rabbit wool industry. The Angora rabbit is the most recognized wool/fur breed. Various genetic loci that contribute to hair characteristics include angora, hairless, naked, rex

(short hair), wirehair, and wuzzy (Castle, 1903; Castle and Nachtsheim, 1933; Rafat et al., 2009; Rogers et al., 2006; Sawin, 1955). Recent analyses have used the scanning electron microscope to better understand morphology and variability of the cuticular scale patterns, hair cortex, medullary arrangement and profile of the hairs (Van den et al., 2001), and the optical fiber diameter analyzer to describe the characteristics of Angora rabbit fiber (Rafat et al., 2007). A genetic study looking at single nucleotide polymorphisms (SNPs) in the fibroblast growth factor 5 (FGF5) gene concluded that this gene could be the potential (or linked to the) major gene affecting wool yield in Angora rabbits (Li et al., 2008).

Size Inheritance (Giants and Dwarfism (*Dw/dw*))

Size inheritance from early breeding studies was conducted to assess various morphological characteristics including dwarfism (*Dw/dw*) (Fox, 1994; Sawin, 1955; Wegner, 1997). Domestic breed sizes range for the largest breeds (German Grey and Flemish Giants, 19lb) to the smallest (dwarf, 1.5–2lbs). Some early breeding studies were conducted on size inheritance during selection of large race (III) and small race (X) strains (Latimer and Sawin, 1959). One example of size changes that may occur from inbreeding studies to produce lines from commercially available New Zealand White (NZW) rabbits that showed differential resistance or susceptibility to a regressive strain of cottontail rabbit papillomavirus (CRPV) found that successive generations showed increasing reductions in size until the lines became non-viable (Breitburd et al., 1997).

Growth, Uterine Capacity, Litter Size, Meat Content, and Milk Production

Domestic rabbit breeds supply a thriving industry for meat, fur, and milk in Europe and the US, and

these factors have sparked considerable interest in the genetic inheritance of these commercially important traits (Ferguson et al., 1997; Hamilton et al., 1997; Lukefahr et al., 1989; Ozimba and Lukefahr, 1991a). Earlier studies looked at inherited features of uterine capacity (an important correlative of litter size), meat content (sometimes referred to as butcher traits), growth rate, embryonic development, milk production and fur content (Argente et al., 2003, 2003a; Blasco et al., 1994, 2003, 2005; Hamilton et al., 1997; Hernandez et al., 1997, 2004; Lukefahr et al., 1983; McNitt and Lukefahr, 1990; Medellin and Lukefahr, 2001; Moce et al., 2004a, 2004b; Ozimba and Lukefahr, 1991b; Peiro et al., 2007; Piles et al., 2004). Most of these studies were conducted by careful breeding studies looking for inherited traits and their dominance/recessiveness in F1 and F2 crosses. Some correlative observations demonstrated that increasing uterine capacity increases uterine length and decreases weights of fetus and fetal placenta in rabbits (Argente et al., 2003b). The purpose of these extensive studies was to maximize the commercial potential of various rabbit breeds for the various industry needs such as meat, milk, fur, and fecundity.

A search for genetic markers for some of these traits has revealed some interesting candidate genes including using the method of polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) for the analysis of the growth hormone receptor (GHR) gene (Deng et al., 2008), and SNPs in the progesterone receptor gene (Peiro et al., 2008) (Table 7.2).

Immunology

Immune function in the rabbit has been a significant area of interest in the area of genetics of immune responses. Blood proteins and blood cells are a convenient source of material for genetic studies on protein polymorphisms in rabbit breeds including both wild (Spanish) and domestic populations of rabbits (Arana et al., 1989; Branco and Ferrand, 2002; Branco et al., 1998), and immune molecules, including serum antibodies, macroglobulins, cytokines, and chemokines as well as immune cells are richly present in these samples. Laboratory rabbits, especially NZW, have been used extensively for the production of specific polyclonal antibodies. The rabbit antibody repertoire is constructed predominantly via gene conversion following initial assemblage from a limited set of VH gene fragments in which most rabbit B-cells have the same VH gene (Knight and Winstead, 1997). This construction contrasts to the development of the B-cell repertoire in humans and mice where VH and VL gene fragments are numerous. Interestingly, specific antibodies that recognize and discriminate between normal and phosphorylated proteins are best developed in rabbits. In the rabbit, these VH regions bear allotypic markers,

and these have provided a focal point in the study of genetic inheritance of antibody allotypes (Gilman-Sachs et al., 1981; Knight and Becker, 1990; Knight et al., 1991; Su and Nei, 1999), and several rabbit strains have been identified that have been used in these analyses (Kelus and Weiss, 1986; Weiss et al., 1982). Another interesting and unique observation regarding rabbit antibody production is that the rabbit genome contains multiple genes coding for the constant regions of IgA heavy chains (Burnett et al., 1989) with associated allotypes (Lammert et al., 1977). These latter observations suggest that lagomorphs may have as many as 13 IgA isotypes and hence a complex secretory immune system (Burnett et al., 1989).

Earlier work looked at the functional properties of a number of blood proteins via red blood cell agglutination assays, and four main blood group antigens were identified (Castle and Keeler, 1933; Keeler and Castle, 1933). These blood group antigens can also be used to study genetic susceptibility of rabbits to rabbit hemorrhagic disease virus (RHDV) infection (Ruvoen-Clouet et al., 2000).

Additional cell-surface immune markers that have been studied in rabbits include the CD1 gene cluster which demonstrate similarities to the CD1 cluster in humans and which both differ from the clusters in inbred mice (Bachvarov et al., 1995; Hayes and Knight, 2001). The genomic sequence of the rabbit has provided the much-needed information in the design of primer pairs for the quantitation of various cytokine mRNAs by Quantitative PCR (Godornes et al., 2007), and will continue to aid researchers interested in the immune responses of domestic rabbits. Production of monoclonal antibodies to various rabbit cytokines and CD markers will also be streamlined and aided by the published rabbit genome.

Genetic Susceptibility and Disease Models

Many mutant rabbit models will be discussed in other chapters. Some interesting models include those for study of inheritance of hyperlipidemia (Kurosawa et al., 1995; Meijer et al., 1992); glaucoma (bu/bu) (Fox et al., 1977); spina bifida (Crary et al., 1966); audiogenic seizures (Asakura and Wada, 1972; Nellhaus, 1958); spontaneous diabetes (Conaway et al., 1980, 1981); megacolon syndrome in homozygous spotted (En En) rabbits (Bodeker et al., 1995); systemic lupus erythematosus (SLE) (Puliyath et al., 2008); congenital simple non-syndromic coronal suture (CS) synostosis (Mooney et al., 1994) (<http://www.hopkinsmedicine.org/craniofacial/Research/RabbitMutation.cfm>); and susceptibility/resistance to infectious diseases such as *Mycobacterium tuberculosis* (Dorman et al., 2004; Lurie et al., 1949, 1952), RHDV (Guillon et al., 2009) and herpes simplex virus (HSV) (Seto et al., 1995). An extensive

TABLE 7.2 Candidate Genes and Genetic Sequences that Correlate with Disease, Markers and Traits in the European Rabbit and Domestic Breeds

Gene or Genetic markers ^a	Disease, Marker or Trait	Genetic Loci, Marker or Probe	Alleles or Genotypes	Reference
CCR5	Myxomatosis susceptibility	Allelic variation with related species	One Unique	(Carmo, et al., 2006)
FGF5	Angora wool yield	SNPs: TCT insert of exon 1 (site A); T/C missense of exon 3 (site B); and a T/C same-sense mutation of exon 3 (site C)	A, B and C	(Li, et al., 2008)
GHR	Butcher traits	two GHR mutation sites (C705T and C810T)	AA, MM, BB, NN	(Deng, et al., 2008)
Progesterone R	Reproductive trait	SNPs (2464G > A, three SNPs in the 5'-UTR exon 1, and a silence SNP in exon 7)	A and G	(Peiro, et al., 2008)
MC1R	Rabbit coat color	Mutants (synonymous and deletions	c.333A > G c.555T > C c.304_333del30 c.280_285del6	(Fontanesi, et al., 2006)
CXCR4	Myxomatosis susceptibility	14 synonymous nucleotide substitutions	polymorphic	(Abrantes, et al., 2008)
rabbit kappa-casein	Intronic differences	PCR-based typing method to distinguish rabbit CSN3 alleles	2 alleles	(Bosze, et al., 2000)
ACP3	Polymorphic marker	Allele 3 in laboratory rabbits; polymorphism	ACP3*1, ACP3*2 ACP3*3	(Branco and Ferrand, 1998)
TIMP-1	Embryo survival	Candidate gene, non-polymorphic	4 new alleles	(Vieira and Ferrand, 1995)
N-acetyltransferase	Drug sensitivity	Candidate gene for rapid and slow acetylators rabbits		(Hein, et al., 1982)
MC4R	Carcass trait	detected by PCR-SSCP; A --> G conversion mutation	A and G	(Jiang, et al., 2008)
OVGP1	Litter size and other fertility measures	triallelic microsatellite (17 polymorphic sites co-segregated into three haplotypes (A, B and C)	g.12944C > G (p.Arg468Gly) g.12944G g.325(GT)(14)T(G)	(Merchan, Peiro et al., 2009)
Microsatellites	Reduced sperm quality	29 microsatellite markers		(Gage, et al., 2006)
Rabbit C repeats	Repeats; comparative marker for evolutionary studies	Consensus sequence (TCCCAGCAACCACATGGGAGGCAGAGA)		(Hardison and Printz, 1985)
Mitochondrial cytochrome b	Repeats; comparative marker for evolutionary studies	233-bp sequence for genetic studies		(Hardy, et al. 1995)
Microsatellites	Aortic atherosclerosis	23 microsatellite markers	2-11 per locus	(Korstanje, et al., 2001b)
Microsatellites	Genetic markers (mono-, di-, tri- and tetranucleotide repeats	181 microsatellites identified via genome database searches		(van Lith and van Zutphen, 1996)

(Continued)

TABLE 7.2 (Continued)

Gene or Genetic markers ^a	Disease, Marker or Trait	Genetic Loci, Marker or Probe	Alleles or Genotypes	Reference
Microsatellites	Polymorphic markers in rabbit breeds	157 rabbit nuclear gene microsatellites; 13 primer pairs polymorphic analyses	2-11 per locus	(van Haeringen, et al., 1996)
AFLP	Regulation of the basal serum HDL cholesterol	12 AFLP markers and 3 LG VI classical markers for quantitative trait loci (QTL)		(van Haeringen, et al., 2001)
LINE 1 repeats	Polymorphic repeat sequences for evolutionary studies	22-bp conserved sequence (beginning of all L1 repeats)		(Price, et al., 1992)
AFLP	Dietary cholesterol susceptibility	15 primer combinations to identify 226 polymorphisms		(van Haeringen, et al., 2002)

^aAbbreviations. CCR5, Chemokine receptor; FGF5, fibroblast growth factor 5; GHR, Growth Hormone Receptor; MC1R, melanocortin 1 receptor; CXCR4, Chemokine receptor; ACP3, ; TIMP1, ; MC4R, melanocortin-4 receptor; OVGP1, oviductal glycoprotein 1; AFLP, amplified fragment length polymorphism.

listing of genetic mutations is presented in summary form by Fox (1994), and a selection of genes and genetic markers that are associated with various mutations and traits are listed in Table 7.2.

Inbred Rabbit Strains

Several inbred strains of rabbits have been developed by various researchers and these rabbits are valuable resources for the research community. Early breeding studies demonstrated some significant difficulties in the establishment of inbred lines due to the well-known phenomena of exposure to deleterious recessive genes, developmental abnormalities, inbreeding depression and inbred lethality (Breitburd et al., 1997; Chai, 1969, 1970; Chai and DeGenhardt, 1962); however, several inbred lines were established initially at the Jackson Laboratory, Bar Harbor, ME (Chai, 1968, 1969, 1970; Chai and DeGenhardt, 1962). Commonly used strategies to establish inbred lines used full-sib mating similarly to the establishment of inbred strains in other laboratory species. Earlier (as well as more recent) studies have established a number of lines as closed colonies with restricted selected breeding to study various diseases and traits (Blasco et al., 2005; Braun, 1974; Braun et al., 1973; Falkenberg and Frensdorff, 1975; Hernandez et al., 2004; Latimer and Sawin, 1955, 1959; Mooney et al., 1994; Rafat et al., 2009). One interesting NZW inbred rabbit line that has been preserved only as tissues is the rabbit that is being used to establish the 7× genome at the Broad Institute, and is known as the Thorbecke strain (Mendez et al., 2008). Some selected inbred lines are discussed below.

B, Y, A, C, and R Inbred Lines (The Jackson Laboratory)

Five inbred strains, B, Y, A, C, and R (the R line has become extinct) were originally developed at the Jackson Laboratory, and maintained by full-sib mating (Chai and

DeGenhardt, 1962; Crary and Fox, 1980). Renamed as inbred lines B/J, R/J, and Y/J, the lines were originally developed by Dr Cohen to study effects on blood group genes (Fox, 1978). Additional inbred strains were developed by Dr Sawin, Dr Chai, and others at the Jackson labs from several stocks that included the partially inbred III line that was used in a study on selected breeding for size traits (Crary and Sawin, 1960; Latimer and Sawin, 1958, 1959; Sawin and Crary, 1956). Inbred lines include the homozygous rapid acetylator inbred strains (B/J, III/J, IIIC/J, III/DwJ, IIIEP/J, and IIIVO/J), and the homozygous slow acetylator inbred strains (ACEP/J, III/cdJ, IIIVO/ahJ, and IIIVO/utJ) (Hein et al., 1982). Additional Jackson inbred strains include AC/J, AX/J, AXBU/J, C/J, OS/J, WH/J, X/J (Usher et al., 1982). Some recent studies with the III/J line looked at levels of liver microsomal progesterone 21-hydroxylase activity (Pendurthi et al., 1990). The audiogenic inbred line, EIII/JC is likely derived also from the original Jackson lines (Hu et al., 2002). The B/Jas line is highly susceptible to HSV encephalitis and thus used as a genetic model to study disease susceptibility to this virus (Seto et al., 1995; Yuasa et al., 1999).

Thorbecke Inbred Line

The Thorbecke inbred line was originally developed from NZW rabbits (>20 generations) by Dr. G.J. Thorbecke, and was previously available commercially from Covance Research Products, Inc. (Denver PA). The strain was used to study susceptibility to *Mycobacterium tuberculosis*, in an aerosolized infection model (Mendez et al., 2008). Thorbecke rabbit tissues have been preserved for DNA source material to establish the 7× genome sequence of the domestic rabbit by the Broad Institute, but this strain is no longer available.

AX/JU and IIIVO/JU Inbred Lines

This pair of inbred lines was originally developed to study the effects of dietary cholesterol in a rabbit model.

Strain IIIVO/JU is not reactive to dietary cholesterol, whereas the other strain (AX/JU) is reactive to dietary cholesterol (de Wolf et al., 2003). These lines make excellent models for various quantitative trait loci (QTL) mapping (Korstanje et al., 2001a, 2001b; van Haeringen et al., 2001, 2002) and for comparative studies such as differential responses to analgesics (Avsaroglu et al., 2003, 2008), polymorphism of biochemical markers (Dieter and Johnson, 1982; Korstanje et al., 2001a), and microsatellites (Korstanje et al., 2001b, 2001c).

WHHL (Watanabe-Heritable Hyperlipidemic) Inbred Line

The WHHL or Watanabe inbred strain was developed initially from spontaneously hyperlipidemic rabbits and is a model for human familial hypercholesterolemia (Bell et al., 1987; Meijer et al., 1992; Shiomi et al., 1987; Watanabe, 1980; Watanabe et al., 1977). Clinical features of this strain include elevated serum lipids, cholesterol (400 +/−70 mg/dl), and beta-lipoprotein levels (Watanabe et al., 1977).

JWY-NIBS and NWY-NIBS Inbred Lines

Two inbred rabbit strains, JWY-NIBS and NWY-NIBS, one derived from Japanese White rabbits, the other from strain III NZW rabbits were established by full-sib mating in the Nippon Institute for Biological Science, Laboratory Animal Research Station (Fukuta et al., 1996; Yazawa et al., 1986). These strains have been used to study comparative traits (Fukuta et al., 1996) including mandible size (Goto et al., 1987).

KHC (Kurosawa and Kusanagi Hypercholesterolemic) Inbred Line

The KHC strain displays inherited and persistent hypercholesterolemia and was produced by inbreeding from mutants discovered in 1985 (Kurosawa et al., 1995). KHC rabbits had serum cholesterol, triglyceride, and phospholipid concentrations that were 8–10 times greater than the concentrations in clinically normal Japanese White rabbits, which were the original source strain for the KHC line (Kurosawa et al. 1995).

Rabbit Lines and Closed Colonies

Various rabbit lines represented as closed colonies, or selected breeding from mutant individuals have been developed that display unique genetic features and which provide important models to study aspects of genetics and immunity. Some selected examples of these lines are listed below.

Gelbsilber (GS) Rabbits

GS rabbits are a closed colony derived from NZW rabbits. These rabbits were found to be high antibody

responders to the lactic dehydrogenase isoenzyme LDH-H4 and thus differ from most New Zealand White (NZW) rabbits which produce no detectable antibody to this antigen (Falkenberg and Frensdorff, 1975; Freyler et al., 1976). It was concluded that in GS rabbits the antibody response to the LDH-H4 antigen is controlled by an Ir gene and that these animals might be a useful model to study genetic control of the immune response in rabbits (Falkenberg and Frensdorff, 1975).

Alicia Rabbits

A mutation of the allotype allele a2 in this rabbit was found and defined as “ali”. The corresponding rabbit strain is called “Alicia” (Allegrucci et al., 1990, 1991; Bernstein et al., 1985; Chen et al., 1993, 1995; DiPietro et al., 1990; Kelus and Weiss, 1986; Sehgal et al., 1998). The mutant ali/ali animals have a deletion in a key variable region gene in the immunoglobulin heavy-chain locus that is present in the related wild-type 2R1/2R1 strain. They are defined for immunoglobulin allotypes, which are allelic variants of antibody heavy and light chains.

Basilea Rabbits

Basilea rabbits contain a mutation in the immunoglobulin genes (Mage et al., 1984; McCartney-Francis et al., 1987; Freedman et al., 1975; Gilman-Sachs et al., 1981), and produce antibodies with lambda light chains when homozygous (Weiss et al., 1977). Basilea rabbits do not produce normal K1b9 light chains but continue to produce immunoglobulins with light chains of the rare K2 isotype and of lambda type (Lamoyi and Mage, 1985; Weiss et al., 1977). These rabbits have been used in earlier studies to characterize immunoglobulin light-chain classes, and to study the genetics of immune responses to various antigens including streptococcal group A-variant polysaccharide (Dubiski et al., 1982; Garcia et al., 1982; Weiss et al., 1977, 1979, 1982; Zhu et al., 1999).

JIRONG Rabbits; Japanese White Rabbits

Countries other than Europe and the US have established their own rabbit strains for research studies. JIRONG rabbits are a line developed in China that originated from European stocks of Rex rabbits. These rabbits have been used in a microsatellite marker study of comparative traits and for parental testing (Han et al., 2005). Japanese White rabbits (Mage et al., 1984) are a breed used as a primary source of several inbred rabbit lines such as the KHC rabbits (Kurosawa et al., 1995) and the JWY-NIBS rabbits (Yazawa et al., 1986).

Research Stocks

NZW rabbits are the major resource rabbit for research laboratories and industry toxicology studies. The main features of this breed that account for this

popularity include the ease of location of surface veins and arteries (especially the ear) for blood collection and the general ease of handling. Other strains available commercially include Dutch-belted and California White. Many research labs have their own unique strains, inbred lines and transgenics, and some of these resources are becoming lost to the research community due to reduced institutional and commercial support. For example, the U. S. National Institutes of Health (NIH) recently advertised competitive research grants to support the maintenance and continuation of unique and valuable antibody allotype-defined rabbit colonies that have been bred and maintained at the NIAID facilities over the past 45 years (<http://grants.nih.gov/grants/guide/notice-files/NOT-AI-08-035.html>). Three valuable strains are included in the colony and are designated as b9/b9, ali/ali (Alicia), and the parental wild-type 2R1/2R1. The b9/b9 rabbits were shown to be optimal for production of chimeric rabbit:human monoclonal antibodies with high affinities and specificity for antigen. These colonies otherwise were to be discontinued. As mentioned previously, the Thorbecke strain used in compilation of the rabbit genome has already been lost. U.S. research facilities that provide rabbits for researchers in the U. S. number more than 700 suppliers (Grannis, 2002).

Genomic Sequence Information and Resources

A variety of genomic databases are now available for the rabbit researcher. Some examples include: EMBL (<http://www.ebi.ac.uk/embl/>), GenBank (<http://www.ncbi.nlm.nih.gov/>), DNA Database of Japan (<http://www.ddbj.nig.ac.jp/>) and the Broad Institute rabbit genome (2X) database (<http://www.broadinstitute.org/science/projects/mammals-models/rabbit/rabbit-genome-data-links>). In addition, several groups have compiled various sets of primer sequences to identify rabbit microsatellite sequences which can then be used for SNPs analyses (Payseur and Cutter, 2006) as well as chromosome “paints” using filter in situ hybridization (FISH) techniques (Hayes et al., 2002; Korstanje et al., 1999), and subsequent cytogenetically anchored rabbit microsatellite maps (Chantry-Darmon et al., 2003, 2005, 2006; Korstanje et al., 2003; Zijlstra et al., 2002). Repetitive DNA sequences can be used in genetic analyses and these include multiple sequences that define a lentiviral subgroup referred to as rabbit endogenous lentivirus type K (RELK) (Katzourakis et al., 2007; van der et al., 2009). Long interspersed repetitive DNA (LINE1 or L1) and short interspersed repeats (SINEs) or C-repeats, represent highly repeated DNA sequences in the rabbit genome that also provide genetic markers for comparative genomic analyses (Demers et al., 1989; Hardison and Printz, 1985; Krane et al., 1991; Price et al., 1992).

Additional repetitive markers (in *Oryctolagus cuniculus*) include mitochondrial DNA that contains a tandem array of 153-bp repeats in the vicinity of the replication origin of the H-stand. Variation among molecules in the number of these repeats results in inter- and intra-individual length polymorphism also known as heteroplasmy (Casane et al., 1994, 1997).

Microsatellite markers have also been identified from the published genome to map various quantitative trait loci (Korstanje et al., 2001b; Mouguel et al., 1997; van Haeringen et al., 2001; van Lith and van Zutphen, 1996), nicely illustrating the value and power of the rabbit genome database. Other research tools for the study of rabbit genetics include rabbit bacterial artificial chromosome (BAC) libraries (Rogel-Gaillard et al., 2001). Together, these data sets and tools provide a wealth of sequence information for comparative genetics for rabbit researchers. A selection of genes and genetic markers that have been linked to various mutations, diseases, traits, and susceptibilities are presented in Table 7.2. We predict that this list will grow more rapidly upon publication of the more complete 7× genome (Abrantes et al., 2008; Bosze et al., 2000; Branco and Ferrand, 1998; Carmo et al., 2006; Deng et al., 2008; Fontanesi et al., 2006; Gage et al., 2006; Hardison and Printz, 1985; Hardy et al., 1995; Hein et al., 1982; Jiang et al., 2008; Korstanje et al., 2001b; Li et al., 2008; Peiro et al., 2008; van Haeringen et al., 1996, 2001; van Lith and van Zutphen, 1996; Vieira and Ferrand, 1995).

TRANSGENIC RABBITS

Introduction

Successful generation of the first transgenic rabbits was reported in 1985 (Hammer et al., 1985), following the creation of the first transgenic mice (giant transgenic mice with overexpression of rat growth hormone) in 1982 (Palmiter et al., 1982). Since then, transgenic rabbits have been generated and used as animal models for a variety of human diseases and as bioreactors for production of pharmaceutical proteins. Although transgenic mice are still dominantly used in biomedical research, it is known that the causative mutations resulting in human diseases do not cause corresponding pathological changes in mice in some instances (Rysinska et al., 1990). Additionally, a number of techniques are difficult to apply to mouse models due to their small size and phylogenetic features, such as for studies of the long QT syndrome (Brunner et al., 2008; Nerbonne, 2004) and hypertrophic cardiomyopathy (Marian et al., 1999; Nagueh et al., 2000). Alternatively, the rabbit (*Oryctolagus cuniculus*) with an intermediate size between rodents and large farm animals and closer phylogenetic features to

primates has attracted more attention in recent years in biomedical and pharmaceutical research. As of July 2009, there had been 180 publications on transgenic rabbits as searched for in Medline, representing an increase of 100% in the last 7 years on the basis of a total of 90 publications between 1985 to 2002 (Fan and Watanabe, 2003). Transgenic rabbits have been most commonly used as models for studies of cardiovascular diseases, such as atherosclerosis, hypertrophic cardiomyopathy, and long QT syndrome, in addition to their use in studies of cancers, AIDS, and other human diseases. Because of their appropriate size and short reproductive cycle, transgenic rabbits have also been used subsequently as bioreactors for production of pharmaceutical proteins. A summary based on current publications obtained from a search of the Medline database focuses on basic methods and strategies for generation of transgenic rabbits, their use as models of important human diseases and application as bioreactors for production of valuable pharmaceutical proteins.

Basic Methods and Strategies for Generation of Transgenic Rabbits

Four methods have been established for generation of transgenic animals, including (1) pronuclear injection of embryos at the single cell stage, (2) injection of genetically modified embryonic stem (ES) cells into blastocysts, (3) sperm-mediated gene transfer, and (4) genetically modified somatic cell and nuclear transfer (cloning). To date, the majority of transgenic rabbits have been produced by microinjection of a DNA construct into the pronucleus of embryos at the single-cell stage although a few labs have reported generation of transgenic rabbits by sperm-mediated gene transfer (Li et al., 2006; Shen et al., 2006) or somatic cell cloning (Skrzyszowska et al., 2006). The production of gene knock-in (targeted insertion of the transgene at a selected locus) or knock-out (elimination of a specific gene) animals by genetically modified ES cell injection into a blastocyst has still been limited only to mice since the true totipotent ES cells essential for this technique have not been available in any other species, including rabbits.

A comprehensive review article has provided a detailed description and discussion for production of transgenic rabbits (Fan and Watanabe, 2003). Until now, DNA microinjection into the pronucleus is still the most reliable method for generation of transgenic rabbit founders. The basic procedures, mostly converted from the method for production of transgenic mice with some modifications, include induction of superovulation (by timed administration of pregnant mare's serum gonadotropin or follicular stimulating hormone and human chorionic gonadotropin), pronuclear

microinjection of a DNA construct, transfer of embryos to the oviducts of a pseudopregnant-induced recipient, identification of transgenic founders, establishment of transgenic lines, and analysis of transgene expression and associated phenotypes. Most of the transgenes are constructed by recombination of a cDNA (lack of introns and regulatory sequences) for a gene of interest and a specific promoter for controlling the transgene expression. A few transgenic rabbit lines carrying yeast artificial chromosome (YAC) or bacteria artificial chromosome (BAC) vectors, which can accommodate large genomic sequences, have been established (Brem et al., 1996; Rouy et al., 1998). It is known that genomic sequences direct higher levels of gene expression and tissue specificity than cDNA (Giraldo and Montoliu, 2001).

Rabbit embryos are larger (160 μ m diameter versus 110 μ m) and slightly more opaque than mouse embryos. The pronuclei in most rabbit embryos are sufficiently visible under phase-contrast microscopy for microinjection. However, pronuclei in approximately 25% of rabbit embryos may not be visible or clear enough for microinjection. One study reported that centrifuging for 10 min at 12000g could make pronuclei of the embryos visible and suitable for microinjection (Hirabayashi et al., 2000). Another characteristic of rabbit embryos is the thicker zona pellucida due to formation of a thin mucin coat. Mucins are high-molecular-weight and heavily glycosylated proteins (glycoconjugates) produced by epithelial cells in the oviducts. Neither the zona pellucida nor the thin mucin coat is difficult to penetrate, nor do they influence microinjection. However, if the mucin coat is too thick, which might be related to delayed harvest of embryos from oviducts, it can make microinjection difficult since the embryo cannot be held stably for insertion of the injection pipette. It is also known that the presence of the mucin coat greatly influences pregnancy by showing a dramatic drop in pregnancy rate in its absence (Moore et al., 1968). Therefore, it is desirable to transfer the embryos to oviducts within 2–3 hours following microinjection to allow formation of the mucin coat prior to the embryos reaching the uterus for implantation.

Microinjected DNA integrates into the host genome at a random site from one to variable multiple copies. The integration site may influence the transgene expression level due to exposure to nearby enhancers or silencers, or enhanced methylation (Jaenisch, 1988). The efficiency of genomic integration following DNA injection for production of transgenic rabbit founders is still quite low, ranging from 0.5–3% (Bosze et al., 2003). Some factors influencing the efficiency of rabbit transgenesis have been addressed (Fan and Watanabe, 2003; Murakami et al., 2002). Several attempts have been made to improve the rate of genomic integration.

A recent study reported that microinjection of DNA into both male and female pronuclei of rabbit embryos significantly increased the transgene integration rate to 8.1% (4/49) in contrast to 3.3% (1/30) in single pronucleus injection (Chrenek et al., 2005). A further study revealed that all founders derived from injection of both pronuclei could transmit the transgene to the next generation in a Mendelian fashion. Results from this preliminary study suggest that injection of both pronuclei might significantly improve the efficiency for production of transgenic rabbit founders.

Another important factor is the stability of transgene transmission to offspring, which is essential for establishment of transgenic lines. Mosaicism in transgenic rabbit founders was a frequent problem involving the transmission of transgenes in early studies (Castro et al., 1999). It has been known that rabbit embryos develop faster at early stages than mouse embryos (Bosze et al., 2003). Therefore, the problem of mosaicism in transgenic rabbit founders could be minimized by precise control of the timing of hormonal administration, mating, embryo collection, and microinjection. To date, none of the transgenic rabbit founders produced in our lab were found with mosaicism as all offspring retained the transgene.

Production of transgenic animals from genetically modified ES cells with addition (knock-in) or deletion (knock-out) of a specific gene through homologous recombination is one of the most important advances in transgenic technology. Unfortunately, this approach is still generally restricted to mice as the only species in which the true totipotent stem cells have been available so far. Although ES-like cells have been derived from rabbits and blastocyst injection of the cells resulted in low chimerism of live-born offspring (Graves and Moreadith, 1993; Schoonjans et al., 1996), no germ line transmission of transgenes from these animals has been reported. More recently, two groups independently reported the generation of rabbit ES cells from blastocysts (Fang et al., 2006; Wang et al., 2007). These ES cells can differentiate into many cell types in vitro and generate teratomas containing tissue types of all three germ layers when injected into SCID mice. However, no study with blastocyst injection of the cells has been reported.

As an alternative to knock-out animals, this limitation has been partially overcome by overexpression of a dominant-negative mutant gene to suppress the expression of an endogenous gene in transgenic rabbits (Brunner et al., 2008). Furthermore, the development of a cloning technique based on nuclear transfer from a cultured and genetically modified somatic cell into an enucleated oocyte has provided a more viable alternative to the ES cell route for production of knock-in or knock-out animals in any mammalian species.

Successful cloning of the first rabbits from somatic cells was reported in 2002 (Chesne et al., 2002). In this study, several healthy and fertile somatic clones of rabbits were produced using freshly collected cumulus cells as nuclear donors. A more recent paper reported the production of cloned transgenic rabbits by a novel technique called chimeric somatic cell cloning (Skrzyszowska et al., 2006). These cloned transgenic rabbits were produced by transfer of skin fibroblast cell nuclei from an adult transgenic rabbit to the cytoplasmic microenvironment of single enucleated blastomeres from two-cell stage rabbit embryos. Results from these studies are very encouraging and have opened a way to the development of knock-in or knock-out rabbits by combining the techniques of culture and genetic modification of somatic cells (as nuclear donor) and cloning by nuclear transfer.

Transgenic Rabbit Models for Major Human Diseases

Transgenic Rabbit Models for Atherosclerosis

Atherosclerosis is a leading cause of death in developed countries. It has been well known that high plasma cholesterol concentration (hypercholesterolemia), especially low-density lipoprotein (LDL) cholesterol, constitutes an important risk factor in atherosclerotic lesion formation. Laboratory rabbits have been widely used for studies of atherosclerosis due to its reasonable size, ease of manipulation, quick response to dietary cholesterol, and similar lipoprotein metabolism to human. Among the animals that have been studied, the rabbit is the only one in which hypercholesterolemia could be induced by administration of a high-cholesterol diet for a few days (Bocan et al., 1993). The cholesterol-fed rabbit has become the classical model for the study of lipoproteins and their roles in atherosclerosis. The unique characteristics of this rabbit model, including low plasma total cholesterol concentrations, high-cholesterol ester transfer protein activity, low hepatic lipase (HL) activity, and lack of an analog of human apolipoprotein (apo) A-II, have been well addressed (Brousseau and Hoeg, 1999). Furthermore, the Watanabe rabbit (Watanabe heritable hyperlipidemic rabbit, WHHL), a genetically altered strain of New Zealand White (NZW) rabbit, constitutes a laboratory animal which mimics human familial hypercholesterolemia and is suitable to study the link between hypercholesterolemia and atherosclerosis (Watanabe, 1980).

In the last decade, use of transgenic rabbits has attracted great interest for studying the pathogenesis of atherosclerosis. Since the report of the first transgenic rabbits in this area (Fan et al., 1994), the number of applications of transgenic rabbit models to the

TABLE 7.3 Transgenic Rabbit Models for Studies of Lipid Metabolism and Atherosclerosis

Transgenes	Major Effects on Lipoproteins and Atherosclerosis	References
Human hepatic lipase	Reduced VLDL, IDL and HDL; attenuated response to diet-induced hypercholesterolemia and atherosclerosis	Fan et al., 1994
Human 15-lipoxygenase	Protection against diet-induced atherosclerosis	Shen et al., 1995
Human apoB-100	Increased LDL; decreased HDL; effect on atherosclerosis not determined	Fan et al., 1995
Human lecithin:cholesterol acyltransferase	Decreased LDL; increased HDL; protection against diet-induced atherosclerosis	Hoeg et al., 1996
Human apoA-I	Protection against diet-induced atherosclerosis	Duverger et al., 1996a
	Impaired endothelium-derived vasorelaxation	Lebuffe et al., 1997
	Increased HDL; ineffective inhibition to atherosclerosis	Boullier et al., 2001
Human apoE2	Development of type III hyperlipoproteinemia and spontaneous atherosclerosis on regular diet	Huang et al., 1997
Human apoE3	Reduced VLDL; increased LDL; effect on diet-induced atherosclerosis not fully determined	Fan et al., 1998
Human apo(a)	Lipoaprotein(a) assembly with rabbit apo B	Rouy et al., 1998
	Increased atherosclerosis on cholesterol-rich diet	Fan et al., 2001a
Human lipoprotein lipase	Protection against diet-induced atherosclerosis at lower plasma cholesterol than controls	Fan et al., 2001b
	Increased small dense LDL and promoted atherosclerosis at equal plasma cholesterol with controls	Ichikawa, 2004
Matrix metalloproteinase-12	Increased atherosclerosis at higher hypercholesterolemia for longer period	Liang et al., 2006
	Accelerated initiation and progression of atherosclerosis by degradation of internal elastic layer or basement membrane	Yamada et al., 2008

VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

studies of lipid metabolism and atherosclerosis has gradually increased. To date, transgenes for nearly a dozen proteins involved in atherogenesis have been successfully expressed in rabbits, including the human apolipoprotein(a) [apo(a)], apoA-I, apoB, apoE2, apoE3, hepatic lipase (HL), lecithin:cholesterol acyltransferase (LCAT), lipoprotein lipase (LPL), 15-lipoxygenase, matrix metalloproteinase-12, and macrophage metalloelastase (Table 7.3). Most of these transgenic rabbit models have been well characterized and discussed in several review articles (Brousseau and Hoeg, 1999; Fan and Watanabe, 2003). These studies in transgenic rabbits have helped to elucidate the metabolic roles of the proteins in atherogenesis.

In 1994, Fan and colleagues reported the generation of transgenic rabbits for studying the effects of human hepatic lipase (HL) in atherosclerosis (Fan et al., 1994). HL overexpression in the transgenic rabbits showed significant effects on plasma lipid and lipoprotein levels. Total cholesterol and triglyceride levels were reduced by 42% and 58%, respectively, in the transgenic rabbits compared to non-transgenic controls.

Lipoprotein analysis revealed a marked reduction of high-density lipoprotein (HDL), very-low-density lipoprotein (VLDL), and intermediate-density lipoprotein (IDL) in the transgenic rabbits. A further study showed that feeding a diet with 0.3% cholesterol resulted in attenuated hypercholesterolemia in the transgenic rabbits compared to non-transgenic littermates (Fan et al., 1995). The attenuated hypercholesterolemia in the HL transgenic rabbits might be associated with a diminished extent of aortic atherosclerosis (Taylor, 1997).

Apolipoprotein(a) [apo(a)] is a component of lipoprotein(a) [Lp(a)], and naturally presents only in Old World monkeys and humans. Apo(a) is bound to apoB-100 by a disulfide linkage resulting in the Lp(a) complex. The elevated plasma level of Lp(a) has been associated with increased incidence of cardiovascular disease, stroke, and restenosis (Maher and Brown, 1995; Utermann et al., 1989). To investigate the Lp(a) assembly and its role in atherosclerosis, transgenic rabbit lines expressing human apo(a) were established by two independent groups (Fan et al., 1999; Rouy et al., 1998).

In the plasma of transgenic rabbits, unlike the plasma of transgenic mice in which human apo(a) can not bind to murine apoB for formation of Lp(a) particles, human apo(a) was shown to be associated with rabbit endogenous apoB for formation of Lp(a) complex. Results from these studies provide new insights into apo(a) expression and Lp(a) assembly. These transgenic rabbits also provide a potential model to further study Lp(a) and its role in promoting atherosclerosis. A further study of these transgenic rabbits showed that the human apo(a) transgenic rabbits fed a 0.3% cholesterol diet for 16 weeks had more extensive atherosclerotic lesions than the non-transgenic controls, although both groups had similarly increased cholesterol levels in their plasma (Fan et al., 2001a). Results from this study suggest that Lp(a) may have proatherogenic effects in the transgenic rabbits fed a cholesterol-rich diet.

Prospective epidemiological studies suggest that HDL cholesterol and apoA-1 limit atherosclerosis development. To study the effect of apoA-1 in the inhibition of atherosclerosis, transgenic rabbits expressing human apoA-1 were generated (Duverger et al., 1996a). When the transgenic rabbits were fed a cholesterol-rich diet (0.48 g cholesterol per 120 g of the diet), it was shown that the plasma level of HDL cholesterol in the transgenic group was twice that of the control group. Correspondingly, the atherosclerotic lesions in the thoracic aorta were reduced by 50% in the transgenic rabbits compared to non-transgenic controls. Results in this study showed that the protective effects of human apoA-1 on cholesterol-rich diet induced atherosclerosis were associated with the increased HDL levels possibly by the mechanism of reverse cholesterol transport (Duverger et al., 1996b). However, a later study showed that human apoA-1 transgenic rabbits with high HDL cholesterol levels were not protected against the development of atherosclerosis when the rabbits were fed a cholesterol-rich diet which induced dramatic hypercholesterolemia (Boullier et al., 2001). Therefore, the role of human apoA-1 in atherogenesis needs to be further characterized.

The development of transgenic rabbits expressing human apoB-100 has also been described (Fan et al., 1995). Analysis of plasma lipid concentrations in the transgenic rabbits demonstrated that total plasma cholesterol and triglyceride levels were 2–3-fold higher than those observed in non-transgenic controls. Nearly all of the cholesterol and human apoB-100 was in the LDL fraction, with striking enrichment of the triglyceride content. It was further found that the transgenic rabbit LDL contained large amounts of apoC-III and apoE, which suggests that hepatic overexpression of apoB-100 might have led to the assembly of nascent triglyceride-rich particles in the animals that had the size and density of LDL rather than of VLDL (Brousseau

and Hoeg, 1999). The atherosclerosis susceptibility was not determined in these transgenic rabbits.

ApoE2 (Cys112, Cys158) is an apoE variant associated with the human genetic disorder, type III hyperlipoproteinemia. Transgenic rabbits expressing high plasma concentrations of human apoE2 were produced and described by Huang and co-workers (1997). Overexpression of human apoE2 (30–70 mg/dl) in transgenic rabbits resulted in a marked accumulation of β -migrating VLDL and significant increases in VLDL and IDL concentrations, the hallmarks of type III hyperlipoproteinemia. Atherosclerosis susceptibility was assessed in both male and female transgenic and non-transgenic rabbits fed a normal rabbit chow. The transgenic rabbits developed spontaneous atherosclerosis in the aortic arch and proximal abdominal aorta. Interestingly, male transgenic rabbits showed more extensive atherosclerosis than transgenic females, which suggests that sex hormones may play a role in modulating type III hyperlipoproteinemia (Huang et al., 1997).

Generation of transgenic rabbits expressing lecithin-cholesterol acyltransferase (LCAT), an enzyme that plays an important role in cholesterol and HDL metabolism, was reported in 1996 (Hoeg et al., 1996). Overexpression of human LCAT in transgenic rabbits resulted in a substantial change in plasma lipid and lipoprotein concentrations. Plasma total, free and esterified cholesterol concentrations, as well as phospholipid concentrations, were significantly increased in both low and high expressors of the transgene compared to control littermates. The elevation in plasma total cholesterol contents was associated with a marked increase in HDL concentration. Analysis of other lipoproteins revealed significantly increased plasma apoA-1 and apoE concentrations, but reduced apoB concentration in the LCAT transgenic rabbits. Furthermore, study of atherosclerosis susceptibility in the rabbits showed significantly reduced atherosclerosis compared to control littermates on a 0.3% cholesterol diet for 17 days (Hoeg et al., 1996).

To study the physiological role of lipoprotein lipase (LPL), a key enzyme in hydrolysis of triglyceride-rich lipoproteins in lipid and lipoprotein metabolism, transgenic rabbits expressing human LPL were established (Araki et al., 2000). In the transgenic rabbits, LPL activity was four times higher than that of non-transgenic controls, and plasma triglyceride was decreased by 80% and HDL by 59%. Analysis of the lipoprotein density fractions revealed that overexpression of the LPL transgene resulted in a remarkable reduction in the levels of VLDL and IDL. The initial study for atherosclerosis susceptibility showed marked protection against diet-induced hypercholesterolemia and development of aortic atherosclerosis (Fan et al., 2001b). However, it should be noted that plasma cholesterol levels in the transgenic rabbits

were significantly lower than those in control rabbits (Ichikawa et al., 2004). To clarify, if the antiatherogenicity was caused by the increased LPL activity itself or if the antiatherogenic effect of LPL was dependent upon the LPL lipid-lowering effect, LPL transgenic rabbits and littermates were fed diets with different amounts of cholesterol (0.3–0.6%) so that their plasma cholesterol concentrations could be adjusted to maintain similarly high levels for 16 weeks (Ichikawa et al., 2004). Interestingly, the subsequent study demonstrated that the transgenic rabbits developed 1.8-fold more extensive aortic atherosclerosis than the control rabbits even though they had the same level of hypercholesterolemia. Overexpression of human LPL in transgenic rabbits reduced remnant lipoproteins (β -VLDL) but concomitantly led to a significant increase of large and small LDLs compared to controls. An in vitro experiment showed that small-sized LDLs of the transgenic rabbits were more susceptible to copper-induced oxidation and had higher affinity to biglycan than large-remnant lipoproteins. Based on the results of these studies, the authors concluded that “LPL exerts a dual function in terms of its atherogenicity, namely antiatherogenicity, through enhancing receptor-mediated remnant lipoprotein catabolism and pro-atherogenicity by the generation of a large amount of small-sized LDLs” (Ichikawa et al., 2004).

Matrix metalloproteinase-12 (MMP-12) was first identified as a potent elastolytic metalloproteinase specifically secreted by macrophages (Shapiro et al., 1993). Increased activity of MMP-12 has been implicated in many inflammatory processes including atherosclerosis (Beaudeux et al., 2004; Watanabe and Ikeda, 2004). It has been demonstrated that expression of MMP-12 was prominently upregulated in atherosclerotic lesions of cholesterol-fed rabbits (Matsumoto et al., 1998). To study the pathophysiological roles of MMP-12 in atherosclerosis, transgenic rabbits with targeted expression of human MMP-12 gene in macrophages were established (Fan et al., 2004; Liang et al., 2006). A preliminary study for atherosclerosis susceptibility found no significant difference in the aortic atherosclerotic lesion size or quality between transgenic and non-transgenic rabbits with lower hypercholesterolemia. In contrast, transgenic rabbits with higher hypercholesterolemia for longer periods developed more extensive atherosclerosis in the aortas and coronary arteries than non-transgenic controls (Liang et al., 2006). Furthermore, increased expression of MMP-12 derived from macrophages was found to be associated with elevated expression of MMP-3. These results suggest that macrophage-derived MMP-12 plays a pivotal role in the cascade action of other MMPs, which may be involved in exacerbating extracellular matrix degradation during the progression of atherosclerosis (Liang et al., 2006). In a subsequent study, the same group investigated macrophage

migration and elastolysis in relation to fatty streaks in MMP-12 transgenic rabbits (Yamada et al., 2008). They found that MMP-12 transgenic rabbits fed a 1% cholesterol diet for 6 weeks developed more pronounced fatty streaks associated with more significant degradation of the internal elastic layer compared to non-transgenic controls. In addition, the transgenic rabbits showed greater numbers of infiltrating macrophages and smooth muscle cells in the lesions than control rabbits. The authors concluded that MMP-12 may be critical to both initiation and progression of atherosclerosis by degradation of the elastic layers and/or basement membrane. These transgenic rabbits may provide a potential system to test specific MMP-12 inhibitors for treatment of progressive atherosclerosis (Yamada et al., 2008).

Other transgenic rabbits which have been used for the study of atherosclerosis include those with modified genes for apoE3 (Fan et al., 1998); apoB mRNA editing enzyme catalytic polypeptide 1 (APOBEC-1) (Yamanaka et al., 1995); and 15-lipoxygenase (Shen et al., 1995). Additionally, generation of double-transgenic rabbits by crossbreeding two different transgenic lines and WHHL transgenic rabbits have been described elsewhere (Brousseau and Hoeg, 1999; Fan and Watanabe, 2003).

Transgenic Rabbit Models for Long QT Syndrome

The long QT (LQT) syndrome is an inherited disorder of cardiac rhythm and is usually associated with autosomal dominant mutations. This disease is characterized by prolongation of the QT interval in the surface electrocardiogram (ECG), spontaneous polymorphic ventricular tachycardia and sudden cardiac death (SCD) in young patients (Moss, 2003). At least nine different forms of the disease have been identified so far, but among the genotyped individuals, mutations in genes encoding repolarizing K^+ channels (LQT1:KCNQ1; LQT2:KCNH2) are found in 90% of affected individuals as the underlying cause and many of the mutations show a dominant negative mechanism (Roden, 2006). In these patients, ventricular arrhythmias and sudden death are often triggered by emotional stress and/or physical exercise. In female LQT syndrome patients, an increase in the incidence of arrhythmias has been reported with menses and during the first postpartum months (Seth et al., 2007).

In some experimental studies, transgenic mouse models of LQT syndrome have been instrumental in understanding the assembly and role of potassium channels in regulating heart repolarization (Nerbonne et al., 2001). However, the mouse heart is small, the resting heart rate is 310 beats/minute, and action potential duration (APD) is very short. Additionally, the main repolarizing currents in mice are carried by

different channels compared with human, which limits the applicability of studies in the mouse to human patients (Nerbonne et al., 2001). In contrast, the rabbit heart is much larger and has a much slower heart rate (130 beats per minute at rest). Most importantly, the rabbit heart is also more similar to the human heart in terms of the contractile proteins that are expressed as well as the ion channels important for repolarization (Vermeulen et al., 1994). Furthermore, the rabbit heart has the same K^+ current components, namely the rapidly activating component (I_{kr}) and the slowly activating component (I_{ks}), which determine the repolarization of the action potential (AP). Thus, the rabbit represents a more physiologically relevant model system to study diseases involving these channels.

Transgenic rabbits expressing pore mutants of the human genes *KCNQ1* (KvLQT1-y315S) and *KCNH2* (HERG-G628S), which lead to LQT1 and LQT2 phenotypes, respectively, have been successfully created and characterized (Brunner et al., 2008). Over-expression of these transgenes is targeted in cardiomyocytes of the rabbit heart under the control of the rabbit β -myosin heavy chain (β -MyHC) promoter. Characterization of the transgenic rabbits revealed that prolongation of the QT interval and APD at 90% repolarization (APD90) were due to the elimination of I_{ks} and I_{kr} currents, respectively. Clinically, the elimination of I_{ks} and I_{kr} has different effects. LQT1 rabbits show QT prolongation but neither spontaneous arrhythmias nor an increase in sudden death. In contrast, LQT2 rabbits have a more pronounced QT prolongation at slow heart rates and show both spontaneous arrhythmias and a significant increase in sudden death after the age of about 6 months, consistent with the end of puberty in rabbits. Additionally, LQT2 females exhibit a high incidence of sudden death during lactation, an observation similar to the increased incidence of arrhythmias in women with the LQT2 syndrome postpartum (Seth et al., 2007). It has been hypothesized that sex hormones modulate cardiac repolarization and arrhythmogenesis in LQT2 females.

This transgenic rabbit model has also been used to study the side effects of anesthetic agents in individuals genetically predisposed to SCD in a subsequent study (Odening et al., 2008). In this study, five common anesthetic agents, including isoflurane, thiopental, midazolam, propofol, and ketamine, were investigated. The results showed that (1) isoflurane resulted in a prolonged QT interval in LQT2 but not in LQT1 rabbits; (2) thiopental prolonged the QT interval in both QT1 and QT2, but less pronounced in LQT1 rabbits; (3) midazolam prolonged the QT duration in both LQT1 and LQT2, but not in controls; and (4) propofol significantly increased the QT interval in both LQT1 and LQT2 rabbits as well as controls. Additionally, signs

of altered repolarization and arrhythmias were noted only in LQT2 rabbits during the monitoring periods under anesthesia. Multiple premature ventricular contractions, which can have a marked effect in humans, occurred in many LQT2 rabbits under midazolam, ketamine, or thiopental. Furthermore, it was also noted that isoflurane and propofol were especially proarrhythmic in LQT2 rabbits and led to sudden death in 30% of LQT2 rabbits. Results from this study indicate the importance of careful study of ECG by anesthesiologists prior to surgery, which may help in selecting more appropriate anesthetic agents. It is anticipated that the transgenic rabbit models could play a major role in identifying mechanisms of SCD and could be used to screen drugs that interact with the Human Ether-à-go-go Related Gene (hERG) potassium channel, causing QT prolongation and potentially fatal arrhythmias.

Transgenic Rabbit Models for Hypertrophic Cardiomyopathy

Hypertrophic cardiomyopathy (HCM) is a genetic disease characterized by cardiac hypertrophy, myocyte disarray, interstitial fibrosis, and left ventricular (LV) dysfunction (Wigle et al., 1995). Certain mutations in genes for sarcomeric proteins have been identified as causes of HCM (Marian and Roberts, 1998). The most common gene responsible for HCM is the β -myosin heavy chain (β -MyHC), in which a point mutation accounts for 35–50% of HCM cases (Watkins et al., 1995). The mechanism and molecular pathogenesis of the mutant sarcomeric proteins, including mutant β -MyHC, in HCM is well understood (Marian and Roberts, 1995). However, a desirable small animal model for identifying new therapeutic targets and diagnostic methods for HCM was not available until the successful generation of a transgenic rabbit model for HCM (Marian et al., 1999). Although transgenic mice expressing a variety of mutant sarcomeric proteins showed the phenotypes of premature death, cardiac myocyte disarray, interstitial fibrosis, and systolic and diastolic dysfunction, the mice did not develop left ventricular hypertrophy, the hallmark of HCM in human patients (Muthuchamy et al., 1999; Oberst et al., 1998; Vikstrom et al., 1996). Additionally, the composition of cardiac sarcomeric proteins between humans and mice are very different, as β -MyHC is pre-dominant in the human ventricle but α -MyHC is pre-dominant in the mouse ventricle (Swynghedauw, 1986). In contrast to the mouse, β -MyHC is the major myosin isoform in rabbit myocardium (Kavinsky et al., 1984), and it has an approximately 98% homology to the human β -MyHC protein (Jaenicke et al., 1990). Since it is known that MyHC isoforms act significantly different in actin-activated My-ATPase activity and cross-bridge kinetics (Sugiura et al., 1998), difference in cardiac sarcomeric

protein composition can affect the phenotypic response of the heart to mutant protein. Therefore, the rabbit provides a more reasonable model for studying HCM compared to mice.

To establish a transgenic rabbit model for HCM, Marian and colleagues (Marian et al., 1999) generated transgenic rabbits by targeted cardiac expression of the mutant β -MyHC-Q403, known to cause HCM in humans (Watkins et al., 1992). Expression of the mutant β -MyHC-Q403 protein in the transgenic rabbits induced virtually identical phenotypes to that of HCM caused by the mutant gene in humans, including cardiac hypertrophy, myocyte and myofibrillar disarray, excess interstitial collagen and high incidence of premature death. The expression of comprehensive phenotype of HCM, plus the feasibility of high-resolution serial echocardiograph, has made the β -MyHC-Q403 transgenic rabbits an ideal model for pathogenic and therapeutic studies of HCM.

In several subsequent studies, this transgenic rabbit model has been used to test a variety of drugs for treatment of HCM. These studies revealed that atorvastatin could prevent development of HCM in young transgenic rabbits (Senthil et al., 2005), and simvastatin induced regression of cardiac hypertrophy and fibrosis and improvement of cardiac function (Patel et al., 2001). Most interestingly, a recent study demonstrated that treatment with N-acetylcysteine, a safe prodrug against oxidation, reversed established cardiac hypertrophy and fibrosis and prevented systolic dysfunction (Lombardi et al., 2009). Furthermore, this transgenic rabbit model has also been used to explore more sensitive and accurate methods for early diagnosis of HCM. It was reported that tissue Doppler imaging could consistently detect myocardial contraction and relaxation abnormalities, irrespective of cardiac hypertrophy in the transgenic rabbits and was shown to be more sensitive than conventional echocardiography for HCM scanning (Nagueh et al., 2000). This finding has been confirmed in human patients with HCM in a study that showed Doppler imaging to be an accurate and sensitive method for an early diagnosis before and independent of hypertrophy (Nagueh et al., 2001).

Transgenic Rabbit Models for HIV-1 Infection and AIDS

A major obstacle to study HIV-1 infection and AIDS development is the lack of a suitable small animal model for testing diagnostic, therapeutic, and preventive modalities. Non-human primate species as the ultimate model are either endangered, not generally available, or expensive. Even among the non-human primates, only the chimpanzee and gibbon ape could be infected by HIV-1 (Agy and Katze, 1993). Native rodents are non-permissive for HIV-1 infection. Even

expression of human CD4 (HuCD4), the known principal receptor for HIV-1, failed to render mouse cells permissive to the HIV-1 infection (Lores et al., 1992). In contrast, the rabbit seems relatively more susceptible to HIV-1 infection compared to rodents, as supported by the reported successful infection of normal rabbit cells (Kulaga et al., 1989) and rabbits (Filice et al., 1988; Gordon et al., 1990; Kulaga et al., 1989; Varnier and Kindt, 1992) although the infection was much less efficient than in human lymphocytes and no consistent signs of AIDS-like disease were observed in the infected rabbits. It was suggested that a rabbit CD4 (RbCD4)-like receptor might be sufficiently homologous to HuCD4 to interact with HIV-1, since soluble human CD4 treatment abrogated infection of rabbit T cells by HIV-1 (Hague et al., 1992). However, sequence comparisons of RbCD4 cloned from a rabbit thymus cDNA library showed that six of the 18 residues implicated in HIV-1 binding by CD4 differ between the human and rabbit proteins, and no correlation between RbCD4 expression by rabbit cell lines and their ability to support HIV-1 infection was seen (Hague et al., 1992). A subsequent study demonstrated that expression of HuCD4 in rabbit peripheral blood mononuclear cells (PBMC) could enhance the infection of the cell by HIV-1 and result in a rapid depletion of the infected cells (Leno et al., 1995). Furthermore, two groups then simultaneously reported the successful generation of HuCD4 transgenic rabbits (Dunn et al., 1995; Snyder et al., 1995). Snyder et al. (1995) reported that HuCD4 expression was restricted to RbCD4 expression lymphocytes, and peripheral blood lymphocytes (PBLs) from HuCD4 transgenic rabbits produced greater amounts of HIV-q p24 core protein following HIV-1 infection in vitro than the cells from non-transgenic rabbits. No in vivo HIV-1 infection study in these transgenic rabbits has been reported, though in vitro studies by Dunn et al. (1995) demonstrated enhanced susceptibility of lymphocytes derived from HuCD4 transgenic rabbits to HIV-1 infection. Furthermore, in vivo HIV-1 infection of the HuCD4 transgenic rabbits was demonstrated by virus isolation, detection of HIV-1-specific DNA in peripheral blood leukocytes (PBLs) and seroconversion to various HIV-1 proteins. However, no signs of AIDS-like disease were observed in the infected transgenic rabbits, indicating that these HuCD4 transgenic rabbits were still not sufficiently susceptible to HIV-1 infection for disease development. One hypothesis was that some co-receptors might be involved in susceptibility to HIV-1 infection, such as the β -chemokine receptor CCR5 which was demonstrated as an essential receptor for HIV-1 entry into cells (Deng et al., 1996; Ghorpade et al., 1998). An in vitro study demonstrated that rabbit cells expressing both HuCD4 and human CCR5 were highly permissive for HIV-1 infection and viral

replication in rabbit cells could approach the level seen in human cells (Speck et al., 1998). Additionally, HIV-1 virions produced in rabbit cells were able to support spreading infection and successfully infected human PBMC. These findings suggested that transgenic rabbits expressing both human CD4 and CCR5 genes might support more reasonable levels of HIV-1 replication in vivo and serve as a useful small animal model. However, such a transgenic rabbit model has not been reported so far, although it has been shown that mice with such transgenes were still insufficient to support HIV-1 infection (Browning et al., 1997). It is very possible that the mechanism for HIV-1 infection might be more complicated than first believed and some additional co-factors might be involved.

Transgenic Rabbit Models for Human Papillomavirus Infection

Papillomaviruses are a group of small DNA viruses associated with neoplasia of skin and other epithelia in some species of animals (Campo, 2002) and humans (zur Hausen, 2002). Due to the restricted species specificity of these viruses, a natural animal model is not available to study the infection and pathogenesis of human papillomaviruses (HPV), most notably the so-called oncogenic HPVs (16, 18, 31, 33) since they are associated with the development of most cervical cancers. Cottontail rabbit papillomavirus (CRPV) has long been used as an animal model to study the pathogenesis and development of prophylactic and therapeutic strategies for oncogenic HPV infection due to its similarity in genomic structure and tumorigenesis (Christensen, 2005; Kreider and Bartlett, 1985).

Epidemiological and molecular studies indicate that, although the oncogenic HPVs have been found in more than 90% of cervical cancers, the viruses alone are insufficient to cause malignant transformation, for which some co-factors (such as inactivation of tumor suppressor genes and/or activating mutation of cellular oncogenes, etc.) could be required (zur Hausen, 2002). Some preliminary studies found that activated Ha-ras, an oncogene found in many human malignant tumors including some HPV 16- or 18-related cervical cancers, was required for malignant transformation of HPV16-immortalized human cervical cells (DiPaolo et al., 1989); and both HPV16 DNA and EJras were required for transformation of primary cells (Matlashewski et al., 1987). To study the interaction between oncogenic papillomavirus and potential cofactors in vivo, transgenic rabbits carrying CRPV genome alone or carrying both CRPV genome and EJras oncogene were generated (Peng et al., 1993). Analyses of these transgenic rabbits showed that the rabbits expressing CRPV E6/E7 alone developed only skin papillomas, but the rabbit expressing both CRPV E6/E7 and EJras oncogene was born

with extensive skin squamous cell carcinomas (SCC). In a subsequent study, transgenic rabbits with targeted expression of EJras in the skin by CRPV upstream regulatory region showed growth of keratoacanthomas in the first week of age (Peng et al., 1999). The keratoacanthomas were morphologically similar to the tumors in humans and spontaneously regressed at about 2 months of age. After complete regression of the keratoacanthomas, the EJras expression was undetectable and there was no growth of new tumors. However, a further study showed that CRPV infection of the skin of the transgenic rabbits at 2 months of age (after regression of keratoacanthomas) could re-initiate the expression of EJras transgene and accelerate tumorigenesis compared to non-transgenic rabbits (Peng et al., 2001). Results from studies of these transgenic rabbits clearly indicate that activated H-ras oncogene could increase the tumorigenesis/carcinogenesis of the oncogenic papillomavirus infection. This synergistic effect has been further confirmed by a recent human study showing that H-ras mutation was identified in 21% of patients with cervical tumors with poor or moderated differentiation (Landro et al., 2008). However, the intrinsic mechanisms of the synergistic effect between activated H-ras and oncogenic papillomaviruses in carcinogenesis need to be further elucidated.

To assess host immune response to HPV infection, preclinical models are required for initial vaccine and immunotherapeutic testing. However, the restricted species specificity of papillomaviruses makes these studies particularly challenging (Fausch et al., 2003). Currently used preclinical models of natural papillomavirus infection include rabbit, dog, and bovine (Campo, 2002). A natural rodent model for papillomavirus infection and testing cell-mediated immune responses is currently not available. The species restriction barriers of papillomaviruses prevent infection and replication of HPVs in all immunocompetent laboratory animals. Among the natural papillomavirus infection models, the cottontail rabbit papillomavirus model offers several advantages as a preclinical model for studying host immunity to papillomavirus infection (Christensen, 2005). One advantage is that papillomas can be generated by direct infection of skin with naked viral DNA (Brandsma and Xiao, 1993; Kreider et al., 1995). This feature provides opportunities for genetic modification of the viral genome by site-directed mutagenesis, which can be used to induce epitopes into the various viral genes for testing specific immunity. It has been shown that an HPV16 E7 T cell epitope can be engineered into the E7 gene of the CRPV genome without influencing the tumorigenesis of the modified virus. To assess immunity to the HPV epitopes in an infection model for papillomaviruses, a transgenic rabbit model expressing HLA-A2.1 gene, a well-characterized human

major histocompatibility complex class I (MHC-I) gene, has been established (Hu et al., 2006, 2007). HLA-A2.1 expresses and co-localizes exclusively with rabbit MHC-I on the cell surface of all transgenic rabbits. The transgenic rabbits vaccinated with a HLA-A2.1-restricted HPV16 E7 multivalent epitope (82-90) DNA vaccine showed complete protection against infection with modified CRPV DNA containing an embedded HPV16 E7/82-90 epitope (Hu et al., 2006). Similar results were observed from testing several HLA-A2.1-restricted epitopes from CRPV E1 in the transgenic rabbits (Hu et al., 2008). Data from these studies indicate that the HLA-A2.1 transgenic rabbit model will provide opportunities to directly test HLA-A2.1-restricted epitopes of HPV proteins in the context of a human MHC class I gene. Furthermore, this transgenic rabbit model may also have potential application in testing CD8⁺ CTL-inducing vaccines against other human pathogens that are permissive or semi-permissive in rabbits, such as human T cell leukemia virus (Franchini et al., 1995), adenovirus (Romanowski et al., 2002), EBV-like viruses (Hayashi et al., 2003), and tuberculosis (Dorman et al., 2004).

Transgenic Rabbit Models for Retinal Degeneration

Retinitis pigmentosa (RP) includes a group of inherited retinal disorders characterized by a progressive loss of rod and cone photoreceptors and eventual atrophy of the entire retina (Hartong et al., 2006). RP is genetically heterogeneous, associated with mutations in several photoreceptor-specific and some non-specific genes (Daiger et al., 2007). To date, mutations in the rhodopsin gene have been identified as the most prevalent class, causing approximately 25–40% of the autosomal dominant RP cases (Dryja et al., 1991).

To develop a model for studying the pathophysiology and developing new treatments for this disease, transgenic rabbits expressing rabbit rhodopsin with a point mutation (Pro347Leu) have been generated (Kondo et al., 2009). In total, six lines of transgenic rabbits were established from pronuclear injection of a BAC (bacterial artificial chromosome) transgenic construct carrying the rabbit rhodopsin mutant gene Pro347Leu. Further studies on two of these lines with high levels of the transgene expression showed rod-dominant progressive retinal degeneration. Retinal histology exhibited a marked regional variation in the loss of photoreceptors with the central retina more severely affected than the peripheral retina. Assessment of retinal function by electroretinograms (ERGs) in the transgenic rabbits indicated early loss of rod function associated with relatively preserved cone function, which is very similar to the clinical signs found in human RP patients with the rhodopsin Pro347Leu mutation (Berson et al., 1991; Oh et al., 2003). Combined

with large eyes and easier handling and breeding, transgenic rabbits may provide a useful animal model for studying the pathophysiology of retinal degeneration and developing novel treatment for this disease.

Transgenic Rabbit Bioreactors for Production of Clinically Useful Proteins

Large and medium-sized transgenic animals, including cows, pigs, sheep, goats, and rabbits, have been used as bioreactors for production of human proteins for potential therapeutic and diagnostic use (Houdebine, 1995, 2000). By controlled tissue-specific expression, large amounts of human recombinant proteins can be produced and purified from milk, blood, or urine of transgenic animals. There are several advantages to obtaining therapeutic proteins from transgenic bioreactors compared to proteins from other sources (Fan and Watanabe, 2003). First, proteins from bioreactors may reduce contamination by pathogens, which is a major concern for using proteins directly isolated from human sources. Second, the native activity of foreign proteins expressed in bioreactors can be better preserved than that of recombinant proteins derived from bacteria or yeast. It has been known that bacteria do not add carbohydrates to polypeptide chains so that not all proteins generated in bacteria can necessarily form their mature native structure. Additionally, the post-synthesis modifications such as carboxylation, glycosylation, and amidation, which are essential for full biological function of many proteins, could be accomplished in the mammary glands of transgenic bioreactors (Houdebine, 1995, 2000). The choice criteria for selecting the most suitable species as bioreactor for production of human proteins is usually based on the quantity of protein needed per year. The production of protein needed in large amounts (tons to hundreds kg per year) should be carried out in cows, sheep, or goats, whereas rabbits can be used as an intermediate producer for proteins needed in moderate amounts (kilograms per year) (Castro et al., 1999). Compared to large farm animals, transgenic rabbits as bioreactors have some unique features. Rabbits have much shorter reproductive cycles and larger litter size; and are ready to breed at most times, including during postpartum, in all different seasons. Therefore, large numbers of rabbits can be produced in a short period of time. Additionally, generation of transgenic rabbit founders is relatively easier and more efficient than generation of other transgenic farm animals. A transgenic lactating doe can yield up to 10kg of milk per year, which can be collected under semi-automatic hygienic conditions (Duby et al., 1993). Rabbit milk, which has been well characterized, naturally contains about 14% protein compared to approximately 3–6% in the milk of goats, cows, and

TABLE 7.4 Transgenic Rabbit Bioreactors for Production of Clinically Useful Proteins

Proteins	Expression site	References
Human interleukin 2	Mammary glands	(Buhler, et al., 1990)
Human α 1-antitrypsin	Blood	(Massoud, et al., 1991)
Human tissue plasminogen activator	Mammary glands	(Riego, et al., 1993)
Human insulin-like growth factor	Mammary glands	(Brem, et al., 1994)
Human insulin-like growth factor	Mammary glands	(Wolf, et al., 1997)
Human insulin-like growth factor	Mammary glands	(Zinovieva, et al., 1998)
Human erythropoietin	Mammary gland	(Rodriguez, et al., 1995)
Human erythropoietin	Mammary glands	(Massoud, et al., 1996)
Human erythropoietin	Mammary glands	(Korhonen et al., 1997)
Human growth hormone	Mammary glands	(Limonta et al., 1995)
Human extracellular superoxide dismutase	Mammary glands	(Stromqvist et al., 1997)
Salmon calcitonin	Mammary glands	(McKee et al., 1998)
Human alpha-glucosidase	Mammary glands	(Bijvoet et al., 1999)
Human nerve growth factor β	Mammary glands	(Coulibaly et al., 1999)
Human blood clotting factor VIII	Mammary glands	(Hiripi et al., 2003)
Human blood clotting factor VIII	Mammary glands	(Chrenek et al., 2007)
Bispecific antibody	Blood	(Grosse-Hovest et al., 2004)
Vaccinating recombinant rotavirus proteins (VP1, VP2)	Mammary glands	(Soler et al., 2005)

sheep (Duby et al., 1993). Furthermore, rabbit colonies can be easily maintained under specific pathogen-free conditions in barrier facilities. All these features make rabbits highly attractive to be used as bioreactors for production of recombinant human proteins, especially the proteins expressed in the rabbit mammary glands.

Medical products which can be expressed in transgenic rabbits may be classified into three major categories: (a) therapeutic proteins, (b) hormones and bioactive peptides, and (c) monoclonal antibodies (Bosze et al., 2003). A number of recombinant proteins have been specifically expressed in the milk or blood of transgenic rabbits (Table 7.4). Among these proteins, human alpha-glucosidase derived from transgenic rabbit milk (Bijvoet et al., 1999) is the first transgenic rabbit product that has been used in clinical trials for the treatment of Pompe's disease (Van den Hout et al., 2000, 2001). Pompe's disease is a lysosomal glycogen storage disorder involving heart, smooth and skeletal muscles, caused by acid alpha-glucosidase deficiency. The rapidly progressive infantile form is the most serious form of this disease and is commonly fatal in the first year of life. There is currently no approved treatment for this fatal disease, but several recent clinical trial studies have shed hopeful light for patients of this disease. In an open-label pilot study, four babies (2.5–8 months

old) with typical symptoms of infantile Pompe's disease and virtual absence of alpha-glucosidase were treated by an enzyme replacement therapy with intravenous infusion of recombinant human alpha-glucosidase (rhGLU) purified from transgenic rabbit milk at a starting dose of 15 mg/kg or 20 mg/kg, and then increased to 40 mg/kg. The study demonstrated that alpha-glucosidase activity reached the normal range in all the patients treated with 40 mg/kg rhGLU for 12 weeks. Histological assessment showed lowered lysosomal glycogen storage and improved tissue morphology (Van den Hout et al., 2000). Furthermore, a long-term follow-up study revealed that all the patients survived beyond the age of 4 years, in contrast to untreated patients who succumbed at a median age of 6–8 months (Van den Hout et al., 2004). Additionally, a separate study investigated the therapeutic effect of rhGLU from the rabbit milk in late-onset Pompe's disease. Three patients aged 11, 16, and 32 years respectively were enrolled in the study. All the patients were wheelchair-bound and two of them were ventilator-dependent with a history of deteriorating pulmonary function. After 3 years of treatment with weekly infusion of the hrGLU, the patients showed stabilized pulmonary function and less fatigue. Most impressively, the youngest and least-affected patient could walk without support and finally

abandoned his wheelchair after 72 weeks of treatment (Winkel et al., 2004). These clinical trial studies provide very optimistic evidence for production of a safe and effective medicine in the milk of transgenic rabbits.

Transgenic rabbits have also been developed for the production (in milk) of important vaccine reagents for infectious diseases such as human rotaviruses. Rotaviruses are the main cause of viral gastroenteritis in young children worldwide leading to approximately 500 000 deaths annually, mostly in developing countries (Glass et al., 2004). For unknown reasons, vaccination with the live attenuated rotavirus has been associated with an increased risk of intussusceptions. To develop a safe and effective vaccine for this disease, production of recombinant VP2 and VP6 proteins in transgenic rabbit milk was reported (Soler et al., 2005). Evaluation of the recombinant viral proteins in a preliminary study showed immunogenic effects and significant reduction in viral antigen shedding after challenge with virulent rotavirus in an adult mouse model. This study has opened a new route for development of vaccines by expressing antigenic proteins in transgenic animal milk.

The rabbit has been commonly used in the production of polyclonal antibodies due to their relatively large body size and blood volume, easy access to the vascular system, and the existent of a large body of information on the purification of rabbit immunoglobulins. For many years, researchers have attempted to produce monoclonal antibodies (mAb) in rabbits since rabbits are known to produce antibodies to many antigens that are not especially immunogenic in mice or rats (Weidle et al., 1991). However, a plasmacytoma fusion partner, essential for production of mAb, was not available in rabbits. To obtain a rabbit plasmacytoma cell line that could be used as a fusion partner, Spieker-Polet et al. (1995) generated transgenic rabbits expressing both c-myc and v-able. These rabbits developed plasmacytomas from which several plasmacytoma cell lines were established. When the cells from a clone of one cell line fused with spleen cells of immunized rabbits, stable hybridomas that secreted antibodies specific for the immunogen were produced. This study demonstrated the possibility of using transgenic rabbits for production of mAb although no subsequent study has been reported. A more recent report presented the efficient production of a complex bispecific single-chain antibody in the serum of transgenic rabbits carrying the bi-scFv r28M DNA construct (Grosse-Hovest et al., 2004). Functional analysis showed that the serum purified bispecific protein, r28M with known anti-human melanoma specificity, was stable and fully active in mediating target cell-restricted T cell stimulation and tumor cell killing. This study further explored the possibility of producing high concentrations of fully active therapeutic molecules in transgenic animals.

CONCLUSIONS

The Broad Institute's Rabbit Genome Project is designed to encompass a deep 7× full coverage that will be both a comprehensive and powerful resource for rabbit researchers and the rabbit industry alike. Comparative genomics, disease traits, selection for resistance/susceptibility to important human pathogens (e.g. HIV, tuberculosis) will likely benefit from this new database. In addition, the domestic rabbit is an important research tool to study other genetic predispositions including inherited hyperlipidemia, atherosclerosis, glaucoma, diabetes, and for the production of polyclonal antibodies and biopharmaceuticals via transgenics. Transgenic rabbit models for studying the genetics of specific diseases will continue to expand our knowledge of these processes in humans and provide excellent preclinical models for therapeutic treatments. Rabbits also are excellent models to study immune responses to various pathogens and are used extensively to make specialized antibody reagents for the research community.

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Anatomy, Physiology, and Behavior

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INTRODUCTION

The rabbit is a popular model in laboratory animal medicine due to its relatively large size and docile nature. Though rabbits are sometimes mistakenly thought of as rodents, they are in their own Order Lagomorpha. Among the many breeds of rabbits, by far

the most popular in research is the New Zealand White rabbit. Research in these rabbits has contributed to advances in cardiology, orthopedics, dentistry, immunology, and more.

This chapter closely follows the outline presented in the anatomy and physiology chapter of the 1994 edition of *The Laboratory Rabbit*. In fact, the two chapters by

Cruise and Brewer have been extensively cited owing to the unchanging nature of anatomy.

more than females and dominant rabbits marking more than submissive rabbits.

EXTERNAL FEATURES

Rabbits have thin, delicate skin that is generously covered with both underfur and guard hairs (Harcourt-Brown, 2002). The rabbit skin, similar to the rat, has blood vessels immediately under the dermis (Cruise and Brewer, 1994). Unlike the rat, however, the fascia superficialis is well differentiated due to the elastic fibers and dense collagen content (Brewer, 2006). Hair grows in waves starting from the ventrum and grows dorsally and caudally. Thick fur covers the feet in place of footpads. The fragile skin can tear easily and must be handled carefully during any manipulation. Females have a large skin fold on the ventral surface of their neck called the dewlap (Figure 8.1). Breeding females pull hair from this area to create a nest before parturition (Meredith, 2000).

Rabbits have three scent glands: the chin glands, anal glands, and inguinal glands. The chin glands are specialized submandibular glands which open onto the ventral surface of the chin. The inguinal glands are pocket-like perineal glands that lay dorsal to the urogenital opening on either side of the anus. Both males and females have these glands, with males marking

Head and Neck

Rabbits have pinnae that are large, vascular and able to move independently of one another. They can represent a large part of the total body surface area (up to 12% in New Zealand Whites) (Brewer and Cruise, 1994). When dilated by heat, the rabbit ear shows the presence of large arteriovenous shunts and is thus an important mechanism for thermoregulation (Gordon et al., 1953).

Rabbit ears are fragile and should not be used for restraint. The dorsal skin is thick and contains many hair follicles, while the ventral skin has few hair follicles. The skin on the ventral surface of the ears contains large sebaceous glands that provide a convenient medium for carcinogen study, while those in the dorsum are smaller. The main vessels are the central ear artery and two lateral veins. The central ear artery runs towards the ear tip and bifurcates into two major branches showing a U-turn curve halfway up to the pinnae. Thus in the rabbit, the direction of blood flow in the rostral and caudal edges of the ear is from the pinnae to the base of the ear (Ninomiya, 2000). The central ear artery can be used for blood sampling as well as direct blood pressure measurements, though the pressure is approximately 10mmHg less than in the common carotid artery. Though the veins and artery in the ear can be used for catheters and blood draws, forceful venipuncture can cause vasculitis, vascular necrosis, and sloughing of the skin.

The rabbit visual field forms almost a complete sphere (Cruise and Brewer, 1994). Eyes are laterally placed, endowing rabbits with a broad field of vision, except for the area under their mouth, which they compensate for with their vibrissae and sensitive lips. The rabbit cornea is large, occupying up to 30% of the globe (Queensberry and Carpenter, 2004). The lens is spherical and large and the ciliary body is small and poorly developed. The fundus of the rabbit is merangiotic, where only part of the inner retina is supplied by retinal vessels. This is in contrast with a holangiotic fundus, seen in dogs, cats, cattle, goats, sheep, pigs, rats, mice, and primates, where the retinal blood supply is from central or cilioretinal arteries (Buyukmihci, 2010). In practical terms, this results in a band of blood vessels and myelinated nerve fibers crossing the retina in a horizontal plane from the optic disc (Williams, 2007) (blood vessels present only laterally and medially to the optic disc) as opposed to a holangiotic fundus that contains blood vessels throughout the retina (Keil, 2010). Because the optic disc lies above the horizontal midline of the eye, it requires the examiner to look upwards during a retinal exam (Harcourt-Brown, 2002). The

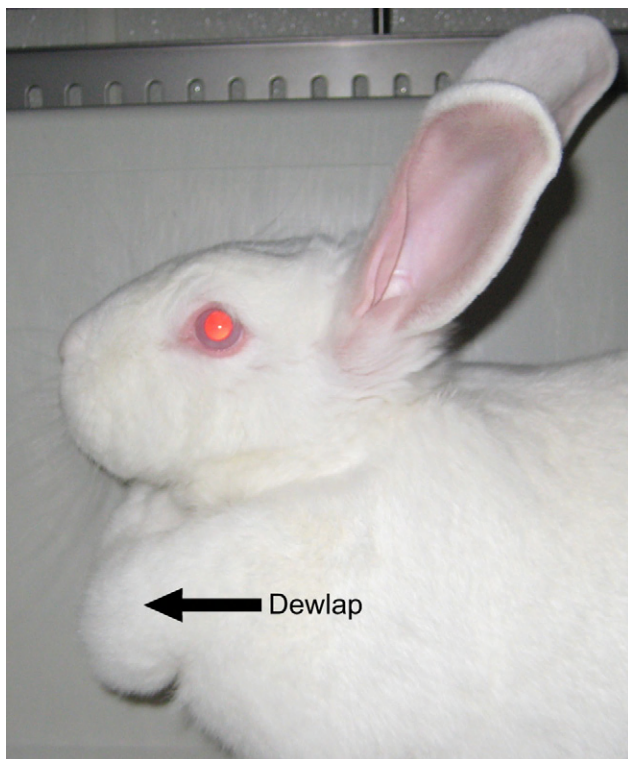


FIGURE 8.1 Female rabbit with prominent dewlap.

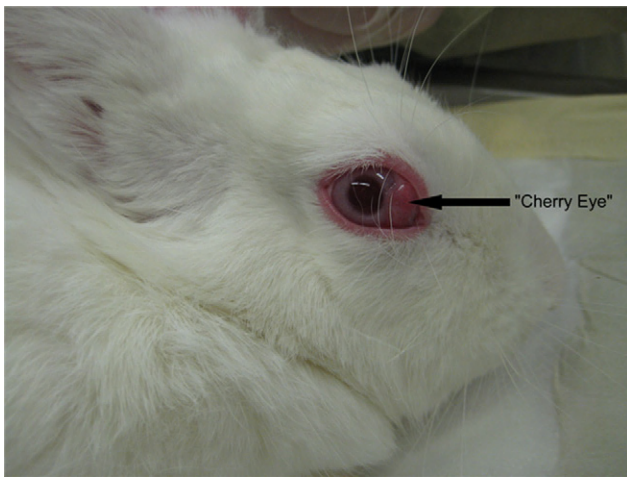


FIGURE 8.2 Rabbit with prolapsed gland of the nictitating membrane.

optic disc has a physiologic cup and the eye does not have a tapetum lucidum, similar to pigs, guinea pigs, primates, and birds (Ollivier et al., 2004).

Rabbits have a third eyelid that moves across the cornea during anesthesia, though it only covers two-thirds of the cornea (Harcourt-Brown, 2002). It also covers a Harderian gland which has a small, white upper lobe and a larger, pink lower lobe. This lower lobe, also known as the deep gland of the nictitating membrane, can prolapse, similar to a “cherry eye” in a dog (Figure 8.2). Because rabbits often freeze on examination, many rabbits fail to exhibit a menace response (Vernau et al., 2007).

Rabbits have an extensive orbital venous plexus, which can make enucleation of the eye complicated. It has been suggested to perform enucleations transconjunctivally, remaining as close to the globe as possible, instead of transpalpebrally, in order to avoid this structure (Williams, 2007). In addition, a large mediastinal mass can cause compression of venous return to the heart, engorging the retrobulbar plexus and resulting in exophthalmos (Wagner et al., 2005). The rectus dorsalis muscle lies several millimeters posterior to the limbus, under the bulbar conjunctiva. Because it is visible through the dorsal conjunctiva, unlike in humans, dogs, or cats (Keil, 2010), it is possible to access this muscle during surgery and use it to stabilize the globe. Other species require dissection to expose the muscle.

The small nasolacrimal duct follows a convoluted path through the lacrimal and frontal bones, ending in a single nasolacrimal punctum on each side. The duct passes close to the molar and incisor tooth roots and can therefore be affected by dental disease (Marini et al., 1996). At the level of the proximal maxillae and the base of the upper incisor, the duct narrows and is a common place for obstruction, resulting in epiphora.

Recent work has detailed the anatomic and structural characteristics of the lacrimal duct system (Ding et al., 2010). Review of published literature has shown that the rabbit lacrimal system more closely resembles that of humans rather than that of rats or mice (Schechter et al., 2010). The smallest branches of the duct system are the intercalated ducts, composed of single-layer cuboidal cells. Several intercalated ducts join to form larger intralobular ducts, which are also cuboidal in shape. Intralobular ducts merge to form interlobular ducts, which drain several lobules and are comprised of simple cuboidal to low columnar cells. From here the interlobular ducts merge into intralobar ducts, which drain individual lobes. The cells here are low to tall columnar cells and range from simple to pseudostratified. Finally, these converge into interlobar ducts, which are of variable size depending on the surrounding tissue. These cells are composed of simple to pseudostratified and stratified columnar cells and the number of cells increases as they approach the main excretory duct (Ding et al., 2010).

Trunk

The trunk is composed of the thorax, abdomen, and back. Females have eight to ten nipples while males have similarly placed rudimentary nipples. The anal opening lies distal to the base of the tail and dorsal to the urogenital opening. The anus is surrounded on both sides by hairless inguinal spaces. The male's urogenital opening continues into a preputial fold which forms a sheath for the penis. In the female, this skin fold forms the vulva, which contains the small clitoris on the ventral wall. In males, the scrotal sacs containing the testes are located on either side of the penis.

Extremities

The forelimb is composed of the shoulder, upper arm, forearm, and hand. The hind limb is divided into the thigh, leg, and foot. The front legs have five digits and the rear legs have four, all of which extend into a long, curved claw.

OSTEOLOGY

The rabbit's skeleton is delicate in relation to its size. The skeleton makes up approximately 8% of their body weight in comparison to a cat, whose skeleton represents 12–13% of its body weight (Cruise and Brewer, 1994). Strong epaxial muscles in the presence of a relatively weak lumbar spine predisposes rabbits to fracture of their lumbar spine (Suckow and Douglas, 1997), usually at the seventh lumbar vertebrae (Queensberry and Carpenter, 2004).

Axial Skeleton

Skull

The rabbit skull has a number of bones with a spongy texture and wide medullary cavities, in contrast to the smooth, hard skull of the dog (Whitehouse and Grove, 1958). The caudal (nuchal) surface of the skull is composed of the occipital bone, which in turn is formed by the supraoccipital, paired lateral exoccipitals, and the ventral basioccipital. The foramen magnum lies within this space and with its lateral occipital condyles creates the articulation for the atlas. The external occipital protuberance creates a sharp ridge that separates the dorsal surface of the skull from the nuchal surface. The dorsal ligament of the neck and occipital muscles attach to this external occipital protuberance.

The dorsal surface of the skull is formed by the dorsal surface of the occipital, the small interparietal, the paired frontal bones, and the nasal bones. The frontal bones extend ventrally to form part of the orbital wells.

The bulla is formed by the tympanic bone and holds the large tympanic cavity and the three ossicles of the middle ear. It continues dorsally into a short wide bony tube with a large opening. This entire tube is the external auditory meatus (Cruise and Brewer, 1994). The rabbit's tympanic bullae are much larger than most mammals, with a longer and more tubular external auditory meatus (Wingerd and Stein, 1985). The tympanic bullae also appear to be rounder in shape and do not project ventrally beyond the level of the occipital bone as they do in the dog and cat (King et al., 2006).

The squamosal bone, which forms a large portion of the lateral wall of the cranium, lies anteriodorsal to the bulla. A prominent protuberance, the zygomatic process, forms the caudal end of the zygomatic arch. The zygomatic arch is flatter than that of the dog resulting in a narrower skull, which aids the rabbit in burrowing (Osofsky et al., 2007).

The mandibular fossa fits the head of the mandible, and is formed by the hollowed out ventral side of the zygomatic process. The alisphenoid is bordered caudally by the squamosal, and cranially by the orbitosphenoid, which is pierced by the optic foramen. The cephalic wall of the orbit is formed ventrally by the maxilla and dorsally by the lacrimal bone, which extends beyond the orbital rim. The maxilla extends rostrally from the orbit and its lateral zygomatic process, from the rostral end of the zygomatic or jugal bone, forms the main portion of the arch. The bilateral alveolar processes or ventral portion of the maxilla contains the roots of the incisors, and creates the rostral, ventral, and lateral boundaries of the nasal bone.

The ventral portion of the cranium comprises the medially located basioccipital, basisphenoid, and presphenoid bones rostral from the foramen magnum.

The basisphenoid is perforated by the round foramen cavernosum which leads into the interior of the bone. The hypophyseal fossa is in the floor of the cranial vault. The ventral aspect of the skull is continued by the palatine bones, where the caudal ends articulate with the two laminae of the pterygoid process of the alisphenoid bone. A thin, dorsal extension of the palatine bone can be visualized from the wall of the orbit. The palatine continues forward medial to the two alveolar processes of the maxilla where it articulates and courses medially to form the caudal portion of the hard palate. The hard palate continues rostrally from the ventral portions of the maxilla and premaxilla.

The nasal cavity is divided into the right and left fossae by the cartilaginous portion of the mesethmoid. It continues caudally with the perpendicular plate of the ethmoid bone which consists of the cribiform plate and paired lateral ethmoidal labyrinths. Caudally, the ventral portion of the cartilaginous septum is supported by the vomer bone. The turbinate bones are located on the lateral walls of the nasal cavity.

The two sides of the mandible join into a fibrous or fibrocartilaginous symphysis. The body, or the horizontal portion, contains the teeth while the caudal vertical portion comprises the ramus.

The hyoid apparatus is comprised of the median hyoid bone and the paired greater and lesser cornua. These are connected laterally by two muscles of the jugular process of the occipital bone. The entire apparatus is enveloped at the base of the tongue and lies between the angles of the mandible.

Vertebral Column

The typical vertebral formula is C7T12L7S4C16. However, the number of vertebrae can vary, with 44% of rabbits having 12T/7L, 33% having 13T/6L, and 23% with 13T/7L. The spinal cord ends within S2 in 79% of rabbits, S1 in 19%, and S3 in 2% or rabbits (Greenaway et al., 2001). All of the lumbar vertebrae have prominent mammillary processes of the cranial articular process, where the powerful lumbar muscles attach to the vertebrae (Whitehouse and Grove, 1958). Unlike other domestic animals, the dorsal aspect of the lumbar vertebral mammillary process is level with and slightly ventral to the spinous process. The first three vertebrae of the sacrum are fused, while the fourth sacral vertebra is variably fused (Whitehouse and Grove, 1958) with the third vertebra.

Ribs and Sternum

The first seven ribs are true ribs and articulate with the sternum, while the last five ribs (false ribs) do not. The heads of the ribs articulate with the corresponding thoracic vertebra and the caudal part of the body of the vertebrae cranial to it. The costal cartilage of the

seventh, eighth, and ninth ribs are attached to each other. The cartilages of the last three floating ribs are not attached to any others and therefore lie free. The sternum is composed of six sternbrae. The first is the manubrium sterni and the sixth is the xiphoid process with a distinct, thin, broad plate of cartilage protruding from the caudal end.

Appendicular Skeleton

Pectoral Girdle and Limb

The pectoral girdle is made up by the scapula and paired clavicles. The only direct attachment to the axial skeleton is the sternoclavicular ligament (Cruise and Brewer, 1994). The other major attachments are via the musculature. The humerus, radius, and ulna are typical of the mammalian family. The carpus is comprised of two rows of bones. The distal row articulates with the five metacarpal bones and there are five digits. The first digit has two phalanges while the four other digits have three.

Sesamoid bones are located on the palmar and plantar surfaces of the feet and occur in transverse pairs at the metacarpophalangeal junction and in linear pairs at the articulations of the second and third phalanges.

Pelvic Girdle and Limb

The ilium, ischium, and pubis fuse to form the pelvic girdle which is attached to the vertebral column at the iliosacral articulation (Cruise and Brewer, 1994). The os acetabuli, a small accessory bone, together with the ilium and ischium, join to form the acetabulum in the pelvic girdle. The femur attaches only to the tibia while the fibula fuses distally with the tibia for half its length. There are six tarsal bones arranged in three rows. The proximal row has two large bones, the middle row one bone, and the distal row three bones. The first metatarsal is very small, while metatarsals two through five are well-developed. The four digits of the hindlimbs each have three phalanges (Cruise and Brewer, 1994). The sesamoid bones in the pelvic limbs are the patella, the three bones located posterior to the stifle joint, and the small ones on the plantar surface of the foot by the metatarsophalangeal joints and between the second and third phalanges.

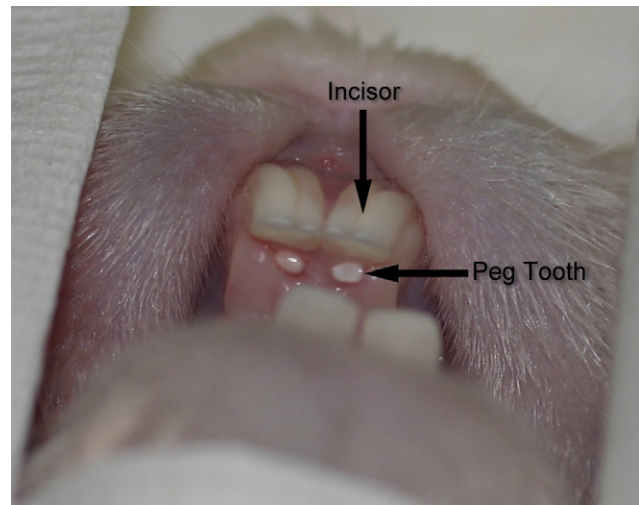


FIGURE 8.3 Peg teeth.

Rabbits have hypsodont dentition and there is a long crown in lieu of a true root. The permanent teeth grow in by 3–5 weeks old. The upper and lower incisors grow 10–13 cm a year (Jenkins, 2001). The grinding action against opposing teeth keeps the teeth worn. Dental problems are a common sequellae if there is malocclusion or dislocation. A pair of small incisors, known as peg teeth (Figure 8.3) lies caudal to the larger incisors in the upper arcade. The upper lip has a divided groove (hence the expression “harelip”) that continues curving up right and left of the nostrils. There are 25–30 tactile vibrissae, which together with the cleft lip allow rabbits to locate and eat their food. Rabbits also have a diastema, a space between the incisors and premolars, which together with the “hare-lip” and rostrally positioned incisors help to cut off short pieces of vegetation. Rabbits chew quickly and thoroughly, with jaw movements up to 120 per minute (Cruise and Brewer, 1994).

Tongue

The rabbit's tongue is large in comparison to the size of the animal. The dorsal surface is covered with small elevations called papillae, which give the tongue its roughened surface. There are four types: vallate, foliate, fungiform, and filiform. All but the last type have associated taste buds. The rabbit's large tongue and small mouth opening together make visual intubation of the trachea virtually impossible without specialized equipment.

Salivary Glands

Rabbits have four pairs of salivary glands: the parotid, the submaxillary (also called mandibular), the sublingual, and the zygomatic (Cruise and Brewer, 1994).

ORAL CAVITY

Teeth

The dental formula of the rabbit is:

$$\frac{I2}{1}, \frac{C0}{0}, \frac{PM3}{2}, \frac{M2-3}{3 \times 2} = 26 \text{ or } 28$$

The larger parotid gland extends below and in front of the ear base between the skin and masseter muscle. The associated duct runs rostrally along the lateral surface of the masseter muscle close to the branches of cranial nerve VII (the facial nerve), and enters the oral cavity opposite the last upper molar. The large size of the parotid gland and the accessibility of the duct make this system a popular research model. The submaxillary gland is oval-shaped and located at the angle of the mandible. The sublingual gland is small in the rabbit. The zygomatic salivary gland, not present in many other animals including humans, lies just below the lacrimal gland in the anteroventral angle of the orbit.

There is continuous secretion of saliva from the mandibular gland in rabbits, unlike in ruminants where saliva is produced from the parotid glands and in cats and dogs, where it is produced from the sublingual glands (Brewer and Cruise, 1994). Amylase is present in rabbit saliva while lipase and urea are only present in trace amounts (Ruckebusch et al., 1991).

Esophagus

The esophagus has three layers of semi-involuntary striated muscles that extend all the way down to the cardia of the stomach, as opposed to in humans, where striated muscle comprises the upper two-thirds of the esophagus, the middle third contains some smooth muscle and the lower third consists of only smooth muscle. There are no mucus glands in the esophagus.

Esophageal integrity can be determined by measuring the difference in electrical potential between the esophagus and the stomach. The rabbit has a relatively low gastric potential difference (-12 mV) in comparison to the esophagus (-26 to -29 mV). The opposite is true in humans (Mecheler and Ingelfinger, 1969).

ABDOMINAL VISCERA

Gastrointestinal Tract

The rabbit's stomach makes up approximately 15% of the volume of the GI tract and is never completely empty (Cruise and Brewer, 1994). In fact, for a rabbit GI tract to become completely empty it would take 9 days. The adult stomach is very acidic, with a pH of 1–2. In contrast, the suckling rabbit has a stomach pH of 5–6.5, which allows bacteria to pass through the GI tract and colonize the hindgut (Harcourt-Brown, 2002). After weaning, the stomach quickly becomes acidic. The cardia is large, thin-walled, non-glandular, and lined with stratified squamous epithelium. The fundus is the major exocrine secretory portion of the stomach. It has gastric pits lined with parietal cells which secrete

acid, and intrinsic factor and peptic cells which secrete pepsinogen. The pyloric region is heavily muscled. The duodenum lies at an acute angle and compression of the pyloric valve in response to any increased pressure, such as hepatomegaly, gas, or trichobezoar may occur. The cardia sphincter is well-developed and arranged in such a way that renders the rabbit, like rodents, unable to vomit.

The small intestine is shorter in comparison to other species and comprises about 12% of the GI tract (Suckow and Douglas, 1997). The bile duct and pancreatic duct enter the duodenum separately. The bile duct opens into the duodenum proximally while the pancreatic duct enters distally. The jejunum is less vascularized and its walls are thinner than those of the duodenum.

The ileum ends in the cecum as the rounded sacculus rotundus, which forms the junction between the ileum, cecum, and proximal colon. It is at the ileum that Peyer's patches first appear. The cecum is the most prominent organ in the abdominal cavity and typically contains 40% of the ingesta. It is a thin-walled organ that folds onto itself three times and has an internal surface made up of a long spiral fold that is continued into the ampulla coli. The distal portion of the cecum ends as a 5-inch, thick-walled, narrow, blind tube known as the vermiform appendix, and contains a large number of lymphoid aggregates. It also secretes bicarbonate ions into the cecum, acting as a buffering agent for the volatile fatty acids formed by cecal fermentation (Cheekem, 1987).

Ileoceccocolic Complex

This complicated section of the GI tract is challenging to describe due to the three-dimensional, interconnected nature of the area. It acts as the meeting point between the end of the ileum, the cecum, and the proximal colon. Simply put, the end of the ileum, which is the sacculus rotundus and the beginning of the colon, which is the ampulla coli, meet at the cecum to form this intersection (Figures 8.4 and 8.5).

The sacculus rotundus appears to have immunological properties and contains lymph follicles that give it a honeycombed appearance. It is therefore often referred to as the ileocecal tonsil and is a common site for foreign bodies (Jenkins, 2000). The ileum lies nested between the body of the cecum and the upper ascending colon in a C-shaped manner and is attached by peritoneal folds.

The colon is characterized by sacculations (haustra) and bands (taeniae). The anatomy of the colon has alternatively been described as the proximal and distal colon or as having an ascending, transverse, and descending colon. Both descriptions use the fusus coli, the "pacemaker" of the colon, as a dividing point since

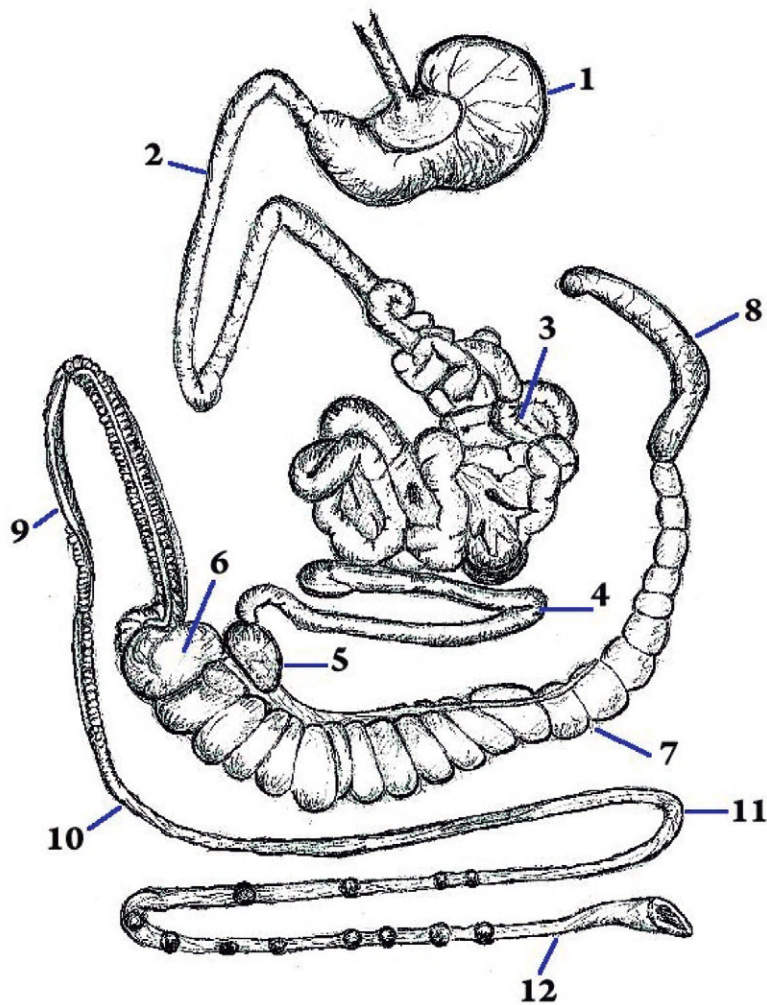


FIGURE 8.4 Schematic drawing of rabbit gastrointestinal tract. 1, Stomach; 2, duodenum; 3, jejunum; 4, ileum; 5, sacculus rotundus; 6, ampulla coli; 7, cecum; 8, vermiform appendix; 9, ascending colon; 10, fusus coli; 11, transverse and descending colon; 12, rectum.

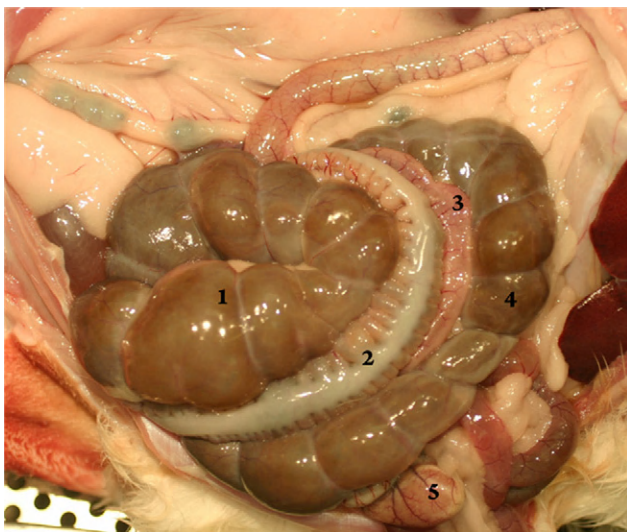


FIGURE 8.5 Photograph of GI tract in situ. 1, base of cecum; 2, colon; 3, ileum; 4, body of cecum; 5, vermiform appendix.

it is a natural anatomic and physiologic demarcation. In this reference the colon will be described as being divided into three sections. The first section, the ascending colon, has five limbs and is separated by flexures (Davies and Davies, 2003). The first limb has three taeniae that form three rows of haustra, which move food along by muscular contractions. Small, wart-like protrusions (about 0.5cm in diameter), called “warzen” (warts) populate the mucosal surface of this section. They act to increase surface area thereby increasing absorption. The three taeniae gradually taper down to one taenia and one haustrum distally in the second and third limb of the proximal colon. The rest of the colon is devoid of taenia. The transverse and descending colon is separated from the proximal colon by the fusus coli. The fusus coli has mucosa that is 4–5 times thicker than that of the descending colon and contains ganglion aggregates (Harcourt-Brown, 2002). This allows the fusus coli to act as a pacemaker to regulate

TABLE 8.1 Summary of GI Tract

Stomach	pH 1–2 Contains 15% volume of GI tract
Duodenum	Bile duct and pancreatic duct enters separately
Jejunum	Less vascularized and thinner than duodenum
Ileum	Has Peyer patches Connects to cecum as sacculus rotundus – honeycombed appearance due to lymph follicles Lies between body of cecum and upper ascending colon in C-shaped configuration Ends in a blind sac called vermiform appendix
Cecum	Typically contains 40% of ingesta
Colon	3 limbs Ascending – 3 taenia form 3 rows of haustra populated by “warzen” Transverse Descending Fusus coli – “pacemaker” of colon – separates proximal colon from transverse and descending colon

peristaltic activity, and controls excretion of the two different types of feces. In New Zealand White rabbits the colon can be a meter long (Cruise and Brewer, 1994). (Table 8.1).

Gastrointestinal Physiology

Rabbits are true herbivores. Rabbits are monogastric hindgut fermenters and require a high-fiber diet (between 14–20%). As a prey species, they rely on the rapid transit time of their unique digestive system to keep their body size and weight low, enabling them to be fast-moving and nimble. In this way, a rabbit's gastrointestinal tract has adapted to the rapid ingestion of large quantities of vegetation (Jenkins, 2001). The transit time of food through the stomach is approximately 3–6 hours and times through the jejunum and ileum are 10–20 and 30–60 minutes, respectively (Carbao and Piquer, 1998). Rabbits mostly eat at dawn and dusk (crepuscular), but eliminate continuously throughout the day. Rabbits chew quickly and thoroughly, preferentially choosing tender, succulent parts of plants as the mainstay of their diets (Queensberry and Carpenter, 2004). High-fiber materials are ingested to stimulate cecocolic motility.

Dietary fiber in rabbits is classified as “digestible” and “indigestible”. Indigestible fiber consists of fibers that are larger than 0.5mm and are quickly expelled as undigested hard, dry feces. The ascending colon separates the large indigestible fiber particles from the smaller, digestible fibers. These digestible fibers are less than 0.3mm and are retropulsed back into the cecum

where they undergo bacterial fermentation (Harcourt-Brown, 2002). The digestible portion, which ultimately becomes cecotrophs, is moved back and forth in the haustra in the cecum and colon. As the large fibers move into the center of the lumen, the digestible portion moves into the wall of the haustras. When the particles move retrograde back into the cecum, fermentation releases volatile fatty acids and produces vitamins and proteins. The constant back-and-forth movement of the cecum and upper third of the colon causes the food particles to separate. Through this motion the digestible and indigestible fiber parts are simultaneously propelled in opposite directions. This, coupled with the fast digestive time, allows the rabbit to function without having to store large amounts of food in the GI tract.

Coprophagy is an important part of the rabbit physiology. The cecotrophs (also known as “night feces”) are ingested directly from the anus and are softer and lighter in color. They are an important source of nitrogen-containing compounds such as niacin, riboflavin, pantothenic acid, and cyanocobalamin (vitamin B12). The cecotrophs have a mucoid membrane that protects the feces from the low pH of the stomach and are ingested whole (Davies and Davies, 2003). The hard waste feces are produced during the first four hours of food ingestion while the cecotrophs are produced 4–8 hours later. Access to the anus for coprophagy is an important point to keep in mind if placing an Elizabethan-collar or similar such device. The term “night feces” is a misnomer as cecotrophs can be ingested at any time of day as it is influenced by light and feeding patterns (Hornicke et al., 1984).

The GI tract of the rabbit provides an important immunologic function. In rabbits, gut-associated lymphatic tissue (GALT) is distributed along the small intestine, sacculus rotundus, and the vermiform appendix (Lanning et al., 2000). GALT is composed of follicles where diversification of the rabbit primary antibody repertoire occurs. The similarity of the rabbit GALT and chicken bursa has led investigators to hypothesize that the rabbit GALT is the functional equivalent of the bursa (Knight, 1994).

Liver and Gallbladder

The liver is comprised of four lobes. The right and left lobes are divided by a deep median cleft. These lobes are then further divided into cranial and caudal lobules (Cruise and Brewer, 1994). The quadrate lobe is a subdivision of the right lobe and lies medial to the gallbladder. The caudate lobe is small, has a narrow attachment to the dorsal hilar region of the liver, and projects dorsally toward the right kidney (Cruise and Brewer, 1994). Torsion of the caudate lobe is possible,

though rare (Queensberry and Carpenter, 2004). The gallbladder is deep within the abdominal cavity in a depression of the caudal surface of the right cranial lobule. The hepatic ducts join to form the common bile duct which receives the cystic duct from the gallbladder and enters the dorsal surface of the duodenum immediately caudal to the pylorus.

The rabbit liver is a typical mammalian liver, consisting of a cribiform sheet of hepatocytes, one cell layer thick (Cruise and Brewer, 1994). Sinusoids drain into a central vein. The perisinusoidal space is formed by the wall of the sinusoids and the wall formed by hepatocytes. In rabbits this space is very narrow so that it is difficult to observe, unlike in humans where it is wider.

Rabbits mainly secrete biliverdin rather than bilirubin in their bile, similar to most non-mammalian species; 25–50% of rabbits also produce atropinase, which can inactivate atropine, and thus it is a poor choice as an anti-cholinergic.

Rabbits secrete a large amount of bile in relation to their size. A 2-kg rabbit can secrete up to 250ml of bile a day, in contrast to the dog, which secretes seven times less on a weight basis (Brewer and Cruise, 1994). Although secretin is a potent choleretic in other mammal species, it has no effect on bile flow in the rabbit (Wheeler, 1968).

Pancreas

The rabbit pancreas is a diffuse organ that is often hidden in the surrounding mesentery. It is also close to the splenic and caudal pancreaticoduodenal vessels. The pancreatic duct empties into the duodenum approximately 35–40 cm distal to the entrance of the biliary duct and appears to be the only laboratory animal with a single, separate pancreatic duct (Brachet, 1935). This duct is actually the accessory duct, as the terminal portion of the main pancreatic duct regresses during embryonic development (Harcourt-Brown, 2002). Pancreatic surgery can be challenging in rabbits and thus must be performed with great care.

Ligation of the pancreatic duct induces atrophy and fibrotic replacement of the pancreatic acini in the rabbit (Catala et al., 1987), similar to other mammalian species. However, unlike many other species, where ligation of the pancreatic duct causes pancreatic insufficiency, rabbits continue to populate the duodenum with proteolytic enzymes. It has been hypothesized that smaller pancreatic ducts communicate directly with the duodenum to supply these enzymes (Arvanitakis and Folscroft, 1978). In fact, it appears that pancreatic duct ligation does not induce long-term visible pathologic signs except for a chronic and progressive diabetogenic state leading to irreversible pathology (Catala et al., 2001).

Spleen

The spleen lies on the left dorsolateral surface of the greater curvature of the stomach and is small compared to that of other species. The rabbit's relatively small spleen has been attributed to the fact that the gut-associated tissue contains about 50% of the total lymphoid tissue mass. At birth, the rabbit spleen lacks follicles and germinal centers and only begins to develop lymphatic follicles at 2 weeks of age (Jeklova et al., 2007).

Rabbits have been a popular model for polyclonal antibody production due to their large body size, good responsiveness to immuno-stimulation, and ease of blood collection. The most popular sites for antigen injection include the skin of the dorsum, biceps femoris, quadriceps, and popliteal lymph node. Intraperitoneal injections are generally not performed in rabbits.

Adrenals

The adrenal glands lie cranial and medial to the kidneys. The left adrenal gland is caudolateral to the origin of the superior mesenteric artery and the right adrenal gland lies close to the caudal vena cava.

UROGENITAL SYSTEM

Kidney

Unlike most other mammals, which have multipapillate kidneys, rabbits have a unipapillate kidney, similar to rodents. The anatomy of a unipapillate kidney, where one papilla and one calyx enter the ureter directly, makes the renal pelvis a direct extension of the ureter (Dwyer and Schmidt-Nielsen, 2003). As is typical in mammals, the right kidney lies more cranial than the left kidney.

The rabbit medulla has narrow ends curled around at the lower and upper poles of the kidney, similar to an orange segment (Sheehan and Davis, 1959). The structures of the medulla, which contain the loops of Henle, collecting ducts, and vasa recta, merge toward the papilla, which projects out from the center of the medulla. The medulla is divided into an outer zone, which is further divided into an outer and an inner stripe, and an inner zone, which forms the papillae (Kaissling and Kriz, 1979). The transition from the inner stripe to the outer stripe occurs at the level of the fornices. All the vascular bundles in the rabbit kidney are of uniform size and have a regular distribution throughout the inner stripe. They do not fuse to form secondary bundles, as they do in the rat and mouse (Kaissling and Kriz, 1979). The total venous system in the medulla is also simpler than in other species (Kaissling and

Kriz, 1979), where the venous vasa recta ascend with the bundles as opposed to ascending independently of the bundles. The simple structure of the rabbit medulla is considered to be a primitive one compared to other species, such as the rat and mouse, which have a more complex medullary architecture and concomitant higher functional efficiency (Kaissling and Kriz, 1979).

The pelvic tissues form two septa that surround the medullary pyramid. The pelvic cavity extends between the septa and the pyramid to the fornix. It then extends outwards to form a series of secondary pouches on the outer surface of the pelvic septa (Sheehan and Davis, 1959). The typical unipapillate pelvis is generally a simple, funnel-shaped space (Dwyer and Schmidt-Nielsen, 2003), but can, as in the rabbit kidney, have elaborate, extensive evaginations. The epithelia of the fornices, at the level of the inner medulla, are composed of a single layer of cuboidal cells, whereas in the outer medullary region the epithelium is squamous. There are two distinct layers of smooth muscle fibers found in the walls of the renal pelvis. The inner layer contains fibers that insert near where the pelvic wall joins the base of the papilla, and are continuous with the ureteral smooth muscle. The outer layer is highly innervated, more diffuse and covers only the renal pelvis. It connects with the inner layers but ends abruptly at the junction of the ureter (Dwyer and Schmidt-Nielsen, 2003). The rabbit kidney is unique in that the tubules can be separated from kidney slices with the basement membrane intact. This makes the rabbit a useful model for renal tubule physiology studies (Brewer, 2006).

Each adult kidney contains approximately 200 000 nephrons (Rytand, 1938). Unlike dogs and humans, the number of glomeruli in rabbits increases after birth (Smith, 1951), similar to rats. Ectopic glomeruli are normal in adult rabbits, about 60 in each kidney (Steinhausen et al., 1990). These ectopic glomeruli often produce small cysts in the pelvic septum and can sometimes be seen with the naked eye (Moffat and Fourman, 1964).

Differences in definition and nomenclature have resulted in a variety of terminology of renal anatomy. This text will refer heavily to the previous work done by Cruise and Brewer as stated above. As in many animals, the rabbit proximal convoluted tubule (PCT) is divided into three segments: the PCT-S1 (first two-thirds of the PCT), the PCT-S2 (last third of the PCT and first part of the pars recta), and the PCT-S3 (the terminal part of the pars recta) (Woodhall et al., 1978). Transitions between segments are gradual compared to other mammals such as the dog and rat (Kaissling and Kriz, 1979). The proximal tubule plays an important role in water reabsorption as it reabsorbs two-thirds of the glomerular filtrate. The epithelium of the proximal tubule is made up of a single layer of

cells supported by a basement membrane (Quigley et al., 2003). The proximal convoluted tubule has a net fluid absorption rate of 1 ml/mm/kg in the adult and around 0.3 ml/mm/min in juvenile rabbits (Quigley and Baum, 1990).

The rabbit loops of Henle are composed of 66% long and 34% short loops, with only 7% of the efferent arterioles from the juxtamedullary glomeruli supplying blood to the medulla (Kaissling and Kriz, 1979). In contrast, other mammals such as the rat, mouse, pig, and human have more short loops than long, while carnivores such as the cat, fox, and dog have only long loops (Cruise and Brewer, 1994). The thin descending limb of Henle's loop is different in short- and long-looped nephrons (Kaissling and Kriz, 1979). In long-looped nephrons it descends through the outer medullary zone into the inner medulla. In short loops, as well as in the thin ascending limbs of long loops, they pass over into the distal tubule. The thin descending limbs also exhibit great differences in luminal diameter and cellular thickness. The larger limbs (outer diameter up to 40 µm) are thought to give rise to the longest loops, the limbs of intermediate size (diameter range from 30 µm to 20 µm) to the intermediate-length loops, and the small thin limbs (outer diameter 20 µm) to the short loops (Kaissling and Kriz, 1979).

Superficial nephrons and upper midcortical nephrons drain separately via a connecting tubule to a cortical collecting duct (Kaissling and Kriz, 1979). Arcades are ascending tubules in the cortical labyrinth that join deep and midcortical nephrons to a collecting tube. These tubules are composed of connecting tubule cells (CNT), collecting duct (CD) cells, and intercalated (IC) cells (Kriz and Bankir, 1988). On average, six nephrons drain into one cortical collecting duct (Kaissling and Kriz, 1979). The IC cells are rich in mitochondria and have many rod-shaped particles in the membranes of cytoplasmic vesicles. The IC cells are prominent in collecting ducts and other transporting epithelia. Unlike most mammals, the rabbit's connecting ducts do not contain typical CD cells, but only CNT cells interspersed with IC cells (Kriz and Bankir, 1988). The rabbit is also unique in that the CNT has a more distinct segment in structure and in function (Knepper and Rector, 1991). The cells of the CNT appear "lighter" on light and electron microscopy and have numerous infoldings of the basal cell membrane (Kaissling and Kriz, 1979). There are IC cells in the inner stripe of the outer medullary collecting duct (OMCD), but these only occur in the outer half of the inner stripe, where they comprise 10–15% of the cell population (Ridderstrale et al., 1988). The simple, clear-cut structural and functional organization of the rabbit distal nephron makes it a useful tool to understand more complex systems in rats, mice, and humans (Loffing, 2002).

Similar to amphibians and neonatal mammals, rabbits have a wide range in the number of active glomeruli at any one time (Salako et al., 1971). Up to a 16-fold increase in water diuresis is possible without a significant change in glomerular filtration rate (Smith, 1951). Even a blood pressure increase of up to 100% will result in little or no change to the glomerular filtration rate (Smith, 1951). This autoregulation, found to some degree in other mammals, but not to the extent found in rabbits, occurs at the level of the afferent arteriole (Steinhausen et al., 1990).

The rabbit only has a limited capacity to concentrate urea, so when the urea load increases, so does the amount of urine production (Knepper and Rector, 1991). In most mammals inulin clearance is equal to the glomerular filtration rate. In rabbits, however, inulin clearance can be changed by altering blood flow of the rabbit glomeruli, specifically at the afferent arterioles (Brewer, 2006). Since the creatinine clearance and inulin clearance are equal in the rabbit, it can therefore be used to measure glomerular filtration rate (Kaplan and Smith, 1935). Rabbits are also not sensitive to the actions of loop diuretics (Ridderstrale et al., 1988), such as furosemide.

Rabbits are especially susceptible to low doses of acid administration. Unlike most mammals, a decreased plasma pH does not stimulate the deamination of glutamine, which is the major mechanism in ammonia synthesis. Only lowered bicarbonate (HCO_3^-) concentration is effective in stimulating this system (Simpson and Sharrard, 1969). The rabbit is also not as efficient in HCO_3^- reabsorption as other mammals. Most mammals avoid alkalosis by neutralizing HCO_3^- catabolism with ureagenesis. If rabbits are fed a diet low in nitrogen, they are unable to titrate enough HCO_3^- through ureagenesis. HCO_3^- is also absorbed through the gut. These two conditions make the rabbit susceptible to a greater HCO_3^- load than other mammals. In addition, carbonic anhydrase, an enzyme that catalyzes hydration of CO_2 to HCO_3^- is absent in the rabbit's thick ascending limbs of the renal tubules unlike other mammals (Ernst, 1975; Iino, 1981).

In the rabbit, the collecting duct is an important site for acid secretion, bicarbonate reabsorption, and active secretion of bicarbonate (Hamm, 1990). The transition between the convoluted part of the distal tubule to the connecting tubule is marked and clear. Here, the homogeneous epithelium of the distal tubule changes into the heterogeneous epithelium of the connecting tubule (Kaissling and Kriz, 1979). In concordance with this change in anatomy is a change in physiology, namely the absorption of NaCl. In this and all subsequent segments the sodium-potassium ATPase activity is significantly lower.

The final degradation product of purine metabolism in the human is uric acid. Different species of mammals handle this differently. Whereas humans, with 700 out of 9000 mg of urate filtered daily, have one of the lowest excretion ratios, the rabbit has one of the highest (Brewer and Cruise, 1994). Since as much as 160% of the filtered load is excreted, it is likely that much of the secreted load is also excreted. In the dog and rat less than 40% is excreted (Brewer and Cruise, 1994). The anion-exchange mechanism, located in the luminal plasma membrane in the dog and rat that affects reabsorption in the proximal tubule does not exist in the rabbit.

Rabbit urine is generally cloudy due to the high concentration of ammonium magnesium phosphate and calcium carbonate monohydrate precipitates (Suckow and Douglas, 1997). Rabbit urine can also vary in color from orange and brown to red, depending on the diet, and can be mistaken for hematuria (Harcourt-Brown, 2002). Though most mammals regulate their calcium levels through the activated metabolite of vitamin D, rabbits' absorption of calcium from the gut makes their calcium levels independent of this mechanism (DeLuca, 1974). Unlike most mammals, urine is the major route of excretion for calcium in the rabbit (Kennedy, 1965). Because of this, rabbits are unusual in that serum calcium levels vary with their diet. Rabbits fed a 10% calcium carbonate diet can excrete almost 60% of the calcium in their urine (Cheeke and Amberg, 1973). A high-calcium diet can result in thick, creamy urine, and prolonged feeding of a high-calcium diet can result in calcification of the aorta and kidney.

Urine color can vary from yellow to red due to porphyrin pigments. Though thought to be derived from the diet, the source of porphyrin has not yet been identified. Urine pH is typically around 8.2 and the rabbit produces 50–75 ml/kg of urine a day (Gillett, 1994).

Male Genital Organs

Male rabbits have two hairless scrotal sacs cranial to the penis. The testis has an elongated oval shape with a blunt cranial pole and a pointed caudal pole. The testes descend at 12 weeks and the inguinal rings do not close. Males have open inguinal canals and can retract their testicles abdominally, which they do during sexual inactivity or periods of inadequate food. Care must be taken to close the inguinal rings during castration.

The nomenclature of the accessory sex glands of the rabbit has been a source of historical confusion due to the use of terminology for other mammalian species as well as poor consensus among anatomists (Holtz and Foote, 1978). For example, in humans the terms seminal vesicle and vesicular gland are used interchangeably. In rabbits, however, they have been described as

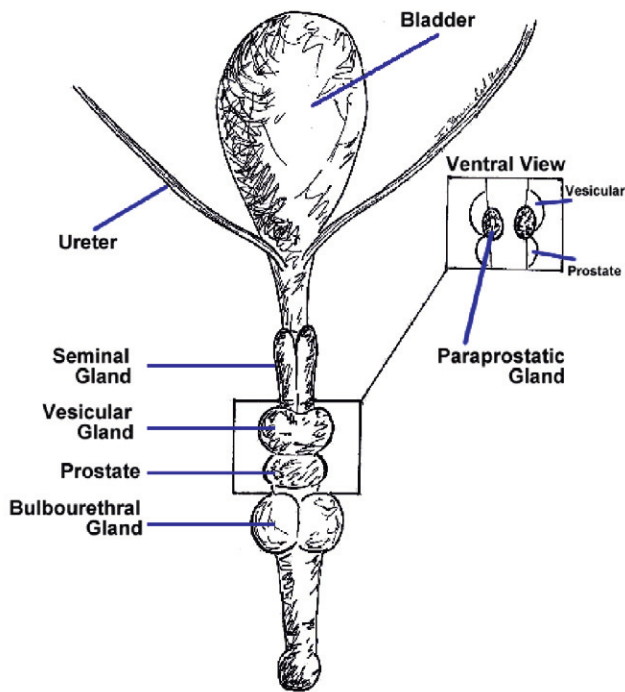


FIGURE 8.6 Dorsal view of the male accessory sex glands. Ventral view in smaller box.

two distinct glands. In addition, different rabbit sources name the glands differently. Most references include the prostate, seminal vesicles, and bulbourethral glands. Other sources include the terms proprostate and paraprostate gland. For the sake of continuity the accessory glands here are listed (roughly cranial to caudal) as: seminal vesicles, vesicular glands, prostate, paraprostatic glands, and bulbourethral glands (Cruise and Brewer, 1994) (Figure 8.6).

The seminal vesicles are the most cranial glands located at the neck of the bladder. Depending on the amount of fluid it contains, the size of the seminal vesicles can vary greatly. The ductus deferens enters the ventral wall of the seminal vesicle dorsal to the bladder (Cruise and Brewer, 1994). The vesicular glands are caudal to the seminal vesicles, cranial to the prostate and separated by a very thin connective tissue septum (Kurtul et al., 2001). The vesicular gland ducts enter the urethra at the seminal colliculus. The prostate overlaps the caudal part of the vesicular glands and can be distinguished anatomically by its lighter color (Kurtul et al., 2001). Four to six small prostatic ducts enter the urethra on both sides and posterior to the seminal colliculus (Cruise and Brewer, 1994). The bulbourethral glands lie caudal to the prostate and are separated from it by a distinct groove. The paraprostatic glands are small, club-shaped glands that are at the ventrolateral aspect of the prostate (Holtz and Foote, 1978).

These glands cannot be visualized dorsally and are very small. All glands are bilobed, except the bulbourethral glands, which are paired.

The penis does not have an os penis, but instead has a free extremity called the pars libera. Dorsolateral to the body of the penis are the white inguinal glands and the associated brown inguinal glands, which empty their secretions into the hairless inguinal spaces. Though some references cite males as not having nipples, it appears that males have very small, rudimentary nipples under their fur.

Female Genital Organs

The female reproductive tract has a bicornuate uterus with two cervixes that open independently into the vagina. The mesometrium can be a major site of fat deposition, making ovario-hysterectomies of overweight does challenging. The urethra enters the vagina at a vestibulum known as the urogenital sinus (Cruise and Brewer, 1994), which extends out into the vulva. The bulbourethral gland, similar to that of the male, lies on the dorsal wall of the vestibulum. The clitoris is located along the ventral surface of the urogenital sinus with the glands clitoris projecting into the urogenital aperture.

Though sexual maturity depends on the breed, in general body weight is more important than age (Suckow and Douglas, 1997). Small breeds can reach sexual maturity at 4–5 months, medium breeds at 4–6 months and large breeds at 5–8 months. New Zealand White rabbit females reach maturity at around 5 months while males reach maturity at 6–7 months. Does mature earlier than bucks, with the bucks producing sperm 40–70 days after reaching puberty (Queensberry and Carpenter, 2004).

Rabbits are induced ovulators and do not have a defined estrous cycle. Ovulation occurs approximately 10 hours after copulation. Pseudopregnancy can occur if coitus does not result in pregnancy, if a doe mounts another doe, or if the rabbit is injected with luteinizing hormone (Kennelly and Foote, 1965) or human chorionic gonadotropin. Pseudopregnancy can last 15–16 days. Does are generally receptive for 14–16 days followed by 1–2 days of non-receptivity.

The placenta is hemochorial and passive immunity is endowed to the young through the yolk sac instead of through colostrum or placenta (Suckow and Douglas, 1997). Breeding life for a doe can be up to 3 years while that of a buck is around 5–6 years. Gestation is usually 31–32 days. Pregnancy can be confirmed at day 14 with palpation, day 11 with radiography and day 7 with ultrasonography.

Semen is deposited into the anterior vagina, where the sperm individually pass through the cervical mucus. During an active corpus luteal phase, such as early

pregnancy or pseudopregnancy, the cervical secretions are thick and mucoid, thereby inhibiting sperm transport.

Reproductive Behavior

Receptivity in a female is denoted by a swollen, moist dark pink to red vulva. Does do not have a defined estrous cycle, but a certain rhythm can be determined. Vaginal cytology is generally not useful. Due to territorial behavior, the doe should always be introduced to the buck's cage. Fifteen to 20 minutes (often much less than that) is usually sufficient to determine if the doe will accept the buck. Receptive females will exhibit lordosis and present the perineum to the buck. Non-receptive behavior includes running away, biting, vocalizing, or cornering. If the doe refuses the buck, the doe should be taken away. This prevents physical trauma to either animal as well as psychological trauma to the buck.

Bucks initiate copulation by sniffing, grooming, nuzzling, tail-flagging (elevation of the hindquarters with a stiff-legged gait and tail flat across the back), and enurination (urine spraying) at the female (Queensbury and Carpenter, 2004). Experienced males often initiate mating within minutes of being introduced to a receptive female. Copulation is quick, generally over after a few thrusts. The male, whose hindquarters are off the ground, falls off and emits a characteristic cry after ejaculation. Robust males will attempt to copulate almost immediately.

Parturition in rabbits is known as "kindling" and typically happens in the early morning hours. This is characterized by the rabbit pulling off fur for nest building and a decreased appetite for 2–3 days beforehand. Both anterior and breech presentations are normal. Kits are born altricial, nurse only once a day, and open their eyes around day 10. Kits can ingest up to 35% of their body weight in milk in less than 5 minutes (Hudson and Distel, 1983). Kits begin eating dry food at 21 days and are usually weaned at 8 weeks. Litter size depends on parity, with primiparous does producing smaller litters. Though textbooks describe eight mammary glands, this can be variable. A kit has total dependence on milk until day 10. Does can become pregnant during the post-partum period and can be lactating and pregnant at the same time.

THORACIC VISCERA

Cardiovascular System

Rabbits have a relatively small heart. In fact, it represents only 0.2% of the rabbit's total body weight in contrast to 0.76% in dogs (Hew and Keller, 2003). The rabbit conduction system is similar to that of other mammals and includes the sinoatrial node, internodal pathways, AV node, His bundle, and bundle branches.

The sinoatrial (SA) node is well-defined, less complex than that of other mammals, and relatively long considering its total heart size (James, 1967). The rabbit's SA node has a very large percentage of P cells, which are not distinctly organized about a central artery (Hew and Keller, 2003). This simplicity allows the exact location of the pacemaker to be delineated, and in fact the rabbit is the first mammal in which the SA node was localized (Bleeker et al., 1980).

Though the rabbit has a right and left coronary artery, most of the heart is supplied by the left coronary artery and its branches (James, 1967). The AV node and the His bundle are relatively small in the rabbit, and their only arterial circulation is supplied by the septal artery (James, 1967). Branches from the septal artery are perpendicular to its long axis and ascend across the right ventricular septal endocardium to the His bundle and the AV node. This sequence is opposite from that of humans.

Rabbits have a left and right cranial vena cava (Pariaut, 2009). The presence of a left cranial vena cava results in an extremely large coronary sinus, which in turn disrupts most of the atrial septal structures cranially. The internal carotid artery is relatively small despite being the main blood supply source to the brain. The pulmonary artery is heavily muscled compared to other species (Cruise and Brewer, 1994).

The primary return of venous blood from the head is the external jugular vein, unlike in humans, where it is the internal jugular vein. Unlike other species, there are few anastomoses between the branches of the internal and external jugular veins. Therefore, ligation of the external jugular vein can cause swelling and protrusion of the eyeball (Hoyt et al., 1994). This usually resolves itself in 24 hours. Ligation of the external carotid artery causes ipsilateral ocular necrosis.

Rabbit heart rates can range from 180–300 beats/min, with higher heart rates often seen due to the increased sympathetic tone secondary to stress (Pariaut, 2009). The right atrio-ventricular valve has two bicuspid leaflets rather than three and the aorta has a rhythmic contraction that is neurogenic in origin.

Most mammal hearts have a mixed population of connective tissue and Purkinje cells. Though this makes the conductive tissue readily distinguishable, this mixture also complicates microelectric recordings (Brewer and Cruise, 1994). Rabbits have little or no connective tissue admixture with the Purkinje cell, which makes the rabbit the species of choice for Purkinje fiber studies (Sommer and Johnson, 1968). In addition, these cells have a long cylindrical shape with wide clefts between them. These clefts, 25–50 times the width found in other species, reduce the problems with resistance and perfusion encountered in other species (Colatsky and Tsien, 1979).

The aortic nerve subserves no chemoreceptors, only baroreceptors (Kardon et al., 1974). The nerve to the carotid sinus subserves both. This nerve joins the afferent fibers from the root of the right subclavian vein to form the depressor nerve. Unlike other laboratory animals where the depressor nerve is free for only a short distance, the depressor nerve in the rabbit runs separately from the vagosympathetic trunk, making it easily accessible for electrode implantation (Cruise and Brewer, 1994). The carotid artery is more compliant than that of the dog and has a greater ratio of elastin to collagen (Cox, 1978).

Due to the fact that rabbits exhibit hypercholesterolemia within a few days of being fed a high-cholesterol diet, they are the most widely used animal in atherosclerosis research (Yanni, 2004). The Watanabe rabbit is a classic model for heritable hypercholesterolemia (Aliev and Burnstock, 1998) and plays an important role in atherosclerosis research. Rabbit lipoprotein metabolism correlates to human lipoprotein metabolism more closely than mice. Rabbit and human lipoprotein profiles are rich in low-density lipoproteins (LDL), while the mouse is rich in high-density lipoproteins (HDL) (Zhang et al., 2009). Rabbits and humans also have abundant plasma cholesteryl ester protein (CETP), whereas mice do not. Rabbits do not develop tissue plaques as humans do (Kolodgie et al., 1996). Instead, early lesions come in the form of foam cells originating from macrophages. In addition, the massive inflammation exhibited by rabbits does not reflect human physiology. Long-term studies of rabbits on a high-cholesterol diet are difficult since the high hepatotoxicity makes long-term survival in rabbits rare (Yanni, 2004). Advanced lesions can be induced in the thoracic and abdominal aorta by combining a high-cholesterol diet with a single or double balloon injury (Abela et al., 1995). These balloon lesions create plaques with lipid cores covered by a fibrous cap and a high density of smooth muscle cells (Yanni, 2004) and are more similar to human atherosclerotic lesions than those produced by solely feeding a high-cholesterol diet (Aikawa et al., 2001). Interestingly, female rabbits accumulate less cholesterol in the aorta than male rabbits when fed a high-cholesterol diet, but this protective effect is not present when the aorta is subject to balloon injury (Holm et al., 1998).

Recently, high-cholesterol-diet-fed rabbits have been used as a model for Alzheimer disease. Amyloid- β (A β), a peptide fragment of the larger membrane spanning β -APP protein containing senile plaques (SP), is found in excessive amounts in the brains of patients with Alzheimer disease (Sparks, 2008). Rabbits fed a high-cholesterol diet exhibited a high accumulation of neuronal A β compared to control rabbits (Sparks et al., 1994). The finding that increased circulating blood cholesterol levels also increases cholesterol levels in the brain (Sparks et al., 1994) has contributed to the more frequent use of the cholesterol-fed rabbit model.

Blood pressure measurements can be obtained with the Doppler method. Blood pressure and heart rate in the rabbit tend to increase during the night (Pariaut, 2009), with the most common systolic blood pressure measurement between 120 and 180 mmHg.

Respiratory System

Larynx and Pharynx

The rabbit epiglottis is a relatively large structure that lies dorsal to the soft palate, thereby allowing air directly from the nasopharynx to the larynx and trachea without entering the oral cavity. It is this anatomy that makes the rabbit an obligate nose-breather and is important to remember when attempting intubation of the trachea (Figures 8.7 and 8.8). The glottis of the rabbit is small and with the relatively large tongue, the glottis remains well covered.

Lungs

Lungs are divided into cranial, middle, and caudal lobes with the right caudal lung lobe additionally divided into the lateral and medial lobes. The thymus does not regress with age and lies in the cranial ventral thoracic cavity. The right cranial lung lobe is larger than the left cranial lung lobe due to the presence of the heart in the left pleural cavity.

The sole blood supply to the pleura is the pulmonary artery. Extensive anastomosis with the pulmonary artery occurs at the level of the hilus in the rabbit, unlike in the dog, cat, or monkey (Cruise and Brewer, 1994). In rabbits the bronchial artery extends to the third division of the bronchi. There is a lack of collateral blood supply beyond this area, which places the rabbit at serious risk if an obstruction to the pulmonary artery occurs.

The rabbit lung does not contain respiratory bronchioles, but instead terminates in vestibules which contain alveoli (Cruise and Brewer, 1994). The air flow volume of the left lung is higher than that of the right due to the lower resistance of the proximal airways per unit volume (Yokoyama, 1979).

BRAIN AND SPINAL CORD

The brain is anatomically similar to other mammals. It is enclosed by the meninges: the outer dura mater, the arachnoid, and the inner pia mater (Wingerd and Stein, 1985). The dura mater adheres to the periosteum of the skull and extends down between the cerebral hemisphere as the falx cerebri. It becomes the tentorium cerebelli (Kozma et al., 1974) at the level of the cerebellum and cerebrum.

The forebrain consists of the telencephalon and the diencephalon. The telencephalon is comprised of the

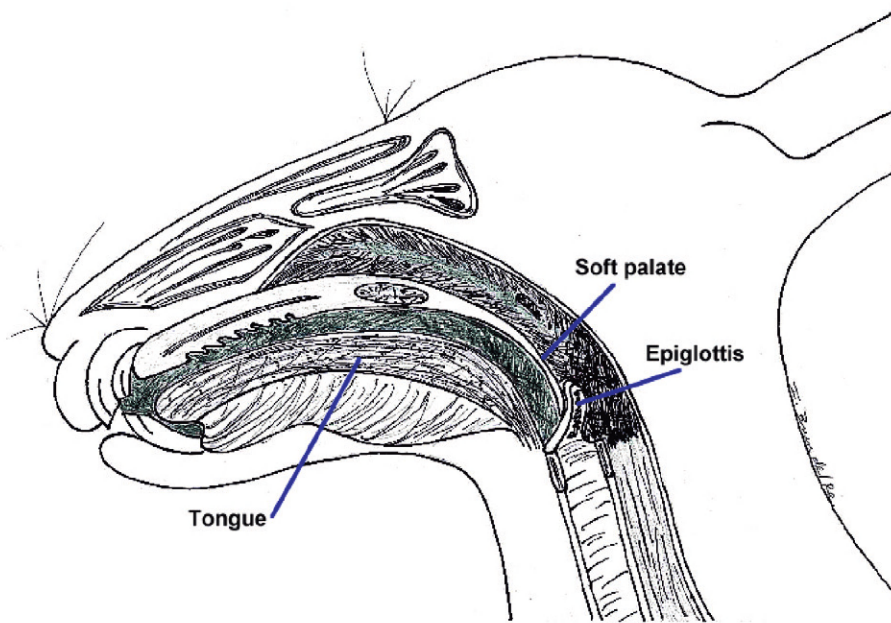


FIGURE 8.7 Sagittal view of the rabbit pharynx. Note the normal position of the epiglottis lying dorsal to the soft palate.

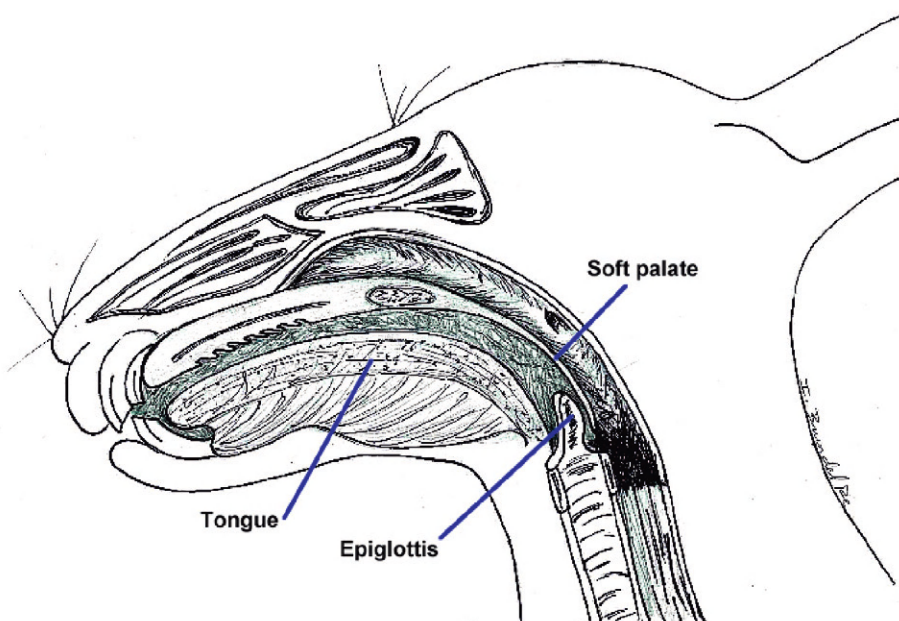


FIGURE 8.8 Same sagittal view with the epiglottis reflected ventrally – position for oral intubation.

cerebrum (cerebral cortices and basal nuclei), and is the largest portion of the brain. The diencephalon is comprised of the metathalamus, thalamus, hypothalamus, pineal gland, and pituitary gland. The optic chiasm lies within the diencephalon (Wingerd and Stein, 1985). The rabbit's cerebral cortex is lissencephalic, or smooth, due to the lack of gyri. The two hemispheres are divided by a longitudinal cerebral fissure (sagittal fissure) and are connected at the bottom by a white matter tract, the corpus callosum. All sensory pathways must travel through the thalamus to reach the

cerebral cortex, with the exception of olfaction (Oliver Jr and Lorenz, 1993). The olfactory bulbs are positioned at the rostral and ventral ends of the cerebral hemispheres, receive the sensory endings of the olfactory nerve, and are relatively large (Osofsky et al., 2007; Wingerd and Stein, 1985). The dorsally located pineal body originates from the corpus callosum and courses caudodorsally, nearly into the caudal end of the cerebral hemispheres.

The midbrain (mesencephalon) lies caudal to the diencephalon and is comprised of the dorsal tectum

(Latin = roof) and ventral tegmentum (Latin = covering) (Osofsky et al., 2007). The tectum consists of the rostral and caudal colliculi, which, unlike dogs and cats, are clearly visible in the rabbit (Craigie, 1951). The rostral colliculi, which are larger than the caudal colliculi (Craigie, 1951), are involved in the visual reflex while the caudal colliculi are important in the auditory reflex. Important structures in the tegmentum include the mesencephalic aqueduct, the red nucleus, and the reticular activating system (Wingerd and Stein, 1985).

The hindbrain (rhombencephalon) consists of the cerebellum, pons, and medulla. The cerebellum and pons form the metencephalon and the medulla forms the myelencephalon, the most caudal part of the brain (Wingerd and Stein, 1985). The thalamus, hypothalamus, midbrain, pons, and medulla make up the brainstem.

Similar to other mammals, the cerebellum consists of the central vermis and the right and left cerebellar hemispheres. Rabbits have prominent paraflocculi, which are ventrolateral extensions of the cerebellar hemispheres (Cruise and Brewer, 1994). The paraflocculus is often damaged when removed due to its being almost completely enclosed by the dorsal portion of the petrosal bone (Cruise and Brewer, 1994). The paraflocculus is separate from the flocculus but has connections at the pyramis and uvula. The flocculus has been extensively studied in rabbits due to its importance in the mechanism of the vestibulo-ocular reflex and in the sensory and sensorimotor integration for retinal image stabilization (Voogd and Wylie, 2004). In the midsagittal plane, the cerebellum is triangular in shape, instead of the round shape seen in other domestic animals such as the dog, cat, horse, and sheep (Osofsky et al., 2007).

The pons lies caudal to the cerebral peduncles. The ventral part of the pons contains the transverse pontine fibers that transverse across the ventral aspect of the brain into the cerebral peduncles (Osofsky et al., 2007). These thick, paired ridges, which are separated by the median interpeduncular fossa, lie just behind the mammillary body. The third cranial nerve emerges from the ventral surface of the peduncle, while the cut end of the proximal portion of cranial nerve V (trigeminal nerve) emerges from the ventral surface. The dorsal part of the pons contains the nuclei for cranial nerve V (CN V). The medulla oblongata is located caudal to the pons and connects the brain and the spinal cord. The rostral medulla contains the nuclei of CN VI (abducens nerve), CN VII (facial nerve), CN VIII (vestibulocochlear nerve), the pyramids, and the trapezoid body. The pyramids are narrow tracts that are ventrally located on each side of the midline from caudal to the pons to the anterior funiculi of the spinomedullary junction. The trapezoid body of the medulla is also located ventrally, but is dorsal and lateral to the pyramids. It is smaller and made up of transverse fibers that extend from the

ventral cochlear nucleus. It lies in between the angle formed by the lateral margin of the pyramid and the caudal margin of the pons.

The sensory and motor roots of CN V lie at the lateral border of the pons. The small abducent nerve arrives along the lateral margin of the anterior end of the pyramid via several delicate rootlets (Cruise and Brewer, 1994). CN VII and VIII arise from the lateral margin of the trapezoid body. CN IX (glossopharyngeal nerve), X (vagus nerve), and XI (accessory nerve) arise from the lateral margin of the medulla by several linearly arranged roots. CN XII (hypoglossal nerve) arises through several roots from the ventral surface of the caudal medulla at the lateral margin of the pyramid.

The cerebellum is visualized by removing the fourth ventricle and consists of the central vermis and the laterally located right and left cerebellar hemispheres (Wingerd and Stein, 1985). The posterior medullary velum supports a choroid plexus. The fourth and third ventricles are connected by the aqueduct of Silvius. The dorsal aspect of the third ventricle can be seen by spreading the caudal tips of the cerebral hemispheres and pulling away the pineal body. At the roof of the third ventricle is another choroid plexus. Small, spindle-shaped masses called habenulae form the lateral margins of the aperture. The two habenulae are united caudally by the habenulae commissure. The thalami are thick masses of nervous tissue that form the lateral walls of the ventricle. The dorsal portion of the thalamus forms an oval projection, the lateral thalamic tubercle, lateral to the habenula, and the anterior tubercle of the thalamus, a small elevation in the angle between the lateral tubercle and the cranial portion of the opening of the third ventricle. The lateral geniculate bodies lie at the most lateral parts of the dorsal surface at each side of the lateral tubercle. The medial geniculate bodies are less pronounced and lie caudomedial to the lateral bodies.

Dividing the cerebrum along the sagittal fissure exposes the midline structures. The lateral ventricle is exposed by removing the dorsal wall of one cerebral hemisphere. The corpus callosum ends rostrally in a club-shaped form and bends downward caudally, forming the splenium, which is attached to the body of the fornix. A pair of curved bands form the fornix, which fuses for a short distance in the midline until it forms the body of the fornix.

Similar to the brain, the spinal cord is enclosed by the meninges. The dura mater, the outermost layer, is a thick fibrous layer that is not firmly attached to the bone, and thereby creates an epidural space. The pia mater, the innermost layer, is a very thick membrane that is continuous with the dura mater. Fine connecting tissue resembling a spider web extends from the arachnoid mater to the pia mater (Osofsky et al., 2007).

The space between the arachnoid and the pia mater is known as the subarachnoid space, which contains the cerebrospinal fluid (King, 1987). Because the spinal cord and vertebral column have different growth rates, spinal cord segments and nerve roots are not necessarily housed in the corresponding vertebrae (Craigie, 1951). There are two enlargements of the spinal cord: the brachial and lumbar intumescences, or enlargements (Kozma et al., 1974). The brachial enlargement is located in the caudal cervical region and contains the spinal cord segments of cervical nerves 4 through 8 and thoracic nerve 1 (Osofsky et al., 2007). These nerves contribute to the brachial plexus. The lumbar enlargement contains the ventral rami of lumbar nerves 4 to 7 and sacral nerves 1 to 3 and contributes to the lumbosacral plexus. These areas contain a large number of neurons and cell bodies that innervate the muscles of the thoracic and pelvic limbs. Due to the fact that the spinal cord continues into the sacral vertebrae, it is important to remember that the chance of accidental puncture of the dura and arachnoid membranes during epidural injection is higher than in other animals (Johnston, 2005).

The formula for spinal nerves is $C_8T_{12}L_7S_4C_6$. The spinal cord contracts to a thin filament at the middle sacrum, where it is called the filum termale, and continues caudally to the base of the tail. The dorsal spinal nerves originate cranial to their exit from the vertebral column, thereby forming the cauda equina which continues down with the filum termale caudally (Cruise, 1994). Because the spinal cord extends more caudally in the rabbit, the cauda equina is less pronounced than it is in humans (Craigie, 1951).

METABOLISM

The fasting metabolic rate of a rabbit is approximately 44–55 kcal/kg/day (Gillett, 1994). The fat content of the neonate is about 16% of body weight, similar to a human infant (Cornblath and Schwartz, 1976). Neonates have a low reserve of gluconeogenic precursors in the liver. However, kits are able to remain normoglycemic without suckling until the glycogen reserves are depleted, about 6 hours postpartum (Shelley, 1961). Kits only nurse once a day during the dark phase (Jilge, 1993), and are a good model for nursing-anticipatory behavior as they show a clear locomotor anticipatory behavior in response to a nursing mother (Caba and Gonzalez-Mariscal, 2009).

Rabbits have a high intake of water compared to body size, approximately 50–150 ml/kg. Rabbits fed fresh greens ad libitum will often not drink water (Cheeke and Amberg, 1972), while high-protein diets increase water needs. When deprived of water rabbits will decrease food intake and gradually become

anorectic. Fasted rabbits also will become polydipsic, eventually ingesting almost 650% of the normal water intake by day three (Brewer and Cruise, 1994). The water source and ability of the rabbit to reach the water source should be ascertained.

Thermoregulation

The normal body temperature of an adult New Zealand White rabbit at rest is between 38.5–39.5°C (101.3–103.1°F) (Ruckebusch et al., 1991). Rabbits are uncomfortable in temperatures over 20°C (68°F) and can be significantly stressed by temperatures over 28°C (82°F). The recommended ambient temperatures (16°C–21°C/60.8–69.8°F), as outlined by the *Guide for the Care and Use of Laboratory Animals*, is significantly lower than that for other laboratory species (Jenkins, 2001).

As mentioned previously, the ears play an important role in thermoregulation due to the countercurrent heat-exchange system present there (Kawato et al., 1989). It has been shown that there is adrenergic and cholinergic innervation of the arteriovenous anastomoses (AVAs) and that the constriction of the AVA walls controls microcirculation (Iijima and Tagawa, 1976). Other examples of AVAs in nature include the pampiniform plexus, nasal mucosa, and counter-current mechanisms in the various appendages of birds, whales, and seals (Ninomiya, 2000). While these heat-exchange networks consist of arteries and corresponding veins, the rabbit ear network is composed of a major artery and a number of capillaries surrounding the vessel (Ninomiya, 2000). This anatomy allows venous blood to drain more slowly, which enhances heat exchange within the narrow skin space.

Rabbits are unable to sweat, and salivation and panting are usually insufficient to dissipate heat. Moderately heat-stressed rabbits can exhibit decreased food consumption and impaired gastrointestinal function (Jenkins, 2001). They will also lie down in a stretched or prone posture versus a resting one (Finzi et al., 1992). Unlike most mammals, the rabbit shows little increase in heart rate in response to heat. Similar to the rat, the rabbit does not have a carotid rete (Daniel et al., 1952), which normally plays an important role in blood cooling in the brain. Blood from the head drains into the external jugular vein without a pathway to keep the brain cool (Ninomiya, 2000). This unique anatomy makes rabbits highly susceptible to high temperatures (Morimoto, 2009), which can ultimately result in death.

BEHAVIOR

Rabbits are social, nocturnal animals that, despite over 2000 years of domestication, still have a highly developed prey instinct. As with most prey species,

rabbits will become immobile when scared or stressed, making them difficult to assess or examine. This defense, sometimes called “tonic immobility”, has been directly related to the proximity of the predator. When tested during the immobility phase, rabbits that are closer to a predator will right themselves more slowly, while those closer to a home cage will right themselves more quickly (Ewell and Cullen, 1981). Another defense adaptation is to run quickly towards a hiding place such as a burrow or hide box (Gunn-Dore, 1997). Rabbits that are handled in the first week of life showed less fear as adults. In fact, simply being exposed to human scent during this time period decreases fear among rabbits (Ducs et al., 2009).

Rabbits are very territorial and will use their scent glands as well as feces to mark their territory. They can also mark with urine and by “chinning” people and their environment. Rabbits can have very complex social structures. They will show dominance by mounting, barbering, and scent-marking (Baysm et al., 2006). Females are aggressive, if not lethal, towards kits not their own. Despite being timid and shy, rabbits are intelligent and cognizant of their surroundings (Jenkins, 2001). They apparently recognize not only other rabbits, but are able to identify individual humans as well (Davis, 2000).

Laboratory animal housing should take into consideration natural behaviors and preferences of the species being housed. The following recommendations do not take into account experimental, space, and budget issues that all facilities face, but rather is a summary of recommendations for optimal laboratory housing collected from the literature.

Whenever possible, rabbits should be socially housed, with compatible pairs or trios being the most effective. Group housing of immature rabbits is possible without risk to injury. Ideally however, groups should be formed soon after weaning (Gunn-Dore, 1997). Rabbits that have been single-housed for more than 6 months should not be group-housed; they may be very fearful and lack motor skills due to prolonged inactivity. Adult male rabbits should not be housed together as they will fight and can sustain serious injury.

When choosing housing it is important that vertical distance is taken into account as well as horizontal space in order to ensure that rabbits can assume a typical lookout posture (Gunn-Dore, 1997). Housing should attempt to mimic a burrow with an open front and closed top bottom and sides (Jenkins, 2001). Though wire bottom cages are common in laboratory rabbit housing, rabbits should be given an area of solid flooring. In addition, rabbits should be given a hide box, which affords them an escape route when stressed. Toys and other types of enrichment, including being fed hay, should also be provided.

Similar to other species of social animals that are frequently housed singly in the laboratory environment, stereotypical behaviors can sometimes be observed in these rabbits. These include chewing/licking/biting of the bars, the floor, the walls, and the water bottles, hair-pulling and eating, nose-sliding, head-swaying and pawing the cage walls, rearing, and self-mutilation (Gunn-Dore, 1997). Some animals will simply become lethargic and will either exhibit poor appetite or, conversely, excessive appetite. Singly housed rabbits should be in visual contact with other rabbits and extra enrichment should be provided.

Rabbits are obligate nose breathers and thus do not like to have their noses touched. This is important to keep in mind when performing oral exams or attempting to medicate or force feed. Grunts, growls, and snorts indicate anger or annoyance. They can also “thump” their back feet when upset. It is best to approach these rabbits carefully as they will scratch and bite their handlers. Fear or pain is exhibited by a high-pitched scream, as well as an extended head, flattened ears, and bulging eyes. A rabbit in pain can be inactive, dull, become hunched, attempt to hide, or excessively scratch and lick. They can also become aggressive and bite.

The most common response to pain is anorexia. Often this is the first clinical sign to be noted. Rabbits will also grind their teeth, especially in response to visceral and dental pain (Johnston, 2005). Most rabbits will sit quietly in the back of the cage with minimal movement when painful. However, some rabbits will go through periods of rapid and uncontrolled movement and will struggle when handled. Some rabbits will breathe slowly with an obvious nasal flare and deep breathing pattern, in contrast to their usual rapid, shallow respiration (Johnston, 2005). A painful rabbit will also stop grooming itself and other cagemates.

Grooming is a normal behavior and healthy rabbits will spend a lot of time grooming themselves and other rabbits. If a rabbit grooms after being handled, this usually indicates that the rabbit has recovered from the interaction. Rabbits exhibit a behavior called “tooth purring”, which is exhibited by fast, light grinding of teeth and quivering whiskers. This is usually a sign of contentment and can be distinguished from bruxism, which indicates GI pain, by the slower, louder sounds produced (Baysm et al., 2006). Rabbits can lick a person to show affection, as well as nip at them to get their attention or solicit petting.

Rabbits tend to urinate and defecate in the same location, thereby making it possible to litter-train them. However, it is best to train them when still juveniles, as adults, especially males, will defecate to mark territory.

It is possible to “hypnotize” a rabbit by placing the animal on its back with its neck stretched out and the belly gently rubbed. The breathing should decrease and

the pupils constrict (Baysm et al., 2006). However, this method appears to be dependent on the individual handler and rabbit and should never be used in place of sedatives or anesthesia.

In general, rabbits are gentle, docile animals that make them an ideal species for the research environment. Proper and careful training of laboratory workers can make the interaction calm and less stressful for both the animal and handler.

Acknowledgments

We'd like to thank Dr Ilaria Brun del Re for the drawings and Dr Tim Lawson for his invaluable support and edits.

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Rabbit Colony Management and Related Health Concerns

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INTRODUCTION

According to 2006 United States Department of Agriculture (USDA) statistics, there were approximately 240 000 rabbits used annually in research within the U.S. (USDA, 2006). Given that there were just under 1100 registered research facilities, this averages out to about 220 rabbits per research facility. Of course, not all USDA-registered research facilities housed rabbits that year. This is in contrast to rats and mice for which estimates put their research numbers between 17–22 million in the U.S. (U.S. Congress, Office of Technology Assessment, 2006). Thus, although rabbits are the most numerous research animals covered by the Animal Welfare Act, they represent only about 1.4% of the total number of animals used in research. Comparing this to the rabbit meat and pet industry (estimated at 9 million rabbits (USDA, 2002)), research rabbits comprise only 2.6% of all rabbits produced in this country.

The statistics on rabbit use support a common concept in “large animal research” in the U.S.: although there may be a few large research institutions using very large numbers of rabbits, there are also a significant number of research facilities which use only a few rabbits. This can present a variety of challenges for facility managers and veterinarians not accustomed to this species in a laboratory setting. The purpose of this chapter is to provide an overview of management of rabbits within a research setting.

LABORATORY MANAGEMENT

The housing of rabbits in the laboratory setting is conceptually the same as housing them for meat production, show, or pet purposes. The primary difference comes in the steps taken to preserve their health status or to contain infectious agents used within these animals for research purposes.

Most rabbits used in research today are purchased through commercial laboratory animal vendors. Many of these vendors certify their rabbits as free of many or all of the common diseases of rabbits. The integrity of the research being conducted with these animals is often contingent upon them remaining disease-free and

distress-free during the experimental period. Therefore, the housing conditions and husbandry procedures must be of sufficient design and quality so as to protect the animals from inadvertent disease exposure and environmental stress.

Equipment

The equipment used in conjunction with rabbit husbandry in the research setting must be of sufficient quality to ensure its proper function without risk to the animals. The applicable regulatory standards and guidelines prescribe vigorous sanitation methods that can damage or destroy equipment made from inferior materials very quickly. This can lead to time delays and expense of replacement, and can also create significant hazards for the animals and personnel in contact with this equipment. The following sections refer to individual or small-group housing of rabbits in conventional laboratory caging systems. Large group and non-conventional housing is addressed further below.

Caging

All caging within an animal research facility must meet the same minimum criteria: functional containment of the animal. The caging must be safe for the animal; therefore it must be without sharp edges or protrusions which may inflict injury. It must meet the minimum space requirements for the animal to be housed; and, it must have provisions for meeting the basic biological needs of the animal (feed, water, waste disposal) while maintaining a sanitary environment.

Rabbits, by nature, are curious animals that actively manipulate their environment. This can present a challenge for containment and requires that caging has sturdy lock mechanisms out of reach of the animals. Caging secured with only a slide pin or simple latch can be dislodged if the rabbit can reach the mechanism with its mouth or by vibration of the cage when hopping or “thumping” the rear feet (a communication behavior usually associated with stress or danger). Fortunately, rabbits usually tend to be fairly timid and will often remain within the cage even if the door is dislodged, especially if not immediately adjacent to the floor.

All surfaces of the cage should be free of sharp edges and rough or pointed protrusions. This should be true for both the inside of the cage (to protect the animals) and the outside of the cage (to protect personnel).

Cage and rack material must be of sufficient quality and design to ensure adequate sanitation. Surfaces must be smooth, impervious to moisture, heat- and chemical-resistant, and potentially able to withstand sterilization. Materials such as stainless steel and high-temperature plastics are recommended, although other “stainless” metals may be adequate (Figures 9.1 and 9.2). If plastics are used, they must be evaluated periodically (as should all caging) to ensure that sanitation has not been compromised due to deterioration of surfaces by chewing.

Racks may be floor-mounted, wall-mounted, or on casters. If racks are mobile, a mechanism to secure the rack should be included as the motion of the animals within the cage can cause rack motion. This is particularly important if there are drains or other floor imperfections that may result in rack instability and put the animals at risk.

Cage flooring has the potential to inflict injury upon the rabbits because they directly contact this surface more than any other and because it bears the pressure of their entire weight. Flooring must function to

support the animal in a comfortable manner while still facilitating waste removal. Animal Welfare Act Regulations (USDA, 2005) require that caging be “constructed and maintained so as to enable the rabbits to remain dry and clean.” Flooring configuration can be either slatted, tubular, wire grid, or plastic with perforations. Ulcerative pododermatitis (“sore hocks”) has long been associated with rabbits housed on wire grid flooring (Ostler, 1961; Templeton, 1962). Plastic has been advocated as providing a smoother resting surface and decreasing animal-related noise. Regardless of the configuration, slots or openings for fecal passage should be approximately 0.5–0.7 inches in at least one direction. This provides adequate space for passage of feces while providing enough support for a comfortable resting surface. Smaller openings collect fecal material and may trap the rabbit’s toes leading to injury. Floor gap width should be given special consideration when housing either very young animals or giant breed adults, and alterations may be required to prevent injury.

Consideration must also be given to the size of any other openings or gaps in the caging such as around the door, between the ventilation grates in the sides and around the water nozzle opening. This is particularly true when housing very young animals which tend to be very curious and may attempt to force their heads into small gaps, leading to injury and possibly strangulation.

Minimum space requirements for housing rabbits are provided in Table 9.1. These requirements are mandated by the Animal Welfare Act Regulations (USDA, 2005) and are similar to those in the *Guide for the Care and Use of Laboratory Animals*, except that the Guide recommends an interior cage height of 16 inches and that larger rabbits may require more cage height so that animals can sit up (ILAR, 2011). Feed and watering systems are usually incorporated into the design of the caging system. These are discussed separately in the next section.



FIGURE 9.1 Standard stainless steel rabbit cage rack with automatic watering coil at top right.

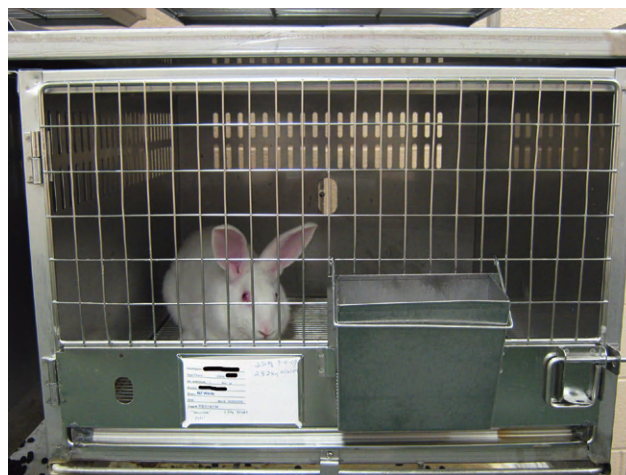


FIGURE 9.2 Rabbit in standard stainless steel rabbit cage.

II. RABBITS

TABLE 9.1 USDA Space Requirements for Laboratory-Housed Rabbits

	Individual Weights		Minimum Floor Space		Minimum Interior Height ^a	
	kg	lbs	m ²	ft ²	cm	in.
Individual rabbits (weaned)	<2	<4.4	0.14	1.5	35.56	14
	2–4	4.4–8.8	0.28	3.0	35.56	14
	4–5.4	8.8–11.9	0.37	4.0	35.56	14
	>5.4	>11.9	0.46	5.0	35.56	14
	Weight of Nursing Female		Minimum Floor Space/ Female & Litter		Minimum Interior Height ^a	
	kg	lbs	m ²	ft ²	cm	in.
Females with litters	<2	<4.4	0.37	4.0	35.56	14
	2–4	4.4–8.8	0.46	5.0	35.56	14
	4–5.4	8.8–11.9	0.56	6.0	35.56	14
	>5.4	>11.9	0.70	7.5	35.56	14

^aThe Guide for the Care and Use of Laboratory Animals recommends 16 inches of interior cage height.

Caging Accessories

Feed and watering systems, enrichment devices, nest boxes, resting platforms, and all other cage accessories must meet the rigorous sanitation requirements of the primary cage or be disposable. If these materials are not disposable, they must be sanitized according to regulatory requirements and periodically inspected to ensure they are not damaged in such a way as to compromise sanitation or pose a hazard to the animals. Specific enrichment devices are discussed below.

FEEDING SYSTEMS

Feed distribution systems may be incorporated into the cage design or food may be provided in separate dishes on the cage floor. Most commercial caging for research use incorporates a “J” type feeder (Figure 9.3). These feeders generally attach and extend through a special slot in the front of the cage or door. This allows the provision of additional food without the need to enter the cage or disturb the animal. These feeders must attach securely to the cage and usually have a retaining clip or other mechanism to secure it to the cage. If not, some rabbits will easily dislodge the feeder as they play with it, potentially leaving an opening large enough for escape of small rabbits or possible injury.

Other caging systems may have feed bowls or troughs attached to the inside of the cage. Some of these systems have special doors or other mechanisms that allow access to the feed bowl without opening the cage. Still others may just have defined areas on the cage floor for food bowls. In any system that uses a separate food bowl, the primary consideration is that it must be secured to the cage in some manner or be heavy enough

and designed in such a way as to prevent the rabbit from tipping it over. Rabbits will play with any loose objects in the cage. Like many other species, rabbits are variable in their food intake, some eating their entire ration quickly while others nibble throughout the day. Having food left in the bowl does not preclude rabbits from playing with the dish and potentially spilling their entire daily ration through the floor grate. Although logic would indicate that the rabbit should be hungrier at the next feeding and therefore likely to finish the ration prior to playing with the bowl, this is often not the case. Rabbits may lose a significant amount of weight due to ration wasting if feed bowls are not secured.



FIGURE 9.3 Common “J-type” rabbit feeder. The feeder fits onto the front of the cage as shown in Figure 9.2.

For attached feeding systems, the food access point for the rabbit should be located 3–4 inches off the floor and the opening should be large enough to ensure that the rabbit's head does not get trapped. This is especially of concern if rabbits are limit-fed as they will force their head into the feeder to reach the last food morsels. Conversely, openings should not be so large as to allow small rabbits to enter the feeder and use the feed pellets as bedding.

WATERING SYSTEMS

Water may be provided via water bottles, automatic watering systems, or water bowls (not recommended). Selection of the appropriate system depends upon the number of animals, the type of research, and the caging design.

Water bottles are commonly used, particularly when small numbers of rabbits are being housed at one time. The bottles can be made of glass (not recommended due to safety hazards of breakage and weight) or highly resistant plastic and must be able to withstand sanitation procedures and possibly steam sterilization. The bottles may use either rubber stoppers or screw-on metal caps (usually stainless steel). Stainless steel is highly recommended for sipper tubes. Rabbits are notorious chewers and will quickly destroy any malleable materials. For this reason if rubber stoppers are used, there should be a metal guard or other device in place to prevent access by the rabbit.

For all water bottle use, the bottles must be securely attached to the cage. Many caging systems have specialized holders built-in to retain the water bottle. If not securely attached to the cage, the rabbits will play with the sipper tube and dislodge the bottle. Sipper tubes should be positioned low in the cage to ensure easy access, particularly for potentially debilitated animals.

Automatic watering systems are particularly useful in large colonies where water bottle usage would be very labor-intensive. These systems incorporate a water distribution piping system that is attached to each rack with a quick-disconnect valve connector built into the cage rack. This plenum distributes water to lick valves (Figure 9.4) which protrude into each cage, usually at the back. The plenum may terminate in a drain valve that is automatically (or manually) drained periodically into a nearby drain or sink. Alternately, the water may be recycled back into the distribution system after retreatment to ensure a sanitary supply. Most automatic watering systems for rabbits are one-pass systems where water is discharged and not recycled. The water in these systems may be treated in a variety of manners as described below.

Automatic watering systems should be used with procedures that ensure adequate sanitation and/or water purity as well as proper mechanical function on

a day-to-day basis. These systems often incorporate a water purification system, pressure reduction system, flush system, and distribution system. All of these mechanical systems are subject to failure, resulting in either widespread or individual failure to provide water. Even a single day without water can significantly affect animal health, well-being, and research results. A system for daily monitoring of automatic watering function must be developed that will ensure proper function at the individual cage level.

Although water bowls can be used with rabbits, this is highly discouraged. Aside from the animals playing with the bowls (thus requiring that they also be secured), rabbits will also quickly soil their water bowls with food slurries and possibly urine or feces. If water bowls are used, it is advisable to conduct multiple daily checks to ensure that potable water is available at all times.

RESTING BOARDS

Although not required by regulatory authority, resting boards may provide an improved environment for the animals, particularly those housed on wire grids or



FIGURE 9.4 Automatic water drinking valve.

for heavy animals. In some cases, resting boards may be incorporated into the caging system with a ramp to provide an elevated resting perch. When provided, these boards must also be chemical- and heat-resistant to withstand sanitation practices. They are usually constructed of durable plastic or metal, although disposable materials have been used to provide additional enrichment, such as autoclaved cardboard. When disposable materials are used, they should be evaluated for potential contamination and ingestion hazard as well as their potential effects on research results.

NEST BOXES

Animal Welfare Act Regulations (USDA, 2005) mandate that a “suitable nest box containing clean nesting material shall be provided in each primary enclosure housing a female with a litter less than one month of age.” Nest boxes must also be constructed of materials that are easily sanitized and durable. In this regard, stainless steel or durable plastic are recommended. Drainage holes for urine are not essential because the doe spends little time in the nest. Careful consideration should be given to the flooring material in nest boxes, as hip dysplasia has been reported in kits using boxes with slick flooring (Owiny et al., 2001). Although there are no minimum size requirements for nest boxes, they should be of adequate design to allow the doe to turn around comfortably without trampling the kits, but small enough to provide a sense of security and privacy to the doe. Bedding selection is discussed above.

ENVIRONMENTAL ENRICHMENT DEVICES

Specific approaches and effects of environmental enrichment are addressed above. As with any other equipment that enters the rabbit’s primary housing environment, enrichment materials must meet the same rigorous sanitation standards as the caging itself. If not, it must be disposable and not utilized for more than a single animal (or group if housed together). Enrichment devices that are not adequately sanitized between animals have a high potential for disease transmission as fomites because oral manipulation of the devices by the rabbit is common. Devices should also be evaluated for the potential to cause external trauma or gastrointestinal disease if ingested.

Specialized Caging

Depending on research needs, rabbits may be housed in a variety of specialized housing systems. Metabolic cages resemble standard housing cages but are equipped with a pan underneath the cage that funnels urine and feces into collection vessels for metabolic measurements. Individually-ventilated and biological-containment housing systems are just beginning to appear commercially

for rabbits. These systems provide either animal protection from the environment, containment of biological agents used within the rabbits, or some combination of both. Containment systems usually involve standardized caging housed within a containment unit, whereas ventilated caging is normally incorporated directly into the housing system. Other types of specialized caging may be encountered in the research environment depending on research protocol requirements. Housing that provides opportunities for exercise, environmental preferences, response/reward systems, and other research activities have been developed, but these are usually developed or adapted in the specific laboratory setting and are not commercially available.

Bedding

Direct bedding is not commonly utilized when housing rabbits in a research setting. Since the majority of the commercial caging systems are designed with a grate system allowing feces and urine to drop away from the animals, bedding within the cage is usually reserved for nest boxes. The exception to this is in group-housing environments which are discussed below.

There are a variety of materials that make suitable bedding for rabbits. Most commercial bedding materials for rodents are adequate, although fibrous materials such as hay, straw, or excelsior may be preferred by the rabbits, especially within nest boxes. These products more closely approximate the grass and straw that rabbits combine with their fur to make nests in the wild. Primary concerns for direct bedding should be animal comfort, low potential toxicity if ingested, and minimal dust (Kaliste et al., 2004).

Indirect bedding may be used within the pans below the cages. In this case, the absorbency of the bedding is most important as animal comfort is not a factor. Paper products, absorbent pads, wood shavings, or a variety of other materials may be suitable. Some products contain odor-absorbing chemicals or antibiotics to break down or decrease ammonia production to help decrease room odor. Another concern with indirect bedding should be that the products not emit volatile odors that may affect research either naturally or when in contact with urine, although this may be of less concern in rabbits than rodents (Ruben et al., 2007).

Physical Environment

Indoor environmental requirements for housing rabbits can be found in both the Animal Welfare Act Regulations (USDA, 2005) and the *Guide for the Care and Use of Laboratory Animals (Guide)* (ILAR, 2011). Requirements are similar to most other species housed in a research setting, with specific exceptions noted below.

Lighting

Light levels are generally set at 30-foot candles 1 meter above the floor as per *Guide* recommendations for all species. This has been shown to be adequate for general husbandry purposes without detrimental effects on the animals (Bellhorn, 1980). Levels above this may cause significant optic alterations which may affect research results, especially for albino rabbits which already have an altered visual threshold compared with pigmented animals (Balkema and Drager, 1991). Bi-level lighting with increased illumination during husbandry duties may allow for more subdued lighting overall. Care must be taken to ensure the high-light override automatically reverts back to low-light levels to prevent accidental overexposure.

The photoperiod should be controlled and is normally set at 12 hours light and 12 hours dark for typical research applications. If breeding or reproductive studies are being conducted, a 16-hour light:8-hour dark cycle has been recommended (Cheeke, 1987). The light cycle should be monitored, preferably continuously, to ensure proper functioning. Alterations in light cycle can have profound effects on research and reproductive parameters (Colasanti and Trotter, 1976; Hasan et al., 1981).

Light fixtures should provide even light throughout the housing environment; should be sealed to the ceiling to prevent the entrance of vermin; and should allow for external sanitation. Although external windows may provide natural lighting and viewing enrichment for the animals, they are not recommended. External windows make photoperiod control challenging; can cause temperature variations within the room; and may pose a security and vermin risk. Although viewing windows indoors may be beneficial in allowing animal monitoring without disturbance, care must be given to prevent room photoperiod disturbance with continuous hallway lighting.

Noise

Excessive noise to the point of potential auditory damage (>85db) is rarely encountered in the normal laboratory housing environment, but sound can be a significant stressor for laboratory animals (Nayfield and Besch, 1981; Sales et al., 1999). Rabbits are keenly aware of noise in their environment and this should be taken into consideration when designing facilities for rabbit housing. Housing rabbits adjacent to other species that are very vocal (e.g., non-human primates, dogs) can provide a source of stress and therefore, potential experimental variation. Constant background noise appears to be less disturbing than intermittent noise and providing a low level of constant noise (white noise) may help mask noises of intermittent interruption.

Rabbits can be a significant source of noise as well. Thumping in metal cages or rattling enrichment devices or cage doors can cause quite a disturbance when numerous rabbits are housed in the same room. This may be a consideration for sensitive species in surrounding housing areas.

Heating, Ventilation, and Air Conditioning (HVAC)

Although the *Guide* (ILAR, 2011) sets temperature recommendations for rabbits at 61–72°F, they appear to be most comfortable at the low end of this range. Temperatures above this range can lead to panting, anxiety, and hair loss. Room temperature selection should take into consideration the type of caging, whether bedding is provided, and whether animals are housed singly or in a group. Room temperatures need to be monitored, preferably continuously, to ensure proper functioning and to maintain animal health and welfare.

Humidity ranges per *Guide* recommendations are set at 30–70% relative humidity. Rabbits appear to be most comfortable at the lower end of this recommended range.

Standard ventilation rates of 10–15 air changes per hour (ILAR, 2011) appear adequate for laboratory-housed rabbits. Rabbit housing rooms are generally set at a negative air pressure differential to adjacent spaces to help contain allergens and the relatively large amount of hair these animals shed. Multiple factors should be considered in determining the appropriate pressure differential. These include the requirements of adjacent spaces, types of research, and health status of the animals.

It is generally recommended that room air supply be 100% fresh air with no recirculation. Although recirculation is allowed with HEPA-filtration (ILAR, 2011) and may offer significant heating or cooling efficiency, this practice should be evaluated carefully. Not only might recirculation of contaminated air expose animals to pathogens, but rabbits release significant amounts of hair and dander which may make filter maintenance challenging. Ooms et al. (2008) demonstrated airborne contaminants within rabbit holding areas to be substantial.

Secondary Enclosure

The secondary enclosure is defined as the space within which the primary enclosure resides; i.e. the room in most laboratory settings. Even though in many housing situations the animal never comes in direct contact with the secondary enclosure, stringent sanitation requirements necessitate careful consideration in design of the space. Surfaces must be smooth, impervious, and able to withstand heat and chemical exposure. Wall and ceiling penetrations should be sealed to prevent vermin entrance. Fixtures, outlets, and switches

must be water-resistant to allow safe room sanitation. Wall fixtures should be protected if heavy, mobile rack systems are used for housing. Floors should be impact-resistant to withstand heavy stationary and mobile racks and dropped equipment (such as cage pans). Access panels, ventilation grates, and other permanent fixtures should be constructed of materials (such as stainless steel) that can withstand physical and chemical abuse without breaking or rusting.

Husbandry

Husbandry in the research environment is more than just meeting the biological needs of the animals. Research is a sophisticated endeavor where inter- and intra-animal variations must be minimized. Experimental results may be affected by changes in diet, chemical exposure, light levels, temperature, and many other environmental factors. It is the challenging responsibility of the husbandry program to provide the most appropriate setting for the animals while maintaining tight control over environmental variables that may affect research.

Food

Specific nutritional requirements of rabbits are discussed elsewhere in this chapter. In most laboratory settings, rabbits are fed a limited ration once or twice per day. Rabbits, particularly the New Zealand White breed, tend to get very obese if fed *ad libitum*. Feed is usually provided without further treatment or processing in the laboratory setting. The appropriate amount to feed depends on the quality of the food, the metabolic state of the animal, and specific research requirements. In general a once-daily feeding of 90–120 g of a high-quality, pelleted rabbit feed will be adequate for a medium-sized adult rabbit (Harkness and Wagner, 1989). An alternative method to prevent obesity is to feed a higher-fiber, lower-energy diet *ad libitum* (Cheeke, 1994), although this will be associated with increased fecal output. A liquid rabbit diet with comparable results to pelleted diets has been described (Latour et al., 1998) and this may be useful for studies requiring oral dosing. Changing diets can have significant impact on gut flora and should be done slowly over several days by gradually increasing the new diet mixed with the old.

Food should be purchased from a reputable dealer where it is stored in an area with appropriate environmental and vermin control. Many animal feeds produced for commercial use contain additives and/or antibiotics which may have significant impact on research studies. Certified, laboratory-grade feeds are recommended and producers should be able to certify not only the ingredients used to make the feed, but also that there has been no contamination of the feed from

products left over from other food production runs. Feed should be transported to the facility in dedicated vehicles which prevent contamination and/or vermin infestation. Feed bags that arrive damaged in any way should not be used as they constitute a contamination risk.

Within the facility, feed should be stored off the floor and away from the walls to facilitate sanitation of the feed holding area. Refrigeration is preferable, but any clean, dry, cool area is adequate. Regardless of storage conditions, feed should not be used beyond 6 months past the mill date (usually stamped on the bag) and perhaps sooner if storage conditions are less than ideal. Within the storage area feed should be rotated so that the oldest feed is used first.

Open feed in use may be stored within the animal room, but should be kept in a clean, sealed container. The mill date (or expiration date) should be clearly visible. Feed storage containers and feeding implements should be sanitized between fillings on a regular basis.

Water

Unless restricted by research protocol, rabbits should have *ad libitum* access to clean, potable water. This is most easily accomplished via an automatic watering system, but bottles are adequate if checked frequently. Poor water quality may impact animal health and research results. Water treatments vary by institution and research requirements and run the spectrum from untreated well water to reverse osmosis and irradiation. The level of water treatment is often dictated by other projects and species being housed. Regardless of source and treatment, water quality should be periodically evaluated to ensure the absence of impurities and infectious agents.

Bedding

Specific bedding selection was discussed above. Selection of a bedding supplier should follow the same criteria as for a feed supplier. A reputable supplier should store and transport bedding materials under very similar conditions as outlined for feed suppliers.

Within the facility, bedding should be stored off the floor and away from the walls to facilitate sanitation. Storage areas should be maintained clean, dry, and monitored for vermin. Although bedding rotation is not critical, it may be appropriate, especially under storage conditions with higher humidity.

Open bedding in use may be stored within the animal room, but should be kept in a clean, sealed container. Bedding storage containers and implements should be sanitized on a regular basis.

Health Monitoring

Monitoring the health and well-being of animals in the research setting is the responsibility of all who enter that setting. A good health-monitoring system ensures

that any abnormalities are promptly reported to the responsible veterinary care staff, up to and including the institutional veterinarian. An alert system (such as a health check notification card (Figure 9.5)) is a good way to ensure prompt veterinary assessment of abnormal conditions. Although there is no federal mandate in the United States for individual health records for rabbits, the *Guide* (ILAR, 2011) highly recommends such records to appropriately document health concerns and treatments, especially for animals on surgical protocols. Even if individual records are not kept, documentation must be sufficient to convey this information for any given animal within the facility.

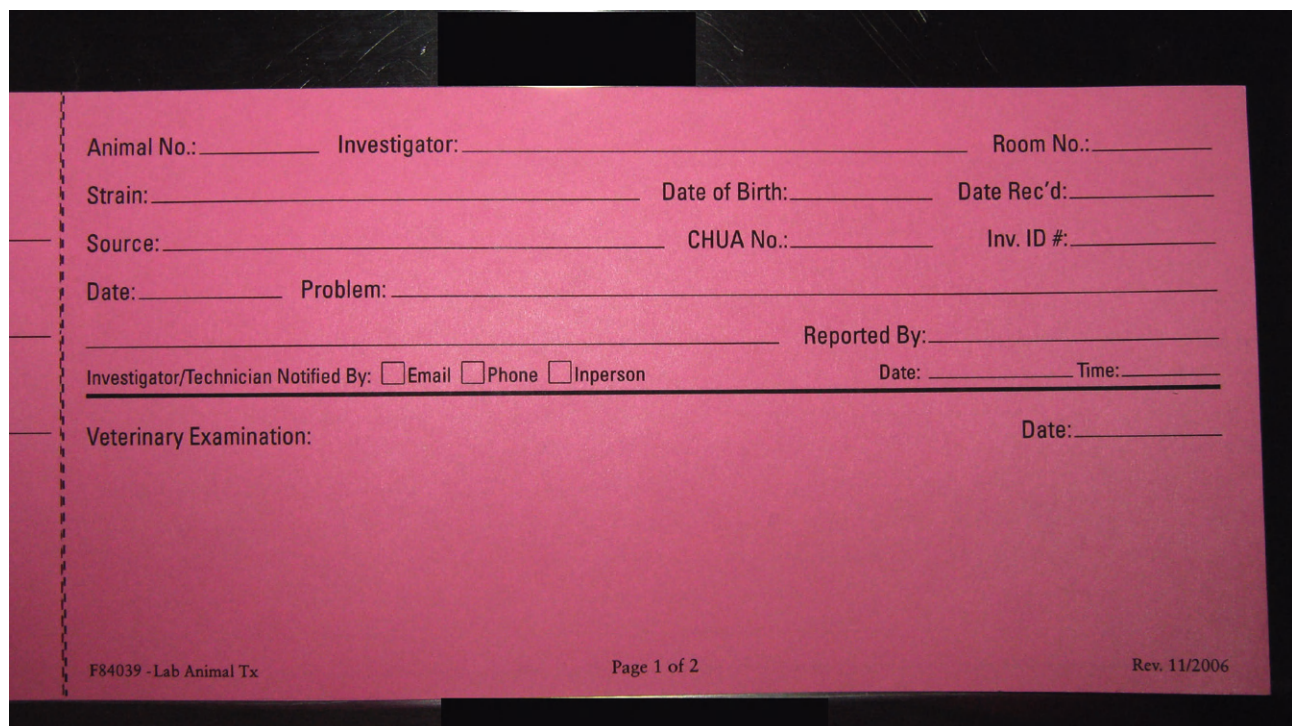
Sanitation

Sanitation standards are one of the few places where the Animal Welfare Act Regulations (USDA, 2005) and the *Guide* (ILAR, 2011) differ. The Animal Welfare Act Regulations require monthly sanitation of primary enclosures for rabbits whereas the *Guide* generally suggests biweekly sanitation (although there is some latitude for professional judgment). Sanitation protocols to be utilized should be compared to any and all applicable standards and the most stringent standards should be followed to ensure compliance. In all cases, sanitation is generally defined as cleaning followed by either chemical disinfection and/or hot water rinse (typically at least 180°F). If chemical disinfectants are utilized,

there must be adequate contact time for disinfection and they must be thoroughly rinsed to prevent animal exposure. Regardless of the methods utilized, sanitation efficacy should be periodically tested to ensure adequate and consistent results. Rabbit cages and pans present a difficult sanitation challenge because rabbits on standard laboratory diets excrete significant amounts of calcium in their urine which forms calcium carbonate scale on cages and pans. Acid wash cycles or pre-soaks may be necessary to remove this scale prior to sanitation because normal alkaline wash cycles inadequately remove the scale. In addition to calcium deposition, rabbit urine may normally be pigmented red or orange and this must be distinguished from hematuria (Cheeke, 1987).

PRIMARY ENCLOSURES

The *Guide* (ILAR, 2011) suggests sanitizing enclosures and accessories, at least once every 2 weeks, although allowance is granted for professional discretion based upon the individual situation. The Animal Welfare Act Regulations (USDA, 2005) require sanitation of the primary enclosure at least every 30 days. Whether individually housed in a caging system or group-housed in pens, the primary enclosure must be emptied and appropriately disinfected at least once per month. Many facilities just default to biweekly cage sanitation to ensure that integrated equipment (such



Animal No.: _____ Investigator: _____ Room No.: _____

Strain: _____ Date of Birth: _____ Date Rec'd: _____

Source: _____ CHUA No.: _____ Inv. ID #: _____

Date: _____ Problem: _____

Reported By: _____

Investigator/Technician Notified By: ☐ Email ☐ Phone ☐ Inperson Date: _____ Time: _____

Veterinary Examination: _____ Date: _____

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FIGURE 9.5 Health check alert notification card.

as feeders) is being sanitized according to regulatory requirements. For directly bedded animals, soiled litter must be removed and replaced with clean litter weekly and collection pans/troughs under grid-type caging must be “cleaned” weekly (USDA, 2005).

SECONDARY ENCLOSURES

There are neither regulatory requirements nor specific recommendations concerning how often secondary enclosures (i.e., rooms) must be sanitized. Though left to professional discretion, the general expectation is that secondary enclosures will be cleaned regularly and disinfected as appropriate to the circumstances. In most facilities the housing room is completely disinfected either between groups of animals or periodically anywhere from monthly to biannually.

ANCILLARY EQUIPMENT

Although the *Guide* (ILAR, 2011) generally leaves sanitation, frequency of feeding and watering equipment up to professional judgment, it does recommend that solid-bottom caging, bottles, and sipper tubes be sanitized at least weekly. The Animal Welfare Act Regulations (USDA, 2005) strictly require that these implements be sanitized at least every 2 weeks. This standard is generally applied to any other equipment directly within the primary enclosure (such as manipulation devices), although the sanitation requirements for these implements are vague.

Animal Procurement

Suppliers

In many areas there are “backyard breeders” and/or agricultural auctions where inexpensive rabbits may be purchased for research use. The procurement of rabbits from these sources should be undertaken with caution because the health status of such rabbits is often substandard or, at best, undefined. Numerous studies have documented untoward effects of rabbit pathogens on research (Baker, 2003). High-quality, pathogen-defined animals purposely bred for research are highly recommended. Although the expense of purchasing and shipping these animals may be significantly higher than local breeders, this is often offset by the time and effort saved in having to repeat studies due to unforeseen variables or even animal loss due to illness. In addition, the need to repeat studies due to animal health-related complications may be considered wasteful from an ethical perspective of animal use. Numerous natural pathogens, such as *Pasteurella multocida* and *Clostridium piliforme*, have been shown to alter a variety of organ system functions and could significantly impact research results (Baker, 2003). Although cost cannot be

discounted in times of limited research funding, the quality of the research produced must always remain a high priority to ensure scientific progress and, thereby, justify animal use.

Quarantine and Stabilization

Animals may arrive in reusable transport cages that need to be returned to the supplier or in disposable transport boxes. Entrance procedures into the facility are dependent upon the health status of the animals, housing conditions, and research needs. These may vary from direct placement into the caging environment where the research will be conducted to strict isolation and quarantine procedures.

At a minimum, animals should be inspected upon arrival to ensure they are free from overt clinical signs of disease or trauma. If acceptable, the animals should ideally be placed in a quarantine holding area. This may not be required if animals are received from a single vendor with high and consistent health standards. The length of quarantine is highly variable and depends upon the expected health status, time requirements for any diagnostic procedures, likely cross-contamination with other colony animals, and incubation times for diseases of concern. If quarantine procedures are utilized, they should be at least as long as the acclimation period.

Even if quarantine is deemed unnecessary due to high health quality, newly arrived animals need a period of acclimation to regain a consistent physiological plane prior to initiation of any research project (Conour et al., 2006; Obernier and Baldwin, 2006). Again, recommendations for the length of the acclimation period are variable, but most institutions adhere to a minimum of 48–72 hours which has been shown to correlate with stress hormone stabilization after shipping (Toth and January, 1990). Acclimation periods may also be varied within a facility depending upon the intended use of the animals; i.e. a survival surgery research protocol may require a longer acclimation period than a non-survival teaching protocol.

Identification

Although there are no specific legal requirements for rabbits in a research facility to be individually identified, it is highly recommended for research recording and clinical health record maintenance. The cage card alone may be sufficient identification for individually housed animals, but other methods that are integral to the animal should be considered and would likely be advantageous when animals are pair- or group-housed. Although small numbers of animals may allow individual animal feature recognition; this may not be adequate when staff are unfamiliar with the rabbits. Feature recognition may also be insufficient when

large numbers of similar animals (such as New Zealand White rabbits) are being housed.

Animal-specific methods of identification include ear tattoos, ear clips or tags, fur dyes, and implantable telemetry devices. Ear tattoos are usually very permanent, but may require anesthesia to place depending upon institutional policy. This may be true for ear clips or tags as well. In addition, clips or tags may tear out causing trauma to the pinna and loss of identification. Non-toxic fur dyes (Figure 9.6) are usually innocuous, but may require repeat application. Subcutaneous transponders (microchips) are being used more commonly as they are relatively easily placed and can provide significantly more information, including telemetry data such as body temperature. The disadvantages are that they require specialized equipment for detection, may require anesthesia for placement, and have been associated with tumor induction in rodents (Blanchard et al., 1999; Blanchard et al., 1997; Elcock et al., 2001; Tillman et al., 1997).

Environmental Enrichment

The *Guide* (ILAR, 2011) stresses the importance of meeting all environmental requirements of laboratory animals, including physical, social, and behavioral requirements. Stereotypic behaviors (Hansen and Bethelsen, 2000), self-injurious behaviors, and even metabolic abnormalities (Potter and Borkowski, 1998)

can result from maintaining rabbits in a mundane environment. Obviously the normal environment of wild rabbits cannot adequately be replicated within the research environment, so one must make approximations of environments and activities that might substitute for normal experiences and promote normal species-specific behaviors (Baumans, 2005; RSPCA, 1993).

For all laboratory species, environmental enrichment must be approached with several important factors in mind: (1) there is animal-to-animal variation in what is considered enriching, what is considered stressful, and what makes no difference at all; (2) food rewards change the nutritional status of the recipient; and (3) enrichment devices are a potential source of disease transmission or other health risk. Although it is desirable for rabbits to live as comfortably as possible, enrichment choices must be evaluated to ensure that they do not detrimentally alter research results. This has been of particular concern in toxicology research facilities, but at least one study (Johnson et al., 2003) has demonstrated that manipulation enrichment can be provided without untoward effects on toxicology studies in rabbits.

Environmental enrichment for rabbits commonly involves the addition of either food supplements (Figure 9.7A,B) and/or a variety of manipulation devices (Figure 9.8A–D) to provide variety and activity to the sterile cage environment. Few studies examining the interaction of rabbits with enrichment devices had

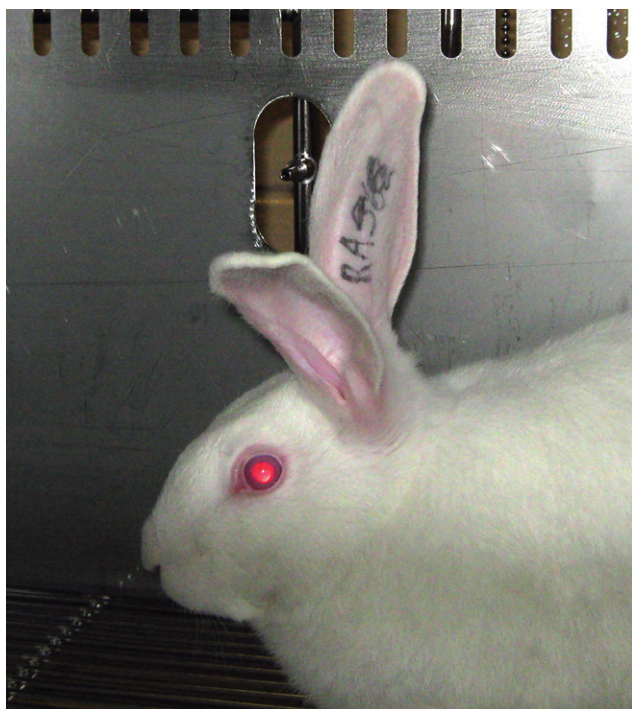


FIGURE 9.6 Non-permanent identification mark on inner surface of rabbit ear.



FIGURE 9.7 Common food-enrichment devices, alfalfa cubes (A) and fruit-flavored gnawing treats (B). Photographs courtesy of Bio-Serv, Inc. (Frenchtown, NJ).



FIGURE 9.8 Common shelter and manipulation enrichment devices for rabbits, stainless steel rattles (A), polypropylene dumbbells (B), polycarbonate hut (C), and hollow polyethylene ball (D). Photographs courtesy of Bio-Serv, Inc. (Frenchtown, NJ).

been conducted prior to publication of this text (Hansen and Bethelsen, 2000; Harris et al., 2001; Huls et al., 1991; Johnson et al., 2003; Lidfors, 1997).

Food supplements and/or diet variations are common methods of enriching the environment for research rabbits. One study (Harris et al., 2001) found that the total interaction time was higher for food supplement-type enrichments than for non-food enrichment devices. Berthelsen (1999) found that bar gnawing and fur chewing were decreased in rabbits supplied hay in the cage environment. The nutritional quality of the supplement should be evaluated to ensure purity and

to determine potential research impact. In addition, some popular supplements may greatly increase caloric intake, leading to obesity or decreased ration intake and potentially resulting in nutritional deficiencies.

Manipulation devices are the other common means of enriching the environment for laboratory rabbits. There are many commercially available products advocated for rabbit enrichment (Figure 9.8A–D) and many laboratories have devised their own homemade devices for this purpose. Such products usually involve something the rabbit can pick up and move about or something attached to the cage that can be rattled to

make noise. Manipulation devices should be carefully evaluated to make sure they do not pose a hazard to the animal either inherently or after damage by the rabbit (Shomer, 2001).

The impact of human social interaction must not be discounted when it comes to rabbit enrichment. Rabbits have been demonstrated to recognize and respond to particular individuals (Davis and Gibson, 2000), particularly when those individuals were associated with positive interactions (food rewards).

Group Housing

Group housing of rabbits in various configurations has been recommended as a preferred alternative to the traditionally single-housed rabbit in stainless steel caging (BVAAWF et al., 1993). Although most rabbits (especially in the United States) are still housed individually or in pairs within standard commercial rabbit caging, group housing in pens on contact bedding is increasing in popularity (Quinn, 2009). Cage housing is perceptually easier from a research control, sanitation, animal-monitoring, and health standpoint. Mixing animals in a pen environment allows for aggression, can make food intake monitoring difficult, and brings up concerns about meeting stringent sanitation requirements. These perceptions aside, as concerns over the social environments of research animals rise, group housing of rabbits will likely become an increasingly selected option or may even become the standard as regulations are revised.

Group housing is normally accomplished by creating a pen arrangement on the room floor (Figure 9.9). Walls may be constructed of plastics or laminates and may incorporate the room walls. Materials must be non-toxic and able to withstand chewing and sanitation

procedures. Pens are usually bedded with straw, shavings, or paper; and hay (often autoclaved) may be provided as forage. Various containers or huts may be provided to allow animals privacy and escape from aggression. Sanitation is accomplished by spot-cleaning daily and complete bedding removal and sanitation at least biweekly.

Stereotypical behavioral disorders, which may occur in single-caged rabbits, have been shown to greatly diminish in group-housed rabbits (Held et al., 2001; Krohn et al., 1999; Love, 1994; Poderscek et al., 1991) and female rabbits have displayed preference to be housed together (Brooks et al., 1993; Held, 1995), although this depended on the configuration of the solitary housing choice.

Some investigators have expressed concern over the influence that group housing may have on research results or control in social animals where dominance hierarchies are likely to develop. As rabbits are commonly used for antibody production, Turner et al. (1997) examined immunological responses between dominant and submissive rabbits in a group-housing environment and found no differences. Another group (Whary et al., 1993) compared humoral and delayed hypersensitivity response, feed intake, growth rate, and selected physiological parameters and found no differences between group-housed and individually caged rabbits. Not only have studies shown that there may be no significant research differences between housing types (Fuentes and Newgren, 2008), Nevalainen et al. (2007) demonstrated that biological parameters can actually be more stable in group-housed animals. Even if group housing is not feasible during research studies, some have advocated keeping rabbits in groups until the research begins (Stauffacher, 1992).

Aggression between male rabbits (and occasionally females) after puberty makes group housing of intact male groups essentially impossible without significant risk of injury. Castrated males have been successfully group-housed with only minor conflicts (Raje and Stewart, 1997).

BREEDING

The age of reproductive maturity is variable by rabbit breed, but usually occurs between 4–8 months with smaller breeds maturing faster than larger breeds (Harkness, 1987). The adult male rabbit is referred to as a buck, the female as a doe, and the young as kits (plural). Does should be bred as soon after maturity as possible as they will become less receptive the longer they go without breeding; especially if they become obese.

Although breeding is a natural activity of rabbits, the laboratory setting is not a natural environment for



FIGURE 9.9 Group housing of rabbits in floor pens. Photograph courtesy of J. Kincer.

this activity. Environmental factors can have significant impact on the willingness to breed and the overall success of a breeding program. Both sexes of rabbits have very specific behaviors in association with sexual activity and understanding these behaviors is crucial to ensuring the successful production of offspring from mating through weaning.

Sexual Behavior in Bucks

Pre-copulation

In the wild, male rabbits display a range of behaviors to attract females and dissuade male competitors. Chasing of both sexes is commonplace in the wild, but usually physically limited in the laboratory environment, therefore other behaviors may be intensified as a replacement. These other activities include tail-flagging, urine spraying (enurination), stiff-legged walking, and intensive cage circling (Patton, 1994).

Tail-flagging is a display behavior commonly seen in males when exposed to receptive females. The tail is held straight up or laid over the back and may be flicked repeatedly with the hindquarters pointed towards the female. Besides display purposes, this behavior may also be associated with the release of olfactory stimuli.

Enurination is the purposeful ejection or spraying of urine. This is a common behavior of bucks in non-breeding situations believed to primarily be a means of olfactory marking. This behavior can intensify in bucks exposed to breeding does and may be directed toward the doe or a competitor buck.

Ambulation within the cage may increase when bucks are exposed to breeding does. They may appear agitated, “thump” the bottom of the cage with the hind feet, circle the cage, increase interaction with enrichment devices, assume specific postures (e.g., elevating the hindquarters), and walk with a stiff-legged gait.

Copulation

Does should always be brought to the buck’s cage for breeding. Rabbits can be very territorial and does will often attack bucks that enter their environment. In the wild, receptive does seek out bucks for breeding and therefore are away from their marked nesting area. In addition, bucks may be more interested in marking the female’s territory than in copulation.

If the doe is receptive, breeding should occur very rapidly. The buck mounts from behind the doe and makes quick, jerky pelvic thrusts. Once intromission occurs, the buck ejaculates quickly and then may fall backwards and cry out. This may appear unnatural and cause concern for observers, but it is perfectly normal and the buck recovers very quickly.

The ejaculate is usually of a gelatinous consistency and the normal volume is approximately 1 ml. Details of normal rabbit ejaculate composition have been published elsewhere (Hammer, 1970).

If copulation does not occur very soon after placing the doe in the buck’s cage, the doe should be removed. This indicates either a disinterested buck or an unreceptive doe and can present an unsafe environment for both.

Post-Copulation

Bucks tend to be aggressive breeders and will often quickly attempt to mount the doe again after copulation. Although this second attempt may be successful, the doe may aggressively reject the buck leading to injury; therefore the doe should be removed shortly after the first successful copulation.

Individual bucks may be used to breed several does in the same day without a decrease in fertility. Commercial breeders will often keep one buck for every 10–25 breeding does, with the buck used to breed several does in a single day and with a day of rest in between sets (Patton, 1994).

Sexual Behavior in Does

Pre-Copulation

Although rabbits do not appear to have a regular estrous cycle, they do display periods of increased receptivity that occur every 4–6 days in domestic does (Meyers and Poole, 1962). In the wild they are much more seasonal with specific periods of receptivity mixed with periods of anestrus. Although there appear to be some cyclic vaginal changes, cytology may be unreliable due to variability associated with environmental cues. Vaginal smears from normal adult rabbits showed no distinct pattern of cellular type in does isolated from males, but showed increased numbers of superficial epithelial cells in does with auditory, olfactory, and visual contact with bucks (Ola and Oyegbade, 2008). Studies on follicular development and estrogen levels indicated a 14–16-day cycle with 12–14 days of higher estrogen (receptive period) followed by 1–2 days of low estrogen (refusal period) (Cheeke et al., 1987). The reproductive cycle of the doe is difficult to define because it is so highly influenced by environmental factors such as buck exposure, nutrition, light, and temperature.

If the doe is receptive, she usually will present her hindquarters to the buck with her tail elevated. Aggressive, confrontational activity should be a clear indicator to remove the doe quickly to prevent injury (see below). Both sexes may display a “preference” for partners. Although one breeding may not be successful, presenting a new partner for either or both may result in successful copulation.

Vaginal examination may give an indication if a doe is ready to breed. Receptive does usually have a dark pink to red, moist vulva. Unreceptive does usually have a light pink to almost white, dry vulva. Because this evaluation is quite subjective, it is probably more productive to simply pair prospective breeders and observe behavioral cues.

Copulation

Receptive does will readily accept the buck mounting and will remain passive during the copulation period. Copulation tends to be very brief; therefore there is little time for distinct behavioral variation in the doe.

Post-Copulation

Once the buck has dismounted, the doe may potentially become aggressive towards the buck, particularly if the buck attempts a second copulation. Once a successful copulation has occurred, the doe should be removed from the buck's cage.

Rabbits are induced ovulators with ovulation usually occurring 10–13 hours after copulation (Patton, 1994). A variety of methods have been developed for inducing ovulation in does for artificial insemination, a common practice in commercial production colonies (Bruni et al., 1982; Michelmann and Paufler, 1973; Staples and Holtkamp, 1966). Artificial insemination of rabbits will not be discussed in this chapter. For a complete review, see Theau-Clément (2008).

Gestation

Pregnancy Determination

Pregnancy is usually determined by gentle palpation of the abdomen. Fetuses can generally be detected between 12–14 days of pregnancy (Cheeke et al., 1987). Radiography can be used after day 11 (Hafez, 1970) and ultrasound can be used reliably as early as 7 days post-breeding (Chavatte-Palmer et al., 2008).

Pseudopregnancy may also occur, although this is not common. This is essentially an unfertile ovulation which can be caused by a sterile mating; mounting activity by other does; or any other environmental stimulant which may replicate copulation. Pseudopregnancy generally lasts 16–22 days and the doe may begin nest-building at the end of this period (Templeton, 1940). After about 18 days the corpora lutea begin involution and the condition resolves.

Gestation Length

Gestation length is generally between 29–35 days, with an average length of 31–32 days (Harkness and Wagner, 1989). Environmental factors, especially in housing situations permitting seasonal cues, may

influence total gestation length (Asdell, 1946). Larger litters tend to have longer gestations than smaller ones.

Physical Changes

Rabbits do not show overt signs of pregnancy until very late in gestation. This is particularly true for longer-haired breeds or obese individuals. New Zealand White rabbits are often overfed in the laboratory setting, which can make the detection of an unexpected pregnancy quite challenging. It is not uncommon for husbandry staff to miss a late pregnancy in a newly arrived animal only to find kits in the cage the next morning. With careful observation, conformational changes can be observed. The caudal abdomen will assume a bulkier shape giving the animal a much more prominent pear-shape when observed from above. As parturition nears, the mammary glands will thicken and should become noticeable on palpation, although milk is unlikely to be expressed prior to birth.

As parturition approaches, the doe will normally begin building a nest. If available, substrates such as hay, straw, paper, or excelsior will be incorporated, but the nest almost always includes copious amounts of hair plucked from the doe's body as a liner. The hair loosens as parturition approaches (Sawin et al., 1960) and this hair loss/plucking can give the doe an unthrifty appearance prompting concern from unfamiliar husbandry staff.

Parturition

Parturition (kindling) generally occurs in the morning (Sawin, 1950) and usually occurs very rapidly (within about 30 minutes). Both anterior and breech presentations may occur and occasionally kits may be born hours or even days apart (Patton, 1994). Natural parturition is accompanied by a sudden release of oxytocin and parenteral administration of oxytocin (1–2 IU) may be used to induce parturition or assist if parturition is prolonged (Hagen, 1974).

Pups are generally born in the nest. Immediately after birth the doe will eat the placental membranes, bite through the umbilical cords, and clean the kits.

Litter Composition

In the research setting, the average number of live New Zealand White kits born in a litter is approximately eight (Patton, 1992). Average litter sizes measured in other settings have been variable (Templeton, 1962) depending on methods of counting; environmental variables such as season; and genetics of the rabbit population being measured (Piles et al., 2006; Rollins et al., 1963; Sittman et al., 1964).

The information available on sex ratios within litters is as variable as it is for average litter size. Some studies

have indicated a slight male bias (Hafez, 1970) while others have demonstrated a small preference toward females (Brambell, 1942). Once again environmental factors may play a significant role in sex determination and this could explain the discrepancies in the published data.

Postpartum Breeding

Does will often be receptive to mating immediately following parturition and this receptivity may last up to 35 days if young are removed (Patton, 1994). If the kits remain with the doe, this receptivity drops off within 1–2 weeks and remains low until after weaning (Hammond and Marshall, 1925). When postpartum breeding is utilized, pregnancy success rates appear to be inversely proportional to current litter size and lowest during peak lactation (at approximately 3 weeks) (Cheeke et al., 1987).

Postpartum Care

Nest Building and Behavior

Pregnant does should be provided a nest box approximately 1 week prior to parturition. The design and selection criteria for use in a laboratory setting were discussed earlier. The nest box should include an appropriate direct bedding material such as hay, straw, paper, excelsior, or other non-toxic product. Several days prior to parturition, the doe will begin plucking loose hair from her body to mix with the bedding substrate to make a nest. The nest will be hollow in the middle and usually lined with her hair.

Kits will be born directly into the nest where they will remain until they are physically able to leave the nest on their own. Maternal retrieval is not typical for rabbit does (Ross et al., 1959), therefore any kits that are displaced from the nest will be left to die of malnutrition and/or hypothermia.

Nursing

Does usually have eight mammary glands distributed evenly along the ventral abdomen and thorax, four on each side. Although the glands will thicken and develop several days prior to parturition, milk is not released until after the doe gives birth. Milk production increases to a maximum at approximately 3 weeks postpartum (Hafez, 1970), then declines until lactation ceases at 5–9 weeks.

Rabbit milk is quite rich, containing 12–13% protein, 10–18% fat, 1–2% lactose, and 2.3% minerals (Coates et al., 1964). This richness allows for the normal rabbit maternal behavior of only nursing the litter once per day, regardless of access to the nest (Zarrow et al., 1965). The nursing behavior is probably instinctual, held over from wild rabbits which spend most of the

day away from the nest foraging. Nursing generally occurs in the morning, but this is variable and usually only lasts from 3–5 minutes.

Orphaned rabbits may be fostered or hand-reared using commercially available rabbit or kitten milk replacers. They are generally fed 2–4 times per day allowing them to consume as much as they will take.

Weaning generally occurs between 4–8 weeks of age, depending on availability of access to solid food for the kits. When compared in commercial production, weaning at 4 weeks was not associated with any increased mortality over those weaned at 6 or 8 weeks (Chen et al., 1978).

NUTRITION

Gastrointestinal System

Anatomy and Physiology

Rabbits are herbivores and are considered “concentrate selectors” which means they tend to choose soft, nutrient-rich vegetative growth over those that are higher in fiber and roughage (Irlbeck, 2001; McNitt et al., 1996). This means that their diets are usually higher in protein and soluble carbohydrate and lowest in fiber compared to other types of herbivores (Cheeke, 1994).

Anatomically, the rabbit is a hind-gut fermenter. Similar to many small herbivorous rodents, guinea pigs, and horses, the rabbit’s gastrointestinal tract is dilated towards the end (in this case the cecum) to create a fermentation vat. Symbiotic microbes (primarily *Bacteroides* spp.) populate the cecum and provide the rabbit with the means of breaking down digestible fiber (Irlbeck, 2001).

Ruminants have the advantage of being able to hold food materials in their rumen for prolonged periods of time. This allows prolonged digestion and enables them to subsist on high-roughage, low-quality feed-stuff. The rabbit cecum does not provide this prolonged storage capacity and functions more like a continuous pass-through organ similar to other parts of the gastrointestinal tract. This would potentially allow for a significant loss of digestible materials if it were not for the unique anatomy of the proximal colon. Immediately past the ileoceccocolic junction the colon has distinct haustrae and a specific peristaltic pattern that separates out large, indigestible particles from smaller, nutrient-rich ones (Bjornhag, 1981). The larger particles are sent distally to become fecal pellets and the smaller particles are pushed retrograde back into the cecum for further fermentation.

CECOTROPHY

Another unique aspect of the rabbit’s digestive process is the production and consumption of cecotrophs.

Since rabbits normally consume highly digestible plant products, much of the nutrients are derived directly via normal gastric digestion and absorption in the small intestine. Indigestible dietary portions are fermented in the cecum. As the majority of nutrient absorption occurs prior to the cecum, the rabbit needs a method for getting these nutrients to the absorptive parts of the gastrointestinal tract. To accomplish this, a special form of feces called a cecotroph is created. Sometimes referred to as “night feces,” cecotrophs are only expelled 1–2 times per day and are comprised of material directly from the cecum coated with mucus. The rabbit ingests the cecotrophs directly from the anus and this allows for further digestion and absorption of these nutrients in the upper gastrointestinal tract. These cecotrophs are excreted in glistening, grape-like clusters and are often mistaken for a clinical indication of disease, such as diarrhea, since they are rarely observed in normal animals. This, in fact, may be a true indication of disease since the rabbit normally will consume all the cecotrophs produced and, thus, lack of consumption may indicate an abnormal state, although this may be influenced by diet quality as well. As a general rule of thumb, high-quality diets are associated with less cecotrophy (Fekete and Bokori, 1985). This also applies to the animal’s metabolic state, but in a direct manner; high metabolic demand is associated with greater cecotrophy (Lorente et al., 1988).

As stated previously, the proximal colon separates out the rough, indigestible feedstuffs and passes them along to be expelled as feces. These would be the usual fecal pellets commonly associated with rabbits. Cecotrophs are expelled in discrete clusters completely separate from fecal pellet expulsion. The control of this separation occurs at another unique anatomical feature known as the *fusus coli*; a muscular constriction separating the proximal colon (with haustrae) from the distal colon (without).

Cecotrophy is a very important aspect of rabbit nutrition as a significant portion of the rabbit’s diet is not digestible via normal monogastric digestion. The fermentation process in the cecum provides significant amounts of protein and B-vitamins, and nutritional deficiencies may occur if animals are prevented from ingesting cecotrophs (Robinson et al., 1985).

Nutritional Requirements

There have been surprisingly few studies on the specific nutrient requirements of rabbits and many specific nutrient requirements are still unknown, especially for rabbits in various metabolic states such as gestation. Lebas (1980) remains as one of the most comprehensive reviews of rabbit nutrition available. Fortunately, research rabbit diets have been available commercially for many years and reports of nutritional deficiency or

toxicity in the laboratory setting are rare (Ringler and Abrams, 1970; St. Claire et al., 2004; Stevenson et al., 1976). These reports usually involve inadvertent miscalculations in the formulation of special research diets.

Although it is common in commercial rabbit production to have several diet formulations for different stages of growth, this is usually unnecessary in the laboratory setting as most animals can be maintained on a generic maintenance diet from weaning through senescence. Obesity is much more of a concern than specific nutrient requirements as the metabolic demands on rabbits in the research setting are often small while being fed excessive amounts of high quality, easily digestible feeds. See above for specific feeding guidelines.

Feeding Patterns

Rabbits primarily eat at night and in the early morning when fed *ad libitum*, but often eat immediately after feeding if limit-fed. They usually eat well, hence the obesity concerns noted above, but occasionally go “off feed” for brief periods of time (1–2 days) without recognizable indications of disease or distress (Poderscek et al., 1991). These animals will usually resume eating without treatment and with no apparent physiological impairment, but should be monitored closely as prolonged anorexia may lead to hepatic lipidosis if untreated (Bergdall and Dysko, 1994). Providing molasses on the feed or supplementing with sweetened breakfast cereals may entice consumption, but care must be observed not to train the rabbit to reject its normal ration in favor of these sweet treats. Fresh fruits and vegetables with higher fiber content may be an effective alternative to induce consumption without the potential enteritis complications associated with sweet feeds.

Environmental factors can affect feed intake as well, particularly temperature. Rabbits have a lower thermal neutral zone than most other laboratory species. At higher temperatures, even those normal for other species, food consumption will often be decreased in rabbits. In cooler temperatures they will consume more to meet metabolic demands to regulate body temperature (Cheeke, 1994).

Food Preferences

Rabbits seem to perform better when fed pellets than when they are fed mixed grains, meal diets, or textured feeds (Cheeke, 1994). Rabbits will preferentially eat pelleted, dehydrated alfalfa (Figure 9.10) over alfalfa in its natural form (Irlbeck, 2001). This may be due to their general dislike of fines and stems. The optimum pellet size for rabbits has been suggested to be 0.63 cm long and 0.47 cm in diameter (McNitt et al., 1996). Small-diameter pellets (<0.25 cm) lower intake while



FIGURE 9.10 Standard pelleted alfalfa-based feed.

larger-diameter pellets ($>0.5\text{cm}$) result in greater food wastage (Maertens and Villamide, 1998).

Rabbits appear to have a distinct preference for sweet feeds, but will also preferentially eat foods laced with bitter compounds like saponins (Cheeke et al., 1977) or specific flavors like thyme (Fekete and Lebas, 1983). Although this may be exploited to entice rabbits to accept test compounds orally, these animals can be particularly fastidious about any dietary changes.

Diet Composition

Unless nutritional studies are being conducted or special diet formulations are required for research purposes, a standard, laboratory-grade commercial rabbit feed is recommended. These diets provide balanced nutrition, detailed compositional analysis, and standardized production methods critical to the controlled research environment.

Most commercial rabbit diets are alfalfa-based, although other types of grasses may be suitable. Dehydrated alfalfa is usually the primary ingredient, but this is usually supplemented with grains and grain byproducts (hulls, middlings, etc.), sugars (molasses), vitamins, and minerals.

Grain-based diets, although often accepted by rabbits in the pelleted form, are usually too high in carbohydrates and too low in fiber to sustain a healthy gastrointestinal system. If such diets are utilized, they should be supplemented with a source of indigestible fiber to promote gastrointestinal mobility.

Fresh greens and other succulent feeds may be consumed by rabbits and may serve as enrichment treats; they should not constitute a significant portion of the diet for laboratory rabbits as their nutritional content and quality are generally unknown or inadequate.

Protein and Amino Acids

Optimal crude protein levels have been studied primarily for commercial rabbit production and recommendations range from 15–19% depending on metabolic demands, lower (15–17%) for growth, higher (17–19%) for reproduction (Omole, 1982; Sanchez et al., 1985). Feeding of diets high in protein ($>20\%$) has been associated with increased cecal ammonia, pH, and enteritis (Morisse et al., 1985).

Rabbits have a better ability to digest forage proteins (such as in alfalfa) than other non-ruminant species such as chickens or swine. This is primarily due to their hindgut fermentation and cecotrophy (Robinson et al., 1985). Forages with high tannin content appear to have much lower protein digestibility than those with lower levels of phenolic compounds (Raharjo et al., 1990).

Microbial protein from fermentation contributes to the overall protein intake for rabbits, particularly supplying the amino acids, lysine, threonine, and the sulfur amino acids (Carabano and Piquer, 1998). Unlike ruminants, microbial protein only plays a minor role in meeting the rabbit's protein and amino acid needs (McNitt et al., 1996). Dietary protein intake influences cecotroph consumption; low dietary protein increases consumption, high dietary protein decreases consumption (Cheeke, 1994). Cecotrophs contain approximately 28% crude protein (Stevens and Hume, 1995), but may be low in certain amino acids (such as methionine) and synthetic amino acids are often added to alfalfa–corn diets due to amino acid deficiencies (McNitt et al., 1996).

Absolute requirements for specific amino acids are still not well delineated for rabbits. Recommendations range from 0.2–1.2%, depending on the specific amino acid and the metabolic stage of the rabbit (Lebas, 1980). Most of this work was conducted in the 1970s and involved determination of maximum weight gains while consuming diets with varying levels of specific amino acids (Adamson and Fisher, 1973). Cheeke (1987) conducted a review of this literature. When formulating diets it is probably most important to understand that amino acid levels should be based on unit of digestible energy instead of percentage of diet since rabbits consume diet based on caloric density of the feed (Colin and Allain, 1978).

ENERGY

Maintenance energy requirements have been estimated at 96 kcal metabolizable energy (ME) per kilogram body weight^{0.75} ($\text{BW}^{0.75}$) for lactating New Zealand White does (Partridge et al., 1983). Further studies estimated pregnant doe requirements to be 79 kcal/kg $\text{BW}^{0.75}$ and 114 kcal/kg $\text{BW}^{0.75}$ for lactating does (Partridge et al., 1986a). Lactation requires the highest energy demands of any normal metabolic state (Lang, 1981) and lactating

does at peak lactation are in a negative energy balance and must mobilize reserves to meet their requirements (Partridge et al., 1983). If lactating does are to be used in the research environment, a higher-energy lactating diet should be considered to maintain body condition. Care must be taken to ensure that this diet is not fed to non-lactating animals as obesity will result.

The two major sources of energy for rabbits are sugars absorbed in the small intestine and volatile fatty acids (VFA) produced during cecal fermentation (Cheeke, 1994). Rabbits do not appear to digest starch as readily in the small intestine as other non-ruminants such as swine (Wolter et al., 1980). High-starch, low-fiber diets increase cecal fermentation and production of VFA presumably due to increased substrate (starch) and cecal hypomotility (low fiber) (Cheeke, 1994).

Fermentation in the rabbit gut produces higher levels of butyrate instead of propionate as seen in ruminants. This is due to the microbial flora which is primarily *Bacteroides* spp. (which are often butyrate producers) compared with *Lactobacillus* spp. which commonly populate the rumen and typically produce propionate (Vernay and Marty, 1984).

Dietary fats can also provide for the energy requirements of rabbits. Vegetable fats contain higher levels of digestible energy for rabbits than animal fats (Maertens et al., 1986), and good sources include soybeans and other oilseeds (Johnston and Berrio, 1985).

Fiber

Rabbit growth is optimal with a dietary crude fiber range of 10–15% (de Blas et al., 1986), but dietary recommendations range from 10–25% (Jenkins, 1999). Given the physiological basis for rabbit digestion described earlier, one would logically presume that feeding a high-nutrition, low-fiber diet would be the most beneficial for rabbits, but this is not the case. Insoluble fiber is a very important regulator of gastrointestinal motility in rabbits and low-fiber diets may lead to hindgut hypomotility, fur chewing, and enteritis (Brooks, 1997; McNitt et al., 1996). In addition, low-fiber diets are less palatable to rabbits and may decrease food intake (Cheeke, 1994).

Crude fibers high in cellulose and lignin (such as in alfalfa and other forages) are poorly digested in rabbits. Non-lignified material (such as citrus or beet pulp) has much higher digestibility, but does not contribute to hindgut motility and therefore may be counterproductive for nutrition and increase the incidence of enteritis (Cheeke, 1987). Fine grinding of lignified fiber increases digestibility, but also leads to hypomotility and potential enteritis. Cecotrophy does not significantly increase fiber digestion since the fiber is separated out of the cecotrophs and passes out as normal fecal pellets (Robinson et al., 1985; Stephens, 1976).

Dietary fiber is very important in the digestive health of rabbits (Stein and Walshaw, 1996). The incidence of enteritis (see below) is increased in animals on low-fiber diets and these diets can also predispose them to fur-chewing and the development of trichobezoars (hairballs) (Brooks, 1997; McNitt et al., 1996).

Minerals

Most mineral requirements are easily met by the normal dietary ingredients with the addition of a trace mineralized salt (Cheeke, 1994). A review of recommended mineral levels can be found in Lebas (1980). Calcium and phosphorus metabolism are the most significant mineral concerns for rabbits, and these will be discussed in detail here.

Calcium levels of 0.4–0.5% of the diet have been recommended for non-lactating rabbits (Cheeke, 1994). Alfalfa meal has a high calcium concentration, therefore alfalfa-based diets are usually sufficient to meet the rabbit's needs. In fact, long-term maintenance on alfalfa-based diets may be associated with excessive calcium intake and may lead to health concerns.

Rabbits are unique in that they have high circulating serum calcium levels (12–13 mg/dl compared to 10 mg/dl for other animals) (Brommage et al., 1988). This is the result of calcium absorption not being vitamin-D-dependent and not relying on activation of calcium binding proteins in the gut (Jenkins, 1999; McNitt et al., 1996). In addition, the excretion of excess calcium occurs primarily through the urine instead of the bile as in most other species. The renal fractional excretion for calcium is higher (2.5 mM) for rabbits than in most other species (1.5 mM) (Buss and Bordeau, 1984). This urinary excretion is commonly observed as a white deposit (calcium carbonate) in the pans of animals kept on commercial alfalfa-based diets.

Rabbits are able to respond to low dietary calcium levels with the release of parathyroid hormone leading to calcium reabsorption and hypocalciuria (Bordeau et al., 1988). During lactation, this response mechanism may be exceeded leading to a calcium-deficient state (milk fever) (Bartlett, 1980). Aside from the increased absorption and urinary excretion of calcium, other mechanisms of calcium/phosphorus control appear to be very similar to most other species, although their homeostatic mechanisms do not appear to be as effective (Cheeke, 1994).

Phosphorus metabolism is vitamin-D-dependent with deficiencies in vitamin D leading to phosphorus malabsorption and impairment of skeletal mineralization (Brommage et al., 1988).

Vitamins

Specific requirements for most vitamins have not been adequately determined, but recommendations can

be found in Lebas (1980). Meeting the dietary vitamin requirements for laboratory rabbits is relatively easy as their normal feed ingredients are usually more than adequate; and rabbits produce additional B-complex and vitamin K by cecal microbes. Water-soluble vitamins are rarely associated with disease in rabbits, and most reported concerns have been related to the fat-soluble vitamins A, D, and E; 10000 IU vitamin A per kg diet appears to be adequate. The minimum level for vitamin D has not been determined since the rabbit's calcium/phosphorus regulation is not dependent on this vitamin as in other species. Toxicity has been the only reported concern with vitamin D. Minimum levels for vitamin E are also unknown, but deficiency has been reported (Ringler and Abrams, 1970) with a level of 16.7 mg α -tocopherol per kg diet.

Vitamin deficiencies or toxicities are rare in the laboratory setting (Besch-Williford et al., 1985; Brommage et al., 1988; Ringler and Abrams, 1971; Stevenson et al., 1976) and are almost always associated with errors in diet formulation.

Water

Water intake in the rabbit is greater than in most other species. Rabbits consume approximately 120 ml of water per kg BW per day (Cheeke, 1994). Potable water should be available ad libitum.

Water consumption is influenced by feed intake, environmental factors, and feed composition. High-fiber diets require more water intake to maintain a fluid mixture in the gut, as many types of fiber absorb water. High-protein diets will result in increased water intake, as well, to promote urea excretion. Rabbits will compensate for food deprivation with increased water consumption, potentially leading to electrolyte imbalances (Cizek, 1961).

NUTRITIONALLY RELATED DISEASES

Enteritis

Enterotoxemia refers to a specific type of enteritis resulting from the release of toxins by infectious organisms such as *Clostridium spiroforme* (Carman and Borriello, 1982), and *Escherichia coli* (Peeters et al., 1985, 1986), but the disease is frequently diagnosed clinically without culture verification, so the term is often used synonymously with generic enteritis in rabbits. It may be used synonymously here as well since the dietary factors predisposing to enterotoxemia may predispose to other types of enteritis not specifically caused by the release of toxins, such as mucoid enteropathy. The primary etiology in nearly all cases is the alteration of the hindgut flora in a manner that changes gut

homeostasis, fluid balance, and/or provides an environment for the proliferation of pathogenic organisms. In fact, a limited model of mucoid enteropathy can be created simply by ligation of the cecum (Hotchkiss and Merritt, 1996). Nursing rabbits appear to be protected and Gallois et al. (2007) demonstrated that maternal milk contained factors that protect kits from enteropathogenic *E. coli* infection. Newly weaned rabbits may develop enteritis spontaneously (Carman and Borriello, 1983), but older animals usually require some additional predisposing condition for the disease to develop. The specific diagnosis is somewhat irrelevant to the discussion of these dietary influences.

Improvement in rabbit health standards, dietary standardization, limit-feeding, and gradual diet transition protocols can greatly decrease the incidence of enteritis in laboratory rabbits. Enteritis may still be seen in newly acquired animals if immediately given full access to a diet formulation significantly different from what they were previously fed. In commercial production colonies enteritis may still account for losses of up to 20% of rabbits born (Cheeke, 1994).

One of the most common dietary factors leading to enteritis is an excessive amount of fermentable carbohydrate (Lelkes, 1986), leading to hyperfermentation which increases the concentration of volatile fatty acids, decreases cecal pH, and inhibits the normal cecal flora (Bradley, 2004; Harcourt-Brown, 2002; Irlbeck, 2001; Reusch, 2005). The lowered cecal pH leads to excretion of mucus to neutralize this change and the common clinical presentation of a mucoid diarrhea. In addition, hyperfermentation leads to increased glucose production in the cecum and it has been determined that *C. spiroforme* requires glucose to produce toxin (Carman and Evan, 1984).

Dietary fiber, particularly indigestible fiber, is extremely important in the prevention of rabbit enteritis. This is presumably due to its ability to stimulate hindgut mobility and cecal emptying. Increasing dietary fiber may help prevent further occurrences of disease during outbreaks.

Dietary protein levels can also affect the incidence of enteritis. When dietary protein levels are elevated, ammonia production is increased in the cecum causing an increase in cecal pH. This pH change may also cause a dysbiosis and allow pathogenic organisms to flourish (Morisse et al., 1985).

Another dietary factor in the development of enteritis is antibiotic administration, particularly if administered orally. Morris (1995) conducted an extensive review of this subject. Certain antibiotics have been associated with induction of enteritis in rabbits, including lincomycin, ampicillin, amoxicillin, procaine, penicillin, cephalixin, erythromycin, clindamycin, tylosin, and metronidazole (Katz et al., 1978; Loliger et al.,

1984). The actual effect from each of the drugs will differ between animals, but it is generally agreed that these compounds may cause deleterious shifts in the intestinal microbial flora (Stein and Walshaw, 1996). In contrast, some antibiotics do not typically cause this microbial shift, including oxytetracycline, virginiamycin, and tetracycline which have been used as growth promoters (Brooks, 1997; Cheeke, 1994); and sulfaquinoxaline which has been used to control coccidia (Brooks, 1997). Although antibiotics are sometimes used as additives in commercial rabbit meat production, extreme care is usually taken to ensure that laboratory-grade diets are antibiotic-free.

The addition of probiotic cultures of yeast and bacteria (such as *Lactobacillus acidophilus*) to the diet may be beneficial in the prevention and/or treatment of rabbit enteritis (Hollister et al., 1989, 1990).

Obesity

Obesity is a common problem in laboratory-reared rabbits, particularly if fed *ad libitum*. Laboratory diets are highly digestible and often high in carbohydrate content. Combined with the limited physical activity of most rabbits within the laboratory environment, it is not surprising that they may become obese. Obesity is easily prevented in the laboratory environment by following the manufacturer's feeding recommendations and limit feeding to maintain the desired body condition.

Obesity has been linked to lower reproductive performance in does. Obese does may have decreased interest in mating and, if they do mate, kit mortality is increased. This mortality has been associated with increased fetal weight combined with fat deposits in the doe's abdomen resulting in dystocia (Partridge et al., 1986b).

Gastrointestinal Hypomotility/Trichobezoars

Fur chewing has been associated with low-fiber diets and may result in the accumulation of hair within the stomach (trichobezoars) (Cheeke, 1987). The association with low-fiber diets indicates that it is unlikely just an excess ingestion of hair, but more likely decreased gastric motility. Indigestible fiber is important for motility of the rabbit gastrointestinal tract, so the low-fiber diet probably allows the normally ingested hair to accumulate. Although this is commonly seen in long-haired breeds (such as Angora), it is not uncommon in laboratory breeds as well. The hair can become matted with mucoproteins within the stomach and form a mass that can neither pass nor be digested, even if the dietary conditions improve. Severe trichobezoars can result in decreased food intake leading to malnutrition and are easily diagnosed by palpation.

Early diagnosis and treatment of gastrointestinal hypomotility are key to a successful outcome (Bergdall and Dysko, 1994). Historically, recommended treatments included oral administration of proteolytic enzymes such as papain, bromelain, or pineapple juice. Although these may be helpful in breaking down the mucoproteins and softening up the trichobezoar for passage, they do not address the primary problem. The most important factor in treatment and future progression is to get the animal well-hydrated and eating a high-fiber, low-carbohydrate diet. This may require extensive supportive care such as nasogastric feeding and/or parenteral fluid and analgesic administration (for gastric distention pain) (Reusch, 2005). In worst cases the trichobezoar may need to be removed surgically if it becomes too large to pass, although the prognosis for recovery is guarded.

Calcium Imbalances

As discussed earlier, rabbits normally have higher serum calcium levels than other species and their calcium absorption is not under the influence of vitamin D. Combined with their rather inefficient serum calcium control mechanisms, hypercalcemia can easily occur in rabbits consuming diets high in calcium (4%) (Kamphues et al., 1986); and this is intensified by high vitamin D levels. In addition, since the majority of calcium excretion occurs through the kidneys in rabbits, renal disease can also result in a hypercalcemic state (Garibaldi and Pecquet-Goad, 1988).

Prolonged hypercalcemia results in calcium deposition in soft tissues within the urinary, nervous, and gastrointestinal systems (Garibaldi and Pecquet-Goad, 1988) or result in formation of urinary calculi. Prolonged hypercalcinuria can lead to kidney damage.

Lactating does may develop a calcium deficit (milk fever) just after parturition. Symptoms include muscle tremors, jerking of the hind limbs, and ear flapping (Bartlett, 1980). Rabbits may become hypocalcemic (5.8mg/dl) and hypophosphatemic (2.9mg/dl) and may clinically improve following intravenous calcium gluconate administration.

Vitamin D Imbalances

Vitamin D deficiency is very unlikely in the laboratory setting due to high-quality diets combined with the microbial production of vitamins and intake via coprophagy. Prolonged deficiency has been reported (Brommage et al., 1988) and the primary effects were impaired bone mineralization and osteomalacia. This deficiency was associated with impaired phosphorus absorption and hypophosphatemia.

Vitamin D toxicity has been reported in rabbits due to dietary formulation errors or contamination (Besch-Williford et al., 1985; Ringler and Abrams, 1970; Stevenson et al., 1976). Clinical signs included emaciation, weakness, decreased appetite, diarrhea, intense thirst, ataxia, paralysis, and death. Calcification occurred in soft tissues similar to primary hypercalcemia.

Vitamin A Imbalances

Both vitamin A deficiency and toxicity (DiGiacomo et al., 1992; St. Claire et al., 2004) have been reported in rabbits. This literature has been extensively reviewed by Cheeke (1987). Both conditions produce similar signs affecting reproduction with low conception rates, fetal resorption, abortion, low kit survival, and hydrocephalus in neonates. Paresis and muscular dystrophy have also been reported in juveniles with vitamin A toxicity and the animals responded to vitamin E therapy (St. Claire et al., 2004). Lorente and Miller (1977) reported that placental transfer of vitamin A is lower than in the rat, and, although this may protect the fetus from toxicity, this may contribute to their sensitivity to deficiency.

Vitamin-A-deficient rabbits may be more susceptible to infectious diseases as well, since vitamin A plays an important role in the integrity of epithelial tissues including mucous membranes.

Vitamin E Deficiency

Several reports of vitamin E deficiency have been documented. Principal signs have included muscular dystrophy with paralysis of the hind legs. In one report (Ringler and Abrams, 1970), rabbits died at 3–10 days of age with marked degeneration of skeletal muscle. Reproductive failure has also been reported (Yamini and Stain, 1989) and can mimic vitamin A imbalances, although muscular dystrophy was also reported and this is not characteristic of vitamin-A-related disease. Vitamin E deficiency has been reported in association with vitamin A toxicosis (St. Claire et al., 2004).

Dietary Toxins

Dietary toxins are generally of low concern in the laboratory setting when high-quality, laboratory-grade feeds are provided. Fungal toxicosis has been reported in commercial rabbitries due to feed contamination (Krishna et al., 1991). Mycotoxins, particularly aflatoxin and the trichothecenes, are the most common concern. These toxins can cause liver damage, gastritis, nephritis, and enteritis (Fekete et al., 1989; Orsi et al., 2007). Teratogenic effects have also been reported (Wangikar et al., 2005).

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Basic Experimental Methods in the Rabbit

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INTRODUCTION

This chapter describes humane methods for handling and restraining laboratory rabbits, as well as techniques for obtaining biological samples; for administering substances; and for specialized procedures. Rabbits have individual temperaments, and a gentle, calm, confident

restraint method and demeanor in working with each animal will decrease stress for both the rabbit and the researcher. Rabbits can easily be injured, and can inflict injury on the handler if proper considerations are not followed to keep the animals secure and to minimize stress. Acclimating the animals in advance to procedure areas and restraint methods can be helpful. Whenever

possible, use of anesthesia or tranquilizing medication can facilitate procedures. Analgesia is provided after procedures that may cause pain or discomfort after the anesthetic has worn off. Finally, each researcher must be willing to acknowledge when a particular method or procedure does not succeed, and seek assistance or an alternative method.

HANDLING AND RESTRAINT

Physical Methods

Rabbits evolved under pressure as a prey species, which may account for their light skeleton paired with large muscles, to facilitate speed in fleeing from predators. This fragile bone structure predisposes these animals to injury from improper handling, in particular fracture or subluxation of vertebrae, or a broken back. Handling and restraint techniques therefore require that the rabbit's front and rear ends be supported, and that the animal not be allowed to kick or struggle. Handler injury may result from scratches; long-sleeved cloth lab coats with knit cuffs reduce the chances of sustaining a scratch from rabbit toenails.

To remove a rabbit from a cage or pen, the loose skin over the shoulders (scruff) is grasped from above in one hand. The rabbit is turned to face the handler so it is less likely to try to leap back into its housing. With the other hand supporting the rabbit's hind-quarters from behind or beneath, the animal may be lifted. To carry short distances, the animal is brought in close to the handler's body, and the head is tucked under the handler's arm (Figure 10.1). The ears are not included in the grip. Most rabbits are somewhat timid, but an

occasional individual may be aggressive, as evidenced by stamping, growling, and boxing at the approach of the handler. These bold individuals may be easily subdued by placing a towel over the entire animal. The animal may be firmly bunched in the towel and removed from the cage, and then placed on a secure surface, where it may then be grasped by the scruff. Animals are again faced toward the handler when returned to housing, so they will not attempt to leap back into the cage or pen.

Animals held snugly tucked under the arm of the handler as described may be safely carried short distances, but if an animal begins to struggle, the handler should gently release the rabbit on a secure surface; struggling animals can injure themselves and the handler. For moving longer distances, use of a plastic transport box is recommended. Boxes that open from the top, rather than from a door on one end, facilitate entry and removal of the animals. Plastic may be easily sanitized between animals.

Restraint of rabbits by the immobility reflex, or hypnosis, is induced by maintaining traction on the spine with the rabbit held in dorsal recumbency in a stretched position until it relaxes. The head is held flexed toward the chest and the hind legs are held in the other hand as the rabbit is stretched. Once the rabbit relaxes, the rear legs may be released but the head must continue to be held in a flexed position. Once the head is released, the rabbit returns to its normal state of awareness. Restraint boards have been designed to hold animals in this position. However, hypnosis is a method with considerable variation in effect and duration in individual animals. Therefore, it is not recommended for routine use, and never for procedures that could cause pain if the animal suddenly emerges from the hypnotic state.

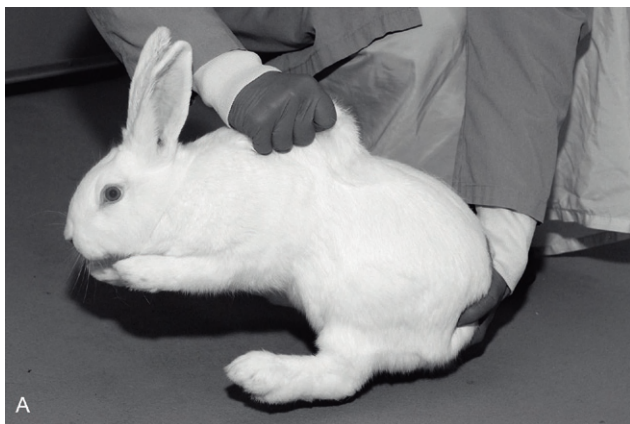


FIGURE 10.1 Restraint for cage removal and carrying. (A) The scruff is grasped and the rear end is supported to keep the spine flexed as the animal is lifted. (B) The rabbit's head is tucked between the handler's arm and body. The animal's hind quarters are held snugly to prevent kicking.

Restraint Devices

A number of commercially available restraint devices are designed for rabbits. Most are designed to give access to the head and ears. Rigid metal and plastic devices are most common (Figure 10.2). Caution is needed to be certain yokes or neck openings are not secured too tightly in models that have an adjustable neck gate or band. Cloth “cat-bags” with Velcro closures can also be used with rabbits, with the advantage of permitting easy access to limbs via zippered side openings in the bags (Figure 10.3). Rabbits can injure their spines or limbs if they struggle in a restraint device. Accustoming them to the device is recommended. Covering the animal’s eyes with a soft drape can help to calm an animal in a restraint device. Absorbent padding may increase comfort if animals are expected to remain in the restraint device for more than a few minutes, and also serve to provide the security of traction for the animals on the slick flooring of

the boxes. Animals should never be left unattended in a restraint device; if the animal panics and struggles, a handler must be nearby to release the restraint and calm the animal.

A simple and effective method of restraint can be achieved with the use of a terry-towel cloth or cloth drape. The animal is snugly wrapped, much in the manner of swaddling a baby, with the final wrap held in place under the rabbit by the rabbit’s own weight (Figure 10.4). In livestock, restraint devices have a calming effect if they provide sufficient even pressure so that the animal feels securely held without pinching or pressure points (Grandin et al., 1989), which may explain why many rabbits are calmed when snugly wrapped (Grandin, 1992). For skittish animals, the handler may sit on a drape on the floor with his legs extended, with the rabbit placed on its abdomen facing the handler with the rabbit’s head at the level of the handler’s knees. A towel or drape is placed over the rabbit’s back, and

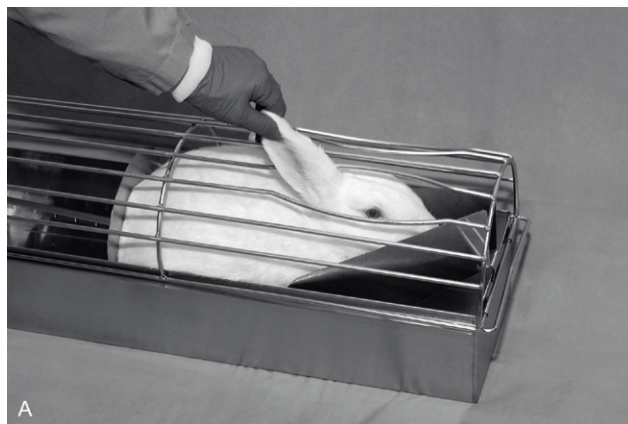
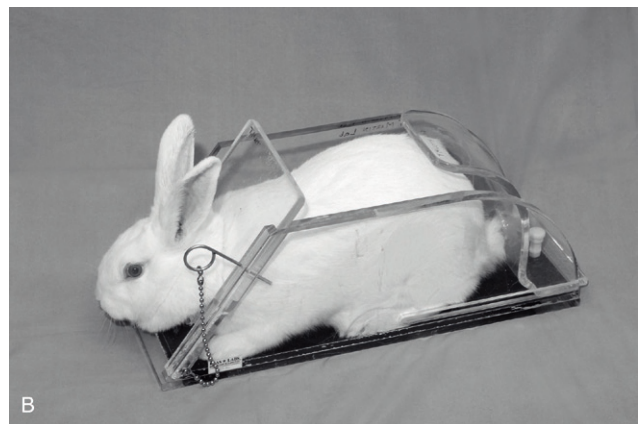


FIGURE 10.2 Restraint devices. Rigid metal (A) and plastic (B) restrainers are designed to permit access to the ears. The rear support should be pressed gently against the animal’s rump. In models with a neck restraint, the neck yoke is fastened last, leaving several cm of space between the yoke and the neck. Floor padding is recommended.



FIGURE 10.3 Cloth cat-bag restraint.



FIGURE 10.4 Restraint in a cloth wrap.

held securely tucked on either side under the handler's lower legs; if the animal struggles, it cannot fall. This restraint position gives ready access to the rabbit's ears. Similarly, the handler may kneel on a towel spread on the floor and place the rabbit between his knees; positioning the rabbit facing the handler allows access to the rear end for placing a rectal thermometer or for administering an intramuscular injection in the lumbar muscles.

Stereotaxic Methods

Rigid fixation for stereotaxic work is accomplished in the anesthetized rabbit. Several atlases of the rabbit brain are published (Fikova and Marsala, 1967; Lavond and Steinmetz, 2003; Monnier, 1961; Shek et al., 1986; Girgis and Wang, 1981; Urban and Phillippe, 1972) that use bregma and lambda coordinates to establish the horizontal and vertical planes. Unlike rodents, where ear-bars are used to secure the head in the sagittal axis, the rabbit ear canals (external auditory meati) are at an oblique angle, requiring specialized adaptation of the stereotaxic frame. Commercial devices are available that adapt stereotaxic frames to fit rabbit skulls, using a tooth bar, nose clamp, and u-shaped clamp that secures the head by clamping at the zygomatic bones (Girgis, 1980; Lavond and Steinmetz, 2003), with additional modifications to permit use of gas anesthesia (Cegavske and Biela, 1980). A stereotaxic frame has been developed that uses the contact plane of the rabbit's mandible as a spatial reference (Kockro et al., 2008). This frame is accurate for operations which require approximate stereotactic guidance, such as the puncture of CSF spaces, but its use in other applications like tumor seeding, biopsy sampling, or lesion generation is dependent on individual accuracy requirements.

SAMPLING TECHNIQUES

Blood Sampling

Blood volume is generally estimated based on body weight. To avoid causing distress and abnormal physiology, a general rule is not to exceed removal of 1% of body weight (10% of blood volume) in any given 2-week interval (Diehl et al., 2001; Joint Working Group on Refinement, 1993; McGuill and Rowan, 1989). This would be the equivalent of 30 ml from a 3-kg rabbit. If larger blood volumes are needed, fluid and cell replacement is necessary. Exsanguination should only be performed as a terminal procedure on an animal that is in a surgical plane of anesthesia; approximately half the animal's blood volume, or about 90–120 ml, can be obtained from a 3-kg rabbit.

Blood samples may be readily obtained from the arteries, veins, and from cardiac puncture. The rabbit ear

provides easy access to a lateral vein or central artery. The ear artery is preferred to the vein for removal of blood volumes of several ml or more. Cardiac puncture is most often a terminal procedure, and allows rapid collection of large volumes of blood. There are a number of methods to accomplish restraint for blood sampling in conscious animals, as described above.

Refinements for blood sampling technique include numbing the ears with local anesthesia, such as a lidocaine and prilocaine cream, applied to the site of vessel puncture about 15 minutes prior to sampling (Figure 10.5) (Flecknell et al., 1990). Gently warming the animal or the ears can produce vasodilation. Use of a tranquilizer that promotes relaxation and vasodilation is also recommended. Approximately 15 minutes prior to sampling, a dose can be given of a phenothiazine tranquilizer such as acepromazine maleate at 0.8 mg/kg subcutaneously, or an opioid combination, fentanyl/fluanisone (Hypnorm) at 0.2 ml/kg intramuscularly. Other traditional methods of inducing vasodilation include topical application to the ear of agents such as xylene (highly irritating, not recommended), d-limonene, 2% nitroglycerin ointment, or oil of wintergreen (irritant). All of these fast-acting agents must be thoroughly washed from the ear after the blood sample has been obtained, using 70% ethanol.

Aural Vessels

For sampling from the ear vessels, a size 25–23-gauge butterfly needle, or a catheter with tubing and syringe adaptor can be used. Butterfly needles have the advantage of flexible tubing, which permits movement of the animal with decreased likelihood of dislodging the needle once it is seated in a blood vessel. Alternatively, a 22-gauge double-headed needle can be used with vacuum collection tubes for sampling from the ear



FIGURE 10.5 Application of topical anesthetic cream to numb the ear for vessel puncture. Note that the cream slicks the hair so that the vessel can readily be seen.

artery (Barrera and Young, 1997). Sampling from the ear vessels does not require that hair be plucked or shaved though this is often done, but the site should be gently cleaned with warm antiseptic solution and dried. Topical anesthetic cream or a lubricant jelly can be used to slick the hair to allow visualization of the vessels. Both the artery and vein are superficial, and are accessed from the dorsal surface of the ear. The needle is placed at a very flat angle, almost parallel to the ear surface, and advanced about half its length (or about 0.5cm), in a direction toward the head (Figure 10.6). For the vein, holding off the vessel at the base of the ear helps to raise the vessel for puncture. Blood flow into the needle hub or tubing is observed with gentle aspiration. When the needle is correctly seated in the artery, blood will be seen pulsing into the syringe or tubing (Figure 10.7). Catheters or butterfly needles may be capped and flushed, and then secured in place with tape, suture, or glue, for repeat sampling. Upon removal of the needle or catheter, hemostasis is achieved by applying gentle pressure with a gauze sponge; pressure may need to be applied for a minute or longer after arteriopuncture to prevent a hematoma.

Jugular Vein

For collecting blood from the jugular vein, the rabbit may be wrapped snugly in a towel, or held securely with one hand on the scruff and one hand on the abdomen. The rabbit is placed in dorsal recumbency by an assistant, who holds the rabbit's neck extended and applies gentle pressure at the base of the neck to distend the vessel. The fur may need to be clipped to facilitate visualization. The blood is then collected via a needle directed in a caudal direction. (Crow and Walshaw, 1997; Nelson et al., 2010). Tranquilization of the animal is recommended for this collection method if the animal is wrapped in a towel for restraint.

Cardiac Puncture

The rabbit must be in a plane of surgical anesthesia prior to cardiac puncture or carotid artery cannulation. Cardiac puncture may result in lethal damage to the heart, lungs, and surrounding tissues, or lead to cardiac tamponade, and so is most commonly used as a terminal procedure; once the blood is withdrawn, the animal is euthanized before it regains consciousness from the anesthesia. The anesthetized rabbit is placed in dorsal recumbency. The location of the heart can be ascertained by palpating the heartbeats at the point of maximum intensity. The heart is pierced with a large 16–18-gauge, 1.5-inch needle, attached to flexible tubing. The approach may be from the side, entering between the ribs at the level of the elbow, or entering from beneath the sternum. The heart is pierced with a single firm thrust, and correct placement into the heart is confirmed by the appearance of blood pulsing into the tubing. If blood does not flow well, the needle may be turned but the tip should not be moved other than in the same line of insertion; repeated probing within the chest may result in death of the animal. If there is no initial blood flow, a fresh start with a clean needle may be attempted. Blood can be drawn into large syringes for collection. A method of euthanasia should be at hand for use once the blood has been collected.

Carotid Artery

Carotid artery cannulation is done under direct visualization. If the animal is to be recovered from the procedure, sterile technique is used. Carotid cannulation may allow for more complete removal of the maximum blood volume for exsanguination than cardiac stick. In the anesthetized rabbit, a cut-down incision is made midline on the ventral neck, to expose the carotid artery running parallel to the trachea. A small segment of the artery is carefully dissected from

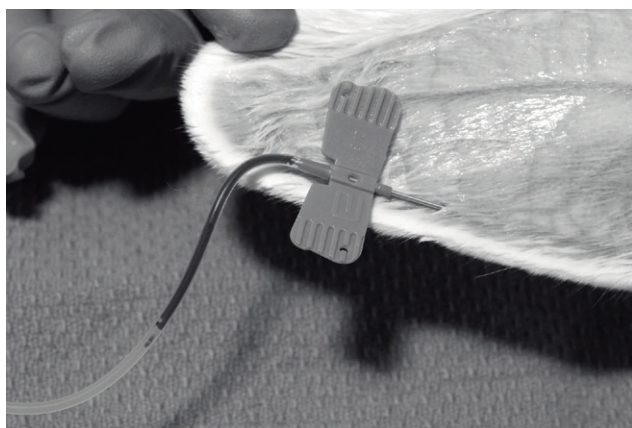


FIGURE 10.6 Sampling from the ear vein with a 25-gauge butterfly needle.

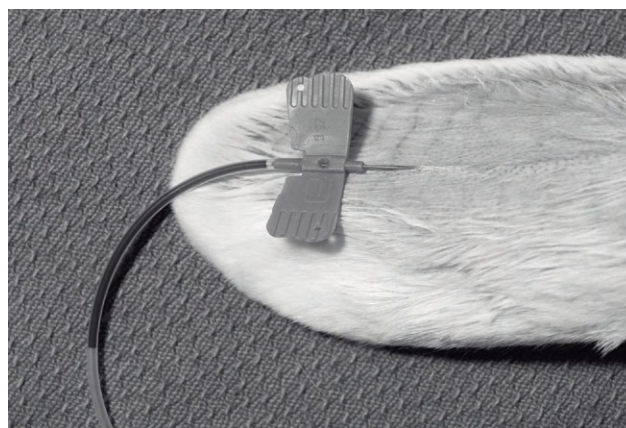


FIGURE 10.7 Sampling from the ear artery with a 23-gauge butterfly needle.

the surrounding connective tissue. A single ligation of the carotid is made cranially, and a temporary clamp is placed caudally on the vessel. A loose ligature is placed around the vessel ready to secure the catheter once it is placed. A small snip is made in the vessel, and a catheter is inserted toward the heart. The catheter is secured in place with the ligature, and the clamp is removed. For exsanguination, if the contralateral neck vessels are exposed and the jugular is cannulated, high flow rate of saline into the jugular vein during the blood collection may help maintain blood pressure and allow increased volume of blood collection.

Indwelling Catheters

For chronic blood sampling over days or weeks, indwelling catheters may be surgically placed into the carotid, jugular, or heart (Cleva et al., 1995; Dennis et al., 1989; Karnabatidis et al., 2006; Nishijima, 2009). Tubing from the catheters can be tunneled under the skin to exit at a site such as the nape of the neck, or tubing is connected to vascular access ports that are completely concealed beneath the skin (Kunta et al., 2001; Swindle et al., 2005). Anticoagulant solutions must be maintained in the tubing to prevent clotting. A technique has been described for using polyethylene tubing to create arterio- or venous catheters that remain patent in the ear vessels for over 3 weeks, and can be used for sampling or infusion (Hungate et al., 1996).

Cerebrospinal Fluid Collection

Performance of cerebrospinal fluid (CSF) collection requires skill and steady hands. During penetration,

damage to the dura mater and atlanto-occipital membrane may cause the sample to become contaminated with blood. For repeated sampling or sampling in the conscious animal, placement of a catheter is needed; a number of approaches to surgical catheter placement have been described (Arkan et al., 1996; Tissot et al., 1995; Vistelle et al., 1994).

For a single tap, the atlanto-occipital (cisterna magna) approach allows harvest of volumes of CSF of 0.5cc, or up to 1.0–2.0cc in larger animals (Wildlife Information Network, 2009). This approach can also be used for measuring intracranial pressure, or for myelography. Equipment needed includes a 22-gauge 1.5-inch spinal needle with stylet (or a hypodermic needle from 25 gauge 5/8 inch to 23 gauge 1.25 inch) and sterile plastic collection vial. Plastic is preferred to glass to minimize adhesion of leukocytes. Aseptic technique is essential during collection, including shaving and antiseptic scrub of the puncture site. A surgical plane of anesthesia is necessary for the rabbit. Ideally, an endotracheal tube is placed to aid in maintaining an open airway as the neck is flexed for the procedure, which may cause airway obstruction.

The animal is placed in lateral recumbency along the edge of a table, with the head flexed toward the chest no more than 90°. Flexion can be accomplished by grasping the ears with the thumb placed on the occipital protuberance (Figure 10.8). Landmarks for needle insertion are the occipital protuberance and the wings of the atlas. The spinal needle with stylet in place is placed midway between these two points, at a 90° angle to the vertebral column, parallel to the table surface. The needle is advanced just a few millimeters

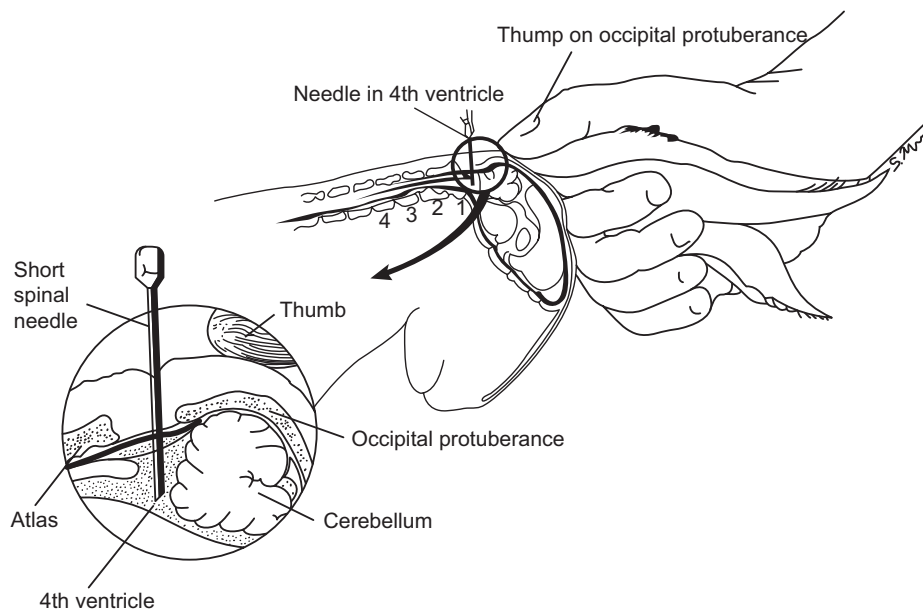


FIGURE 10.8 Cerebrospinal (CSF) fluid collection technique.

at a time in a cranial direction toward the rabbit's nose. Penetration of the dura and subarachnoid membranes may be felt as a "pop", and appearance of CSF in the needle hub when the stylet is removed confirms placement in the 4th ventricle. If the needle hits bone, it may be redirected cranially or caudally, or removed and replaced after reassessing the site location.

A manometer may be attached to the needle to assess intracranial pressure. Care should be taken not to move the needle during this manipulation. To collect CSF, fluid may be allowed to drip into a collection tube. Application of negative pressure with a syringe can cause damage; a 1-ml syringe may be used to collect CSF at no more than 1 ml per 30 seconds.

A lumbar approach may also be used to collect a small volume of CSF, and this is the preferred site to administer contrast agents for myelography. The anesthetized animal is shaved and scrubbed with an antiseptic over the lumbar spine, and placed in either lateral or ventral recumbency. If the rabbit is in lateral recumbency, the spine is flexed and padding is placed under the animal to support the spine so that it is straight and parallel to the table. The needle is directed cranially and inserted between a 45–90° angle to the spine at the cranial edge of the dorsal spinous process of vertebra L6. It is important not to apply negative pressure with a syringe to harvest CSF at this site.

Urine Collection

Metabolic cages designed to collect urine separately from feces are commercially available. If urine samples free from cage contamination are needed, then urine can be collected by manual expression of the bladder, cystocentesis, or urethral catheterization.

Manual urine expression may be best accomplished in an anesthetized rabbit. The full bladder can be palpated and pressure applied in a firm, steady manner. Excessive pressure can cause bladder rupture. Cystocentesis is performed on an anesthetized rabbit, placed in dorsal recumbency. The full bladder is palpated and pressed between the fingers and thumb, up against the abdominal wall. A 20-gauge needle is inserted directly through the skin into the bladder. A 30–60-ml syringe is used to aspirate the urine.

Catheterization of the urethra can be accomplished most readily in male rabbits. A subcutaneous dose of acepromazine at 0.8mg/kg will tranquilize the animal and encourage protrusion of the penis. The buck is supported in a sitting position, tilted slightly back; a seated handler can restrain the animal held in the handler's lap with one hand holding the scruff and the other restraining the rear legs. A sterile, flexible, lubricated catheter, size 9 French is passed into the urethra in a downward direction for about 2cm. The catheter

and penis are manually depressed, and the catheter tip is then gently advanced into the bladder. To catheterize a doe, the sedated rabbit is placed in ventral recumbency. The catheter is first directed vertically in the caudal part of the vagina. The catheter is then brought to a horizontal position to enter the urethral opening in the ventral vagina. A cupped hand can be used to apply abdominal pressure to express urine through the catheter.

Urine collection in neonatal (12-hour- to 10-day-old) rabbits can be accomplished by stroking the abdomen. The kit is held gently but firmly on its back in one hand, while the other hand strokes from the stomach area to just beyond the bladder, with most pressure in the bladder area. After 10 days of age, muscular control of the bladder and increased size of the kit makes it more difficult to use this method, and a longer period of stroking is needed. Up to 5ml of urine can be collected in this manner (Kurien et al., 2004).

Milk Collection

Transgenic rabbits have been produced to secrete into their milk specific proteins which are harvested for medical and research use. A doe can produce 250ml of milk per day of lactation, but only 100–150ml is collected each day, for a yield of 10–15 liters of milk per year. Milking machines for transgenic rabbits have been developed, but the designs remain proprietary (Bioprotein Technologies, 2009). Does typically nurse their young for about 5 weeks. Milk is collected from the females from days 5 through 25 post-partum, to correspond with the peak milk production time.

Rabbit does typically nurse their litters once per day, most commonly in the early morning. Milk collection is done prior to allowing the kits to nurse (Maertens et al., 2006). A milking machine can be devised from a small suction pump (such as a Gomco portable pump). A T-connector is attached to the port of the suction machine. Commercially available DeLee suction catheters with mucus traps are used to collect the milk. The DeLee catheter tip is cut at a 30° angle and inserted into the narrow half of a cleft-palate nipple. This nipple serves as the funnel for milk collection and is cut to fit the size of the rabbit's nipples (Figure 10.9).

A sedated doe can be treated with an intramuscular dose of oxytocin (6IU/kg) to stimulate milk letdown. The doe is restrained in lateral recumbency, and the nipples gently cleansed. Each nipple is inserted into the rubber nipple on the DeLee catheter and rhythmically squeezed gently at the base to express milk in a steady stream, which is collected in the mucus trap by 12.5cm of continuous vacuum. An average of 43.5ml (range 23–114ml) of milk can be collected from each rabbit with this technique (Marcus et al., 1990).

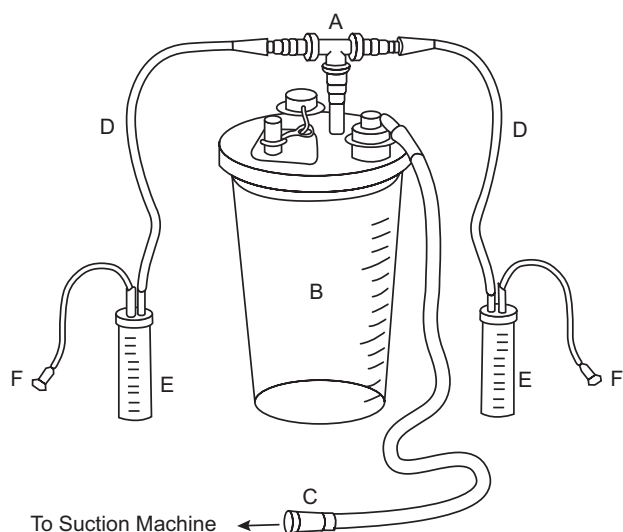


FIGURE 10.9 Milking machine. (A) T-connector, (B) suction trap, (C) tubing to suction pump, (D) DeLee catheters, (E) mucus trap where milk is collected, (F) nipple.

Bone Marrow Collection

Marrow samples are obtained only from anesthetized rabbits. To collect an aspirate for cytology, the proximal humerus or proximal femur can be accessed using a spinal needle with stylet or 18-gauge Rosenthal pediatric biopsy needle (Wildlife Information Network, 2009). The site is shaved and prepped for a sterile procedure. The subcutaneous tissue and periosteum to be penetrated are infiltrated with 2% lidocaine or 0.25% bupivacaine. In the humerus, the needle is inserted at an angle to follow a line between the elbow and the greater trochanter of the humerus. For the femur, the needle penetrates via the trochanteric fossa between the first and third trochanter, at a 45° angle to the femur's long axis. The needle is pushed through the skin, and then into the marrow cavity using a drilling motion. As the needle enters the marrow cavity there is typically a loss of resistance. The stylet is removed and a sterile syringe attached. Negative pressure is applied until about 0.5ml of marrow has been aspirated. The negative pressure is released, and the syringe detached from the needle before the needle is pulled from the bone. Post-procedure analgesia may be necessary, as bone marrow collection can be a painful procedure. If a biopsy is needed for histopathology, a 1.5-inch 18-gauge needle without stylet is used to collect the sample, and no syringe is needed. If samples are needed for both cytology and histopathology, the aspirate is collected first.

COMPOUND ADMINISTRATION

There is great variation in the literature on recommended maximum volumes of substances for administration to rabbits (Diehl et al., 2001; Hawk et al., 2005;

Morton et al., 2001). Paramount consideration goes to avoiding volumes that would cause pain or distress, as well as changes in physiology or pathology that would compromise the goal of the research. Maximum volumes of administration are listed in the sections describing each route, but the reader is cautioned that while these maximal volumes may be tolerated, *the smallest volume that can be given is considered best practice for animal welfare.*

Considerations for substance administration include: warming the substance so as not to chill the animal, particularly when large volumes are to be administered; avoiding substances with extreme pH (<4.5, >8.0) which cause tissue damage; use of isotonic solutions such as 0.9% saline, or giving hypertonic solutions intravenously in a large vessel to promote rapid dilution and decrease chance for irritating the vessel wall; using the smallest needle that will allow ease of administration for the volume to be given, viscosity of the substance, shear-damage to cells passing through the needle, and toughness of the tissue to be penetrated; and using sterile solutions, needles, and syringes and following aseptic technique, including gentle yet thorough disinfection of the tissue at the site of skin penetration.

Oral Route

Feed

Mixing compounds into rabbits' food or water is the simplest method of oral substance administration, when exact quantity and timing of ingestion are not of concern. Several commercial producers of laboratory animal diets will create custom compound diet formulations for rabbits. Rabbits will refuse food or water that has disagreeable odor or flavor. To administer small quantities of liquid, the rabbit is restrained wrapped in a towel, and a syringe is introduced into the corner of the mouth. The liquid is slowly dispensed as the rabbit is observed to ingest it (Figure 10.10). Mixing a substance with honey or sweetened yogurt may increase its palatability.

Gavage

To directly administer precise quantities of liquid, a flexible tube (oral gavage tube) is passed directly to the stomach. Rabbits should not be anesthetized for this procedure, because of the risk of inadvertently passing the tube into the respiratory tract. A size 8–10 red French rubber urinary catheter or infant feeding tube works well. The tube length is measured from the tip of the rabbit's nose along its side to the level of the last rib; when passed this distance, the tube should have entered the stomach. A mark is put on the tube so the correct distance can be readily gauged. A mouth speculum is needed to prevent the animal from chewing on the tube. A speculum can be readily fashioned from a



FIGURE 10.10 Oral administration using a syringe.

plastic syringe barrel; holes big enough for the tube to pass through are cut on opposite sides of the center of the barrel, and the cut edges are smoothed with a file or flame. The rabbit may be restrained in a towel or cat bag, and held in an upright or tipped-back position. A special restrainer has been developed to facilitate oral dosing without an assistant (Abell et al., 1995).

The mouth gag is placed behind the incisor teeth at the diastema, above the tongue. A small amount of lubricant may be placed at the end of the tube. The tube is passed through the mouth gag, and should easily pass the entire pre-marked distance into the esophagus, meeting no resistance. Correct tube placement into the stomach must be assured before any liquids are instilled; liquid placed into the respiratory tract will most often prove fatal to the rabbit. To assure correct placement, the chest may be auscultated for normal breathing, or one can place the open end of the stomach tube into a small beaker of water and observe for bubbles that would be seen if the tube is mistakenly placed in the respiratory tract (Ogden, 2007). A syringe is used to administer the liquid, followed by several ml of water or carrier vehicle to clear the tube. The syringe is kept in place on the tube, or the end of the tube is clamped to stop fluid from exiting the tube as it is withdrawn, to prevent possible fluid inhalation.

Substances in an aqueous base are better tolerated by gavage in large volume than substances in an oil base. Maximum volume recommendations for oral gavage vary from 10–15 ml total, to 10–15 ml/kg (Diehl et al., 2001; Hawk et al., 2005; Morton et al., 2001). A smaller volume generally means a safer and less potentially distressful procedure.

Pharyngotomy Tube

For frequent or chronic dosing, a tube can be surgically placed via a pharyngotomy (Figure 10.11). In an anesthetized rabbit, a 1-cm skin incision is made on

the left side anterior to the larynx. A stomach tube is passed through the mouth into the stomach. Forceps are introduced through the mouth and the tube is grasped and pushed against the pharynx so that externally it can be seen bulging at the site of the skin incision. A cut is made to allow the oral end of the tube to pass through the pharynx. The tube is anchored at this site, and then passed subcutaneously and exteriorized at the base of the ear, where it is again anchored. Pharyngotomy tubes have remained in place and patent for 6–12 months (Harcourt-Brown, 2004; Rogers et al., 1988).

Nasogastric Tube

Nasogastric tubes can also be placed (Brown, 2010; Harcourt-Brown, 2004) but may be stressful to rabbits, and run the risks of nasal cavity irritation and bleeding. The nasal mucosa is first numbed with a few drops of topical anesthetic such as proparacaine or 2% viscous lidocaine, and the rabbit's head is tipped up to allow the drops to coat the nasal passages. If the rabbit struggles during tube placement, use of a sedative may be necessary as well. A size 5–8 French flexible feeding tube is premeasured for the distance from the nose to the last rib, and the length is marked. To pass the tube, the animal's head is held in ventral flexion, taking care not to compress the trachea. The end of the tube is lightly lubricated, and the tube is inserted into a nostril, aimed ventrally and medially. The tube should pass with no or minimal resistance all the way to the premeasured mark. Correct placement should be confirmed as described for a gavage tube. A tape tab is placed on the tube where it exits the nostril, and is glued or sutured in place. The remaining length of tube is secured behind the neck with a light wrap. An Elizabethan collar or padded collar is used to prevent the rabbit from removing the tube. Tubes can remain in place for weeks.

Capsule

Oral dosing with capsules or small pills is difficult in rabbits, but may be accomplished with a small animal balling gun or pill gun. The rabbit is restrained as for oral liquid dosing, and the pill or capsule is placed as far back on the tongue as possible. A pill gun can be fashioned from a plastic 3-cc syringe. A razor is used to cut off the end of the syringe barrel at the 0-ml mark, and the surface is then smoothed with a file or flame. A size 00 gel capsule may be used to hold medication. Once placed into the gun, the pill or capsule may be lubricated with a small amount of mineral oil immediately prior to being placed into the oral cavity. Alternatively the substance may be crushed and mixed with a palatable substance such as honey or flavored yogurt and administered via a syringe placed into the mouth.

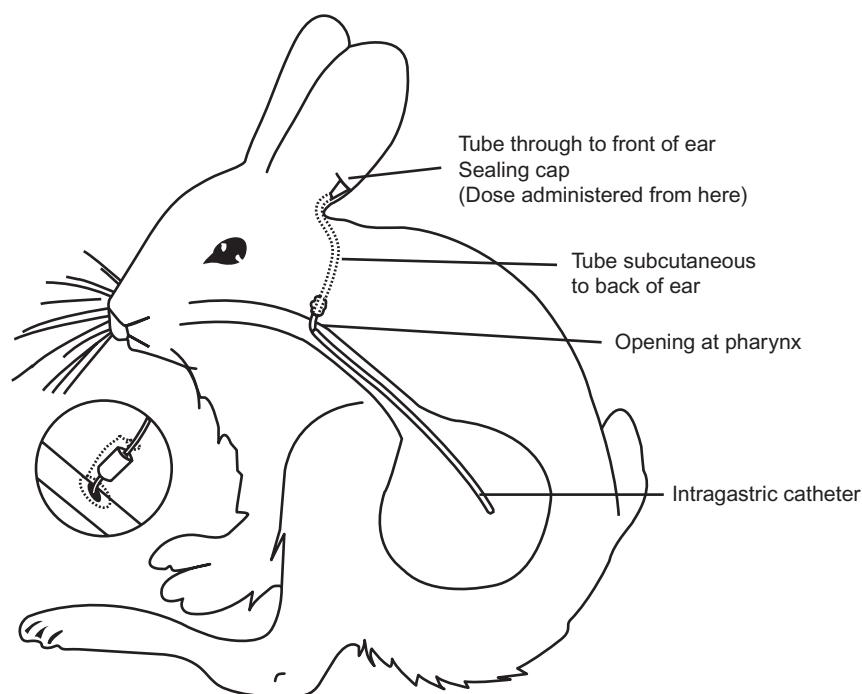


FIGURE 10.11 Pharyngotomy.

Subcutaneous Route

Subcutaneous injections can be accomplished without removing the rabbit from its housing. The animal may be gently pushed up against a cage or pen wall, and the scruff grasped and raised. The needle is held parallel to and pointed away from the hand holding the scruff, and inserted into the loose skin; one should not point the syringe at the hand holding the scruff, to decrease the chance for self-injury if the rabbit should move suddenly. If irritating substances are being injected, such as adjuvants for antibody production, the scruff is not the ideal site, as this area must be grasped for restraint. Alternative restraint methods include wrapping the animal in cloth, or placing it into a cat bag or an open-top plastic carrier or restraint box. The skin at the scruff may be thick, and a 23–25-gauge needle may be needed to penetrate the skin. Maximum volume recommendations are up to 5 ml/kg. If large volumes are being given, such as fluid for rehydration, the fluids should first be warmed.

Intravenous Route

The marginal ear vein is the most readily accessible site for intravenous injection in the conscious animal. In larger rabbits (3 kg or more), cephalic and saphenous veins may be used, but are more easily accessed in an anesthetized or sedated animal. The puncture site should be gently cleaned with warm disinfectant

solution. A refinement for venipuncture technique is to numb the site with a local anesthetic, such as a lidocaine and prilocaine cream, applied to the site of vessel puncture about 15 minutes prior to sampling. Hair may need to be clipped from the cephalic or saphenous sites, but the lateral ear vein can be visualized without clipping or plucking the hair, simply by slicking down the hair with petrolatum or anesthetic cream. A butterfly needle is easiest to use, as the flexible tubing is forgiving of animal movement once the needle is seated in the vessel (Figure 10.6). A 23–25-gauge needle or over-the-needle catheter can also be used. Needles and catheters can be secured in place with tape or glue or a single suture for repeat administration. A drop of surgical glue works well to temporarily secure a wing of a butterfly needle to the hair on the ear (Harcourt-Brown, 2004). The rabbit is restrained in a cloth wrap or cat bag or rigid restrainer. The vessel is held off near the base of the ear to promote distention, and the needle is advanced in the direction of the head for at least 1 cm. A flash of blood into the needle hub or tubing when gentle aspiration is applied confirms placement in the vessel.

For long-term venous access, a catheter can be surgically implanted, as previously described for sampling techniques. For constant intravenous infusion, rabbits can be maintained on a swivel-tether system, which permits the animal to remain unrestrained and mobile within its cage unit (Yu et al., 1995). Swivel-tether systems for rabbits are commercially available from several

sources, as are jackets or harnesses to secure the system to the animal. These systems may be used with catheters in the ear vessels, or with implanted catheters.

Recommended maximum volumes run to 5ml/kg given as a bolus over several minutes, or 10–20ml/kg for slow infusion over 10 minutes or longer. Lower volumes are given of substances with pH or tonicity that could cause damage. Fluids should be warmed.

Intramuscular Route

Sites for intramuscular injection include the large dorsal muscles of the anterior thigh (quadriceps) and the dorsal lumbar muscles. For quadriceps injection, with the rabbit resting on a table, the handler's forearm holds the rabbit snugly against the handler's body with the rabbit's head toward the elbow, and the hand holding the rear leg to be injected. The large quadriceps muscle group can be easily palpated dorsal to the femur. A short (5/8-inch), 25-gauge or smaller needle is placed parallel to the table surface, perpendicular to the side of the rabbit's thigh.

The lumbar muscles are accessed lateral to the spine, between the readily palpated landmarks of the last rib and the point of the hip (wing of the ileum). The handler's forearm holds the rabbit snugly against the handler's body with the rabbit's head toward the elbow and the hand cupped around the rabbit's rump. The needle is placed parallel to the spinal column, at about a 45° angle to the animal's back.

Volume of injection should be minimized to avoid the pain of muscle fiber separation. If a dose is split into more than one site, consider that multiple injections in a single limb may cause lameness. Recommendations for maximum volume are up to 1.0ml per site, or 0.05ml/kg (Diehl et al., 2001; Hawk et al., 2005; Morton et al., 2001).

Intraperitoneal Route

Intraperitoneal injection carries the risk of causing internal injury or of misplaced injection into a blood vessel or an organ, particularly the large thin-walled cecum or urinary bladder. The injection should be made in the lower (caudal) left quadrant of the abdomen, to avoid the more cranially located organs such as the liver, and the cecum, which tends to be on the right. The rabbit can be restrained, head downward, with its back toward the handler and with its body clasped between the handler's knees while the rear legs are held upward to expose the ventral abdomen. Alternatively, the animal may be held in a sitting position with one hand on the scruff and one hand holding the rear legs. A short (5/8- to 3/4-inch) needle is placed at a 45° angle to the body wall. Gentle aspiration on the syringe prior to

injection may help to confirm that the needle is not in a blood vessel or organ; caution must be used not to change the angle or location of the needle during the aspiration and subsequent injection.

A 23–25-gauge needle is generally used. Maximum volume limits range from 10–20ml/kg (Diehl et al., 2001; Hawk et al., 2005; Morton et al., 2001).

Intradermal Route

The injection site selected is most often along the flanks or loin area where a rabbit cannot readily scratch with its rear feet. The area is clipped, treated with a depilatory cream to remove remaining hair, and finally cleansed gently with an antiseptic scrub solution. The skin is stretched taut, and a tuberculin syringe with a 25–30-gauge needle is inserted, bevel up, into the dermis at a very shallow angle, almost parallel to the skin surface, just until the bevel disappears. Injection of a maximum volume of 0.1ml should produce a bleb. If seepage occurs when the needle is removed, then the needle may be inserted somewhat further on subsequent injections, and held in place for several seconds after injecting the substance.

Endotracheal Route

Endotracheal or transtracheal instillation of substances is used to create experimental models and to treat pathology of the lungs and respiratory tract. Endotracheal intubation techniques are discussed in greater detail in Chapter 2. The animals must be anesthetized or heavily sedated prior to intubation. Rabbits are prone to laryngospasm, and an application of topical anesthetic to the laryngeal membranes is necessary prior to intubation. Rabbits have a small oral cavity, and visualization of the laryngeal openings for placement of an endotracheal tube is possible but challenging.

A number of "blind" intubation techniques may be used, in which the person placing the endotracheal tube listens for breath sounds or observes moist air pulsing in the tube to determine when to advance the tube through the laryngeal opening; stethoscopes with adaptors can be attached to end of the tube (Figure 10.12) (Conlon et al., 1990). Correct placement of the endotracheal tube can be confirmed by auscultation. Substances can readily be introduced to the lungs via the endotracheal tube, by passing a small catheter through the tube lumen, and can be targeted to specific lung lobes (Frevert et al., 2000). Another option is to place a naso-tracheal tube. This technique is performed in an anesthetized rabbit, using an uncuffed, lubricated, endotracheal tube measuring 14.5cm in length and 2.0–2.5mm in inner diameter. (Stephens De Valk, 2009).



FIGURE 10.12 Endotracheal tube placement in an anesthetized rabbit, assisted by an attached stethoscope. The tube is advanced to the larynx, and breath sounds are readily heard through the stethoscope. The tube is advanced into the trachea when the laryngeal openings are widened, as detected by the sound of inhalation. Though not visible in this figure, note that the stethoscope tubing has a 3/4-cm hole cut just past the attachment to the endotracheal tube, to permit air to enter and exit the tube as the rabbit breathes.

Tracheostomy may also be accomplished in an anesthetized rabbit (Irazuzta et al., 1997). Pediatric transtracheal airway catheters are commercially available that are inserted much like over-the-needle catheters used in blood vessel cannulation. Simplest to perform is a transtracheal injection. The anesthetized rabbit is placed in dorsal recumbency. The head is extended and the cricoid prominence is palpated in the midline of the neck. The ventral neck is clipped and scrubbed with disinfectant. A hypodermic needle is placed through the skin 1–2 mm caudal to the cricoid prominence, and through the cricoid membrane, then directed caudally into the trachea. Aspiration of air confirms correct placement (Venkatesh et al., 1988). This technique may be more challenging in females with large dewlaps.

Intra-Articular Route

The anatomy of the joint selected should be reviewed prior to the procedure. The animal is anesthetized. The joint is palpated to determine the site of entry. The site is shaved and scrubbed for this aseptic procedure. A local anesthetic such as 1% lidocaine or 0.25% bupivacaine is infiltrated subcutaneously over the site of penetration. The joint is held steady. Stifle joints are held in a flexed position and entered from either side of the patellar tendon. A 22–25-gauge 3/4- to 5/8-inch needle with attached syringe is inserted carefully into the joint space. Aspiration of synovial fluid can confirm proper placement. Injection volumes of up to 0.5 ml can be made, depending on the size of the joint. If aspiration is done, negative pressure on the syringe is released

before pulling the needle from the joint. Complications include hemorrhage into the joint, trauma to the cartilage, and infection (Crow and Walshaw, 1987; Washington State University, 2009).

Administration to Neonates

Newborns present challenges in substance administration due to their size and fragility. Intravenous administration can be accomplished in the jugular vein, using a 30-gauge 5/8-inch needle. Maximum suggested volume to administer is 0.001 ml/g of body weight. Intraperitoneal injections are given in the lower left quadrant of the abdomen about 3 mm off the midline, using a 27-gauge 5/8-inch needle. Maximum suggested volume of injection is 0.01 ml/g of body weight. Subcutaneous injections may be made in the dorsal neck area with a 27-gauge needle, with a maximum volume of 0.01 ml/g of body weight. Oral gavage is accomplished with an 18-gauge feeding needle, using extra care to prevent traumatic injury. Intratracheal administration of up to 0.3 ml may be tolerated (Venkatesh et al., 1988). Maintaining warmth and hydration are essential when newborns are removed from the nest. Neonates should be handled with gloves and returned to the nest as soon as possible.

SPECIALIZED RESEARCH TECHNIQUES

Telemetry

Telemetry for remote data collection from freely moving animals is a refinement in experimental technique, freeing animals from the stress of repeated restraint and procedures. Small biocompatible transponders surgically implanted in animals can transmit biological data to receivers located outside the cage or pen. Telemetry devices for research animals, and associated computer software, are available from several commercial sources. Rabbits, like any small mammal, can be implanted with telemetry devices to monitor heart rate, blood pressure, body temperature, and electrocardiogram. Implantation of the device subcutaneously in the neck with cannulation of the carotid artery with complete occlusion of the artery is recommended as minimally invasive, with rapid recovery and device longevity of a year or more. (D'Aubioul et al., 2008). Telemetry has also been used to remotely follow intraocular pressure in the rabbit (Vogel, 2002).

Artificial Insemination

Semen can be collected about four times per week from a buck without reducing sperm count or libido. Most bucks can be readily trained to ejaculate into an

artificial vagina. A simple device can be made from 3" T-connector polyvinyl chloride (PVC) pipe with $3/4" \times 3/4" \times 1/2"$ openings, two rubber chair leg tips (25mm and 32mm inner diameters respectively), a rubber band, and a silicone condom (Naughton et al., 2003). The $1/2"$ opening of the PVC pipe is covered with the 25-mm chair tip. The condom is placed straight through both $3/4"$ openings in the PVC pipe. The open end of the condom is folded around one opening and secured in place with the rubber band, to create the vaginal entrance. The closed end of the condom is pulled through the other $3/4"$ opening, and is folded over outside the open $3/4"$ end and held in place while warm water is introduced into the pipe to fill the space between the condom and inside of the pipe. Once filled, the remaining chair tip is put over the opening including the folded-over closed end of the condom, to contain the water and to keep the condom stretched within the length of the pipe. A non-spermicidal lubricant jelly can be placed into the condom opening.

The artificial vagina is held under a dummy made from female rabbit hide that is placed over the handler's arm; the buck mounts the handler's arm. Alternatively, the artificial vagina can be held beneath a sedated female rabbit. The dummy or the doe is brought to the buck's cage. Bucks will generally mount and ejaculate quickly. Recommendations on the ideal temperature of the water vary from 98–113°F (Morrell, 1995; Naughton et al., 2003). If the water temperature is too hot, the buck may contaminate the sample with urine, but if it is too low, the buck will not ejaculate. Rinsing the condom ahead of time with 2–4ml of the extender that will be used to preserve or dilute the semen will help avoid loss of sperm that may adhere to the liner.

Ejaculate volumes range from 0.2–2ml, varying with breed. A quick subjective assessment of sperm motility can be made on a warm slide viewed under a microscope. Samples with less than 70% of sperm showing forward motility are not likely to be fertile (Morrell, 1995). If the semen is to be used immediately, a diluent such as physiological saline can be used. The semen should be maintained at 37°C if it is to be used right away, and should be used within 30 minutes of collection. Cryoprotectants must be added if the semen is to be frozen. The reader is advised to consult assisted reproduction references for discussion of semen extenders and preservation (Daader and Zeidan, 2008).

Does have an irregular estrous cycle with seasonal variation in receptivity. Females should be housed separately for at least 19 days prior to artificial insemination, to assure that they are not pseudo-pregnant. Swelling and color of the vulva can be used to gauge the likelihood of female receptivity, though this method is not always accurate; a swollen, dark pink or purple color is generally indicative of estrus. Lordosis response, an

elevation of the hindquarters to expose the perineum to facilitate mating, is also an indicator of estrus. A doe can be placed into a restrainer with a non-skid floor surface, and pressure gently and firmly applied to her rump with the palm of a hand, to gauge lordosis response (Contreras et al., 2008). An unreceptive doe will clamp her tail firmly against the perineum and remain seated.

Does are induced ovulators, but the mechanical stimulation from artificial insemination is not sufficient to induce ovulation in most females. To induce ovulation, the doe can be mated with a vasectomized buck, or treated with hormones. If a vasectomized buck is used, the artificial insemination should occur 1–4 hours prior to the mating so that the ejaculate does not mix with the artificially placed semen; occasionally vasectomized males develop anti-sperm antibodies in the seminal plasma, which could interfere with the sperm placed via artificial insemination. Does generally ovulate 9–13 hours after coitus. To induce ovulation with hormones, the doe can be given an injection of human chorionic gonadotropin (hCG) at 20 micrograms intramuscularly, or a gonadotropin releasing hormone (GnRH) analog such as Buserelin at 0.8 microgram, subcutaneously (Morrell, 1995). The GnRH analog can be given at the time of insemination, to induce ovulation some 10–12 hours later. hCG is not recommended for repeated use in a doe as antibodies may develop to the hormone (International Rabbit Reproduction Group, 2005). Some studies suggest that the same dose of GnRH analog may be mixed directly with the sperm given for artificial insemination, eliminating the need to provide it by injection (Quintela et al., 2004).

Insemination is accomplished with approximately 0.25ml of dilute semen, containing at least 5 million motile spermatozoa. The aliquot must be placed in the cranial aspect of the vagina, which requires an insertion angle of about 45° upward to negotiate the rim of the pelvis. A 1-ml glass laboratory pipette may be heated and then bent 45° about 8cm from the end, to fashion an easy-to-use delivery pipette (Morrell, 1995). The pipette is warmed to 37°C in a water bath just prior to use. Pregnancy detection via commercial ELISA progesterone tests designed for use in other species can be used with rabbits at about days 17–18 of gestation (Morrell, 1995).

Ovariohysterectomy

Ovariohysterectomy in the doe is performed like the standard veterinary procedure for a cat, with several key differences. Extra care is taken while making a ventral midline incision from cranial to the pelvis to the umbilicus, as the bladder and the cecum may lie directly beneath the body wall. Each ovary is located and ligated twice at the pedicle, and the pedicle is transected. Does have unusual anatomy in having separate uterine horns, each with its own cervix, that open into

a single vagina. Each horn should be ligated separately just cranial to its cervix. A circumferential and transfixation suture is recommended to ligate each horn. Uterine vessels running beside each horn should also be ligated. The horns are transected and removed. Closure of the incision occurs in three layers: muscle wall, subcutaneous space, and skin. Subcuticular closure of the skin may be used, assisted with superglue, or staples may be used (Mitchell and Tully, 2009; Wildlife Information Network, 2009). Laparoscopic ovariectomy has been described in the rabbit (Al-Badrany, 2009).

Orchiectomy

Orchiectomy in the buck is performed like the standard veterinary procedure, with several key differences. One should not pluck hair from the site as this may cause the skin to tear. The inguinal canal is open in bucks, and the testes can move between the scrotum and abdomen. Any of three surgical approaches can be taken: Scrotal (incision in each side of the scrotum); prescrotal (a single midline incision anterior to the scrotum); or abdominal (ventral midline incision). The procedure may be done with either an open or closed tunica albuginea. If an open castration is done, the tunica must be closed separately to assure closure of the canal. Scrotal incisions may be closed with a subcuticular pattern and/or with surgical glue (Mitchell and Tully, 2009, Wildlife Information Network, 2009).

Bile Collection

Long-term bile collection can be accomplished in the unrestrained rabbit (West et al., 2002). Bile flow is diverted via an implanted, exteriorized polyethylene Tygon tube that returns the bile to the enterohepatic circulation. In a sterile surgical procedure using an abdominal midline approach, the gallbladder is removed, and one end of a 40-cm length of tubing inserted into the cystic duct to collect the bile. The distal end of the same tubing is inserted into the common bile duct, to return the collected bile to the gut. The large loop of tubing exits the body wall, is tunneled under the skin, and exteriorized at the shoulder region. The exteriorized loop of the tubing is then cut and the bile can be directed to a collecting bag, or the ends can be reconnected to return bile to the duodenum. The rabbit is fitted with a small commercially available jacket to protect the tubing and hold the collection bag.

Decerebration

Decerebration is elimination of cerebral brain signal integration by separation of connection with the

hind brain (brain stem) and spinal cord. Depending on where the separation is made, specific reflexes are abolished that require integration in the brain. Decerebrate models have been created in rabbits to study control of movement, righting reflexes and proprioception, as well as physiology and pharmacology in the absence of stimulatory effects. Decerebration can be achieved in the anesthetized animal by physically cutting through the brain at a specific site, or running an electric current between probes inserted at specific sites in the brain (coagulation). Depending on the procedure and the anatomic site of the separation, animals may be able to survive the procedure for long periods, or may need to be euthanized in the course of the procedure. For specifics on the procedures, readers are referred to literature describing surgical methodology (Musienki et al., 2008) and coagulation methodology (Koller, 1969).

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Polyclonal Antibody Production

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INTRODUCTION

Prepared antibodies are critical reagents in a wide variety of research methodologies. The antibodies used may be either polyclonal or monoclonal, with each having distinct advantages and disadvantages. The exquisite specificity of a homogeneous monoclonal antibody limits non-specific reactions but also may limit the usefulness of the antibody for a variety of different research procedures. Polyclonal antibodies are heterogeneous and bind to several different antigen epitopes. While decreasing the specificity and potentially increasing non-specific reactions, the polyclonal antibody is also more likely to successfully bind to the specific antigen in a variety of different test conditions. The production of polyclonal antibodies remains and will likely

continue to remain, a common research procedure involving vertebrate animal use.

Rabbits are the most commonly used laboratory animal for the production of polyclonal antibodies. The rabbit offers many advantages over other species. The adequate body size of the rabbit and the ready accessibility of the marginal ear vein and central auricular artery affords a technically easy procedure for the collection of large blood samples. The availability of specific-pathogen-free animals ensures a healthy animal that will focus its immune response upon the presented antigen. The excellent responsiveness of the rabbit to a wide variety of antigens, the presence of a single primary Immunoglobulin G isotype, the vast amount of information available on the production and purification of rabbit immunoglobulins, and the ready



FIGURE 11.1 Intradermal injection of Freund's complete adjuvant. The viscous water-in-oil emulsion requires the use of larger-gauge needles than is typical for intradermal injections. The needle is passed with the bevel up through the skin into the subcutaneous tissues and then advanced back into the dermis.

availability of secondary reagents further enhance the reputation of the rabbit in polyclonal antibody production.

The response to an immunogen is a complex and dynamic phenomenon involving the interaction of different cell populations, cytokines, and chemokines potentially resulting in the production of antibodies, the activation of a CD4 cell-mediated response, and in many cases a CD8 cytotoxic lymphocyte response. Factors impacting the immune response are extremely varied and include the immunogen itself, the immunogen dose, the route of exposure, the host species, the immune status of the host species, and the use of adjuvants.

THE IMMUNOGEN

An immunogen is any molecule capable of generating an immune response when injected into an animal. Polypeptides, polysaccharides, glycoproteins, glycolipids, and lipoproteins with molecular weights above 10000 are typical immunogens since these are the types of molecules generally presented on the surface of pathogens. Immunogens contain one or typically more molecular sites (antigenic determinants or epitopes) to which antibodies are produced and bind. Antigens, in subtle contrast to immunogens, are molecules that contain a site (epitope) that can be recognized and bound by an immunoglobulin or T-cell receptor. While all immunogens are antigens, smaller compounds such as peptides with molecular weights <10000 may contain an epitope but not be capable of inducing an antibody response when injected alone (haptens). These haptens may become immunogenic when bound to carrier compounds, either in the laboratory or in vivo.

Thymus-Independent Antigens

Some antigens are capable of directly activating B cells and inducing a humoral antibody response without the involvement of helper CD4 T cells. These T-independent antigens are generally large molecules associated with bacterial infections including lipopolysaccharides, polymerized flagellin, and pneumococcal polysaccharides or closely related analogs such as dextrans, polyvinylpyrrolidone, and Ficoll (Clark, 1986). These antigens either bind to the B-cell receptor and other receptors on B cells (TI-1 antigens such as lipopolysaccharides of Gram-negative bacteria) or they cross-link multiple B-cell receptors resulting in activation of the B cell (TI-2 antigens such as pneumococcal polysaccharide or polymerized flagellin). Many of these T-independent antigens are potential non-specific mitogens for B cells and are referred to as polyclonal B-cell activators.

The humoral immune response to T-independent antigens is not dependent upon antigen processing and presentation by helper CD4 T cells and is thus a more rapid response. The T-independent response, however, is limited, as primarily IgM is produced and there is no potential for increasing antibody affinity through this mechanism. It has been suggested that this response evolved and persisted to permit a rapid humoral response to the initial bacterial invasion while the specific immune response, normally requiring helper CD4 T cells, is developing. While potentially beneficial for immune protection, the humoral immune response to T-independent antigens is not a significant factor in the experimental production of polyclonal antibodies.

Thymus-Dependent Antigens

The production of antibodies to thymus-dependent antigens involves the interaction of specific antigen, B-cells, professional antigen-presenting cells, and CD4 T cells in the secondary lymphoid tissues. Antigen is either transported to the lymphoid tissues via dendritic cells or the antigen arrives via either the draining lymphatics or direct contact and is phagocytosed by macrophages. In both cases the antigen is processed by the professional antigen-presenting cells and is presented to antigen-specific CD4 T-cells along with MHC class II molecules. This interaction results in the activation of antigen-specific helper T-cells. As B-cells pass through the secondary lymphoid tissue T-cell areas, those recognizing their specific antigen are bound and then activated through specific interactions with antigen-specific helper T-cells. The result is the formation of a primary focus of rapidly multiplying B lymphoblasts in the T-cell area of the lymphoid tissue. Some of the rapidly

multiplying B lymphoblasts relocate to the medullary cords and mature into antibody-producing plasma cells. Other B lymphoblasts relocate to the primary follicles attached to their respective helper T-cells, forming the germinal center. These B lymphoblasts, now referred to as centroblasts, are large metabolically active cells with a rapid mitotic rate. The centrocyte, a group of B-cells expressing an altered surface immunoglobulin with a range of affinities to the antigen, is the end product of the rapid division and somatic hypermutation of the centroblasts. The centrocyte competes with other centrocytes for antigen access and antigen-specific helper T-cells thus selecting for cells with increasing affinity to the antigen. This maturation of the antibody results in the selection of centrocytes with the highest antigen affinity for continued differentiation into antibody-producing plasma cells and long-lived memory B-cells.

The use of a rabbit to produce antibody to a single immunogen has been the typical approach for polyclonal antibody production. The rabbit is capable of mounting an antibody response to multiple immunogens simultaneously, producing a final product reactive with multiple antigens. In specific cases, immunizing a rabbit with multiple immunogens provides an opportunity to conserve resources and improve efficiency. Care must be taken to ensure that the antibodies produced to the respective immunogens will not interfere with the end-use of each antibody or affinity purification techniques can be used to isolate monospecific antibodies. The expansion of antibody-based proteomics is an example of a field where the immunization of rabbits with multiple immunogens followed by affinity purification to obtain monospecific antibodies is well documented (Larsson et al., 2006).

IMMUNOGEN DOSE

The immunogen dose may affect both the level of the antibody response (quantity) and the affinity of the antibody produced (quality). Very low immunogen doses may be inadequate to stimulate antibody production though the low doses may result in the production of memory B-cells that can be stimulated by a secondary exposure to the immunogen. Excessive immunogen doses may result in the production of low-affinity antibodies or the development of tolerance with no antibody production.

A number of studies have compared the antibody response to varying immunogen doses and detailed the dramatic variations. Siskind et al. (1968) compared the antibody response to doses of dinitrophenylated bovine γ -globulin (DNP- γ G) ranging from 0.05–50mg emulsified in Freund's Complete Adjuvant (FCA) in rabbits and had the optimum antibody response with the



FIGURE 11.2 Typical intradermal injection of Freund's complete adjuvant with distention of the dermis and a pale central region (0.05 ml inoculation volume).

0.5-mg doses. Immunizing doses of 5 and 50mg resulted in an initially greater antibody response at 13 days post-immunization but by 41 days post-immunization the antibody response to the 0.05-mg immunizing dose was three times greater. Goidl et al. (1968) immunizing guinea pigs with either 50 μ g or 1mg of DNP-bovine serum albumin (DNP-BSA) showed both an increased quantitative antibody response at 2 weeks and an increased antibody affinity at 2 months post-immunization in the animals immunized with 50 μ g of DNP-BSA. In developing antibodies to viomycin for enzyme immunoassay development, Hu et al. (Hu et al., 1989a, 1989b; Hu and Kitagawa, 1990) determined that the optimal initial immunization dose was 10 μ g with FCA as the adjuvant, 200 μ g with Freund's Incomplete Adjuvant (FIA), and 10 μ g with aluminum hydroxide (alum).

Determining the optimal immunogen dose is not a simple matter. The specific immunogen (immunogenicity, purity, pH, contaminants, etc.), the adjuvant used, the frequency and number of immunizations, and the route of immunization are all variables that directly affect the optimal immunogen dose. The optimal dose of highly immunogenic proteins with robust adjuvants may be as low as 10 μ g while less immunogenic proteins, small peptides, or polysaccharides along with less robust adjuvants may require greater doses to generate an optimal immune response. Since the primary goal is generally to produce a functional polyclonal antibody for other purposes and not to determine the optimal dose, the immunogen dose is generally estimated based upon the experience of the investigator, references in the literature with similar compounds, and the anticipated immunogenicity of the compound. Generally, 100–250 μ g quantities of proteins in adjuvant are adequate to produce a high-titer antibody response in rabbits (Cooper and Paterson, 2009; Harlow, 1988).



FIGURE 11.3 Freund's complete adjuvant intradermal injection sites (25 μ l/injection site) at 4 weeks post-immunization. Injection sites are enlarged, firm, and raised from the surface with central ulceration of the overlying epidermis. There is no indication of local hyperemia, tenderness, or pain upon palpation of the masses.

ROUTE OF IMMUNIZATION

The route of immunization, in large part, determines the distribution and presentation of the immunogen and has a profound effect on the immune response. The ideal immunization route should result in the exposure of the antigen to a large number of professional antigen-presenting cells (APCs) with secondary exposure of a large proportion of lymphoid tissues via the draining lymphatics and blood circulation. Additionally, the immunization site should be chosen such that the inflammatory reaction to the immunogen/adjuvant results in minimal functional impairment or pain and distress to the host animal. A wide variety of immunization sites have been described in polyclonal antibody production. Intra-articular, intrasplenic, and intralymph node injections have been described but these routes provide little advantage over the more routinely used routes of immunization (Holborow and International Association of Microbiological Societies, Permanent Section of Microbiological Standardization, 1970; Horne and White, 1968; Horne et al., 1970). Rabbits lack true foot pads, and coupled with the large body weight supported by the feet, the use of the foot pad as an immunization site is unacceptable in rabbits (Amyx, 1987). Intraperitoneal immunizations, extensively used in rodents, have been associated with pain and distress in mice when FCA is used (Toth et al., 1989; Leenaars et al., 1998; Wanstrup and Christensen, 1965) and are rarely used in rabbits. The intravenous route of immunization can be used with particulate immunogens and for booster injections of soluble immunogens in saline though anaphylaxis is a potential complication (Herbert, 1973). The intravenous injection of water-in-oil emulsions is associated with significant lesions in multiple organs (Brooks et al., 1978; Edwards and Slauson, 1983; McIntosh et al., 1974) and should not be used for polyclonal antibody production. The primary routes of immunization in the rabbit are

subcutaneous, intradermal, transdermal, and intramuscular. Each route has potential advantages and disadvantages and the choice of immunization route is heavily dependent upon the immunogen, the adjuvant, and the immunization schedule.

Intramuscular and subcutaneous injections are both technically quite easy to perform in the rabbit and permit the injection of greater volumes of immunogen/adjuvant per site than the intradermal route. Subcutaneous injections of immunogen-adjuvant mixtures are generally made on the dorsal back of the rabbit with a maximum volume for water-in-oil emulsions of 0.25 ml per site and 1 ml total volume (Canadian Council on Animal Care, 1991, 2002) though multiple injections of smaller volumes per site (e.g. 0.1 ml) may result in a reduction of potential complications. Subcutaneously injected immunogen/adjuvant mixtures tend to diffuse and may result in fistulous tracts that eventually open and drain distant from the initial injection site (Broderson, 1989). Intramuscular injected immunogen/adjuvant is generally placed in a single site within the quadriceps muscle group or the biceps femoris of the thigh. The maximum recommended volume in the rabbit is 0.5 ml with a 0.25-ml recommended single-site limit (Canadian Council on Animal Care, 1991, 2002). Intramuscular immunizations with robust adjuvants have been associated with granuloma formation that extends through both the muscle and fascial planes with possible nerve involvement (Leenaars et al., 1998; Stills and Bailey, 1991).

The intradermal route of immunization, while technically more challenging, offers some distinct advantages (Figures 11.1 and 11.2). The injected immunogen is localized with minimal diffusion, providing some protection from rapid antigen destruction and potentially enhancing the depot effect. The exposure of the Langerhans cells, professional antigen-presenting dendritic cells in high density within the epidermis, ensures efficient immunogen processing and an extensive distribution of activated immune cell populations in the draining lymph nodes. The intradermal route also has drawbacks including the limited recommended injection volume at any single site, the potential for pain with any injection into a closed space (Canadian Council on Animal Care, 2002), and the tendency for oil adjuvants to produce localized ulcerative lesions (Figure 11.3). Maximum single-site intradermal injection volumes are 50 μ l for saline-based immunogens and 25 μ l for water-in-oil adjuvant mixtures (Canadian Council on Animal Care, 2002; Halliday et al., 2000).

Transdermal immunization using Particle Mediated Epidermal Delivery, the commercial name for the current gene gun technology, delivers gold beads coated with DNA vaccine plasmids encoding for the specific immunogen into the epidermis of the skin (Wang et al.,

2008; Williams et al., 1991). Low-volume jet injection similarly targets the epidermal layer but without the particle component (gold beads) (Cartier et al., 2000; Furth et al., 1995; Ren et al., 2002). Both methodologies rely upon the ability of the vaccine plasmids to penetrate the cytoplasm of the targeted dendritic cells of the epidermis and dermis resulting in the DNA being processed and the resulting proteins being presented to T-cells (Donnelly et al., 1997). Both the gene gun and the low-volume jet injection methods are reported superior to the routine intradermal injection procedure in producing an immune response to DNA immunogens (Ren et al., 2002). In both cases, adjuvants can be added to the immunogen to enhance the immune response (Donnelly et al., 1997).

DNA immunization is an attractive alternative to the traditional protein immunization because plasmid DNA is relatively easy and inexpensive to generate and avoids the need to generate specific peptides or purify proteins for immunization. Transdermal DNA vaccination methodologies are still evolving and significant differences in behavior between species and injection sites on the same species have complicated development (Scheerlinck and Greenwood, 2006). While the DNA vaccination strategies have great promise for both therapeutic vaccinations and polyclonal antibody production, they presently have only limited application in routine polyclonal antibody production.

IMMUNIZATION SCHEDULE

The frequency of re-immunization or boosters is a significant variable in the production of polyclonal antibodies. The goal of the booster immunization is to re-stimulate the immune system and increase the circulating antibody level. To be most effective, the booster immunization should be administered after the initial immunogen level has decreased (i.e. where antibody titers have measurably waned), allowing selection for B-cells producing antibody of a higher affinity for the antigen. Appropriately timed booster injections stimulate the expansion of high-affinity memory B-cells resulting in the production of high levels of high-affinity antibody. Boosters administered too quickly after the initial immunization maintain high immunogen exposure levels, delay the selection of high-affinity B-cells, and fail to induce the desired secondary antibody response.

As with other aspects of polyclonal antibody production, the determination of an optimal interval for booster injections is complicated by the variations introduced by immunogen type, immunogen dose, route of immunization, and the adjuvant used. Without a "depot"-forming adjuvant the antibody titers tend to peak at 2–3 weeks following immunization and rapidly

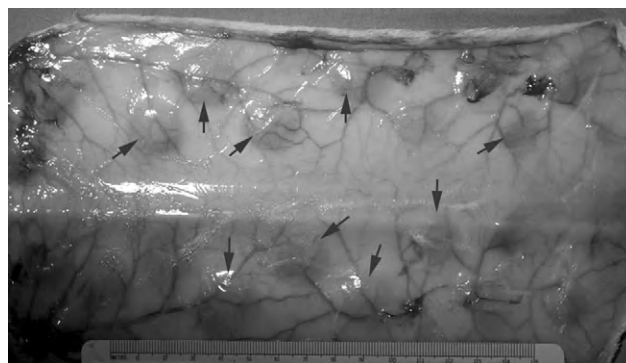


FIGURE 11.4 Freund's complete adjuvant intradermal injection sites (25µl/site) at 15 weeks post-immunization. The skin has been reflected to show the confinement of the granulomatous inflammation to the dermis (arrows).

decline. With "depot"-forming adjuvants antibody titers frequently peak 3–4 weeks or later following immunization and, dependent upon the adjuvant and immunogen, often remain elevated for many weeks.

ADJUVANTS

Adjuvants are best described as compounds that augment the immunological response to an immunogen. Purified proteins are poor immunogens and seldom result in a robust immune response when injected without an adjuvant. One of the prerequisites for a good immune response to an antigen is an inflammatory response. Inflammation results in recruitment of inflammatory cells and the activation of macrophages and dendritic cells, an essential component in the development of an immune response. The inflammation is not without consequence, resulting in variable degrees of tissue damage and pain, in large part dependent upon the characteristics and extent of the inflammatory response. Of all the components of the production of polyclonal antibodies, the choice of adjuvant is one of the most important and frequently the most contentious.

In choosing an adjuvant the overall goal is a critical component. Prophylactic or therapeutic vaccines are designed to produce a protective immune response with a minimum of side-effects. The desired response is one that will protect the recipient from developing serious disease following exposure to the agent and may require a humoral antibody response, an activated CD4-cell-mediated response, or a CD8 cytotoxic lymphocyte response. Experimental antibody production, however, is primarily directed towards the efficient and economical production of a high-titer and high-affinity antibody to the specific immunogen. While minimizing the side-effects is a consideration it is not nearly as critical

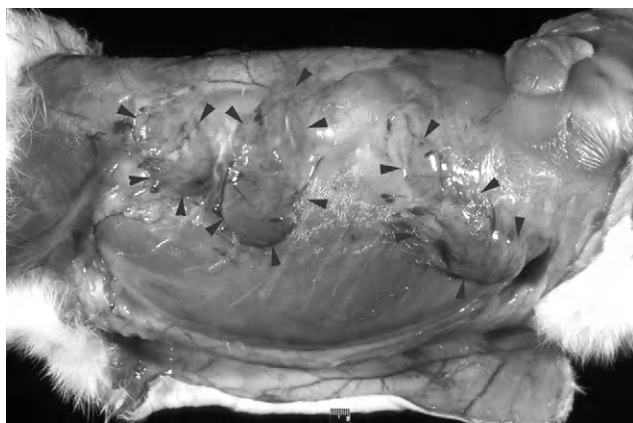


FIGURE 11.5 Freund's complete adjuvant subcutaneous injection sites (100 μ l/site) at 15 weeks post-immunization. Granulomas extend ventrally from each injection site along the dorsal back (arrows).

as with prophylactic vaccines while the production of CD4-cell-mediated responses or CD8 cytotoxic lymphocyte responses, often a critical component in prophylactic vaccine development, is of minimal concern.

The mechanism of adjuvant stimulation of the immune response is becoming better defined as the aspects of antigen recognition and immune response are more fully understood. Adjuvants may have up to five specific mechanisms of action including the "depot" effect, an antigen presentation effect, an antigen distribution effect, an immune modulation effect, and a cytotoxic lymphocyte induction effect (Cox and Coulter, 1997). Of these effects, the depot activity and the immunomodulatory effects are critical for the induction of a robust humoral immune response to a specific immunogen.

An adjuvant with "depot" effect protects the immunogen from both dilution and degradation by the host. "Depot" adjuvants localize and slowly release the immunogen resulting in a prolonged exposure of the immune system to a low level of the immunogen. The significance of the "depot" effect was demonstrated by Herbert in several studies. First, he immunized animals daily for 50 days with either antigen in saline or antigen in a water-in-oil emulsion. While both groups developed a high antibody titer, the titer remained elevated in the animals injected with the emulsion but it rapidly dropped in the animals injected with the antigen in saline (Herbert, 1967). In another experiment he inoculated mice with a single subcutaneous injection of ovalbumin in either saline or a water-in-oil emulsion. The water-in-oil emulsion produced antibody titers nearly 500 times that of the saline-injected ovalbumin and they remained at peak levels for approximately 1 year following the single injection (Herbert, 1968).

Emulsions are the most commonly used type of "depot" adjuvants in experimental immunizations for

the production of polyclonal antibodies. The most common emulsions are either water-in-oil or oil-in-water emulsions although more complex emulsions do exist. A number of emulsifiers have been used with either highly purified non-biodegradable mineral oils or biodegradable oils such as squalene and squalane. Other compounds that may serve as adjuvant "depot" agents include aluminum compounds (alum), microparticles, liposomes, and nitrocellulose.

In addition to protecting and slowly releasing the immunogen, a "depot" adjuvant should also preserve the conformational integrity of the immunogen so as to present the antigenic site to the effector cells. Water-in-oil emulsions maintain the immunogen in the aqueous phase while oil-in-water emulsions tend to localize the immunogen in the oil phase. Oil-in-water emulsions tend to work better with hydrophobic or amphipathic immunogens while water-in-oil emulsions tend to be more effective with hydrophilic or amphipathic immunogens (Hanly et al., 1995). Aluminum salt adjuvants bind immunogens via electrostatic forces and ligand exchange, an interaction that is dependent upon the appropriate pH, the buffer used, the type of aluminum salt, and the ratio of aluminum salt to immunogen. Optimizing this binding for the maximum presentation of the immunogen to effector cells requires reaction conditions specific for each immunogen-aluminum adjuvant mixture (Baylor et al., 2002; Hansen et al., 2007, 2009; Lindblad, 2004).

While protecting and slowly releasing an immunogen, an adjuvant must also ensure that the immunogen is processed and distributed widely throughout the immune system to maximize immune response. Many adjuvants form large multimolecular aggregates with the immunogen encouraging uptake and processing of the immunogen and any associated immunomodulators by macrophages and professional antigen-presenting cells (dendritic cells). Adjuvants may incorporate carbohydrate moieties or molecular configurations specific for macrophage or dendritic cell receptors to increase uptake and processing (Abel et al., 1989; Bonifaz et al., 2004; Chinnah et al., 1992; Jiang et al., 1995; Ribí et al., 1984). Following initial processing of the immunogen, the dendritic cells and macrophages relocate to the regional lymph nodes and spleen where further processing results in the production of antigen-specific B-cells and plasma cells. This processing and transport to the regional lymphoid tissues occurs quite quickly with both emulsion and aluminum salt adjuvants and surgical removal of the initial injection sites within days of the immunization has little effect on the animal's immune response even though antigen is still present in the excised injection site (Freund, 1951; Holt, 1950).

Immunomodulators are used in many adjuvants to stimulate and often alter the immune response of the

host. Immunomodulators have historically consisted of microbial cells (i.e., killed mycobacteria and *diphtheria pertussis*), microbial cell components (i.e., mycobacterial cord factor, cell wall skeleton, muramyl dipeptide, lipopolysaccharide, and N-acetyl-glucosaminyl-N-acetylmuramyl-L-alanyl-D-isoglutamine (a soluble glycopeptide from *Lactobacillus bulgaricus*)), and modified microbial products (i.e., monophosphoryl lipid A and t-muramyl dipeptide). These immunomodulators are recognized by the innate immune system as associated with infection and are referred to as pathogen-associated molecular patterns (PAMPs). Pattern recognition receptors (PRRs) such as the toll-like receptors (TLR), the nucleotide-binding oligomerization domain receptors (Nod-like receptors or NLRs), and Dectin-1 specifically bind to these PAMPs stimulating and directing the adaptive immune system. Immunomodulators may also be included as contaminants in proteins used as carriers for small peptides such as keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA), ovalbumin, etc. (Palm and Medzhitov, 2009) or the immunogen can be conjugated with a PAMP such as flagellin (Cuadros et al., 2004). To be effective, the immunomodulator and the immunogen must be delivered together and preferably to the same phagolysosome (Blander and Medzhitov, 2006).

The recruitment and activation of the dendritic cells and macrophages by adjuvants that is essential for immunogen processing also results in a number of other inflammatory sequelae. Activated macrophages secrete a number of proinflammatory cytokines including interleukin (IL)-1 β , tumor necrosis factor (TNF)- α , IL-6, IL-8, and IL-12. These cytokines recruit additional inflammatory cells, increase vascular permeability, cause local tissue destruction, and result in systemic effects including pyrexia and the production of acute-phase proteins by the hepatocytes. These local and systemic effects are potentially undesirable effects that may result in pain and distress to the host. Balancing the requirement for immune activation for antibody production against the resultant inflammatory reactions and potential pain and distress to the host is a critical consideration in adjuvant selection for experimental polyclonal antibody production.

Water-In-Oil Emulsion Adjuvants

Freund's Adjuvants

Since the original descriptions of its formulation and efficacy (Freund, 1951, 1956; Freund and McDermott, 1942; Freund et al., 1937, 1948), Freund's complete adjuvant (FCA) has remained the most widely used and most effective adjuvant for polyclonal antibody production. The immunostimulatory capabilities of FCA are generally unsurpassed (Altman and

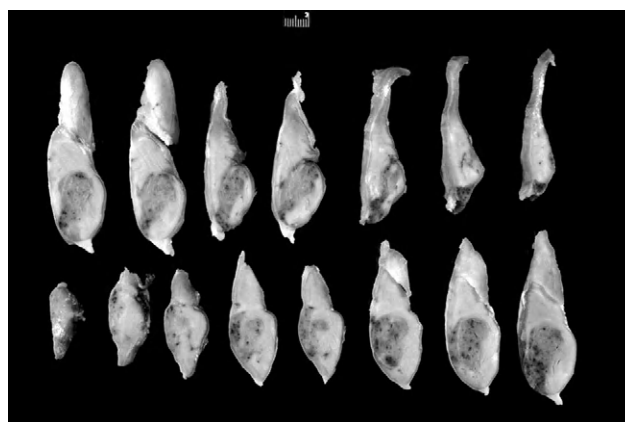


FIGURE 11.6 Transverse sections of the biceps femoris and adjacent muscles 15 weeks following an intramuscular injection of Freund's complete adjuvant. Multifocal areas of hemorrhage and granulomatous inflammation extend the entire length of the biceps femoris.

Dixon, 1989; Munoz, 1964; Smith et al., 1992; Warren et al., 1986) and FCA remains the "gold standard" to which newly formulated adjuvants are compared. The use of FCA has also been associated with a variety of lesions including localized injection site granulomas (Broderston, 1989; Johnston et al., 1991; Kleinman et al., 1993; Leenaars et al., 1994, 1998a, 1998b; Leskowitz and Waksman, 1960; Steiner et al., 1960; Stills, 1994, 2004; Stills and Bailey, 1991; Toth et al., 1989; Warren et al., 1986); granulomas in a variety of organs distant from the injection site (Broderston, 1989; Steiner et al., 1960); necrotizing dermatitis (Leenaars et al., 1998a; Steiner et al., 1960); and spinal cord damage from granuloma formation (Hill et al., 2006; Kleinman et al., 1993). The serious lesions produced by FCA prevent the licensure of this adjuvant in either human or veterinary medicine for prophylactic vaccinations and have resulted in numerous regulatory guidelines (Canadian Council on Animal Care, 1991, 2002; United States Department of Agriculture, 1997) and recommendations (Grumstrup-Scott and Greenhouse, 1988; Jackson and Fox, 1995; Leenaars et al., 1999; Stills, 2000, 2007) regarding its use in experimental polyclonal antibody production in animals.

FCA is composed of a light mineral oil, mannide monooleate (a surfactant), and heat-killed and dried mycobacterial cells. Freund's incomplete adjuvant (FIA) lacks the heat-killed and dried mycobacterial cells but is otherwise identical. The present FCA and FIA adjuvants are far more refined and vastly less toxic than the original formulations. The original Freund's adjuvants used a crude mannide monooleate (Arlacel A[®]) surfactant and an oil that contained a variety of organic hydrocarbons and contaminants, including paraffins, olefins, aromatic hydrocarbons, and cycloparaffins, many of

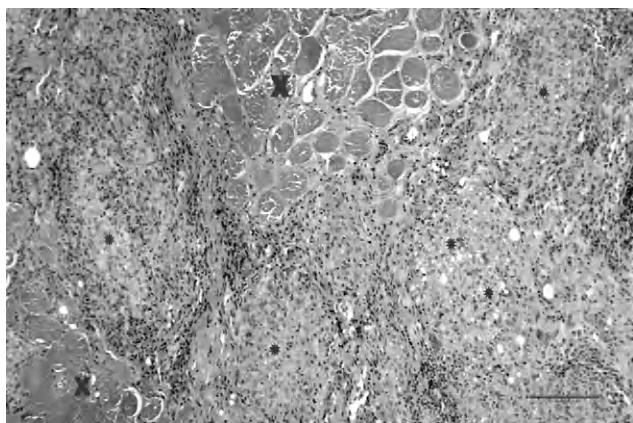


FIGURE 11.7 Chronic granulomatous inflammation in the biceps femoris 15 weeks following the intramuscular injection of Freund's complete adjuvant. Multifocal areas of macrophage infiltration (*) with necrosis of the adjacent muscle fibers (X). Residual mineral oil appears as clear vacuoles. Hematoxylin and eosin; bar = 100 μ m.

which were cytotoxic and inflammatory. Contaminating short-chain hydrocarbons were both immunosuppressive and responsible for the acute toxicity associated with the adjuvants (Shaw et al., 1964; Stewart-Tull et al., 1976; Wilner et al., 1963). The development of mineral oil-based influenza and poliomyelitis vaccines for human use in the 1940s and 1950s resulted in specific and more stringent requirements for both the oil and the surfactants to minimize adverse reactions (Berlin, 1962; Friedewald, 1944; Henle and Henle, 1945; Salk, 1953; Salk and Laurent, 1952; Salk et al., 1951, 1952). Changes in oil-refining procedures after 1970 and improved purification techniques have removed most of the contaminants from the light mineral oil presently used (Stewart-Tull, 1998). The type and amount of killed and dried mycobacterial cells in FCA has also been changed from 10mg/ml of virulent *Mycobacterium tuberculosis* in the original formulation (Freund and McDermott, 1942; Freund et al., 1937) to 0.1mg/ml to 1mg/ml of relatively avirulent *Mycobacterium tuberculosis* H37Ra or *Mycobacterium butyricum* (Stewart-Tull, 1995).

Both FCA and FIA are very efficient and effective adjuvants, generally producing high-affinity and high-avidity antibody to a wide range of immunogens. The mycobacterial components of FCA tend to produce a strong delayed-type hypersensitivity with a bias towards a Th1 profile (Lindblad, 2000). The delayed-type hypersensitivity reaction generally prevents reimmunization with FCA due to the severe resultant reaction. Both FCA and FIA are water-in-oil emulsions that when properly prepared, slowly release their immunogens over many weeks (Halbert et al., 1946). Glass Luer-lock syringes are used with double-hubbed needles or three-way stopcocks to emulsify the water-in-oil emulsion by repeatedly passing the

mixture through a small orifice. Properly prepared, the thick and viscous water-in-oil emulsion does not diffuse when a drop is placed on the surface of cold water (Garvey et al., 1977; Herbert, 1973). Improperly prepared emulsions and oil-in-water emulsions of Freund's adjuvants are much less effective in producing a robust immune response.

There are numerous reports characterizing the lesions associated with the use of Freund's adjuvants. The typical lesion is focal necrosis with a granulomatous inflammatory response with oil-filled "foamy" macrophages (Broderson, 1989; Leenaars et al., 1994, 1998b; Stills, 1994; Stills and Bailey, 1991; Wiedemann et al., 1991). Intradermal injections are associated with large palpable granulomas that frequently ulcerate (Broderson, 1989; Leenaars et al., 1994; Stills, 1994; Stills and Bailey, 1991) (Figures 11.3 and 11.4). Subcutaneous injections frequently migrate from the injection, forming granulomatous fistulous tracts that may open and drain remotely from the initial injection site (Broderson, 1989) (Figure 11.5). Intramuscular injections result in granuloma formation that extends through the fascial planes of the muscle with possible secondary nerve involvement (Altman and Dixon, 1989; Leenaars et al., 1998a; Stills, 1994; Stills and Bailey, 1991) (Figures 11.6 and 11.7). Beyond the reactions associated with the injection site, granulomatous lesions are often detected in the draining lymph node, spleen, lung, kidney, and other organs where microdroplets of the emulsion are distributed by the circulatory and lymphatic systems (Waksman and Bullington, 1960).

While there is an extensive literature on the associated lesions, there are few studies evaluating pain and distress associated with the use of FCA or FIA in rabbits. Amyx (1987) first addressed the issue and concluded that under some conditions the local inflammatory lesions likely resulted in pain or distress. A few reports of the pathological lesions associated with the use of FCA and FIA in rabbits have included some information on the clinical assessment of pain or distress (Johnston et al., 1991; Leenaars et al., 1994; Stills and Bailey, 1991) while others have presumed the presence of pain or distress based entirely on the observed lesions without any clinical or behavioral evaluation (Jennings, 1995; Mallon et al., 1991). The few prospective studies to evaluate both physiologic and behavioral changes in rabbits injected with FCA or FIA via the subcutaneous or intramuscular route have failed to detect any changes directly attributable to the adjuvant-immunogen injection (Halliday et al., 2000, 2004; Leenaars et al., 1994, 1998a). In contrast there are a number of studies indicating that pain or distress is associated with the intraperitoneal injection of FCA-immunogen in rodents (Leenaars et al., 1998b; Toth et al., 1989; Wanstrup and Christensen, 1965) and high dosages of intraperitoneal FCA in rats reliably

produce acute inflammation (Geisterfer and Gauldie, 1996; Geisterfer et al., 1993; Giffen et al., 2003; Olivier et al., 1999), indicating that Freund's adjuvants have the potential for inducing pain and distress in animals under specific circumstances.

An additional approach to evaluating the potential of pain and distress associated with the injection of Freund's adjuvants comes from reports of human injections. Water-in-oil adjuvants similar to FIA were used in human influenza vaccines in the early 1960s prior to discontinuance due to safety concerns. Reports of injection site pain were uncommon and generally mild (Alving, 2002; Lindblad, 2000; Salk, 1953; Salk and Salk, 1977; Salk et al., 1952) but rare side effects (Cutler et al., 1962; Stuart-Harris, 1969) included cystic swellings and muscle indurations which persisted for up to 1 year and histologically consisted of oil granulomas. Long-term follow-up studies on United States military personnel vaccinated with FIA-influenza failed to identify any additional risk associated with the vaccination (Beebe et al., 1964, 1972). In spite of FIA's low incidence of adverse reactions and its proven effectiveness, attempts to reintroduce FIA in human vaccines in the late 1960s were unsuccessful (Davenport, 1968). Reports of human administration of FCA are far fewer than with FIA. Hughes and colleagues (Hughes et al., 1970) used repeated injections of FCA in early human cancer immunotherapy studies and reported that severe reactions were only seen after repeated injections, after the patients developed sensitivity to the tuberculin antigen. Chapel and August (1976) reported severe pain in five of nine cases of accidental FCA injection in humans and only in those that were tuberculin-sensitive prior to the injection. These reports, coupled with the fact that many human diseases characterized by chronic granulomatous inflammation, including tuberculosis, leprosy, sporotrichosis, and "Buruli" (*Mycobacterium ulcerans*) cutaneous ulcers are relatively painless until quite advanced (Kumar et al., 2005; Pfyffer et al., 2003), suggests that the initial granuloma produced by FCA is not necessarily associated with significant pain.

Specol

Specol (ID-DLO, Lelystad, The Netherlands) is a water-in-oil adjuvant composed of a purified light mineral oil (Markol 52) with the emulsifiers Span 85 and Tween 85 (Bokhout et al., 1981) developed as a less toxic alternative to Freund's adjuvants. All of the ingredients in Specol are individually FDA approved for veterinary use and can be purchased individually (Vogel and Powell, 1995). Studies comparing the antibody responses to different immunogens emulsified in either Specol or FCA/FIA have generally reported similar results in rabbits (Leenaars et al., 1994, 1998b), pigs (Oonk et al., 1998), and mice (Leenaars et al., 1995,

1998b) although there are a few reports with self-antigens (Leenaars et al., 1995) and small peptides (Ferber et al., 1999) where FCA/FIA was shown to be more effective. Studies comparing the histologic lesions following the use of Specol or FCA/FIA are limited, inconclusive, and have been primarily conducted in mice. Leenaars et al. (1994, 1998a, 1998b) reported fewer histologic lesions associated with the use of Specol than FCA/FIA while Ferber (Ferber et al., 1999) reported more severe lesions with Specol. Studies comparing pain and distress are very limited but it is likely that the pain and distress are similar with the use of either Specol or FCA/FIA.

Montanide ISA Adjuvants®

The Montanide Incomplete Seppic Adjuvants (ISAs) (Seppic, Paris, France) consists of a series of adjuvants composed of a variety of oils, emulsions characteristics, emulsifiers, and immunomodulators. The Montanide ISA50V, ISA51, and ISA70 adjuvants are water-in-oil emulsions based on a purified mineral oil and are very similar to FIA. The few studies comparing these adjuvants and FCA/FIA report a similar antibody response with equal or less inflammation (Johnston et al., 1991; Leenaars et al., 1994, 1995, 1998b). The other Montanide adjuvants are primarily prophylactic adjuvants composed of a variety of mineral and metabolizable oils designed for use in human and veterinary vaccines (Stewart-Tull, 2003).

TiterMax® and TiterMax Gold®

TiterMax and TiterMax Gold (CytRx, Norcross, GA) are water-in-oil emulsion adjuvants consisting of the metabolizable oil squalene, an emulsifier (sorbitan monooleate 80), a patented block copolymer (CRL8941 or CRL8300, respectively), and microparticulate silica. The high-molecular-weight non-ionic block copolymers consist of linear chains of hydrophilic polyoxyethylene flanking linear chains of hydrophobic polyoxypropylene (Hunter and Bennett, 1986) producing an adjuvant effect presumably through enhanced antigen presentation, complement activation, chemotaxis, and macrophage activation (Howerton et al., 1990; Hunter and Bennett, 1984; Hunter et al., 1994). The immunomodulatory effects of block copolymers have made them very promising candidates in the development of human prophylactic vaccines (Hunter, 2002).

TiterMax and TiterMax Gold adjuvants were developed as alternatives to the use of FCA/FIA in polyclonal antibody production capable of equal or better antibody production with less reported tissue toxicity (Bennett et al., 1992; Jennings, 1995). Since their introduction, numerous studies have compared the TiterMax adjuvants with other adjuvants using a variety of species, immunogens, and routes of administration.

Bennett et al. (1992) reported comparable antibody responses with TiterMax and FCA in rabbits, mice, and goats with reduced lesions. Other reports, however, have indicated reduced inflammatory lesions in comparison to FCA but also reduced antibody production in rabbits (Leenaars et al., 1994, 1998b; Smith et al., 1992) and guinea pigs (Robuccio et al., 1995). Studies in mice have indicated comparable antibody production to FCA following intraperitoneal administration but severe lesions were also produced while subcutaneous administration resulted in minimal lesions but also poor antibody production (Leenaars et al., 1998b).

Oil-In-Water Emulsion Adjuvants

Oil-in-water adjuvants differ from the water-in-oil adjuvants in that they are typically formulated with only a small amount of oil dispersed in an aqueous phase in contrast to the water-in-oil emulsions that typically have $\geq 50\%$ oil. Oil-in-water emulsions tend not to form a localized depot like the water-in-oil adjuvants but rather are rapidly transported from the injection site to the draining lymphatics, reducing the potential for localized chronic inflammation at the injection site. The reduction in potential injection site complications has made the oil-in-water emulsion adjuvants much more likely candidates than the water-in-oil emulsion adjuvants for the development of human prophylactic vaccines (Allison, 1999; Alving, 2002).

RIBI Adjuvant System[®]

The RIBI Adjuvant System[®] (RAS[®]) (Corixa Corporation, Seattle, WA) is a commercial adjuvant system first available for experimental antibody production in 1985. The system is based upon the use of a small quantity of metabolizable squalene oil and Tween 80 surfactant into which the antigen is incorporated prior to emulsification with the aqueous phase (Ribi et al., 1975). The resulting emulsion is much less viscous than typical water-in-oil emulsions and can be sterilized by filtration and administered easily by injection (Saha and Mondal, 2000; Stills, 1994). Immunostimulants must be added to the emulsion as alone, the emulsion is a poor adjuvant (Altman and Dixon, 1989). Three different immunostimulants are used to produce the three different research formulations of the RAS[®].

Trehalose 6,6'-dimycolate (TDM) is the non-immunogenic lipid component of the mycobacterial cord factor that is found in virulent strains of the mycobacteria (Altman and Dixon, 1989). TDM is included in all RAS[®] formulations and augments both the humoral and the cell-mediated immune responses (Azuma et al., 1974; Ribi et al., 1975, 1982) though it is reportedly less effective in the rabbit than in rodents (Altman and Dixon, 1989). Monophosphoryl lipid A (MPL[®]) is a

chemically modified form of lipid a, the component of bacterial lipopolysaccharide that mediates endotoxic shock. MPL[®] serves as an agonist for TLR-4, retaining the immunostimulatory effect, but has greatly reduced toxicity (Ribi et al., 1984). MPL[®] is a component of two of the RAS[®] formulations, only absent in the formulation recommended for use in mice with strong antigens. A second mycobacterial-derived compound, cell wall skeleton (CWS), is present in the RAS[®] formulation recommended for use in rabbits, goats, and primates. The immunostimulatory activity of CWS is primarily due to muramyl dipeptide (MDP), which is responsible for much of the adjuvant activity attributable to whole mycobacterial cells (Ellouz et al., 1974).

Studies comparing the antibody response to specific immunogens with RAS[®] formulations and FCA/FIA have, with one exception in mice (Lipman et al., 1992), generally indicated substantially higher antibody production with the FCA/FIA adjuvant regimen (Johnston et al., 1991; Leenaars et al., 1995, 1998a). Studies comparing the pathological lesions produced by RAS[®] have yielded widely varying results from lesions similar to those seen with FCA/FIA (Johnston et al., 1991; Leenaars et al., 1994, 1998a) to only minimal lesions with the RAS[®] adjuvant compared with FCA/FIA (Deeb et al., 1992; Leenaars et al., 1998b).

Syntex Adjuvant Formulation (SAF)[®]

The Syntex Adjuvant Formulation (SAF, SAF-1, SAF-m)[®] (Chiron Corporation, Emeryville, CA) is a microfluidized oil-in-water emulsion consisting of 5% squalane, 2.5% Pluronic[®] L121, 0.2% polysorbate 80 (Tween 80), and phosphate-buffered saline with threonyl muramyl dipeptide (t-MDP) (Vogel and Powell, 1995). The synthetic block copolymer Pluronic[®] L121 and t-MDP, a synthetic analog of muramyl dipeptide (MDP), serves as the immunostimulants (Allison and Byars, 1986; Howerton et al., 1990; Allison, 1999; Hunter and Bennett, 1984).

The Syntex Adjuvant Formulation was developed as a prophylactic vaccine adjuvant and has been evaluated in humans and a number of other species for efficacy and safety as a prophylactic vaccine adjuvant. Adjuvant-related complications in a human cancer immunotherapy trial consisted of injection site erythema, tenderness, induration, myalgia, arthralgia, and pyrexia (Hsu et al., 1997). As with other adjuvants, studies comparing the effectiveness of SAF with FCA and other adjuvants in antibody production have reported mixed results. A study comparing influenza immunization with SAF or FCA as the adjuvant in mice (Hjorth et al., 1997) reported SAF to be equal or superior to FCA. A similar influenza immunization study in guinea pigs, however, found SAF to be inferior to FCA, TiterMax[®], RIBI[®], and aluminum phosphate for antibody production (Robuccio et al., 1995).

Particulate Adjuvants

Aluminum Salt Adjuvants

Absorption of immunogens onto aluminum phosphate or aluminum hydroxide (Alhydrogel) is the most common method of producing prophylactic vaccines for both human and veterinary use (Galloway et al., 2004; Gupta et al., 1993, 1995). Aluminum salt adjuvants bind immunogens via electrostatic forces and ligand exchange providing a relatively short-lived depot effect with peak antibody titers at 3–4 weeks post-injection (Altman and Dixon, 1989). Aluminum adjuvants also exhibit some immunomodulatory properties, inducing secretion of CCL3, CCL4, and CXCL8 by human monocytes and macrophages (Seubert et al., 2008), CCL2, CXCL1, CCL11 (Kool et al., 2008), and IL-1 β (Kool et al., 2008) in mice. Aluminum adjuvants tend to promote a nearly exclusive Th2 type of immune response (Lindblad, 2004) with little or no cell-mediated Th1 or CD8 cytotoxic lymphocyte response (Reed et al., 2009).

Aluminum adjuvants have been extensively used in human vaccines and have a proven safety record. They do rarely result in side effects, primarily localized erythema and granuloma formation at the injection site (Baylor et al., 2002; Gupta et al., 1993) and this appears to be more commonly seen with subcutaneous administration (Pittman, 2002). While they will continue to be used extensively in prophylactic vaccines, aluminum salt adjuvants are not extensively used for primary immunizations for experimental polyclonal antibody production purposes. Aluminum adjuvants do, however, have a number of characteristics that warrant their consideration as adjuvants for both primary and booster injections in polyclonal antibody production (Stills, 1994).

Immune-Stimulating Complexes

Immune-stimulating complexes (ISCOMs) are 40–100-nm honeycomb complexes composed of immunogen, cholesterol, phospholipids, and a saponin from the South American soap tree (*Quillaja saponaria*) (Dalsgaard et al., 1995; Liang et al., 2006; Morein and Bengtsson, 1999; Morein et al., 1984; Sun et al., 2009). The saponin is a heterogeneous mixture of glycosides that is essential in the formation of the ISCOM and serves as a potent adjuvant, presumably increasing uptake of the ISCOMs by dendritic cells (Liang et al., 2006). There are a number of commercial semipurified or purified *Quillaja* saponin preparations including Quil A (Superfos AS), Spikoside (AdVet), and Iscoprep 703 (Isotec AB). Quil A has been extensively used in veterinary vaccines but the hemolytic activity of Quil A on parenteral administration has prevented its use in human vaccines (Vogel and Powell, 1995). ISCOMs are classically formed by reacting the saponin, cholesterol,

and phospholipids with a hydrophobic or amphipathic antigen in the presence of a suitable detergent. Hydrophilic immunogens such as small peptides must be modified by incorporating hydrophobic regions to be effectively used in ISCOMs. The preparation of the ISCOM matrix is dependent upon the ratio of saponin to immunogen, the hydrophobicity of the immunogen, the phospholipid component, and the preparation method, making proper formulation of the immunogen-adjuvant mixture problematic and evaluation of the final product difficult (Myschik et al., 2006; Sun et al., 2009). Many difficulties associated with the use of ISCOMs for experimental polyclonal antibody production were addressed by the development of the preformed ISCOMs (ISCOM-matrix) that could be readily associated with the chosen immunogen (ISCOMATRIX[®], CSL Behring, King of Prussia, PA). While developed primarily for use in prophylactic vaccines, the ISCOM-matrix provides an easy-to-use adjuvant for the development of experimental polyclonal antibodies.

ISCOMs and ISCOM-matrix adjuvants have been used extensively in veterinary vaccines (Morein et al., 2004; Scheerlinck and Greenwood, 2006) and in a number of experimental vaccines in a variety of species (Sun et al., 2009). The ability of ISCOMs to induce a CD8 cytotoxic lymphocyte (CTL) response has made them a valuable adjuvant, especially for prophylactic vaccines against viral diseases (Scheerlinck and Greenwood, 2006). ISCOMs are efficiently endocytosed by antigen-presenting cells with processing for both MHC class I and class II presentation (Reed et al., 2009; van Binnendijk et al., 1992). While ISCOMs have an excellent safety record as veterinary vaccines, studies evaluating the inflammatory response to ISCOMs in mice immunized by intraperitoneal injection (Smith et al., 1999) have documented a rapid influx of neutrophils and mast cells followed by macrophages, dendritic cells, and lymphocytes with an increase in the levels of IL-1, IL-6, IL-12, and interferon- γ peaking at 24–72 hours post-injection.

Studies comparing ISCOMs or ISCOM-matrix adjuvants with other adjuvants for experimental polyclonal antibody production have been quite limited and reached varying conclusions from the production of significantly greater antibody (Browning et al., 1992), being comparable to FCA (Leenaars et al., 1995; Sjolander et al., 1993), to producing a significantly lower antibody response (Levi et al., 1993; Lockyer et al., 1993; Sjolander et al., 1993). In all cases where the histologic lesions were compared, significant histologic lesions were minimal with the ISCOM adjuvants except in the case of intraperitoneal injections (Leenaars et al., 1995). The use of ISCOMs in experimental polyclonal antibody production suffers from the complicated adjuvant preparation

and the necessity of immunogen modification to ensure binding with the ISCOM-matrix or incorporation in the ISCOM. As stated by Leenaars et al. (1995), the complicated preparation is a significant disadvantage.

Gerbu® Adjuvants

The Gerbu® adjuvants (GERBU Biochemicals GmbH, Am Kirchwald 6, 69251 Gaiberg, Germany) are based on the use of the immunomodulator N-acetyl-glucosaminyl-N-acetylmuramyl-L-alanyl-D-isoglutamine (GMDP), a soluble glycopeptide from the cell wall of *Lactobacillus bulgaricus*. All Gerbu® adjuvants contain cimetidine as an immune enhancer and most also contain saponin, another immunomodulating compound. A Gerbu® adjuvant specifically designed for monoclonal antibody immunizations in mice (Gerbu Adjuvant MM®) additionally contains poly(adenylic)-poly(uridylic) acid heteroduplex (poly A:U) and theophylline as additional immunomodulators. Gerbu® adjuvants are aqueous adjuvants with suspended ultrafilterable particles composed of a paraffin mixture containing dimethyldi(stearoylhydroxyethyl)ammonium chloride (Esterquat 1) in conjunction with mannide monooleate, glycerol, L-proline, and ciprofloxacin.

Developed primarily as adjuvants for prophylactic vaccines, the Gerbu® adjuvants have evolved and changed in an effort to improve response and minimize complications. Evaluations and comparisons of studies using the Gerbu® adjuvants are complicated by the many variations of the adjuvant and the tendency to refer to all products as "Gerbu adjuvant." Studies that have evaluated and compared Gerbu® adjuvants to others have consistently shown Gerbu® to be well tolerated with few lesions but generally failing to produce antibody titers equivalent to those produced using other adjuvants. Studies in sheep and avians have shown Gerbu® adjuvants to be effective for prophylactic immunization but deficient in antibody production compared to FCA, FIA, and others (Beck et al., 2003; Shu et al., 2001). Ferber et al. (1999) reported the antibody response to immunization with small synthetic peptides in Gerbu adjuvants to be equivalent to the response using FCA/FIA and superior to that using PolyA:PolyU, TiterMax®, and RIBI while other reports indicate superior responses using FCA, FIA, and TiterMax® (Seetharamaiah et al., 1996).

SUMMARY

Polyclonal antibody production will remain an essential research activity and rabbits will continue to serve as one of the primary species used in polyclonal antibody production. The schedules and methodologies used to immunize rabbits and produce polyclonal antibodies will continue to vary dependent upon the

immunogen, the adjuvant, and the end purpose of the antibody. The choice of adjuvant and immunization schedules are critical components of the polyclonal antibody production process that are frequently overlooked as researchers resort to standard published methodologies (Cooper and Paterson, 2008, 2009; Harlow, 1988) that may or may not be applicable to their needs. While FCA remains overall the most effective adjuvant for polyclonal antibody production, the extensive histologic lesions it produces will continue to stimulate the development of alternative adjuvants producing less tissue destruction and potential pain and distress.

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Toxicity and Safety Testing

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INTRODUCTION

This chapter discusses the use of domestic rabbits in toxicity and safety testing of substances such as drugs, chemicals, and medical devices that can impact human safety. While non-clinical testing also benefits animals as with development of veterinary drugs, the examples and procedures described in this chapter represent those commonly used in research and regulated non-clinical safety testing settings. Toxicity and safety studies should always be conducted in compliance with

applicable regulations, laws, policies, and guidelines as well as the requirements specified by the facility's Institutional Animal Care and Use Committee (IACUC).

Domestic rabbits, *Oryctolagus cuniculus*, belonging to the order *Lagomorpha* and the family *Leporidae*, are easily housed and handled. Rabbits are small relative to other species (minipigs, dogs, and monkeys) evaluated in toxicity and safety studies, but large enough to easily conduct ocular and dermal irritation and embryo-fetal evaluations. Breeds frequently used in non-clinical studies include New Zealand White (NZW) and Japanese

albino rabbits (3–5 kg or 6.6–11 lbs) and the smaller, pigmented Dutch Belted (0.9–1.8 kg; 2–4 lbs). Dutch Belted rabbits provide an advantage with respect to the amount of test material required (approximately 40% less compared with albino rabbits). They also provide the ability to test for potential effects of drugs and chemicals on melanocyte development and for potential sequela of drugs that have a propensity to bind melanin in pigmented retinal epithelium and skin (Spence, 2003). Himalayan rabbits have been used in developmental toxicity studies, primarily in Europe, and several decades of information exists demonstrating consistent inter- and intra-laboratory reproductive data with no relevant differences compared with reproductive data in other rabbit breeds (Viertel and Trieb, 2002). Other breeds of rabbit are sometimes used such as the Flemish Giant, Polish, and Red Burgundy to name a few (Gratwohl et al., 1986; Salamon et al., 2007).

Housing and food requirements for rabbits are well-known (Salamon et al., 2007). Several characteristics should be considered when designing toxicity and safety studies and establishing criteria for appropriate supportive care and endpoints. Gastrointestinal flora of rabbits are easily disrupted, leading to diarrhea and poor health, and common laboratory procedures include step-wise acclimation to a specified (e.g., 120 grams) once-daily limited feeding regimen. Coprophagia (ingestion of feces) is normal behavior in rabbits. Ingestion of soft fecal pellets excreted in the early morning is essential because bacterial syntheses of certain B vitamins in the cecum are excreted at this time. Decreases in activity and food consumption are important indicators of intolerance and toxicity. In contrast, decreased food consumption and increased activity, including pulling hair from their ruff to make a nest, are normal changes observed in pregnant rabbits prior to delivery. Normal rabbit urine can resemble purulent discharge; it is often cloudy, even paste-like, ranging from light yellow to deep orange or red brown in color because of the diet. Modifications to housing conditions and habituation to humans have been shown to result in normal circadian body temperature profiles and less variability in the behavioral response of rabbits to experimental procedures such as handling for dosing and blood collection (Verwer et al., 2009).

Test materials can be administered to rabbits by a variety of routes including dermal, ocular, oral, inhalation, intranasal, intramuscular (IM), intraperitoneal, intravenous (IV, including continuous IV infusion), subcutaneous (SC), sublingual, and vaginal. Best practice techniques for administration and dose volume limits as well as vehicle considerations have been described in several publications (Dali et al., 2006; Diehl et al., 2001; Gad, 1994; Morton et al., 2001; Salamon et al., 2007). Blood is relatively easy to obtain from rabbits, and procedures for collection from various sampling sites,

limits for blood sampling volumes, and recovery periods between sampling have been described (Diehl et al., 2001; Salamon et al., 2007). Techniques have been established for collection of urine (Salamon et al., 2007) and semen (Christian, 2008; Foote and Carney, 2000). The larger spleen of rabbits, compared with rodents, has expanded the capacity for screening highly specific monoclonal antibodies. Rabbits recognize more antigens and epitopes than mice or rats making rabbits a good choice for production of monoclonal antibodies against site-specific histone modifications (Guo et al., 2006).

Rabbit anatomy and physiology, including reproductive physiology, have been characterized (Christian, 2008; Foote and Carney, 2000; Salamon et al., 2007). Rabbits are induced ovulators requiring stimulation of the cervix which occurs when a buck copulates with the female. Artificial insemination, less frequently used than natural mating, requires ear vein injection of human chorionic gonadotropin (hCG) to compensate for reduced natural cervical stimulation. Rabbits have a short gestation period and are sensitive to teratogens. In some cases, rabbits respond to test agents in a manner closely approximating those of humans as with thalidomide, which causes similar birth defects in rabbits and primates (including humans), but not in other commonly used laboratory species such as rats (Neubert and Neubert, 1997; Palmer, 1968). Much is known about rabbit placenta and embryo-fetal and postnatal development and comparisons have been made with other species, including humans (Beaudoin et al., 2003; Christian, 2008; Leiser and Kaufmann, 1994; Pentšuk and van der Laan, 2009).

TOXICITY TESTING

Exposure to drugs and chemicals can result in toxicity manifest in specific organs, known as target organs of toxicity. Factors contributing to the susceptibility of an organ to toxicity include pharmacokinetics, metabolism of the drug or chemical, and the ability of the organ to respond to toxic insult (Pfaller and Gstraunthaler, 1998). Typically, mechanistic toxicity testing is conducted retrospectively to understand mechanisms of action and relevance to human safety. These studies provide information applicable to early diagnosis or prevention of target toxicity in humans and may contribute to development of metabolites or analogs of the drug or chemical with the same or better efficacy but with reduced side-effects.

Hepatotoxicity

Tuberculosis (TB) remains an unmet medical need due, in part, to complicating factors such as HIV

co-infection, drug resistance, and variable efficacy of the bacille Calmette-Guerin vaccine (McMurray, 2001). Rabbits, one of the animal models for TB are also animal models to investigate liver toxicity produced by drugs used in the chemoprophylaxis and treatment of TB in humans. The widely used TB treatment isoniazid (INH), the hydrazide of isonicotinic acid, causes hepatotoxicity ranging from elevated liver enzymes in up to 20% of individuals to liver damage (predominantly hepatic necrosis) in 1–2% of individuals (Sarich et al., 1999). Subcutaneous injections of INH in NZW rabbits produces hepatotoxicity similar to that observed in humans, characterized by hepatic necrosis, hepatic steatosis (hepatic fat accumulation), hypertriglyceridemia (elevated plasma triglycerides), and elevated liver enzyme activity (Sarich et al., 1995, 1996, 1998, 1999). Initial studies in rabbits implicated acetylhydrazine and hydrazine, INH metabolites, as the causative hepatotoxins. Further investigation demonstrated hydrazine, and not INH or acetylhydrazine, is most likely involved in the pathogenic mechanism of INH-induced hepatic necrosis. Treatment of TB with the antibiotic rifampicin, an inducer of mixed-function oxidase, also causes hepatotoxicity. The incidence of hepatotoxicity is higher with INH and rifampicin in combination compared with INH alone (Kalra et al., 2007). In albino rabbits, rifampicin potentiates INH-induced hepatotoxicity, and high doses of cimetidine, an inhibitor of the cytochrome (CYP) 450 enzyme system, ameliorated elevations in liver enzyme activity and histopathologic changes (Kalra et al., 2007). Co-administration of silymarin, a polyphenolic flavonoid derived from milk thistle, antagonized INH-induced hepatotoxicity in rabbits suggesting a role for pre-treatment with an anti-oxidative agent in the process of hepatoprotection (Maryam et al., 2010).

As reviewed by Ulrich et al. (2001), idiosyncratic toxicities, defined as those not predicted by dose, duration of exposure, or mechanism of action, are difficult to predict due to their low incidence and differences in species sensitivity. Panadiplon, an experimental non-benzodiazepine anxiolytic, was withdrawn from development due to hepatic toxicity indicated by elevations of serum transaminases in a few Phase 1 clinical trial volunteers not predicted in non-clinical toxicity studies in rats, dogs, or monkeys. Development of a second-generation compound required a suitable animal model to understand the mechanism of panadiplon-induced hepatic toxicity. The Dutch Belted rabbit was selected based on hepatic changes of lipid accumulation observed in a developmental toxicity study (Ulrich et al., 1995). The mechanism of panadiplon-induced hepatic toxicity was investigated by comparing results from in vitro studies in hepatocytes from the sensitive species (rabbits) to those from insensitive species (rats and monkeys) and results from in vivo studies in the

sensitive species (rabbit) to those from an insensitive species (rat). Hepatic toxicity observed in rabbits resembled Reye's syndrome-like changes produced by a variety of mitochondrial fatty acid oxidation inhibitors. However, not all rabbits showed evidence of hepatic toxicity, suggesting other factors such as individual differences in metabolism or nutritional status were necessary to produce the complete toxicological profile including cell death and subsequent liver pathology.

Immunotoxicity

A variety of medical (e.g., latex gloves, condoms, and catheters) and everyday items (e.g., balloons, tires) derived from natural rubber can induce immediate type I or delayed type IV, IgE-mediated hypersensitivity allergic reactions of local contact urticaria, asthma-like symptoms, and systemic anaphylactic reactions (Gad and McCord, 2008a; Rodgers et al., 1997). The source and processing of latex protein in the finished product have been identified as two factors contributing to the high frequency of type I allergic reactions to latex gloves. Studies conducted with rabbit anti-latex sera indicate fresh, unprocessed latex sap contains the most complete presentation of all potential allergenic proteins in comparison to raw latex containing ammonia and finished latex products (Rodgers et al., 1997).

Tampons were known to cause mild and transitory vaginal infection but not severe medical issues until 1978 when toxic shock syndrome (TSS), an acute-onset multi-organ illness, was described (Gad and McCord, 2008d). This occurred a few years after a super-absorbent tampon was test-marketed and subsequently withdrawn from the market. Causes of menstrual TSS are the *Staphylococcus aureus* superantigen toxic shock syndrome toxin-1 (TSST-1) and staphylococcal enterotoxin B or C (Brosnahan et al., 2008). In general, superantigen-secreting *S. aureus* localized at the vaginal surface must penetrate the vaginal mucosa and interact with underlying immune cells to cause TSS. As reviewed by de Azavedo (1989), establishing a suitable animal model to study TSS was difficult and had to take into account: (i) the ability to induce infection with a suitable strain of *S. aureus*, (ii) the ability of the host to mount a specific antibody response to TSST-1, and (iii) the presence of a foreign body to increase the potential to contract TSS. Only rabbits and baboons have been shown to exhibit TSS- or TSST-1-like symptoms following infection with *S. aureus* (Reeves et al., 1986). The response appears dependent upon the age and strain of rabbit with the NZW strain more susceptible to TSST-1 than the Dutch Belted strain (de Azavedo, 1989). Methods employed to induce TSS-like changes in rabbits, and provide an animal model for studying the

pathogenesis of TSS, include: (i) direct administration of TSST-1 via SC infusion over 7 days (Parsonnet et al., 1987), (ii) implantation of micropore diffusion chambers into the uterine horns to permit passage of host proteins into and bacterial products out of the chambers (de Azavedo, 1989), (iii) implantation of flattened polyethylene whiffle balls into the dorsolumbar region for 4–8 weeks to allow leukocyte infiltration prior to inoculation with a *S. aureus* strain (Scott et al., 1983), and (iv) a vaginal tampon model to evaluate the interaction of host, bacteria, and tampon composition on production of TSST-1 and TSS (Melish et al., 1989).

Fungal contamination of feed and feedstuffs reduces nutritional value and causes mycotoxicosis associated with acute and chronic toxicity and death in livestock and humans. Mycotoxins have immunosuppressive properties that lead to increased susceptibility to infection, and several mycotoxins including ochratoxin A (OTA) and citrinin (CIT), both found in improperly stored foods, also cause nephrotoxicity in animals (Kumar et al., 2008). An investigation of OTA- and CIT-related immunotoxicity in NZW rabbits, a species susceptible to mycotoxicosis, revealed significantly lower humoral and cellular immune responses in OTA- and OTA plus CIT-treated animals compared with CIT-treated animals. Based on results of skin hypersensitivity tests and lymphocyte proliferation assays indicated the interaction between these two mycotoxins was severe and additive (Kumar et al., 2008).

Nephrotoxicity

Rabbit in vitro and in vivo models are used to investigate nephrotoxicity. In vitro methods allow mechanistic studies of epithelial transport and alterations brought about by metabolism, hormones, drugs, and chemicals (Blais et al., 1993). Isolated perfused kidney, isolated perfused tubules, isolated glomeruli, renal cortical slices, and proximal tubule cell culture models developed in rabbits have been used to study mechanisms of action of a variety of nephrotoxins such as anticancer drugs (e.g., cisplatin and other platinum analogs) and antibiotics (e.g., gentamicin and cephalosporin) (Aleo et al., 1989; Blais et al., 1993; Pfaller and Gstraunthaler, 1998; Phelps et al., 1987; Romero et al., 1992; Rush et al., 1992; Williams et al., 1990b).

The rabbit renal system is sensitive to contrast agents; thus, in vivo rabbit studies can be used to evaluate toxicity of contrast agents being developed for use in radiographic, ultrasound, or computed tomographic imaging procedures (Pettersson et al., 2002).

Rabbits have been used to study nephrotoxicity induced by administration of the antibiotic cephalosporin. Due to differences in renal accumulation, rabbits are more sensitive to the nephrotoxic effects of

cephalosporins compared with mice and rats (Halligan et al., 1995). Single IV injections of one of three cephalosporins to NZW rabbits produced changes in urine (e.g., glycosuria, lactic aciduria, and aminoaciduria) and bilateral necrosis of the proximal convoluted tubules in cephaloridine-treated rabbits (Halligan et al., 1995). No urinalysis or histopathologic changes were observed in rabbits given cefoperazone or cephalothin. Nephrotoxic effects of cephaloridine were confirmed and minimal effects of cefoperazone and cephalothin were identified using ^1H NMR spectroscopy urinalysis. This study demonstrates the importance of the analytical methods used in conjunction with the appropriate animal model.

Nephrotoxicity is a dose-limiting toxicity associated with administration of cyclosporin (CsA), an immunosuppressant agent that inhibits proliferation of T cells. Rabbits are an established animal model of immunosuppressant-induced nephrotoxicity, although initial toxicity studies of chronic CsA-induced toxicity was challenging in this species. As an example, SC administration of CsA for 60 days to NZW and Red Burgundy rabbits produced clinical toxicity (e.g., weight loss, reduced movement, death) and increased serum creatinine but no histologic changes in kidneys (Gratwohl et al., 1986). The outcome was unrelated to the route of administration or the strain. In contrast, IV administration of CsA at doses in the upper level of the human therapeutic range given to NZW rabbits for 30 days produced histopathologic and ultrastructural changes in the proximal and distal tubules, but not glomeruli, consistent with changes observed in renal biopsies of patients with chronic CsA nephrotoxicity (Thliveris et al., 1991, 1994). In addition to the morphological similarities between CsA-induced renal toxicity in rabbits and human, concentrations and profiles of the major CsA metabolite were similar in NZW rabbits and humans (Venkataramanan et al., 1988). In a modification of the animal model, heterotopic cardiac transplanted NZW rabbits given rapamycin IV for 60 days had significantly less renal toxicity than rabbits given CsA under the same conditions (Thliveris et al., 1995). Because CsA also is used for short periods, a model of acute CsA-induced toxicity was developed in rabbits (Prevot et al., 2000). In this model, SC administration of CsA for 5 days induced vasomotor-related acute renal failure characterized by decreased glomerular filtration rate, decreased renal blood flow, and increased renal vascular resistance; effects partially reversed after discontinuation of treatment.

Neurotoxicity

The pathogenesis of Alzheimer disease (AD) is characterized by extracellular deposition of amyloid beta,

formation of neurofibrillary tangles, selective neuronal loss, and cognitive impairment including loss of memory for recent events. Of the animal models developed to study the AD disease process and potential drug therapies, rabbits are considered relevant because the mammalian order (*Lagomorpha*) closely resembles primates and shows consistent production of neurofibrillary pathology and associated cognitive changes (Bharathi et al., 2006). The role of aluminum (Al) in AD is controversial, and not all animal models (e.g., transgenic mice) are sensitive to Al-induced neurotoxicity. In contrast, rabbits are sensitive to Al-induced neurotoxicity, developing AD-like histopathologic and cognitive changes dependent upon age, dose, route of administration, and specific aluminum complex administered (Bharathi et al., 2006; Savory et al., 2006). Behavioral studies in rabbits using a water maze or a classic conditioning paradigm of nictitating membrane extension (Yokel, 1983, 1985, 1987) determined that changes in learning and memory were not observed after SC administration of Al lactate to neonatal and immature rabbits. A biphasic effect in which learning was facilitated by a low dose and learning and memory impaired by a high dose was seen in pregnant rabbits exposed during gestation and learning deficits alone were seen in adult (6–8-month-old) rabbits (Yokel, 1983, 1985, 1987, 1989). Al-maltolate was shown as the more effective aluminum compound in producing AD-like neuropathology, with effects more pronounced in aged rabbits (Bharathi et al., 2006, 2008).

A cholesterol-fed NZW rabbit model for AD was developed to evaluate disease mechanisms and test potential disease-modifying treatments (Woodruff-Pak et al., 2007). In this model, modification of the rabbit diet by addition of 2% cholesterol alone or with trace amount of copper (0.12 mg/L water) for 10 weeks produced neuropathologic changes in the frontal cortex, hippocampus, and cerebellum resembling those in human AD. Eyeblink conditioning, a form of learning impaired in AD, was also impaired in this rabbit model. Galantamine, a drug approved for treatment of mild to moderate AD, given SC (3 mg/kg) for 15 days ameliorated eyeblink conditioning. This rabbit model of AD provides a means to investigate new drug therapies from both a neuropathological and cognitive perspective.

Embryo-fetal Toxicity

Methotrexate (MTX), a structural analog of folic acid, is indicated for treatment of certain types of cancer, rheumatoid arthritis, and other rheumatic conditions, including systemic lupus erythematosus and juvenile arthritis (Lloyd et al., 1999). Through a well-defined mechanism, MTX depletes the pool of tetrahydrofolate

which limits the availability of one-carbon fragments necessary for the synthesis of purines, ultimately disrupting deoxyribonucleic acid (DNA) synthesis causing rapidly dividing cells to arrest and die (Wolfgang, 2007). Thus, MTX toxicity forms the basis of both its therapeutic and teratogenic effects in humans. A rabbit model has been developed to understand the mechanism of teratogenicity and test protective strategies. Embryo-fetal effects are dependent upon the period of maternal administration of MTX. Administration on gestation days (GD) 8 and 9 leads to litter resorption; administration on GD 10, 11, or 12 causes anomalies (e.g., limb and digit, micrognathia, cleft palate, hydrocephalus, microphthalmia); and administration on GD 12–15 causes distal limb dysplasias (Jordan et al., 1977; Lloyd et al., 1999). A model of MTX-induced teratogenicity was established in NZW rabbits where a single IV dose (19.2 mg/kg) of MTX on GD 12 consistently produces malformations of the head, limbs, and trunk in nearly all surviving fetuses (DeSesso and Jordan, 1976). Focusing on changes in limb-bud structures, a series of experiments indicated these anomalies were caused by transient inhibition of cell division, rather than by cell death, and administration of compounds capable of transferring one-carbon units, whether structurally related to folate or not, provided some protection against formation of the anomalies (DeSesso and Goeringer, 1991, 1992; Lloyd et al., 1999).

Acetazolamide, a carbonic anhydrase inhibitor, and hydroxyurea (HU), an antineoplastic drug that reduces DNA synthesis, are mammalian teratogens. The MTX rabbit model was adapted to investigate embryo-fetal limb-bud development subsequent to maternal SC administration of acetazolamide (1500 mg/kg) or HU (650 or 750 mg HU/kg) on GD 12 (DeSesso, 1981; DeSesso and Jordan, 1976). External and histologic evaluation of fetuses obtained 2–32 hours postdose and on GD 29 demonstrated these drugs did not produce limb anomalies by the same mechanism. Acetazolamide produced bilateral retarded ossification or possible aplasia of the first metacarpal of the forelimb and talus of the hindlimb without apparent alterations in limb-bud morphology. For HU, a series of events led to extensive mesenchymal cellular death resulting in increased intercellular space among remaining viable cells in fetal limb buds. Compared to the sequence of changes induced by MTX, it was concluded that HU and MTX both disrupt intercellular contacts and breakdown of intercellular communication by different mechanisms of action: HU through cytolethality and MTX via disruption of intercellular junctions.

Co-administration of the phenolic antioxidant propyl gallate, the structurally different anti-oxidants ethoxyquin and nordihydroguaiaretic acid, and D-mannitol, a scavenger of hydroxy free radicals, all delay onset of

necrosis and ameliorate the teratogenic effects seen in term rabbit fetuses (DeSesso, 1981; DeSesso and Goeringer, 1990a, 1990b; DeSesso et al., 1994). In a series of studies, vehicle, propyl gallate, or D-mannitol were injected into implantation sites followed by injection of the maternal animal with HU and examination of embryos and fetuses at various times. Amelioration of HU-induced embryotoxicity was reflected in delayed onset of necrosis due to interactions between HU and propyl gallate or D-mannitol within the embryo, results consistent with the hypothesis that hydroxyl free radicals are the proximate reactive species in HU-induced early embryonic cell death.

SAFETY TESTING

Non-clinical studies are conducted to provide information relative to human safety when exposed to a variety of substances including drugs, industrial and agricultural chemicals, specific medical devices, residues of veterinary drugs in human food, and food and color additives. Non-clinical safety tests are conducted (i) in accordance with requirements and guidelines developed and adopted by global regulatory agencies specific to type of test or test material with rationale provided for when these strategies are not followed; (ii) in adherence with the International Organization for Standardization (ISO; <http://www.iso.org/iso/home.html>) and/or Good Laboratory Practices (GLP) specific to the region (e.g., Japan GLP 2008, US GLP 2001) to ensure quality and reliability, although screening, pilot, and dose range-finding studies may be conducted in a non-GLP manner, and (iii) in compliance with United States Department of Agriculture (USDA) requirements specified by the IACUC.

Regulatory agencies recognize the need to reduce duplication of studies and unnecessary use of animals in order to meet regional specific marketing requirements. Guidelines issued by the International Conference on Harmonisation (ICH) addressing safety tests for pharmaceutical drugs have been adopted by the United States Food and Drug Administration (FDA), European Union, and Japan (<http://www.ich.org>). The Organisation for Co-operation and Development (OECD) issues methods for assessing the safety of chemical products that have been adopted for use by government, industry, and independent laboratories (<http://www.OECD.org>). ISO has published standards for testing strategies for medical devices (<http://www.iso.org>). The Office of Prevention, Pesticides and Toxic Substances (OPPTS; <http://www.epa.gov/ocsp>) has harmonized testing guidelines in order to minimize variations among testing procedures required by the United States Environmental Protection Agency (EPA) and replace guidelines issued under the

EPA Toxic Substances Control Act (TSCA) and Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Regulatory agencies also recognize the value of refining animal testing strategies. Some of these approaches include (i) testing only one species for potential embryofetal toxicity if there is more than one relevant species and it can be shown the selected species is a relevant model for the human as outlined in the 2005 ICH S5(R2) guideline; (ii) delaying conduct of some in vivo tests until late in development as described in the 2010 ICH S9 Guidance for Nonclinical Evaluation for Anticancer Pharmaceuticals; (iii) using a tiered approach to toxicity testing (Cooper et al., 2006; Hurrt et al., 2003); and (iv) using step-wise testing strategies such as those in the OECD and EPA OPPTS guidelines for ocular and dermal irritation tests.

Efforts have been made to introduce meaningful, validated alternatives to non-clinical safety testing. In some cases, humans can be ethically tested, eliminating the need for animal studies as with comedogenicity testing (Nigam, 2009). Use of computer models such as LabHEART, a computer model of rabbit ventricular myocytes, simulates conditions such as ventricular tachycardia (Puglisis and Bers, 2001). Information from validated structure-activity relationships (SAR) models can be used to predict toxicity (e.g., ocular, dermal, and reproductive) of some test substances, eliminating the need to test in vivo. In vitro models may be used to screen drugs and chemicals, contributing to go/no go development decisions and providing perspective on the relevance of animal data to human safety. Per OECD and EPA OPPTS guidelines, no in vivo testing is required for substances with predictable corrosive properties such as strongly acid or alkaline substances. The European Centre for Validation of Alternative Methods (ECVAM; <http://ecvam.jrc.ec.europa.eu>), created in 1991, and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM; <http://iccvam.niehs.nih.gov>), formally established in 2000, work to support development, validation, and acceptance of testing methods that could reduce, refine, or replace use of laboratory animals. However, as detailed in comprehensive reviews on this topic, the complexity of safety and risk assessment for toxicity, reproduction, sensitization, and toxicokinetics, to list a few areas, present considerable scientific and regulatory hurdles (Debbasch et al., 2005; Genschow et al., 2002, 2004; Lilienblum et al., 2008; McNamee et al., 2009; Spielmann and Liebsch, 2001).

A detailed review of all regulations and guidelines applicable to safety tests discussed in the following sections is beyond the scope of this chapter; several will be discussed to provide examples of types of safety tests conducted in rabbits. The timing of non-clinical safety tests relative to clinical studies should be appropriate to

support exposure of humans to the test material. This topic is discussed in various documents such as the ICH M3(R2) Guidance for Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals.

Cardiovascular

Safety pharmacology studies evaluate potential undesirable pharmacodynamic effects of a drug or chemical on physiological function, including cardiovascular, in relation to exposure at or above the intended therapeutic range. It is important to identify drugs and chemicals that produce drug-induced, or drug-aggravated cardiac arrhythmias or that prolong the QT interval of the electrocardiograph. Prolongation of the QT interval can lead to ventricular tachyarrhythmia, particularly torsade de pointes which can degenerate into ventricular fibrillation, a life-threatening event that can be caused by administration of anti-arrhythmic and non-cardiovascular drugs. The 2005 ICH S7B guidance proposes an integrated risk assessment based on conduct of *in vitro* and *in vivo* non-clinical tests in conjunction with follow-up mechanistic studies, as necessary. Types of tests include those measuring (i) ionic currents in heterologous expression systems for cloned human ion channels or in isolated cardiac cells, (ii) action potential duration (APD) parameters in isolated cardiac preparations or anesthetized animals, (iii) electrocardiograph (ECG) parameters in conscious or anesthetized animals, and (iv) proarrhythmic effects in isolated cardiac preparations or animals. Rabbits are recognized as an appropriate species for many of these testing strategies, and the following paragraphs provide a broad overview of rabbits models used to assess cardiovascular risk.

In Vitro

Action potential (AP) models use rabbit cardiac ventricular myocytes to investigate the ionic basis of electrophysiological properties linked to arrhythmic risk (Romero et al., 2009) and to replicate cardiac dynamics at rapid heart rate, as would be relevant to tachyarrhythmias (Mahajan et al., 2008).

The rabbit Purkinje fiber model is a multicellular preparation comprised of a single cellular population homogeneously expressing the major ionic channels (Roche et al., 2010). As a surrogate assay for the *in vivo* ECG, this *in vitro* model (i) profiles a drug or chemical candidate's effect on ion channels contributing to the genesis of the AP, (ii) predicts the potential for drugs and chemicals to induce QT prolongation, and (iii) is used to refine assessments of proarrhythmic risk (Aubert et al., 2006; Lu et al., 2002; Roche et al., 2010).

The arterially perfused rabbit ventricular wedge preparation, which preserves cell coupling and is characterized by long viability, is used to assess electrophysiological properties of drugs and chemicals and as a model for testing induced proarrhythmias including ventricular fibrillation, ventricular tachycardia, and torsade de pointes (Roche et al., 2010; Wang et al., 2008). Rabbit ventricular wedge preparations are considered better models compared with guinea pig or dog because rabbit hearts are more prone to early afterdepolarizations, a characteristic likely related to the unique fast recovery of the calcium channel from inactivation (Wang et al., 2008).

The isolated whole-heart rabbit model exhibits characteristics of class III (potassium channel blockers) drug-induced ventricular arrhythmia associated with QT prolongation and increased monophasic AP during late repolarization (Johna et al., 1998). This model allows manipulation of ionic and pharmacologic conditions while preserving the functional anatomy of the heart without interference of cardiovascular reflexes. SCREENIT, a rabbit isolated perfused heart model that records parameters such as monophasic AP, is used to identify drugs and chemicals that induce APD prolongation and proarrhythmia (Hondeghem and Hoffmann, 2003; Lawrence et al., 2006). This model is considered useful for screening drugs and chemicals intended for life-threatening diseases where a tolerance for cardiovascular risk is less of a concern and for defining safety margins above which adverse effects may appear in humans and as added value to an integrated QT risk assessment (Lawrence et al., 2006).

In Vivo

Cardiovascular risk may be assessed prior to initiation of clinical studies based on results from an *in vitro* hERG (human ether-a-go-go related gene) study in combination with *in vivo* ECG and blood pressure measurements from conscious dogs or monkeys, typically the non-rodent species tested in repeat-dose toxicity studies. Rabbits can also be used for these *in vivo* assessments because the ion channels controlling ventricular repolarization are similar in rabbits and humans (Bynum et al., 2009) and prolongation of the QT interval leads to torsades de pointes (Farkas et al., 2004), with females more sensitive to drug-induced prolonged QT than males (Lu et al., 2001). Telemetry-instrumented rabbits have been validated for use in safety pharmacology electrophysiology studies (Bynum et al., 2009). An anesthetized rabbit model using three surface ECG leads has been developed to measure QT and JT interval dispersion, proposed markers of heterogeneous ventricular repolarization and electrical instability (Lu et al., 2004). The advantage of this model is that it provides valuable information in a simplified

manner compared with the technically challenging 6 and 12 surface lead approach. Because several factors, including a high heart rate and short cycle length, contribute to variable ECGs and difficulty measuring the QT(U) interval, guidelines have been proposed to support reliable and reproducible measurements from anesthetized rabbits (Farkas et al., 2004).

Development and Reproductive Toxicity Testing

Assessment of the potential for developmental and reproductive toxicity (DART) is critical in evaluating the safety of drugs, industrial and agricultural chemicals, specific medical devices, residues of veterinary drugs in human food, and food and color additives. As reviewed by Barrow (2009), the US FDA published guidelines in 1966 for teratology testing of medicinal products in response to the high incidence of a severe, recognizable pattern of birth defects in children exposed in utero to thalidomide, a drug used by pregnant women in Europe and Canada as a sedative to treat morning sickness. Other countries also issued reproductive toxicity guidance; however, differences in testing requirements and methodology meant separate reproductive toxicity studies were necessary to meet marketing submission requirements in each of the three major regions: US, Europe, and Japan. Discussions on harmonization of regulatory requirements during the 1980s led to formation of the ICH in 1990 followed by issuance of the S5A guideline for Detection of Toxicity to Reproduction for Medicinal Products in 1994. Last revised in 2005, the ICH S5(R2) guideline (Detection of Toxicity to Reproduction for Medicinal Products and Toxicity to Male Fertility) outlines a three-segment strategy covering six stages of reproduction that should be addressed to fully evaluate effects on maternal reproductive function and on viability, growth, morphogenesis, and function of the developing offspring. At the time the ICH S5A guideline was written, rats were acknowledged as appropriate for all three segments with rabbits an accepted second mammalian species in the conduct of embryo-fetal toxicity studies. Although not often selected, rabbits can be evaluated in fertility and pre-postnatal studies (Teo et al., 2004a, 2004b). Rabbits may also be a relevant species for testing biopharmaceuticals (e.g. monoclonal antibodies) because IgG crosses the yolk sac by a neonatal Fc receptor (FcRn) transport mechanism during late organogenesis similar to non-human primates and humans and in contrast to rodents where transfer occurs earlier in pregnancy (Martin et al., 2009; Pentšuk and van der Laan, 2009).

Currently, regulatory guidelines addressing tests for reproductive toxicity have been issued by ICH, FDA, EPA OPPTS, and OECD. The various guidances

are intended as starting points for investigators who are encouraged to design testing strategies in accordance with knowledge of the drug, chemical, device, etc., and state of the art. The discussion in this section refers to the 2005 ICH S6(R2) guideline as the study designs and concepts are similar with those in the other guidance documents. The ICH S6(R2) guideline emphasizes all endpoints of developmental toxicity (e.g., embryo-lethality, reduced weight, functional or behavioral changes), not just malformations, as important outcomes. Sixteen to 20 adult animals/group are an acceptable minimum number using the mating pair, when both sexes are treated, and the litter, not individual conceptuses, as the unit of comparison when employing inferential statistics. Dose levels should be selected to provide no adverse developmental effects at the low- and mid-dose levels, and minimal maternal toxicity at the highest dose, avoiding increases or decreases in body weight compared with concurrent controls, body weight loss, exaggerated pharmacology, and marked lethality of the pre- and postnatal offspring. Administration of 1 g/kg/day is an adequate limit dose. The statistical power of DART tests to discriminate random from treatment-related effects is poor, and the presence or absence of a dose-related trend can be a critical means of determining the probability of a treatment effect. Four groups, a control and three drug-treated groups usually suffice to define the relationship of findings to dose; however, in the presence of a steep dose-response, a fourth dose group may be necessary to avoid excessive dosage intervals. Selected dose levels should provide data that can be used to determine a no observed adverse effect level (NOAEL) for maternal toxicity, reproductive function, and embryo-fetal development. While statistical significance should be used to support interpretation of results, findings may be biologically relevant in the absence of statistical significance. The guideline discusses data presentation and consistent conventions for reporting data have evolved over time.

The convention of data presentation has evolved to presentation of maternal data on discrete days and over intervals such as organogenesis, postdosing, and overall for the study with calculations for evaluation of body weight change, corrected body weight and corrected body weight change. Cesarean-section parameters are used to calculate values such as mean number ($\pm$ standard deviation) of corpora lutea, implantations, live fetuses per pregnant female, mean percent ($\pm$ standard deviation) pre- and postimplantation loss per pregnant female, and mean percent ($\pm$ standard deviation) early and late resorptions, and dead fetuses per litter. Fetal evaluations can be presented by region (e.g., torso, limb, head) and selected findings combined (e.g. cervical vertebral variations).

Generally, positive control groups are not included in DART studies. Interpretation is made by comparison to the concurrent controls, supplemented with the testing facility's historical control data in the same species and strain used as necessary. Efforts to compile historical control data across facilities have provided databases intermittently supported by various organizations.

In vitro alternatives to animal testing for developmental toxicology have been proposed, ranging in complexity from cell and organ cultures to embryo cultures, the latter approach closely representing embryogenesis from cellular proliferation and differentiation to pattern formation. The possibilities for their application as toxicity screens have been investigated in various validation studies, with rat whole embryo culture (WEC) validated by ECVAM as a screening and prioritization tool for pharmaceutical, agricultural, and industrial chemicals (Genschow et al., 2002, 2004; Piersma, 2004). Use of rabbit WEC is discussed below.

Fertility

Assessment of effects on fertility and general reproductive performance encompasses ICH stages A and B: premating to conception (adult male and female reproductive functions, development and maturation of gametes, mating behavior, and fertilization), and conception to implantation (adult female reproductive functions, pre-implantation development, and implantation). This study also is referred to as a Segment I study or a fertility and early embryonic development study.

Reproductive function in male rabbits can be monitored by analysis of ejaculated semen. Evaluation of ejaculated versus epididymal sperm allows for assessment of alterations in accessory sex gland secretions and measurement of drug or chemical levels in seminal fluid (Perreault et al., 2008). Serial semen samples obtained from bucks allows longitudinal assessment of morphologic, biochemical, and fertility parameters (Morton, 1988; Perreault et al., 2008; Williams et al., 1990a). Using ejaculated semen and inducing ovulation in females, male fertility can be assessed by artificial insemination (AI) of females with known numbers of sperm and effects on embryo-fetal development studied in relation to the paternal animal. Intra- and inter-individual variability of sperm number and concentration is reduced by collecting ejaculates at regular intervals from the same male buck (Perreault et al., 2008) and when measured in four ejaculates collected within a short period of time rather than a single ejaculate (Williams et al., 1990a). Use of automated computer-assisted sperm analysis (CASA) allows for high-speed, objective analysis of many characteristics of rabbit sperm (Farrell et al., 1995) such as motility patterns of spermatozoa obtained from testes, vas deferens, and various sections of epididymis (Pérez-Sánchez et al., 1996).

Segment I fertility studies, generally conducted in rodents or non-human primates, can be conducted in rabbits. As an example, a Segment I study of thalidomide has been conducted in NZW rabbits (Teo et al., 2004a). Therapeutic use of thalidomide is increasing yet little is known about the effect of this drug on human reproductive function and early embryonic development. NZW rabbits were selected for the study because this species is more sensitive than rodents to thalidomide-induced developmental effects. Following conduct of a dose range-finding study that included determination of plasma concentrations of thalidomide in male and female rabbits, a pivotal study was conducted in which four groups of female NZW rabbits (25/group) were given up to 100mg/kg of thalidomide via oral gavage for 14 days prior to mating with untreated males and through GD 7, with cesarean sections conducted on presumed GD 29. Following the initial mating with treated females, male NZW rabbits (25/group) were given up to 500mg/kg/day of thalidomide, 15 times the average human exposure, via oral gavage for 14 days and mated with untreated female NZW rabbits that were cesarean-sectioned 29 days after the last attempted mating. Dosing of the males continued during the mating period and until the day before euthanasia, for a minimum of 56 days. In addition to standard evaluations for viability, clinical observations, body weight, food consumption, cesarean section parameters, and gross evaluation of viscera at necropsy, evaluations in treated males included histopathology of reproductive organs and CASA analysis of sperm motility and count. Administration of thalidomide had no effects on mating and fertility in males or females, pregnancy parameters in females. No thalidomide-related external fetal anomalies occurred in treated or untreated female rabbits. Litter averages for corpora lutea, implantations, litter sizes and viable fetuses decreased mainly in the high-dose female group. It was postulated that the resorptions could be due to thalidomide's anti-angiogenic activity on fetal neovascularization or to thalidomide's immunomodulatory properties. Bilateral small testis in the mid- and high-dose male groups and histopathologic changes (e.g., increased incidence of multinucleated cells in the lumen of the seminiferous tubule and loss of round, elongated spermatids) in the high-dose male group were not associated with effects on sperm motility, count, or concentration at Day 56.

Embryo-fetal Development

Assessment of embryo-fetal development encompasses ICH Stages C and D: implantation to closure of the hard palate (adult female reproductive functions, embryonic development, and major organ formation). This study is also referred to as a Segment II, developmental toxicity, or teratogenicity study.

IN VITRO

As previously stated, *in vitro* techniques enable screening of new drug or chemical candidates and provide a means to investigate mechanisms of action. *In vitro* techniques in rabbits include limb bud cell micro-mass and palatal organ culture systems, in which undifferentiated cells transition to differentiated cells in culture, and postimplantation WEC (Foote and Carney, 2000). Advantages to WEC are (i) use of intact embryos of the same species tested *in vivo*, (ii) testing over a defined period (i.e., 48 hours) during embryogenesis, (iii) control of the environment (e.g., hyperthermia), and (iv) exclusion or introduction of variables (e.g., metabolites) into the culture medium (Chapin et al., 2008).

This section focuses on rabbit WEC, and comprehensive reviews have been published by Chapin et al. (2008) and Ellis-Hutchings and Carney (2010). Briefly, methods first developed in chicks during the early 1900s were applied to mammalian embryo cultures, and, by 1966, techniques were available to explant rat embryos on GD 9.5 or 10 and maintain them up to 48 hours in an *in vitro* environment supporting normal growth and development from the presomite to limb bud stages of organogenesis. Reliable methods for explanting and culturing rabbit embryos during the same early gestation timeframe were developed in the late 1990s. Subsequently, refinements by Carney and Tornesi (2003) demonstrated that chorionic tissue could be completely removed and rabbit embryo, amnion, and attached visceral yolk sac cultured as in rodent WEC. This became the standard method for rabbit WEC and enabled direct comparison of rat and rabbit embryo responses under identical culture conditions. Quantitative endpoints for evaluating effects of treatment on development in cultured embryos include (i) viability, assessed by the presence of a heart beat and by visualization of blood cells circulating through the vitelline vessels, (ii) growth, measured by somite count, (iii) crown-rump length, and (iv) biochemical measures such as total protein. A quantitative morphological scoring system was established in rodents in which developmental landmarks were assigned numerical scores reflecting progression of development. This was followed by a morphological scoring system for rabbit postimplantation embryos (Pitt and Carney, 1999a, 1999b) later modified to align with the system for rats (Carney et al., 2007).

Compared to rodents, rabbit extraembryonic membranes more closely resemble humans and differences in maternal-embryonic exchange contribute to understanding mechanisms of action for developmental toxicants. Rat and rabbit WEC often are studied in conjunction to investigate mechanisms that might explain discordant responses between *in vivo* rat and rabbit developmental toxicity studies and provide a framework for determining the relevance of animal data

to human safety (Ellis-Hutchings and Carney, 2010). Ninomiya et al. (1993) used WEC to study the mechanism of trypan blue-induced teratogenesis, hypothesizing a role for the visceral yolk sac. In culture, trypan blue induced neural tube anomalies, suppression of growth in rat embryos, and concentration-dependent accumulation of trypan blue in the yolk sac. In contrast, no effects on cultured rabbit embryos or yolk sac were observed, suggesting that the species-specific response to trypan blue was related to yolk sac, not maternal factors.

Hansen et al. (1999) investigated the hypothesis that thalidomide alters the amount of reducing and oxidizing equivalents in cells (redox status) inducing oxidative stress resulting in teratogenesis. Using rat and rabbit WEC, species resistant and sensitive, respectively, to thalidomide-induced malformations, Hansen et al. evaluated alterations in levels of glutathione (GSH), a tripeptide with a central role in detoxification processes, and cysteine, the rate-limiting precursor amino acid in GSH formation. Rabbit conceptuses displayed lower baseline GSH and cysteine levels and less ability to recover following *in vitro* exposure to thalidomide compared to rat conceptuses. Limb buds appeared normal on gross examination, but *in situ* hybridization demonstrated severe downregulation of limb differentiation factor FGF-10 in rabbit but not rat embryos exposed to equivalent concentrations of thalidomide. Hansen et al. concluded that rat visceral yolk sac more efficiently handled the toxic insult of thalidomide, and the sensitivity of rabbit embryos to thalidomide-induced GSH depletion resulted from lower visceral yolk sac detoxication capacity allowing more toxicant/oxidant to reach the embryos. Additional support for the validity of these WEC findings in the mechanism of thalidomide-induced teratogenicity comes from the growing body of information supporting the role of redox-sensitive processes in developmental programming (Hansen, 2006) and identification of a thalidomide-GSH conjugate in human liver microsomes (Chowdhury et al., 2010; Ellis-Hutchings and Carney, 2010).

IN VIVO

Numerous *in vivo* developmental toxicity studies have been conducted in rabbits, an accepted, commonly tested species for this evaluation based on accumulated knowledge, availability, and practicality. This section discusses considerations for study designs and evaluation of endpoints important in assessing developmental toxicity.

Conduct of a preliminary, or dose range-finding, developmental study is necessary if (i) no or not sufficient data are available from other non-clinical studies to select dose levels for the pivotal study or (ii) preliminary development toxicity data from two

species will be used to support inclusion of women of child-bearing potential using adequate birth control into a clinical trial of short duration. In the absence of prior data in rabbits, it is prudent to first conduct a tolerability study in a few non-pregnant rabbits, using clinical signs and food consumption as indicators of tolerance. An up-down scheme can be used wherein a leader dose is evaluated to ensure tolerance, followed by a higher dose or a lower dose, depending upon initial results. This approach minimizes the numbers of animals tested and precludes testing too high of a dose.

In a preliminary developmental study, seven or eight presumed pregnant animals/group are dosed and evaluated to achieve the recommended minimum six pregnant rabbits/group. Duration of dosing varies across laboratories ranging from GD 6 or 7 through 18, 19, or 20 to encompass the period from implantation to closure of the hard palate. Initiation and completion of implantation has been shown to range from GD 6.5 to 9 in NZW, Japanese White, and Harlan Porcellus Dutch rabbits (Garside et al., 1996; Nishimura, 2001; Sivaraman et al., 2008), and closure of the hard palate is complete by GD 19 with soft palate closure also complete in many fetuses (Sivaraman et al., 2008). Dosing may extend to GD 28 to ensure inclusion of organogenesis of genitalia (Wise et al., 2009). Dosing over the entire gestational period, GD 0 through 29, is acceptable under the EPA OPPTS 870.3700 guideline (1998). Evaluations are the same as for a pivotal study with the exception that maternal body weight and food consumption may be obtained daily, fetal skeletal examinations are not conducted, and developmental toxicity is assessed based on fetal body weight, numbers of resorptions, and fetal external and visceral examinations. Fetal morphology may not be evaluated in groups where unexpected, severe maternal toxicity occurred in the study, particularly if this leads to death, early euthanasia, or maternal body weight loss lasting more than a couple of days. In this scenario, embryo-fetal findings may be confounded by excessive maternal toxicity, and the dose level that produced the severe toxicity would not be tested in the definitive study.

For the pivotal study, dose levels are selected to evaluate a dose-response. The numbers of presumed pregnant animals assigned to each group should ensure evaluation of 16–20 litters/group. Average litter sizes range from 6.5–8.5 (Wise et al., 2009), although larger litters occur. Studies with one control and three drug-treated groups are typical, with initiation of dosing staggered across groups to control the number of cesarean sections and same-day fetal evaluations. Dosing and study termination are as described above. All animals are observed for mortality and clinical signs. Maternal body weights should be obtained on GD 0 (day of observed mating or artificial insemination) and

every 2 or 3 days to monitor health and adjust the dose. A final body weight at the time of the cesarean section is necessary to calculate adjusted (corrected) maternal body weight (maternal body weight minus gravid uterine weight). Food consumption generally is recorded weekly (four times) by measuring food consumed over a 24-hour period or multiple days and then calculating grams consumed per day for each pregnant female. Optional parameters are maternal clinical pathology, toxicokinetics, and organ weights and embryo-fetal toxicokinetics. Some laboratories add a subgroup of animals to obtain blood samples; however, techniques such as dried blood spot on paper, which requires only 20 µl blood, may eliminate use of additional animals in the future (Wise et al., 2009).

The focus of the cesarean section is to conduct a gross examination of the maternal thoracic and abdominal organs and initiate embryo-fetal evaluations. The gravid uterus is removed, weighed, and opened. The number of corpora lutea, early and late resorptions, live and dead fetuses, fetal sex and weights, and placental observations are recorded. Fetuses are individually identified so all findings ascertained in each fetus by different techniques (e.g., body weight, external, visceral, and skeletal examinations) can be correlated to detect patterns of anomalies. Methodology for fetal evaluations has been reviewed (Christian, 2008; Wise et al., 2009). Each live fetus is maintained in a warm environment during the time, weight, sex, and external observations are recorded, followed by humane euthanasia. Visceral examinations are conducted in each fetus on the day of cesarean sectioning using a free-hand technique, with special attention to the head and heart to ensure evaluation of the brain, hard palate, and cardiac ventricular septum. Skeletal examinations require decalcification and application of stain, alizarin red for bone and, in the case of double staining, alcian blue for cartilage. An alternative to staining, X-ray micro-computed tomography (micro-CT), has been shown to produce high-resolution, 3D images of skeletal structures (Guldberg et al., 2004; Winkelmann and Wise, 2009; Wise and Winkelmann, 2009; Wise et al., 2010). Some attributes of micro-CT are reduction in time and hazardous wastes associated with staining. Regardless of the technique, evaluations of low- and mid-dose group fetuses may not be necessary if there are no relevant differences between the control and high-dose groups.

Efforts to harmonize terminology for fetal anomalies have been underway since the 1980s. The second revision (update to the 1997 version) of Internationally Harmonized Terminology of Developmental Abnormalities (Makris et al., 2009) addressing findings in mice, rats, and rabbits is available on-line at <http://www.devtox.org>. The document's focus is to provide flexibility in application of terminology balanced with

consistency in the approach to describe, rather than interpret or diagnose, fetal observations. The lists of fetal observations are organized as external, visceral, and skeletal abnormalities with the finding designated as a structural or ossification term. Categorization of observations as malformations, variations, or incomplete or delayed skeletal ossification is left to the discretion of each testing facility. Typically, malformations are a permanent structural change likely to adversely affect survival or health of the animal and variations are changes unlikely to adversely affect survival or health of the animal (Wise et al., 2009). Incomplete ossification is a partial ossification, typically assessed by reduced alizarin red uptake at a given site compared with concurrent controls at the same site, and does not imply any change to the bone precursor. Makris et al. (2009) also discussed use of descriptive (e.g., long/large, short/small) terms, alternative terms, combined terms and syndromes, and maternal–fetal anomalies frequently observed for amniotic fluid, umbilical cord, and placenta.

Pre- and Postnatal Development

Assessment of effects on pre- and postnatal development examines fetal development, maternal parturition and lactation, and offspring development and maturity. This study, also referred to as a Segment III study, encompasses ICH stages D through F: closure of the hard palate to the end of pregnancy (adult female reproductive functions, fetal development and growth, and organ development and growth), birth to weaning (adult female reproductive functions, neonate adaptation to extrauterine life, and preweaning development and growth), and weaning to sexual maturity (postweaning development and growth, adaption to independent life, and attainment of full sexual function).

Rabbits have not been considered an appropriate species for this type of study based on their tendency for maternal rejection of neonates and lack of an established behavioral testing battery (Bailey et al., 2009). However, the Segment III study can be modified to ensure evaluation of appropriate endpoints in this species as done by Teo et al. (2004b) in a pre-postnatal assessment of thalidomide following a design similar to that outlined in ICH S5(R2) with the exception that behavioral testing was not conducted in offspring of treated does. Briefly, four groups of rabbits (25 time-mated first generation (F_0) rabbits/group plus an additional six rabbits per group for assessment of concentrations of drug in plasma and milk) were given up to 500 mg/kg of thalidomide, 15 times the average human exposure, via oral gavage from GD 19 through lactation day 28. The F_0 rabbits were allowed to litter, and the offspring (F_1 generation) observed through sexual maturity at which time they were mated (12/sex/group) and cesarean sections conducted on GD 29 to allow external evaluation of

the second-generation (F_2) fetuses. Key findings in this study were increased abortion, changes in some natural delivery and litter parameters (e.g., increased numbers of does with stillborn pups, decreased pup survival on postnatal days 1–4), and limb splay in F_1 -generation pups. The ability of the F_1 generation to reproduce was unaffected by maternal (F_0) administration of thalidomide; and no external observations were seen in F_1 - or F_2 -generation offspring.

Vaccines

Vaccines encompass a range of biological products designed for prophylaxis or treatment of infectious disease, chronic viral disease, cancer, allergy, contraception, addiction, obesity, AD, and bioterrorism. Categories of vaccines include live attenuated or whole killed microorganisms that introduce the pathogen to elicit an immune response, protein subunit vaccines containing purified antigens, and DNA vaccines introducing genes coding for antigens into the hosts' cells (Ramachandra, 2009), vaccines deliver or generate antigens that confer protective (active) immunity by stimulating the immune system to produce antibodies or B cells (humoral immunity) or T cells (cell-mediated immunity). Adjuvants may be added to or used in conjunction with vaccine antigens, particularly with subunit vaccines, to augment or potentiate the specific immune response to the antigen.

Vaccines are given to large numbers of healthy people, including women of child-bearing potential, pregnant women, and children, typically as a limited number of doses separated by months or years. Regulatory and safety issues related to vaccine development, safety testing, and consistency in product regulation have been reviewed by Verdier (2002, 2005), Brennan and Dougan (2005), Ramachandra (2009), and Klinman et al. (2010). Numerous regulatory guidelines either address specific categories of vaccines or are general in nature. Guidance documents have been issued in Europe by the World Health Organization (WHO; <http://www.who.int/en>), European Medicines Agency (EMA; <http://www.ema.europa.eu/ema>), specifically the Committee for Medicinal Products for Human Use (CHMP) and its Vaccine Expert Group; and in the US by the FDA Center for Biologics Evaluation and Research (CBER; <http://www.fda.gov/BiologicsBloodVaccines/default.htm>), specifically the Office of Vaccines Research and Review (OVR) organization.

Non-clinical vaccine studies characterize the nature and duration of the immune response by evaluating local reactogenicity (e.g., irritation, granuloma, abscess, necrosis, and regional lymphadenopathy) and systemic changes (e.g., anaphylaxis, pyrogenicity, organ toxicity, and immune-mediated toxicity, teratology, and

carcinogenicity). Criteria for selecting the appropriate test species include (i) sensitivity to the pathogen, (ii) induction of antibodies and development of an immune response similar to that expected in humans, (iii) ability to develop exacerbation reactions, and (iv) ability to assess local and systemic responses. Rabbits are an accepted species for evaluating vaccines, particularly those designed to stimulate antibody responses, because most human vaccines are immunogenic in this species, a full human dose can be easily administered by a variety of routes, and a large body of background data exists (Brennan and Dougan, 2005; FDA, 2006; Klinman et al., 2010; Verdier et al., 2003). Rabbits are suitable for assessing reproductive toxicity of vaccines because, as with humans, most of the antibody is transferred prenatally versus rodents in which most antibody is transferred postnatally in milk (Brennan and Dougan, 2005; Martin et al., 2009; Verdier et al., 2003). Rabbits are also used to demonstrate drug efficacy as with evaluation of different amounts of the antigen of pertussis whole-cell vaccines because the weight and sensitivity of rabbits to endotoxins are comparable with that of infants (Verwer et al., 2009) and as an inhalation model for testing vaccines against *Bacillus anthracis* (Phipps et al., 2004).

Non-clinical safety study designs should be prepared following appropriate guidance, modified on a case-by-case basis, and reviewed by the applicable regulatory group (e.g., CBER) prior to initiation of animal studies. In general, a non-clinical repeat-dose vaccine study should mimic the clinical plan with respect to dose level, dosing regimen, route of administration, and study duration. Where possible, one full human dose (i.e., the highest dose planned for human use) or the most immunogenic dose level, if this differs from the human dose, should be administered, not scaled to body weight, using the same or comparable lot as intended for use in the clinical trial. The number of doses should be one more ($n + 1$) than the clinical dose schedule. Additional groups could include a placebo control; each valence in the case of multivalent vaccines; and, if the vaccine is formulated with adjuvant, a group that receives adjuvant alone. Three to five rabbits are the minimum number tested per group. Study duration includes time between vaccinations to allow a host immune response to develop (e.g., 2 weeks between doses), and effects of short-term and persistent toxicity should be evaluated in separate cohorts 2–3 days and 2–3 weeks after the final inoculation, respectively. Assessments include routine in-life analysis (e.g., clinical observations, body weight, and food consumption), local reactogenicity (e.g., visual inspection of injection sites or mucosa following oral or nasal administration), impairment of limb use after each injection, immunotoxicology via clinical pathology, immunology

assessments after the first vaccination and at scheduled necropsies, and pharmacokinetics. Postmortem evaluations include histopathology of injection sites and a comprehensive gross and microscopic evaluation of immune organs (e.g., thymus, spleen, and lymph nodes) or, for novel adjuvants, a full tissue list. On a case-by-case basis, it may be necessary to evaluate other immunological parameters such as anti-drug antibodies, cytokines, and cytotoxic T cells. For parenteral vaccines, separate studies in rabbits may be required to examine the presence and persistence of the vaccine antigen or adjuvant at the injection site and draining lymph nodes (Brennan and Dougan, 2005).

Developmental toxicity studies are conducted for many vaccines because prophylactic and therapeutic vaccination programs often include women of child-bearing potential and pregnant women leading to concern for immunomodulatory effects on gestation and development of the fetal immune system (Brennan and Dougan, 2005; Martin et al., 2009). Additionally, use of some vaccines has increased as with the US military anthrax vaccination program which has widened the scope of population receiving BioThrax® to include women of child-bearing age and, inadvertently, pregnant women (Franco et al., 2009). As described in the 2006 FDA guidance for Considerations for Developmental Toxicity Studies for Prevention and Therapeutic Vaccines for Infectious Disease Indications, non-clinical vaccine developmental toxicity studies evaluate effects induced or manifested in the embryo-fetal and postnatal periods. The guidance does not address male reproductive toxicity or male and female fertility, and acknowledges there are times when developmental toxicity studies are not required, such as when a vaccine in development is similar to or of the same product class as an investigational or already licensed product or when tests were previously conducted on individual components in a combination vaccines. When developmental toxicity studies are required, one species, including the rabbit, is acceptable. No suitable species for assessment of appropriate endpoints shows an immunogenic response, studies would be conducted in a non-reactive species. The 2006 FDA guidance on developmental toxicity studies of vaccines refers to the ICH S5(R2) guideline as a point of reference with additional, relevant features added (e.g., the vaccine-induced immune response). Information about the onset and duration of antibody response can be obtained from literature and previously conducted single- and repeat-dose studies in non-pregnant animals. If antibody formation is expected to differ in pregnant versus non-pregnant females, a pilot study in a small number of pregnant animals may be necessary to evaluate antibody formation in relation to exposure and placental antibody transfer to the fetus.

As in a non-clinical repeat-dose study, vaccine developmental toxicity studies assesses a single full human dose or the most immunogenic dose level, not scaled to body weight, administered by the intended clinical route using the same or comparable lot as intended for use in the clinical trial. Additional groups are added as necessary (e.g., placebo and/or adjuvant controls). Forty animals are recommended for each group allocating 20 animals each to cesarean-section and littering subgroups. Additional animals may be necessary if the study design includes dosing prior to implantation or if effects on pregnancy are anticipated. The dosing period should provide exposure to the antigen during organogenesis and at later stages of pregnancy (cesarean subgroup) with growth and development observed in offspring until weaning (littering subgroup). A longer (e.g., 6–8-week) postnatal assessment period may be necessary to evaluate potential long-lasting or permanent changes. Daily dosing may result in immune tolerance; the exact number of doses administered depends on the onset and duration of the expected response. Based on the short, 29-day gestational period of rabbits, it may be necessary to administer priming doses to the doe prior to conception to elicit a peak antibody response during the period of organogenesis. Additional doses may be necessary (e.g., around the time of implantation) to maintain high levels of antibody throughout gestation. Evaluations include maternal, cesarean-section, fetal, and postnatal assessments, plus endpoints demonstrating an immune response in the pregnant female and verification of exposure of the fetuses to maternal antibodies. This latter can be accomplished by collecting serum from maternal animals prior to administration of the vaccine and on the day of the cesarean sectioning to assess development of antibodies. Umbilical cord blood samples are recommended to assess placental transfer of maternal antibodies. On a case-by-case basis, it may be necessary to evaluate other immunological parameters such as anti-drug antibodies, cytokines, and cytotoxic T cells.

Rabbits are also used to support release of vaccine and adjuvanted vaccine formulations through tests contributing to characterization of each lot's potency, pyrogenicity, immunogenicity, and real-time stability (FDA 21CFR610). Dose titration studies are used to confirm the immune response is proportional to the antigen delivered at all doses and to determine the optimal vaccine dose conferring 100% protection. Stability tests consider antibody generation and toxin neutralization. In this type of study, rabbits are inoculated with production vaccine or reference vaccine and serum samples evaluated by enzyme-linked immunosorbent assay (ELISA) and compared for their ability to generate protective antibodies and for toxin neutralization. The

number of rabbits required for release testing depends upon the scope of the project.

Biological Reactivity Tests

Biological reactivity tests evaluate responses to elastomers, plastics, and other polymeric material with direct or indirect contact, or by injection of specific extracts prepared from the material under the skin (United States Pharmacopeia (USP) <88>, 2009). Typically, the systemic injection test is conducted in albino mice, and the intracutaneous and implantation tests are conducted in healthy, young albino rabbit. General procedures in studies in rabbits include clipping fur from the testing site avoiding mechanical injury or trauma, removing loose hairs by vacuum, applying, injecting, or implanting material, and scoring observations at prescribed times according to a weighted scale from 0 (none) to 4 (severe) as described by Draize et al. (1944).

Intracutaneous Test

The USP intracutaneous test (USP <88>, 2009) evaluates local responses to extracts of materials following intracutaneous injection into thin-skinned, albino rabbits. Test material (Samples) and Blanks are diluted in a prescribed manner. On the day of study, fur is clipped closely on both sides of the spinal column and each Sample tested in two rabbits with intracutaneous injection of a Blank on the opposite side of the spinal cord. If results will not be affected, more than one extract from a material can be tested in each rabbit. Up to five test sites/rabbit can be tested, dosing 200 µl/site. Animals are observed 24, 48, and 72 hours after injection. The injection sites should not be touched between observation periods except to discriminate between edema and oil residue. Fur can be re-clipped and test sites lightly swabbed with diluted alcohol to facilitate the examination. Each injection site is examined and scored for evidence of tissue reactions including erythema and eschar formation, edema, and necrosis. After the 72-hour observation, all scores for each Sample and Blank are separately totalled and divided by 12 (2 animals × 3 scoring periods × 2 scoring categories) to determine the overall mean score for each Sample versus each corresponding Blank. The test requirements are met if the difference is 1.0 or less. The test is repeated in three additional animals if the average reaction to the Sample is questionably greater than the average reaction to the Blank at any scoring interval.

Implantation Test

Details of various implantation tests used to evaluate local and systemic responses to plastics and other

polymeric materials in direct contact with living tissue are found in the USP (USP <88>, 2009) and ISO 10993, Part 6 (2007), and have been reviewed by Gad and McCord (2008b).

The USP implantation test is conducted in two rabbits with paravertebral muscles sufficiently large enough to allow implantation. The fur is clipped up to 20 hours prior to the test, and the rabbits anesthetized to prevent muscle movement, including twitching. Proper preparation and placement of the implant strips are critical to the study design. In each rabbit, four Sample strips are implanted on one side of the spine 2.5–5 cm from the midline, parallel to the spinal column, and approximately 2.5 cm apart from each other. Two Blanks are implanted in a similar manner in the opposite paravertebral muscle of each animal. A duplicate strip is implanted at another site if excessive bleeding is observed. Animals are euthanized after 120 hours, and the tissue around the implants grossly examined and scored for hemorrhage, necrosis, discoloration, infections, and encapsulation, the width of which is scored on a scale from 0 (none) to 4 (greater than 2 mm). Histopathology, while not required, is generally conducted. Test requirements are met if the difference between average scores for the Sample and Blank sites does not exceed 1.0, or if the difference between Sample and Blank mean scores for more than one of the four implant sites does not exceed 1.0 for any implanted animal.

The 2007 ISO 10993-6 standard applies to materials that are solid and non-biodegradable; degradable and/or resorbable; non-solid, such as porous materials, liquids, pastes, and particulates. This ISO standard may be applied to medical devices intended for topical use in clinical conditions where the surface or lining may have been breached, in order to evaluate local tissue response. Implantation tests evaluate local short-term (up to 12 weeks) or long-term (up to 104 weeks) effects in the selected species; however, rabbits are generally not tested beyond 52 weeks. The ISO standard contains details applicable to each type of implantation test. In general, the test Sample is implanted into the tissues most relevant to the intended clinical use of the material (e.g., dorsal subcutaneous tissue, paravertebral muscle, or bone). The test period and interim necropsies are selected to evaluate the steady-state biological response in the selected implantation site and based on the nature of the test material (e.g., degradable/resorbable or non-resorbable solid). The biological response is determined via macroscopic and histopathological responses as a function of time comparing responses of the test Sample to those obtained at the control sample or sham-operated sites. A description of the degree of degradation, including material characteristics (e.g., free particles, fiber

formation, amorphous gel, and crystallinity) is provided for degradable materials.

Local Tolerance

These short-term studies evaluate effects of a test substance (e.g., drug, chemicals, or medical devices) in the immediate region of tissue contact. In general, the objectives of the tests are to determine whether the test substance causes irritation (i.e., changes shown to be fully reversible during the defined time period) or corrosion (i.e., tissue damage not fully reversible during the defined time period). Comprehensive discussion and details of local tissue tolerance testing, including differences across regulatory agencies, have been reviewed by Salamon et al. (2007), Blazka and Hayes (2008), and Gad and McCord (2008c). While this test can be conducted in rats or guinea pigs, healthy, young adult albino rabbits from a single strain, generally weighing not less than 2 kg, are preferred based on their size, ease of handling, skin permeability, and extensive data base.

The OECD and EPA OPPTS guidelines employ a sequential testing strategy. For example, no testing is required for material (i) with an extremely acid or basic pH, (ii) an acid/alkaline reserve (i.e., buffering capacity) that suggests corrosivity, or (iii) deemed corrosive or a severe irritant based on prior information. When tests are conducted, a set amount of test substance or a known, moderately toxic dose determined based on a review of all available information is evaluated in a step-wise manner. Initially, one animal is tested and further testing discontinued if a corrosive or severely irritating effect is observed. A negative or irritant response is confirmed in up to two animals, tested simultaneously or one at a time based on the results of the initial test. Additional animals may be needed to confirm weak or moderate irritant responses. Control animals usually are not necessary for tissue tolerance testing; opposite or adjacent tissues of each animal serve as the control. The duration of the study is sufficient to evaluate the reversibility or irreversibility of effects. Effects of the test substance are evaluated at specific intervals and scored according to a weighted scale (e.g., 0 (none) to 4 (severe)) based on set criteria. As reviewed by Hackett and McDonald (1996), the concept of testing a specified amount of material and use of a weighted scale was proposed in the 1940s by Freidenwald et al., Carpenter and Smyth, and Draize et al. and modified over time (e.g., Draize, 1959) to a scoring system that accounted for ocular and dermal irritation and production of reversible inflammatory changes or irreversible tissue damage following application of the test material. The Hackett and McDonald scoring system, a modification

of the McDonald-Shadduck system, was developed for recording findings of slit lamp (biomicroscopic) examinations in studies evaluating topical ophthalmic medications because the Draize Test was considered poorly suited for evaluating medications and devices intended for use in the eye (Hackett and McDonald, 1996; Munger, 2002). Regardless of the scoring system used, personnel performing the observations need to be adequately trained in the scoring system to support appropriate interpretation of observations and promote harmonization across testing laboratories. Also, individual scores do not represent absolute standards for irritant properties of a test substance. The scores should be viewed as reference values evaluated in combination with all other observations from the study, such as the nature and severity of lesions and reversibility or lack of reversibility.

Ocular Irritation and Toxicity

Ocular irritation testing is required by regulatory agencies for hazard assessment of test substances (e.g., drugs, chemicals, medical devices, and cosmetics) applied into or around the eye or which may come in contact with the eye from the environment or in the workplace. Results from ocular irritation tests contribute to hazard classification in accordance with various international regulatory schemes (reviewed by Blazka and Hayes, 2008). Non-clinical test guidelines vary with respect to the number of animals tested, the observation times, and whether or not local anesthetic and irrigation are used (Blazka and Hayes, 2008; Gad and McCord, 2008c). For example, ocular testing of cosmetics that follow Draize et al. (1944) use six to nine rabbits observed for 72 hours after application of the test material, with some tests lasting 7–18 days to assess reversibility (Nigam, 2009) whereas OECD and EPA OPPTS test paradigms use three rabbits followed by observations through 72 hours and out to 21 days to assess reversibility.

The OECD 405 guideline (2002) is commonly used to evaluate drugs, chemicals, and medical devices. Testing for pump sprays and aerosols, not detailed in this section, are described in this guideline. Liquids are tested at a dose of 0.1 ml. Solids, pastes, and particulate substances are tested at a volume of 0.1 ml or a weight of not more than 100 mg with test material ground to a fine dust and the volume of the solid measured after gentle compacting. The test substance is placed into the conjunctival sac of one eye of each animal after gently pulling the lower lid away from the eyeball, instilling the test material, holding the lids together gently for 1 second to prevent loss of material, and evaluating the absence or presence of lesions at specific intervals up to 21 days to assess the reversibility or irreversibility of effects. A local anesthetic may be used prior to

instillation of the test substance; the control eye is similarly treated. Irrigation (washing) of the eyes should not be done for at least 24 hours following instillation of the test substance. Exceptions are solids where the eye can be rinsed 1 hour post-instillation if material has not been removed by physiological means and in case of immediate corrosive or irritating effects. If scientifically justified, the influence of washing is evaluated in two rabbits. Eyes are examined 1, 24, 48, and 72 hours after instillation and 7, 14, and/or 21 days in order to determine reversibility or irreversibility. Ocular reactions are scored according to a weighted scale for changes in the cornea (opacity on a scale from 0 to 4), iris (hemorrhage/gross destruction/no reaction to light on a scale from 0 to 2), and conjunctiva (redness on a scale from 0 to 3), and for chemosis (conjunctival swelling on a scale from 0 to 4). Other effects in the eye and adverse systemic effects are described to provide a complete evaluation. Examination of reactions can be facilitated by use of a binocular loupe, hand slit-lamp, or other suitable device. After recording the observations at 24 hours, the eyes may be further examined with the aid of fluorescein, a stain that highlights corneal changes. The test is terminated sooner than 21 days if animals have no lesions, show continuing signs of severe pain or distress without expectation of reversibility, or if reversibility is observed before 21 days.

A 21-day irritancy test in rabbits is conducted to evaluate potential local irritancy and systemic effects of contact lenses and disinfecting and cleansing solutions (reviewed by Gad and McCord, 2008d). Sixteen rabbits are allocated to two groups to evaluate effects of test and control lenses, or lenses soaked in test and control disinfectants. The lenses are inserted into the right eyes up to 8 hours daily for 21 days. Eyes are examined prior to testing, before each daily application and 24 hours after the last application. Responses are graded on a weighted scale for changes in the cornea (opacity), iris (congestion), and conjunctiva (redness, chemosis, discharge).

Although not discussed in detail in this section, rabbits are commonly used to test novel delivery techniques for drugs in development or medications intended for use in the eye (e.g., treatments for glaucoma) because the rabbit eye is large enough to perform accurate ocular injections or delivery by other methods (Short, 2008). Non-pigmented rabbits (e.g., NZW) are acceptable when combined with studies in a pigmented species (dog, primate). Pigmented rabbits such as Dutch Belted or New Zealand Red are recommended, especially by European regulatory agencies, if the drug binds to ocular melanin. Repeat topical dose application studies may be necessary with ocular tolerance studies conducted first in one to three rabbits.

A basic approach is to start with a single application (20–30 µl) with hourly observations and weighted scoring, followed by an increase in concentration or frequency over several days and evaluation by slit-lamp biomicroscopy. Typical parameters in a pivotal study include ophthalmic examinations, including biomicroscopy to examine the anterior segment, indirect ophthalmoscopy to examine the posterior segment, tonometry to measure intraocular pressure measurement, corneal thickness measurement, electroretinograms (ERG) if the drug reaches the retina, and histopathology. Systemic, not ocular, toxicokinetics is determined. Specialized procedures including fluorescein angiography, ocular computed tomography, or vitreal pharmacokinetics may be required.

Dermal Irritation and Toxicity

Dermal irritation tests evaluate local tissue reactions initiated or aggravated by substances in contact with skin. The OECD 404 (2002), OECD Draft 434 (2004), EPA OPPTS 870.1200 (1998), and EPA OPPTS 870.2500 (1998) guidelines apply to drugs and chemicals. Per EPA OPPTS 870.2500, data on primary dermal irritation are required to support the registration of each manufacturing-use product and end-use product. For medical devices, testing is in accordance with the regulations of the Consumer Product Safety Commission (<http://www.cpsc.gov>) or ISO 10933 – 10 (2002) and American Society for Testing and Materials (ASTM; <http://www.astm.org>) standards to evaluate potential dermal irritation to intact and abraded skin (to simulate wound tissue) of rabbits.

Several procedures are common to the guidelines. Fur is removed from the dorsal area or the trunk approximately 24 hours prior to administration of the test substance. Unless specified otherwise by the specific test, rabbits with abraded skin are not used as this alters permeability. The test substance is applied uniformly to a defined area of skin covering as much area as possible with a thin uniform film. Solids may be pulverized if appropriate, and moistened with the smallest amount of water or, where necessary, a suitable vehicle to ensure good contact with the skin. When a vehicle is used, the influence of the vehicle on penetration of skin by the test substance should be taken into account. Test substances are held in contact with the skin with a porous gauze dressing and non-irritating tape throughout the exposure period. As directed by the specific guideline, the test site may be further covered in a suitable manner to retain the gauze dressing. A collar may be used to prevent ingestion of the test substance, but complete immobilization is not recommended. At the end of the exposure period, residual test substance should be removed, using water or an appropriate solvent.

Acute dermal toxicity studies assess short-term, 24-hour exposure to a solid or liquid test substance. The OECD Draft Guideline 434 (2004) proposes a limit test design to provide information on hazardous properties and rank and classify substances in accordance with the Globally Harmonised System. Under this guideline, one or more fixed, moderately toxic doses ranging from 50–2000 mg/kg is tested in one sex, usually females. Higher doses (e.g., 2000–5000 mg/kg) may be evaluated if the results are directly relevant for protecting human or animal health or the environment or if prior information indicates the test substance is not likely toxic. A stepwise testing paradigm includes an initial, sighting test of one dose followed, if necessary, by a main, pivotal study. A lethal dose in the sighting study is not retested in the main study. Based on results after the first dose in the main study, the test may terminate and the appropriate hazard classification category may be assigned or a higher or lower fixed dose tested. In the main study, each dose level is tested in five animals of one sex; doses can include the one tested in the sighting study. Animals are observed immediately after dosing (at least once during the first 30 minutes), periodically during the first 24 hours with special attention given during the first 4 hours, and daily thereafter for a total of 14 days. Administrations are separated by 3 or 4 days to observe delayed toxicity. Observations should characterize the nature, time of onset, and duration of clinical signs. Other endpoints include body weights and a gross necropsy in all animals. Microscopic examination of organs showing evidence of gross pathology in animals surviving 24 or more hours after the initial dosing may also be conducted.

The primary dermal irritation test per EPA OPPTS 870.2500 (1998) and OECD No. 404 (2002) also evaluates the potential for a test substance to produce irritant and corrosive effects, reversible and irreversible inflammatory changes, respectively, on mammalian skin. This test typically is conducted in three or four rabbits in two phases using a decision-tree approach to guide the next step. Initially, up to three test patches are sequentially applied to one rabbit. The first patch is removed after 3 minutes. If no serious skin reaction is observed, a second patch is applied and removed after 1 hour. If the observations indicate exposure can be humanely extended, a third patch is applied and removed after 4 hours. The test terminates immediately if corrosive effects are observed after any of the three sequential exposures. In the absence of a corrosive effect after removal of the last patch, the animal is observed for 14 days unless corrosion develops at an earlier time point. If the test substance is not expected to produce corrosion but may be irritating, a single patch is applied to one animal for 4 hours. If a corrosive effect is not observed in the initial test, the irritant or negative

response is confirmed in up to two additional animals, each with one patch, for an exposure period of 4 hours. If an irritant effect is observed in the initial test, the confirmatory test may be conducted in a sequential manner, or by exposing two additional animals simultaneously. In the exceptional case, in which the initial test is not conducted, two or three animals may be treated with a single patch, which is removed after 4 hours. When two animals are used, no further testing is needed if both exhibit the same response. Otherwise, the third animal is tested. Equivocal responses may need to be evaluated using additional animals. In each test, the material is administered as an undiluted 0.5ml liquid or as a 0.5-g solid or paste applied to a small area (approximately 6cm²) of skin which is covered with a gauze patch. If direct application is not possible (e.g., liquids or some pastes), the test substance is applied to the gauze patch which is then applied to the skin, ensuring good contact and uniform distribution of the substance on the skin. At the end of the exposure period, normally 4 hours, residual test substance is removed, where practicable, using water or an appropriate solvent without altering the existing response or the integrity of the epidermis. All animals are examined 30–60 minutes and 24, 48, and 72 hours after patch removal, and dermal responses for erythema and eschar formation and edema formation are scored according to a weighted scale from none (0) to maximum (4). In addition, local toxic effects, such as defatting of the skin, and systemic effects (e.g., effects on clinical signs and body weight) are described and recorded. Histopathological examination should be considered to clarify equivocal responses. The study duration is sufficient to evaluate fully the reversibility of the effects observed, typically up to 14 days after removal of the patches. The test is terminated sooner if animals show continuing signs of severe pain or distress or if reversibility is seen before 14 days.

Assessment of skin irritation resulting from chemicals released from medical devices is detailed in ISO 10933 – 10 (2002). Single or repeated exposures (up to 21 days) are tested in healthy young adult albino rabbits of either sex. One animal is tested first if irritation is anticipated. As with the primary dermal irritation test, unless a well-defined positive response is observed, testing is repeated in a minimum of two additional animals. If no response is expected, testing is conducted in three animals. Additional rabbits are tested based on an equivocal response in the three rabbits. Unlike the primary dermal irritation test, negative and positive control groups are included to demonstrate sensitivity of the assay, and a maximum of two test materials may be applied together with the control materials if the same vehicle is used. In preparation for testing, fur on the animals' backs is clipped a sufficient distance (approximately 10cm × 15cm) on both sides of the spine for

application and observation of all test sites. Depilatories may be used if the process has been validated at the testing facility. Four patches are applied in pairs (one control and one test substance) on each side of the spine. Solids, powders, or liquids are applied directly to the skin, and application sites covered with a non-occlusive dressing (e.g., absorbent gauze patch). Extracts and extract vehicles are applied to absorbent gauze patches. In all cases, the application sites are wrapped with a semi-occlusive or occlusive bandage. After a minimum of 4 hours, the dressings are removed and the site positions marked with permanent ink. Tests are scored at 1, 24, 48, and 72 hours following removal of patches. Extended observations for reversibility or irreversibility may be necessary for up to 14 days. Dermal responses include erythema and eschar formation and edema formation graded on a weighted scale. The means of the single- and repeat-dose dermal response scores are correlated with a response category to derive a Primary Irritation Index and Cumulative Irritation Index, respectively.

Mucosal Irritation

Rabbits are used to test for potential irritation of mucosal tissues including vaginal, rectal, and penile (ISO 10993-10:2002). No tests are conducted with material shown to be a skin or eye irritant or with an extremely acid or basic pH. When conducted, tests use six rabbits: three controls and three treated. Additional testing may be necessary if the initial test is equivocal. Animals are evaluated for discharge, swelling, and other evidence of infection, irritation, and injury prior to each treatment. The test solution or suspension, typically 1.0ml, is gradually introduced into the specified orifice or onto a 1-cm area of the penis, sheath retracted. For vaginal and rectal tests, the solution or suspension is introduced into the orifice via a soft catheter or blunt-tipped cannula attached to a syringe moistened with the control or a suitable lubricant; an inert bung may be installed immediately after introduction of the test material. Animals should be prevented from licking the test site and confounding the primary irritation by secondary factors.

The period of exposure typically mimics the projected human duration. Test and control materials are introduced at 24-hour intervals for a minimum of five consecutive days in the vaginal and rectal irritation tests, and every hour for 4 hours in the penile irritation test. Tissues and surrounding areas are evaluated for appearance and signs of discharge, erythema, eschar, edema, and irritation 1 hour (penis) or 24 hours (vagina, rectum) after the initial application and immediately prior to each treatment. In the acute penile irritation test, observations also occur 1, 24, and 48 hours after the last application. Twenty-four hours after the last dose,

animals are euthanized and applicable tissues examined macroscopically. Histologic evaluations are conducted and irritant effects graded by a pathologist for evidence of changes in epithelium, leukocyte infiltration, vascular congestion, and edema. The microscopic grades for all animals in the test group are averaged to derive an Irritation Index ranging from none to severe.

Parenteral Irritation

Parenteral routes are those by which material can be injected or infused. Safety concerns for parenterally administered drugs and chemicals and, in some cases, medical devices, include tissue and muscle irritation, pyrogenicity, blood compatibility, and sterility. Per the 2010 ICH M3(R2) guidance, local tolerance can be confirmed by clinical signs and macroscopic and histopathologic evaluations of injection sites in animals tested in the toxicology studies. However, a single-dose local tolerance study in a single species is considered appropriate to support limited human administration by non-therapeutic routes (e.g., a single IV dose to assist in the determination of absolute bioavailability of an oral drug). The formulation delivered for local tolerance need not be identical but should be similar to the clinical formulation.

As reviewed by Gad and McCord (2008c), three tests conducted in male albino rabbits evaluate parenteral irritation or tolerance to medical devices. In the acute IM irritation study, test material and vehicle are injected once into the left and right vastus lateralis muscle, respectively, of nine male rabbits (1.0ml/site). Euthanasia and evaluation of lesions are performed in three rabbits each at 24, 48, and 72 hours post-treatment. The left and right vastus lateralis muscles from each rabbit are excised, and lesions scored for muscle irritation on a numerical scale from 0 to 5 in accordance with Shintani et al. (1967). The average score for all nine rabbits is calculated and an irritancy category determined over a range of none (0) to severe (5).

Acute vascular irritation studies are conducted in six male rabbits per study. Test material and vehicle are injected into the left and right veins, respectively, of the vastus lateralis muscle at 1.0ml when testing via the IM route (four rabbits), the cervicodorsal subcutis at 1.0ml when testing via the SC route (same four rabbits), and the auricular vein at 0.5ml when testing via the IV route (two other rabbits). Gross observations of injection sites are scored 24 and 72 hours post-treatment for all routes. Euthanasia and scoring of lesions occurs 24 and 72 hours post-treatment (two rabbits/time point) for the IM and SC tests and 72 hours post-treatment for the IV test. Reactions are scored over a range of none (0) to severe (5) following IM and SC administration and from none (0) to severe (3) following IV administration.

An intracutaneous irritation test evaluates local responses to extracts of a biomaterial. As in a dermal

irritation test, fur is clipped from the skin of albino rabbits on both sides of the spinal column and loose hair removed; only rabbits without evidence of trauma from the clipping or other causes are used. The test material (Sample) and control (Blank) are diluted in an appropriate vehicle. More than one extract from a given material can be given to each rabbit if it has been determined test results will not be affected. Up to five sites per animal can be injected in a grid pattern injecting the Sample and Blank into the left and right side of the spinal column, respectively, at 200µl per site. Injection sites are observed 24, 48 and 72 hours post-treatment. The skin may be swabbed lightly with diluted alcohol and the fur clipped to facilitate examination of the injection sites. Observations are scored according to weighted scale from none (0) to severe (4) for evidence of erythema and edema, and necrosis and eschar are noted, if present. The test is repeated in three additional animals if the average reaction to the Sample is questionably greater than the average reaction to the Blank at any scoring interval.

Comedogenicity Test

Comedogenicity is the ability of certain drugs or agents, such as anabolic steroids, to produce comedones (acne), dried sebum and keratin plugging sebaceous follicular openings. Comedogenicity testing in rabbit ears is considered more sensitive than human skin because agents mildly comedogenic in rabbit ears were often negative in humans (Puhvel, 1985). The test in rabbits is conducted in two stages. In the first stage, comedones are induced in the inner surface of both ears of three rabbits (e.g., 0.5–1 ml of 1% coal tar, 50% oleic acid, or 50% tetradecane). This generally takes 2 weeks followed by the second stage, which is application of the test substance to one ear daily for 2 weeks; the other ear serves as a control. At the end of the treatment period, full-thickness biopsies of the treated and control ears are examined histologically, and sections are graded for the amount of reduction of intrafollicular keratin (Kligman and Kwong, 1979; Puhvel, 1985).

Pyrogen Test

A pyrogen is a substance causing induction of a febrile response (elevation of body temperature, fever) which can be fatal in humans and animals. The causal substance is usually a bacterial endotoxin, the lipopolysaccharide complex associated with the outer membrane of Gram-negative pathogens including, but not limited to, *Escherichia coli*, *Salmonella*, and *Shigella*. Pyrogenicity is associated with microbiological contamination of parenteral products, including those that have been sterilized, biosynthetically produced materials, vaccines and adjuvants, and medical devices

introduced into the fluid path. Regulatory agencies require evaluation of these products for pyrogenic potential, either by in vitro methods or the in vivo rabbit test, as a means to limit acceptable risk of febrile reaction in humans to administration, by injection, of the specified product.

The in vitro Limulus Amebocyte Lysate (LAL) test is a clotting reaction produced by a small amount of endotoxin in aqueous extracts of circulating amebocytes of the horseshoe crab (*Limulus polyphemus*). Because the LAL test cannot detect pyrogens except endotoxin, the USP (USP <85>, 2009) only recognizes the LAL method for assaying lipopolysaccharides produced by Gram-negative micro-organisms. Other in vitro alternatives to the rabbit pyrogenicity test have been proposed (Hartung et al., 2001), and ECVAM and ICCVAM consider five in vitro methods based on cytokine release from human blood cells to be validated replacements for the evaluation of materials or products where the objective is to identify and evaluate pyrogenicity produced by Gram-negative bacterial endotoxins but, as with the LAL method, not for other pyrogens.

The in vivo pyrogenicity test is conducted in healthy, adult rabbits by evaluating the rise in rectal temperature after IV injection of a test solution (test product reconstituted, if necessary) into the ear vein (European Pharmacopeia (EP), 2009; Gad and McCord 2008c; USP <151>, 2009). The test is designed for products that can be tolerated by rabbits at a dose not to exceed 10 ml/kg injected IV for 4 minutes (EP) or 10 minutes (USP). No rabbit should be used for pyrogen testing more frequently than once every 48 hours or prior to 2 weeks following a rise in temperature of 0.6°C or more or following its having been given a test specimen judged pyrogenic. Up to 1 week ahead of the test, rabbits are conditioned to all study procedures including injection per EP, and except injection per USP. The pyrogen test is conducted in three rabbits, injecting a sterile, warmed solution that may be dissolved or diluted with acceptable liquids (e.g., sodium chloride). Temperatures are recorded at baseline (30 minutes prior to injection) and at 30-minute intervals up to 3 hours post-injection, although EP and USP differ slightly in the exact times. The UPS and EP also differ with respect to criteria for absence of pyrogenicity. The test material is not pyrogenic if no rabbit shows an individual rise in temperature of $\geq 0.5^\circ\text{C}$ above its respective control temperature. If any rabbit's temperature increases $\geq 0.5^\circ\text{C}$, five other rabbits are tested. Requirements for the absence of pyrogenicity are met if not more than three of eight rabbits have an individual rise in temperature of $\geq 0.5^\circ\text{C}$ and if the sum of the maximum rise in temperature of the eight rabbits does not exceed 3.3°C.

Per the EP, the test material passes if the summed response (i.e., difference between the highest

temperature 3 hours post-injection and baseline temperature for the three rabbits) does not exceed 1.15°C, and fails if the summed response exceeds 2.65°C. If the test fails, it is repeated in three additional rabbits and their response plus the responses of the first three rabbits (i.e., summed responses of the six rabbits) determines if the product passes (summed response does not exceed 2.80°C) or fails (summed response exceeds 4.30°C). Up to 12 rabbits may be tested with pass/fail criteria based on whether or not the summed temperatures are less than or greater than 6.60°C.

Other findings in the rabbit resulting from smaller injection amounts can include increased capillary permeability, increased numbers of white blood cells, hemorrhages, and changes in venous pressure. In some cases, the response to the pyrogen is a severe hemorrhagic reaction with localized necrosis, known as the Schwartzman's phenomenon or reaction. This response is demonstrated in rabbits by SC administration of a bacterial pyrogen followed 24 hours later with IV of the same pyrogen and noting if the site of the later injection turns blue at the center and red at the periphery. Pyrogen tolerance develops when the animals are given repeated injections of a pyrogen. A reduced sensitivity to the same and other pyrogens develops and the febrile response diminishes.

In some cases, a blended testing approach is recommended. For example, the US Center for Veterinary Medicine requires testing the first three batches manufactured using both the LAL and rabbit pyrogen tests to determine whether other types of pyrogenic substances are present (PPM 1240.4122, 2000). This approach takes into account the possibility that pyrogenic materials present in the product are not lipopolysaccharides. Testing the first three commercial batches demonstrates if pyrogen contamination other than lipopolysaccharides is present in the final drug product. If rabbit pyrogen testing in the first three commercial lots is negative, the LAL test can be utilized for release testing.

METABOLISM AND TOXICOKINETICS

Metabolism

The term metabolism, often used synonymously with biotransformation, describes the total fate of a drug or chemical. Rabbit metabolism, primarily studied in the latter half of the 1990s, has been summarized by Calabrese (1988) and Salamon et al. (2007).

Percutaneous absorption and distribution studies in rabbits contribute to developing topical formulations for therapeutic use (Lu et al., 1997; Wu et al., 1996). Rabbits approximate humans with respect to absorption of aspirin (Kergueris et al., 1988). The full range of

hepatic CYP450 isoenzyme activity is present in rabbits (Salamon et al., 2007), and rabbit microsomes have been shown to metabolize diverse agents including retinoids, arachidonic acid, prostaglandins, alcohols, imidazole, trichloroethylene, acetone, prazole, and isoniazid (Anderson and Henck, 1994). Rabbits and humans display N-acetyltransferase activity for isoniazid and several other arylamine drugs, including sulfapyridine, hydralazine, and procainamide, and both display genetic polymorphism with respect to the activity of this enzyme (Calabrese, 1988). In a study of arylamine carcinogens, Glowinski et al. (1978) determined that, in general, acetylation activity levels of rabbits were in the range displayed by fast and slow acetylators humans, making the rabbit a viable animal model for this pharmacogenetic phenomenon. Rabbits are similar to humans in hydroxylation of the antidepressant vinoxazine (Thomare et al., 1992). As reviewed by Calabrese (1988), hepatic aryl hydrocarbon hydroxylase activity is similar and hepatic levels of epoxide hydrolase activity is lower in rabbits compared with humans. Activity of β -glucuronidase in rabbits is slightly higher in liver, lower in kidney, and comparable in the gastrointestinal tract compared with humans (Calabrese, 1988). N-dealkylating, UDP-glucuronyltransferase, and glutathione transferase activities are variable depending upon the substrate, and N-hydroxylase activity is mediated predominantly via the polycyclic hydrocarbon-induced CYP450; and sulfate conjugation is low to medium in rabbits (Salamon et al., 2007).

Toxicity is influenced by factors other than metabolism. For example, dose-dependent disposition of prednisolone in humans is due, in part, to nonlinear serum protein binding, and the binding profile in rabbits is more similar to humans compared with rats and dogs (Rocci et al., 1980). Tesserommatis et al. (2003) investigated the binding of clonidine to rabbit liver protein in the presence and absence of the non-steroidal anti-inflammatory drugs (NSAIDs) flurbiprofen, ketoprofen, ibuprofen, and acetylsalicylic acid. The NSAIDs significantly decreased protein binding of clonidine in homogenized and intact liver slices except for ibuprofen which has a different affinity for liver protein compared to the other NSAIDs.

Metabolism in Pregnant Rabbits

During pregnancy, maternal metabolic adjustments provide an increased supply of nutrients to the fetus. Serial sampling from catheterized NZW rabbits before and during pregnancy show glucose concentration decreases and lactate concentration increases during pregnancy with no significant changes in free fatty acids and ketoacid levels (Gilbert et al., 1984a, 1984b) and the term gravid uterus is a site of high glucose consumption (Gilbert et al., 1984b). Late pregnancy is

characterized by hyperinsulinemia and euglycemia, during which time hepatic glucose production is partially suppressed and peripheral glucose utilization impaired relative to the non-pregnant state (Pere et al., 1992). Using NZW rabbits, Pere et al. investigated the influence of pregnancy on hepatic handling of nutrients after food ingestion in pregnant (GD 30) and non-pregnant rabbits implanted with catheters in the right hepatic vein, portal vein, and femoral artery. The magnitude and duration of arterial hyperglycemia and hyperinsulinemia was greater in pregnant versus non-pregnant animals. The increased glucose levels were attributed to higher gut glucose production and insulin resistance. Higher levels of free fatty acids in pregnant rabbits also contributed to the hyperglycemia. The investigators proposed the glucose was diverted to other sites such as uterus.

Metabolism studies in pregnant rabbits contribute to understanding mechanisms of teratogenicity. Retinoids, used as a chemopreventive and to treat dermatological disorders and induce embryotoxicity and teratogenicity in animals and humans, with two geometric isomers of vitamin A acid, all-*trans*-retinoic acid (RA) and 13-*cis*-RA (isotretinoin) displaying similar effects in laboratory animals (Tzimas et al., 1994). Rabbits have been shown more susceptible to the teratogenic action of 13-*cis*-RA than mouse or rat with teratogenicity of all-*trans*-RA comparable in these three species. In a study to investigate these species- and structure-related differences, Tzimas et al. (1994) administered the two isomers to pregnant Swiss hare rabbits from GD 7–12 and evaluated transplacental pharmacokinetics from the maternal plasma samples and embryos collected at various intervals after the final dose. The primary metabolites of all-*trans*-RA and 13-*cis*-RA were identified as all-*trans*- β -glucuronide, and 13-*cis*-4-*oxo*-RA, respectively. Key findings were slower elimination of 13-*cis*-RA and its metabolites from maternal plasma with accumulation of 13-*cis*-RA in maternal plasma, and more efficient placental transfer of all-*trans*-RA and all-*trans*-4-*oxo*-RA to rabbit embryos reaching concentrations similar to that in plasma. Based on these results, the sensitivity of rabbits to the teratogenic effects of 13-*cis*-RA was attributed to prolonged exposure to the drug and its metabolite and not to isomerization to all-*trans*-RA.

Toxicokinetics

Metabolism and Toxicokinetics

Toxicokinetics describes what an animal's body does with a drug or chemical or its metabolites following administration of the parent drug, typically at a high dose relative to the therapeutic or effective dose. The 1994 ICH S3A guideline identifies toxicokinetics as an

integral part of a non-clinical testing program because it supports species selection and the treatment regimen, enhances the toxicity data, and provides comparisons with clinical data, which is important in assessing risk and safety in humans. The guidance also notes the role for toxicokinetics in interpretation of similarities and differences across species, which may result from differences in protein binding, tissue uptake, receptor properties, and metabolic profiles. Both ICH S3A and the ICH S5(R2) guidelines suggests toxicokinetic data be obtained prior to initiating reproductive studies, and ICH S5(R2) notes information on kinetics in pregnant or lactating animals may also be required.

Toxicokinetics from in vitro studies contributes to understanding mechanisms of action and species differences. For example, Carney et al. (2008) used WEC to investigate why high-dose gavage exposure to ethylene glycol (EG) is teratogenic in rats but not rabbits. Maximal levels of unchanged EG in rabbits were comparable to those reported for pregnant rats while maximal levels of the teratogenic metabolite, glycolic acid, in rabbit maternal blood and embryo were 46% and 10% of the respective levels in rats. The toxicokinetic profile in rabbits indicated the lower levels of glycolic acid was due to a slower rate of maternal metabolism of EG to glycolic acid, slow uptake of the metabolite into the yolk sac cavity fluid surrounding the embryo, and little transfer to the embryo via the visceral yolk sac placenta. Based on a comparison of these data to that known for humans, the authors concluded rabbit is a relevant model for human EG exposure based on the negligible role of the visceral yolk sac (which humans lack) in placental transfer and similar rates of EG metabolism and extraembryonic fluid turnover.

Acknowledgments

The authors thank Ms. Mary Ballweg, Librarian, for her valuable advice and assistance in response to our many questions and requests. Thanks also to our veterinarian colleagues who have enriched our scientific approach to conducting toxicity and safety studies.

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Bacterial Diseases

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INTRODUCTION

Bacteria are responsible for an important group of naturally occurring diseases of rabbits. These diseases cause substantial morbidity and mortality, and thus interfere with the use of rabbits for many purposes. Even when subclinical, bacterial infections may render rabbits unsuitable for certain types of research. Bacterial diseases of rabbits most often involve the respiratory and gastrointestinal systems. This review emphasizes spontaneous diseases affecting these systems, including pasteurellosis, enterotoxemia, Tyzzer's disease, and colibacillosis. Experimentally induced bacterial diseases are discussed in detail elsewhere in this text, and will be addressed here only to the extent that they shed light on naturally occurring diseases.

PASTEURERLOSIS

Background

Pasteurella multocida is the most common bacterial pathogen of laboratory rabbits. The term pasteurellosis can be used to refer to several conditions caused by this organism. Most often, *P. multocida* is harbored in the nasal cavity of rabbits, resulting either in rhinitis or an asymptomatic chronic infection. Occasionally, infection extends from the nasal cavity to other parts of the body. This can result in a variety of clinical manifestations, including pneumonia, otitis media, conjunctivitis, abscesses, genital tract infections, and septicemia. The importance of pasteurellosis in rabbits is underscored by the fact that many of the clinical syndromes are refractory to antibiotic therapy, and there is no reported vaccination strategy that can prevent all the manifestations of the disease.

The earliest descriptions of rabbit pasteurellosis are those of Davis (1913, 1917), who described a *Haemophilus*-like bacillus associated with rabbit abscesses, septicemia, and respiratory disease. These descriptions formed a basis for the most definitive work on rabbit pasteurellosis, a series of ten papers by Webster (1924a–e, 1926a–b, 1927), Smith and Webster (1925), and Smith (1927). These are classic studies in which the etiologic agent, the clinical and pathologic forms of the disease, and factors important in pathogenesis were identified. Many papers on pasteurellosis have been published since this work, but none has contributed as much toward a basic understanding of the disease.

The cause of rhinitis, the most common form of pasteurellosis, was not known until the publication of Webster's studies. Earlier reports suggested that most cases were caused by *Bordetella bronchiseptica*, with *P. multocida* (then called *Bacterium leprosepticum*) and

Staphylococcus aureus contributing occasionally (Ferry and Hoskins, 1920). McCartney and Olitsky (1923) tried to reproduce the condition by challenging rabbits with these organisms, but their attempts were unsuccessful and the etiologic agent remained unclear. Eventually, Webster (1924c) studied the nasal flora of rabbits and proposed that *P. multocida* was the causative organism. This was based on culturing *P. multocida* from virtually all rabbits with rhinitis, while recovering *B. bronchiseptica* from only 30–40%. Webster (1924e) then reproduced the disease by intranasal inoculation of *P. multocida* in *Pasteurella*-free rabbits.

Webster also recognized that *P. multocida* was a principal cause of pneumonia, otitis media, abscesses, and septicemia. He isolated the organism from clinical cases of pneumonia and reproduced the disease by intranasal, intratracheal, and intravenous inoculation (Webster 1926a, 1926b). Otitis media was observed by Smith and Webster (1925) during necropsy of 100 rabbits, few of which had clinical signs suggestive of otitis. Many rabbits had rhinitis and otitis media concurrently, thus implicating *P. multocida* as the cause of otitis media. This was confirmed when intranasal inoculation with *P. multocida*, but not *Bordetella bronchiseptica*, resulted in otitis media. Abscesses and septicemia were also recognized by Webster (1924a) as manifestations of pasteurellosis. He proposed that septicemia was a potentially fatal sequel to other forms of pasteurellosis, especially rhinitis and pneumonia.

Smith and Webster (1925) stated a basic principle governing the pathogenesis of pasteurellosis: the disease usually begins in the nasal cavity following a carrier state, then spreads to other parts of the respiratory system by direct extension, or to other parts of the body by the blood (Figure 13.1). Smith and Webster also

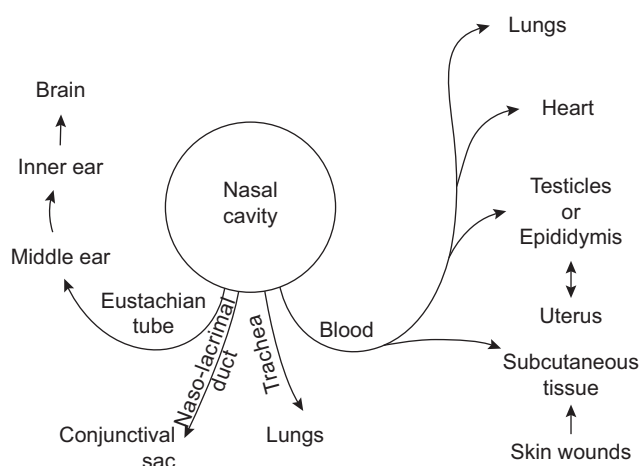


FIGURE 13.1 Possible routes of spread of *Pasteurella multocida* in the rabbit.

realized that development of clinical signs and spread of infection within the rabbit depended on interactions between the host and the organism. They suggested that host resistance was a function of genetic and environmental factors, whereas microbial virulence was strain-dependent.

Etiologic Agent

Pasteurella multocida is a Gram-negative, non-spore-forming, non-motile coccobacillus or short rod that shows bipolar staining. *Pasteurella multocida* is the type species of the genus *Pasteurella*. *P. multocida* includes three subspecies designated *P. multocida* subsp. *multocida*, *P. multocida* subsp. *septica*, and *P. multocida* subsp. *gallicida* (Mutters et al., 1989, 2005). Approximately 80% of rabbit isolates are *P. multocida* subsp. *multocida* (Stahel et al., 2009). *Pasteurella multocida* is a member of the family *Pasteurellaceae* and closely related to the genera *Actinobacillus* and *Haemophilus*. The three genera are heterogeneous, and the exact relationships among the various species are not clear. Many of the species in the family have been reclassified on the basis of genomic relatedness, especially 16S rRNA sequences, rather than phenotypic characteristics.

Pasteurella multocida grows well on blood or dextrose-starch agar but not on MacConkey's agar. After aerobic incubation for 24 h at 37°C, colonies are circular, convex, smooth, and non-hemolytic. Most isolates have large capsules, which imparts a viscous or mucoid appearance to the colonies. Less often, isolates have small or absent capsules, which gives the colonies an iridescent or blue appearance. Encapsulated isolates produce colonies 2–2.5 mm in diameter, whereas non-encapsulated isolates produce colonies about half as large (Brogden, 1980). Rarely, isolates with unusual growth requirements have been reported. In one instance, a strain with an increased carbon dioxide requirement was isolated from a rabbit abscess (Targowski and Targowski, 1979a).

Biochemical characteristics of *P. multocida* have been described by Heddleston (1976) and summarized for rabbit isolates by Manning et al. (1989). Virtually all isolates are positive for catalase, oxidase, indole, hydrogen sulfide, and hexose fermentation and are negative for urease production.

Serological typing has long been used for identification of strains of *P. multocida*. A capsular typing scheme based on hemagglutination was developed by Carter (1955) and later refined by Sawada et al. (1982). Five capsular serotypes have been identified (A, B, D, E, and F). Serotypes A and D are often associated with pasteurellosis in rabbits (DeLong and Manning, 1994; Dziva et al., 2000). Serotype F has also been reported (Jaglic et al., 2004, 2008).

A somatic antigen typing scheme based on a gel diffusion precipitin test identifies 16 somatic antigens designated 1–16 (Heddleston et al., 1972). These antigens are presumed to be lipopolysaccharide (LPS) molecules. These molecules are of low molecular weight with at least two types of antigenic determinants: non-serospecific determinants that may represent partially conserved core LPS components, and serospecific determinants that represent more variable regions (Carey et al., 1984; Manning, 1982, 1984; Manning et al., 1986).

DNA-based methods of typing *P. multocida* provide alternative methods of characterization that overcome the classic phenotypic methods described above. Methods used in characterizing rabbit isolates of *P. multocida* include (i) restriction endonuclease analysis, (ii) ribotyping: restriction endonuclease digestion with subsequent hybridization with a standard probe, (iii) RAPD: random amplification of polymorphic DNA, and (iv) multiplex capsular polymerase chain reaction (PCR) typing (Dziva et al., 2008).

Restriction endonuclease analysis was used to characterize isolates obtained from rabbits in Ohio. The enzyme *Hpa*II was used and 12 distinct DNA profiles were observed (El Tayeb et al., 2004).

Ribotyping involves hybridizing restriction enzyme digested genomic DNA with a 16S or 23S rRNA-based probe. RAPD (random amplification of polymorphic DNA) involves PCR amplified fragments that are resolved by agarose gel electrophoresis; in some cases this method may offer more discriminatory power than ribotyping. The two methods of typing were compared in a study by Chaslus-Dancia et al. (1996). Both methods were used as tools for epidemiologic investigations of patterns of *P. multocida* infection in rabbits in France. Results of the RAPD assays were in general agreement with those of ribotyping.

The multiplex PCR typing method of Townsend et al. (2001) has largely replaced the traditional indirect hemagglutination assays for capsular serotyping.

Single primer PCR fingerprinting with minisatellite and microsatellite primers has also been used to characterize rabbit isolates. This has been described as a technique that can be performed on boiled bacterial extracts as well as genomic DNA and can reliably differentiate among *P. multocida* isolates from rabbits with pasteurellosis (Dabo et al., 1999, 2000).

Epizootiology

Infection with *P. multocida* can occur at a very young age; organisms may be transmitted from infected dams to their offspring during birth or lactation. Perhaps because of maternally acquired antibody, many rabbits remain uncolonized for the first several weeks of

life, but the prevalence of infection increases rapidly with age (Glass and Beasley, 1989). Soon after weaning over 75% of rabbits nursing infected dams are positive by pharyngeal culture (Holmes et al., 1984; Nakagawa et al., 1986). Interestingly, nasal cultures performed at the same age are less likely to be positive (Deeb et al., 1990; DiGiacomo et al., 1983a). This suggests that the pharynx is the earliest site of colonization, with spread to the nasal passages occurring later.

The proportion of rabbits infected with *P. multocida* increases as rabbits mature, and up to 90% of adult rabbits from endemically infected colonies may harbor the organism in the nasal passages (DiGiacomo et al., 1983a; Deeb et al., 1990; Duclos et al., 1986; Garlinghouse et al., 1981; Hagen, 1967; Holmes et al., 1983a, 1983b, 1986; Kawamoto et al., 1990a; Lu et al., 1978; Lukas et al., 1987; Mraz et al., 1980; Nakagawa et al., 1986; Weisbroth and Scher, 1969a). Additional animals may be free of *P. multocida* in the nasal passages, yet still harbor the organism in the pharynx (Holmes et al., 1986). Many infected animals develop clinical pasteurellosis, but the majority remain asymptomatic carriers.

Transmission of *P. multocida* usually occurs by the oral or respiratory route. The organism is passed most readily from animals with acute infections (DiGiacomo et al., 1987a). Direct contact between infected and uninfected animals is the most rapid means of spread, resulting in transmission in as little as 8 days. Aerosol transmission can also occur but it is considerably slower, taking up to 12 weeks when uninfected and infected animals are housed in separate cages in the same room (DiGiacomo et al., 1987a; Lelkes and Corbett, 1983). This slow rate of spread suggests that transmission might be avoided by housing infected and non-infected rabbits in separate rooms, but long-term studies have shown that housing rabbits this way may still result in transmission within 3–6 months (DiGiacomo et al., 1983a; Scharf et al., 1981). It is unclear what mediates spread of infection under these conditions, but fomites could be involved. Rabbits with rhinitis frequently contaminate watering devices with *P. multocida*, and other objects may act as fomites as well, but the length of survival of the organism on inanimate objects is not known (Holmes et al., 1983b; Kawamoto et al., 1990a).

Transmission of *P. multocida* can also occur by way of the reproductive system. Venereal transmission of *P. multocida* was first reported by McKennedy and Shillinger (1938), who observed genital tract infections in does bred to a buck with a testicular abscess. Does frequently carry the organism in the vagina, and thus may act as a source of infection for other rabbits during mating and parturition (Holmes et al., 1983a, 1984).

Studies of *P. multocida* transmission and epizootiology can be facilitated by use of the serologic and DNA-based typing methods described above.

Clinical Manifestations

Background

There are many clinical manifestations of *P. multocida* infection, including rhinitis, pneumonia, otitis media and interna, conjunctivitis, subcutaneous abscesses, genital infections, and septicemia. Many rabbits have several syndromes concurrently. On the other hand, it is common for infected animals to remain carriers for long periods and show no clinical signs. Whether or not overt disease occurs depends on the resistance of the host and the virulence of the organism.

Rhinitis

Rhinitis (“snuffles”) is the most common manifestation of rabbit pasteurellosis. The primary sign of rhinitis is nasal discharge, which may be serous, mucoid, or mucopurulent. The discharge may cause the rabbit to rub the nares with the medial aspect of the forepaw, which results in dampness, matting, and yellowish discoloration of the fur. Occasionally, the external nares have no apparent discharge, and matting is the only evidence that exudate is present. Rabbits with rhinitis may breathe audibly and sneeze or cough, but unless infection has extended beyond the nasal passages there are no other signs of respiratory distress.

Rhinitis is usually a chronic condition with high prevalence in conventionally managed rabbit colonies. The percentage of rabbits affected may be as high as 60%, with the incidence peaking in the spring and fall months (DiGiacomo et al., 1983; Webster, 1924a, 1924c). It is most common for clinical signs to wax and wane while infection persists, but recovery from rhinitis and elimination of *P. multocida* has been claimed to occur (Webster, 1924d). Progression to one of the clinical entities listed below occurs occasionally, with conjunctivitis, abscesses, and otitis media occurring in decreasing frequency (DiGiacomo et al., 1983a).

About 50% of rabbits harboring *P. multocida* in the nares show no clinical signs of rhinitis (DiGiacomo et al., 1983a; Webster, 1924d). In part, this may be a reflection of differences in bacterial virulence. However, the same serotypes are isolated from asymptomatic rabbits and those with rhinitis (DiGiacomo et al., 1983a). Host factors probably also influence the response to infection. Although there has been little experimental investigation of host factors, there is extensive anecdotal evidence that clinical signs can be precipitated by stressful conditions such as shipping, inclement weather, and experimentation. There is also evidence that the incidence of rhinitis is higher in some breeds of rabbits than others (Webster, 1927).

Pneumonia

Pneumonia occurs frequently in rabbit colonies and is sometimes accompanied by pulmonary abscesses,

pleuritis, and pyothorax. Webster (1926) showed that *P. multocida* is the most common etiologic agent. Later investigators supported this finding by isolating *P. multocida* in pure culture from lungs of rabbits with acute pneumonia (Alexander et al., 1952). Flatt and Dungworth (1971b) also concluded that *P. multocida* was responsible for pneumonia. Although they isolated *P. multocida*, *B. bronchiseptica*, and *S. aureus* from pneumonic lungs, they noted that the latter two organisms were also present in normal lungs and were therefore probably not the primary cause of pneumonia. Under experimental conditions, however, *B. bronchiseptica* can induce pneumonia, and it may occasionally act as a copathogen with *P. multocida* (Glavits and Magyar, 1990; Watson et al., 1975).

Although acute cases and epizootics sometimes occur, pneumonia is usually chronic and asymptomatic. In severe cases rabbits are anorectic and depressed, but respiratory distress is seldom observed. Because of the lack of specific clinical signs, the diagnosis of pneumonia is usually made at necropsy. In apparently healthy 8–10-week-old animals, approximately 20% had macroscopic lesions of pneumonia (Flatt and Dungworth, 1971a).

Otitis Media and Interna

Clinical signs are usually absent in rabbits with otitis media. If the inflammatory process extends to the inner ear, torticollis can occur (Figure 13.2) (Fox et al., 1971; Smith and Webster, 1925; Snyder et al., 1973). The prevalence of otitis media at necropsy in conventionally managed rabbit colonies can approach 33%, whereas the number of animals with torticollis is usually under 5% (Smith and Webster, 1925; Snyder et al., 1973). In both clinical and subclinical cases, *P. multocida* is usually isolated in pure culture, but *B. bronchiseptica*, and staphylococci may also be found in 5–10% of cases (Fox et al., 1971).

Sometimes rabbits with torticollis are free of lesions of otitis media/interna, and *P. multocida* is not recovered by culture. In these cases the torticollis may be due to granulomatous encephalitis caused by *Encephalitozoon cuniculi* (Kunstyr and Naumann, 1985). There is no evidence to indicate that ear mites cause otitis media, but affected rabbits may have torticollis due to concurrent infection with *P. multocida*.

Conjunctivitis

Conjunctivitis is a common manifestation of *P. multocida* infection, but it has been rarely mentioned in the literature (Figure 13.3). DiGiacomo et al. (1983) reported that the incidence of conjunctivitis in one rabbitry was second only to rhinitis. While experimental studies are lacking, cultures obtained from rabbits with conjunctivitis usually yield *P. multocida* (Flatt, 1974).



FIGURE 13.2 Pasteurellosis: torticollis secondary to otitis media/interna.



FIGURE 13.3 Pasteurellosis: mucopurulent conjunctivitis.

Signs of conjunctivitis include ocular discharge, chemosis, and conjunctival hyperemia. Extension from the nose via the nasolacrimal duct is the probable route of access to the conjunctiva. Occasionally, the only sign is epiphora; in this instance infection may be restricted to the nasolacrimal duct (Jones and Carrington, 1988).

Abscesses

Webster (1924a) and Griffin (1952) recognized that abscesses are often caused by *P. multocida*. Subcutaneous abscesses are the most common type, and can be recognized by the presence of a firm swelling which may have a soft center. Aspiration of the swelling reveals thick tan-to-creamy-white exudate. Common sites for

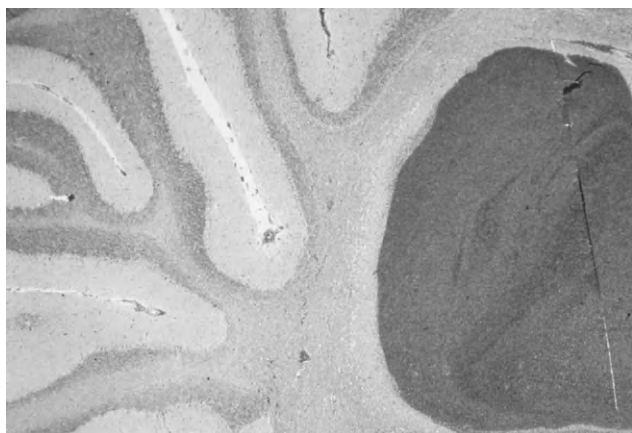


FIGURE 13.4 Pasteurellosis: cerebellar abscess occurring as a result of extension of otitis media.

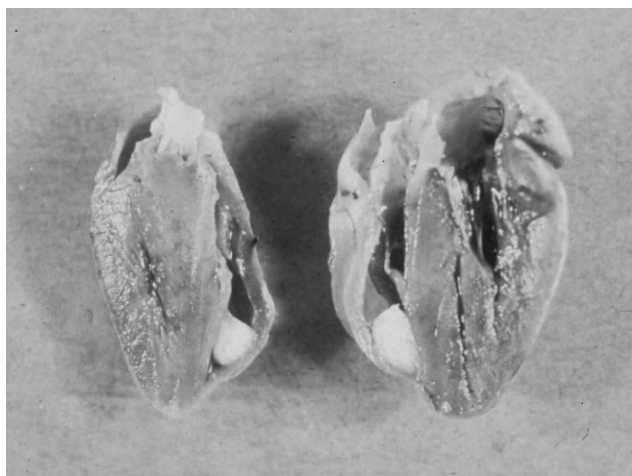


FIGURE 13.5 Pasteurellosis: heart with abscess in right ventricle.

the development of subcutaneous abscesses are the areas around the head, neck, and shoulders. Abscesses may also occur in internal organs, including the lungs, reproductive tract, brain, and heart (Figures 13.4 and 13.5). Specific clinical signs are rarely present and the diagnosis is usually made at necropsy. As in other species, abscesses in rabbits develop by bacteria gaining access to the site by one of several routes: direct extension, hematogenous spread, and wound contamination.

Genital Tract Infections

Infections of the reproductive tract can occur in both bucks and does. Any part of the tract may be affected, but metritis, orchitis, and epididymitis are most common. These infections are usually acquired by venereal transmission, but hematogenous spread from other sites may occur as well (Flatt, 1974). Many genital infections are chronic and asymptomatic, but in breeding animals low conception rates can be seen. Does may have vaginal discharge, especially in acute infections. In

chronic infections, pyometra and massive uterine dilatation are common, sometimes resulting in rupture of the distended uterus (Bjotvedt et al., 1979). Pyometra must be distinguished from uterine adenocarcinoma, which also causes uterine enlargement. Bucks with orchitis or epididymitis may have swelling of the testicles or epididymis in the acute phase of the disease; in later stages abscessation may occur (Flatt, 1974).

Septicemia

Septicemia was recognized by Webster (1924a) as a potentially fatal sequel to other forms of pasteurellosis, especially rhinitis and pneumonia. Clinical signs are not usually observed because death occurs rapidly, but other clinical forms of pasteurellosis may be present concurrently. Diagnosis depends on recovery of *P. multocida* from the blood or parenchymal organs.

Miscellaneous Infections

P. multocida can be associated with infection of any system or organ. Examples of unusual infections include osteomyelitis and meningoencephalomyelitis without coexisting otitis media (Chaffee et al., 1975; Liebenberg and Badger, 1984; Murray et al., 1985). Clinical signs depend on the organ or tissue involved, plus the extent of involvement.

Pathology

Gross and microscopic lesions of pasteurellosis are typical of acute or chronic suppurative inflammation. Grossly, exudate is usually present. The exudate is most often purulent, but may be serous or mucoid depending on the duration of infection. Microscopically, there are large numbers of neutrophils in affected tissues.

Rhinitis is characterized grossly by edema and hyperemia of the upper respiratory mucosa, sometimes accompanied by mucosal erosions. Mucopurulent exudate is present in the nasal cavity and sinuses, and the nasal turbinates may be eroded or atrophic (Figure 13.6). Microscopically, there is vascular congestion, edema, focal mucosal erosion, and an inflammatory cell infiltrate composed primarily of neutrophils. Turbinate bones may be abnormally small and irregular, with increased numbers of osteoblasts and osteoclasts (DiGiacomo et al., 1989, 1991b; Webster, 1924b).

In cases of pneumonia, affected areas of the lungs are dark red and consolidated (Figure 13.7). The cranioventral lobes are most often affected, and there is frequently a sharp line of demarcation from the adjacent normal tissue. Sometimes there are accompanying areas of atelectasis or abscesses (Flatt and Dungworth, 1971a, 1971b; Hagen, 1958). Histologically, the most typical acute lesion is fibrinopurulent necrotizing bronchopneumonia (Figure 13.8). Fibrinopurulent exudate



FIGURE 13.6 Pasteurellosis: purulent rhinitis, nasal cavity, sagittal section.

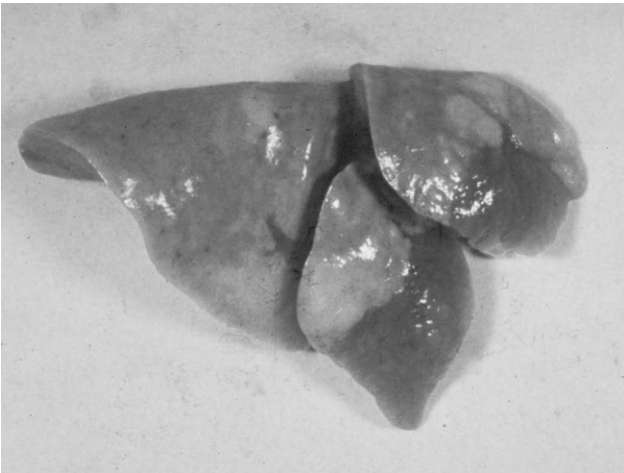


FIGURE 13.7 Pasteurellosis: consolidation of cranioventral lung lobes.

is present in airways and alveoli; neutrophils are the primary inflammatory cell but there are also abundant macrophages and erythrocytes. In chronic cases abscesses surrounded by fibrous tissue are common, as are dense accumulations of lymphoid cells around blood vessels. Airway epithelium is necrotic and necrosis may extend into adjacent lung parenchyma. Frequently, there is accompanying fibrinopurulent pleuritis, pericarditis, and pyothorax (Flatt and Dungworth, 1971a, 1971b).

Early gross lesions of otitis media include reddening of the tympanic membrane and accumulation of

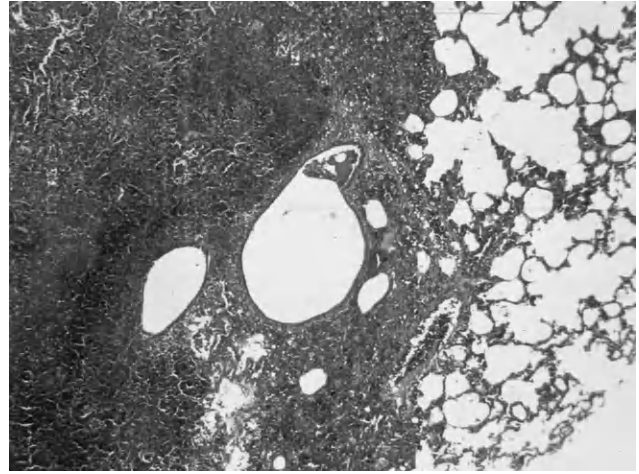


FIGURE 13.8 Pasteurellosis: acute bronchopneumonia.

serosanguineous fluid in the middle ear. Later, the middle ear becomes filled with thick, creamy exudate, which becomes inspissated in chronic cases. Exudate in the middle ear is the most common lesion and occurs in up to 30% of rabbits with no clinical signs of ear disease (Smith and Webster, 1925; Snyder et al., 1973). Histologic lesions of otitis media consist of suppurative inflammation of the tympanic bullae with massive accumulations of neutrophils and necrotic cell debris in the tympanic cavity. In the submucosal tissue of the bulla there is a mixed cell infiltrate of neutrophils and lymphocytes (Snyder et al., 1973). Occasionally, infection of the middle ear extends to the inner ear and brain, resulting in suppurative meningoencephalitis (Figure 13.7).

Other forms of pasteurellosis (except septicemic) are characterized by accumulations of purulent exudate in the affected tissue or organ. The amount and consistency of exudate vary with the severity and duration of the infection. Abscesses may become large and encapsulated; genital tract infections may progress to abscesses of the testicles or pyometra (Figure 13.6). Microscopic lesions are characterized by suppurative inflammation. In organs lined by epithelium there may be neutrophils and cell debris in the lumen, ulceration of the epithelial layer, and infiltration of the underlying tissue by inflammatory cells. In subcutaneous tissue the area of inflammation may be surrounded by a thick fibrous capsule (Flatt, 1974).

In septicemic pasteurellosis lesions are seldom seen, although other forms of the disease are often evident. Grossly and microscopically, there may be congestion of visceral organs and focal serosal or subcutaneous hemorrhages.

Ultrastructural pathology of the respiratory tract of rabbits infected with *P. multocida* serotypes A:3 and D:1 has been reported by Al-Haddawi et al. (1999, 2000,

2000a, 2001). Serotype A:3 attached to cells of the upper respiratory tract and caused damage to ciliated epithelium and goblet cell hyperplasia. In the lungs, thickening of alveolar septa due to hyperplasia of type II pneumocytes was observed. In both locations, coccobacilli were present in membrane-bound vacuoles in the cytoplasm of epithelial and endothelial cells, suggesting that *P. multocida* can invade host cells and cause degenerative ultrastructural changes. Serotype D:1 was associated with similar lesions, but intracellular bacteria were not reported. Another difference was more extensive vascular involvement, with congested subepithelial capillaries, intravascular fibrin deposition, platelet aggregation, and adherence of organisms to endothelial cells. Serotype D:1 proved more pathogenic than serotype A:3. The pronounced vascular lesions were indicative of septicemia and may be due to release of bacterial endotoxin. Dermonecrotic exotoxin, which is often produced by serotype D:1, apparently exacerbated the lesions and facilitated colonization.

Pathogenesis

Dissemination of Infection

Smith and Webster (1925) and Webster (1926a) proposed that pasteurellosis usually begins in the nasal cavity, and then spreads to contiguous tissues by direct extension or to distant sites via the blood (Figure 13.1). They observed that intranasally infected rabbits developed otitis media, and concluded that direct extension through the auditory tube was responsible. They also noted that intravenous, intratesticular, and subcutaneous inoculations of *P. multocida* resulted in pneumonia, and interpreted this as evidence for hematogenous spread.

Studies have confirmed that dissemination within the host is by direct extension and hematogenous spread. When rabbits are inoculated nasally or orally with *P. multocida*, the result is often pneumonia or otitis media (Chengappa et al., 1980; Lu and Pakes, 1981; Lu et al., 1982; Sokkar et al., 1987; Watson et al., 1975). Intravenous or intratracheal challenge also results in pneumonia (Flatt and Dungworth, 1971b; Percy et al., 1986).

Host-parasite Interactions

As in most infectious diseases, the outcome of infection with *P. multocida* depends on interactions between the host and organism. A subclinical state precedes but does not necessarily progress to overt disease. The extent of progression is governed in part by host response, which is in turn related to the genetic background of the host and environmental factors. The most resistant rabbits tend to remain carriers, while less resistant animals are prone to rhinitis or pneumonia.

Progression is also influenced by bacterial virulence. Strains of *P. multocida* vary in their ability to produce disease; some are primarily associated with rhinitis, others with septicemia and pneumonia. Therefore, the ultimate expression of clinical disease is “an index, if perhaps only a rough one, to the resistance of the rabbits and the virulence of the microbe” (Webster, 1924e).

HOST RESPONSE

Both genetic and environmental factors affect host susceptibility. Webster (1927) first reported the influence of genetic factors, noting that the incidence of rhinitis and the mortality rate from pasteurellosis were three times higher for the Chinchilla breed than the Blue Beveren breed. He attributed wide variation of individual response within a breed to “variables inherent in each individual concerned.” Webster and Burn (1927) investigated environmental influences on host response by studying the effects of temperature fluctuation and dietary supplementation. Daily fluctuations between 55 and 90°F caused significant increases in mortality and incidence of rhinitis. Dietary supplementation with fresh vegetables resulted in fewer asymptomatic carriers of *P. multocida*, but there was no reduction in rhinitis or mortality in the supplemented group.

The effects reported by Webster and Burn are examples of how stress can influence the course of pasteurellosis; recent studies confirm and extend these findings. Ammonia in ambient air (50 ppm) or hydrocortisone treatment increase the susceptibility of rabbits to intranasal challenge with *P. multocida* (Lu et al., 1982; Patton et al., 1980). Similarly, the incidence of spontaneous pasteurellosis increases when immunosuppression is induced by intradermal challenge with a malignant variant of Shope fibroma virus (Corbeil et al., 1983).

Humoral immunity is one mechanism of host resistance to pasteurellosis. Infection with *P. multocida* is followed within 2–3 weeks by development of specific IgM and IgG antibodies (DeLong et al., 1992; DiGiacomo et al., 1990). There is evidence that these antibodies can prevent lethality and the development of severe clinical signs. For example, hyperimmune serum from either mice or rabbits protects mice against challenge with homologous organisms (Collins, 1977). Similarly, hyperimmune rabbit serum prepared against extracts of *P. multocida* protects rabbits against intranasal challenge (Lu et al., 1987).

Protection appears to be mediated largely by antibodies against the outer membrane proteins of *P. multocida*. This is suggested by two lines of evidence. First, a monoclonal antibody against the 38.5-kDa outer membrane protein of *P. multocida* protects rabbits against intranasal challenge with homologous organisms (Lu et al., 1991b). Second, affinity-purified rabbit antibodies against outer membrane proteins, but not

antibodies against lipopolysaccharide, inhibit pulmonary proliferation of *P. multocida* in mice (Lu et al., 1991a).

The mechanism by which humoral immunity protects against pasteurellosis is unknown. Immune serum is not directly bactericidal for rabbit isolates in vitro and it is not significantly better than non-immune serum at bacterial opsonization (Hofing et al., 1979; Rush et al., 1981). It is possible that the bactericidal or opsonic activities of specific antibodies are enhanced in vivo, perhaps by interaction with cell-mediated immunity and non-specific immune mechanisms.

The protective effects of antibody appear to be limited to pneumonia and other severe manifestations of pasteurellosis. Neither passively nor actively acquired antibody protect against the carrier state or rhinitis; the organism can persist in the presence of significant levels of serum IgG and secretory IgA (DeLong et al., 1992; Lu et al., 1987). It seems likely that *P. multocida* has developed mechanisms to circumvent the host antibody response, at least at the mucosal level.

The importance of cell-mediated immunity in protection against pasteurellosis is unclear. Rabbits infected with a malignant variant of Shope fibroma virus have depressed T cell activity and are susceptible to severe pasteurellosis, but B cell function is depressed as well (Corbeil et al., 1983). In mice, cell-mediated immunity seems to have a minor role, since immune lymphocytes do not protect non-immune recipients (Collins, 1977). On the other hand, in avian pasteurellosis cell-mediated immunity appears important for protection (Baba, 1984).

Non-specific host responses such as fever and neutrophil response may be important defenses against *P. multocida* infection. A febrile response is correlated with increased survival when rabbits are challenged intravenously with *P. multocida*. When febrile rabbits infected with *P. multocida* are given antipyretics, there is increased mortality, decreased blood leukocyte counts, and greater numbers of bacteria isolated from the spleen and liver (Kluger and Vaughn, 1978; Vaughn et al., 1981). It appears that fever enhances bacterial clearance, perhaps by activation of granulocytes or the monocyte-macrophage system.

Neutrophil function appears to be unaffected by immunization or infection with *P. multocida*. It is therefore unlikely that altered neutrophil function is important in the pathogenesis of pasteurellosis. Granulocytes from immunized, infected, and uninfected rabbits are equally effective in phagocytosing and killing *P. multocida* in vitro (Hofing et al., 1979). Similarly, in infections induced experimentally in subcutaneous chambers, there is no difference in neutrophil response between immunized and non-immunized rabbits (Petkus et al., 1979).

Other non-specific host responses associated with *Pasteurella multocida* include increased expression of

vascular cell adhesion molecule-1 (VCAM-1) by aortic endothelium (Richardson et al., 1997). Interestingly, increased expression can occur even when infection is apparently restricted to a remote site such as the lungs. Another non-specific response is reactive thrombocytosis, which can occur in response to a variety of bacterial infections including pasteurellosis. In a study of experimental *P. multocida* infection, reactive thrombocytosis was a consistent finding but there was no correlation between the magnitude of thrombocytosis and the severity of disease (Ruble et al., 1999b).

BACTERIAL VIRULENCE

The importance of bacterial virulence in pasteurellosis was recognized by Webster (1926b) when he inoculated rabbits intranasally with strains designated as either mucoid or type D. The mucoid strains usually produced an asymptomatic carrier state. In contrast, the D strains tended to be cleared from the nasal passages, although they sometimes caused marked clinical disease. Webster concluded that mucoid strains are better able to colonize the nasal passages than non-mucoid strains; other studies support this finding (DeLong et al., 1992).

The capsular serotypes of rabbit isolates are usually A, D, or non-typeable, and somatic serotypes are usually 3 or 12 (Brogden, 1980; Chengappa et al., 1982; DiGiacomo et al., 1983a, 1991a; Kawamoto et al., 1990b; Lu et al., 1978, 1983; Lukas et al., 1987; Mushin and Schoenbaum, 1980; Percy et al., 1984; Pestana de Castro et al., 1980). Recently, capsular type F has been shown to occur in rabbits. It is unknown whether some isolates previously reported as non-typeable may actually be serotype F. In general, capsular type A and somatic type 12 cause less severe disease than capsular type D and somatic type 3. Serotype F can also be highly pathogenic for rabbits, and is associated with pneumonia and septicemia under conditions of experimental infection (Jaglitz et al., 2008). The predominance of these strains suggests that they produce products such as toxin(s), antiphagocytic substances, or adhesins, which promote infection and enhance virulence (Carter, 1967).

P. multocida virulence factors that have been the subject of research in various species include the bacterial capsule, lipopolysaccharide (also referred to as endotoxin), adhesins, toxins, iron-regulated and iron-acquisition proteins, and sialidases (Harper et al., 2006). These are discussed in detail below.

Capsular composition and structure is governed by one or, at most, two areas on the genome. Capsule is believed to play a significant role in resistance to phagocytosis. In rabbit pasteurellosis, this is suggested by persistence of the organism in infected tissues, despite the presence of large numbers of neutrophils. Studies have shown that some rabbit isolates of *P. multocida*

can in fact resist phagocytosis and killing, but there is no clear correlation between resistance and virulence (Anderson et al., 1984; Rush et al., 1981). This is probably a reflection of the multifactorial nature of virulence and the possible loss of some virulence factors caused by in vitro cultivation.

Lipopolysaccharide (endotoxin) from *P. multocida* can cause fever, central nervous system depression, and shock. In septicemic rabbits, free endotoxin is present in plasma, but it is unclear whether endotoxin plays a significant role in clinical disease, especially in the most common syndromes (Anderson et al., 1984). Lipopolysaccharide stimulates humoral immunity, and antibodies to it convey some level of protection. Since lipopolysaccharide is presumed to be responsible for somatic serotype, the predominance of somatic serotypes 3 and 12 suggests that certain properties of lipopolysaccharide are important in pathogenesis. Attempts to reproduce clinical syndromes of pasteurellosis in birds and ruminants with intravenous injection of lipopolysaccharide from various serotypes have not been consistently successful (Harper et al., 2006).

Another bacterial property that may be important in pasteurellosis is the production of adhesins. Rabbit pasteurellosis usually begins with colonization of the upper respiratory tract, which could be facilitated by production of bacterial adhesins. As might be expected, isolates of *P. multocida* obtained from rabbits adhere better to rabbit cells – especially buccal and pharyngeal cells – than do isolates obtained from other species (Bonilla-Ruz and Garcia-Delgado, 1993). Differences in adhesiveness may explain the predominance of specific serotypes in this disease. Type A strains adhere better to pharyngeal epithelium than types B, D, or E; this observation correlates with a higher rate of isolation of type A organisms from the upper respiratory tract (Glorioso et al., 1982). Type D strains appear to adhere well to tracheal epithelium and aorta endothelium (Al-Haddawi et al., 2000, 2000a). This pattern is in contrast to the pattern seen with type A strains and may explain the ability of type D strains to cause septicemia.

Exotoxin production is uncommon in *P. multocida* with the exception of capsular type D strains. Type D isolates are sometimes highly virulent and capable of causing septicemia or severe pneumonia (Okerman et al., 1979). The virulence of these strains is in part mediated by exotoxin, which is recognized as an important cause of atrophic rhinitis in pigs. In rabbits, purified toxin can induce pneumonia, pleuritis, hepatic necrosis, lymphoid atrophy, and possibly nasal turbinate atrophy (Chrisp and Foged, 1991). Nasal turbinate atrophy in rabbits may not be as closely associated with toxin production as it is in swine; in one study, serotype A:12 isolates appeared to cause turbinate atrophy in rabbits, but evidence of toxin production was equivocal

(DiGiacomo et al., 1993). The toxin produced by type D strains has a molecular weight of 145 kDa and acts by activation of alpha-subunits of heterotrimeric G proteins. G protein activation occurs via deamidation of a specific glutamine residue present on alpha subunits of the proteins (Orth et al., 2009).

Iron acquisition proteins are produced by most bacteria, including *P. multocida*. Analyses of the complete genome sequence of an avian isolate revealed more than 50 genes with a potential role in iron acquisition and metabolism (May et al., 2001). The presence of iron in the environment determines the level of expression of these proteins, so they are referred to as iron-regulated outer membrane proteins. Inactivation of these proteins has been shown to attenuate virulence in mice (Harper et al., 2006). The exact role in virulence is not certain, but may simply relate to the ability of bacteria to survive in the host environment. Bacteria grown in iron-poor conditions express these proteins at a relatively high level and induce a better immune response than bacteria grown in iron-replete conditions. Therefore, strains grown under these conditions may be useful for developing immunization strategies (Confer et al., 2001).

In many species of bacteria, production of virulence factors is regulated by plasmids, but this does not appear to be true for *P. multocida*. Isolates may possess a single small plasmid, but there is no apparent correlation between presence of the plasmid and bacterial virulence (Gunther et al., 1991).

Diagnosis

Isolation

Pasteurellosis may be diagnosed by the observation of clinical signs, coupled with isolation of the organism from infected tissues. If control of pasteurellosis in a colony is desired, it is also necessary to detect subclinically infected animals. This can usually be done by nasal culture. A calcium alginate urethral swab is inserted 1 cm into both nares, then plated onto blood agar with clindamycin (2 µg/ml). Clindamycin inhibits the growth of most rabbit nasopharyngeal flora, but allows growth of *P. multocida*. Identification can then be made on the basis of colony morphology, without additional biochemical tests (Garlinghouse et al., 1981). Samples should also be plated on plain blood agar, to ensure detection of the few strains of *P. multocida* that are inhibited by clindamycin. As an alternative to nasal culture, nasopharyngeal cultures may be obtained by passing the swab 4–5 cm through the nares. Since colonization of the pharynx sometimes occurs before colonization of the nasal cavity, this method may detect infected animals that are negative by nasal culture (Holmes et al., 1987).

If nasal cultures require storage or transport, Cary-Blair medium or Leibovitz medium no. 15 (L-15) have been shown to allow 80–90% recovery after 4 days (Kawamoto et al., 1997).

Cultures of the nares are inexpensive and easy to perform, but may not detect organisms which are few in number or localized to other parts of the body. Studies evaluating the reliability of nasal cultures suggest that approximately 30% of infected animals may not be detected by a single culture (Holmes et al., 1986; Manning et al., 1987). It would be prudent to perform three sequential cultures before accepting an animal as *Pasteurella*-free.

Serodiagnosis and Polymerase Chain Reaction

The limited sensitivity of culture for detection of carriers of *P. multocida* has prompted the development of several serologic tests, including agar gel diffusion precipitin tests (GDPT) (Heddlestone et al., 1972), enzyme-linked immunosorbent assays (ELISA) (Holmes et al., 1986; Klaasen et al., 1985; Lukas et al., 1987; Sanchez et al., 2004; Zaoutis et al., 1991), a capture ELISA assay (Peterson et al., 1997) and ELISA-based immunoblot assays (Manning et al., 1987; Zimmerman et al., 1992). The GDPT technique is more labor intensive than ELISA and slightly less sensitive. ELISA can detect antibody in up to 98% of nasal culture-positive animals, as opposed to 86% for GDPT. Both approaches detect antibody in about 10% of rabbits that are negative by nasal culture, suggesting false-negative culture results for these animals (Kawamoto et al., 1994).

ELISA and immunoblot assays can detect antibody to *P. multocida* in infected rabbits that are nasal culture-negative, but careful selection of bacterial antigen preparations is important for adequate sensitivity and specificity. Boiled cell extracts (Klaasen et al., 1985), potassium thiocyanate extracts (Lukas et al., 1987), polypeptides isolated from whole cell preparations (Zimmerman et al., 1992) and lipopolysaccharide mixtures (Manning et al., 1987) have been reported to provide adequate results. Specificity can be enhanced by using well-defined antigens, such as the 37-kDa protein employed in the capture ELISA described by Zimmerman et al. (1992). However, this approach may result in loss of sensitivity; prevalent serotypes of *P. multocida* such as A:3 and A:12 express this protein, but less common ones may not.

An approach to serodiagnosis that is designed to maximize sensitivity and specificity involves a recombinant antigen ELISA designed to detect antibody to sialidase encoded by the bacterial gene *NanH* (Sanchez et al., 2004). Sialidases are extracellular bacterial enzymes that have glycolytic activity on mucin, thereby providing a carbon source and facilitating mucosal colonization. The host typically produces detectable IgM and

IgG responses, even in the absence of clinical signs. The sialidase produced by *P. multocida* is believed to be ubiquitous in *P. multocida* isolates and lack homology with sialidases from other organisms. Therefore, this technique may minimize problems of sensitivity and specificity that can occur with other serologic diagnostic techniques.

Any serologic assay used in screening for *P. multocida* infection can result in false negatives in the early stages of infection. Therefore, tests several weeks apart are necessary to ensure *Pasteurella*-free status. Seroconversion is usually detectable 2–4 weeks after infection (DiGiacomo et al., 1990; Lukas et al., 1987; Manning et al., 1987).

Another consideration in using serologic screening is that false-positive results can occur when passively acquired antibody is present in juvenile animals. The disappearance of passively acquired antibody to *P. multocida* has not been studied in rabbits, but data from other infections suggest that up to 8 weeks may be required (DiGiacomo and Thouless, 1986). Thus, serologic tests may be unreliable in animals under 2 months of age.

A *P. multocida*-specific polymerase chain reaction (PCR) assay has been described (Kumar et al., 2004). The amplified product is approximately 460 base pairs in size and is present in all confirmed *P. multocida* isolates, regardless of host species. In rabbits as in other species, the assay has been shown to perform satisfactorily with a variety of substrates, including bacterial colonies, bacterial cell lysates, and purified genomic DNA.

Prevention and Therapy

Prevention

Control of pasteurellosis depends mainly on prevention, since therapy is only marginally effective. Several strategies for prevention have been tried, including barrier housing, vaccination, and antimicrobial prophylaxis. Barrier housing is the most reliable strategy.

BARRIER HOUSING

Barrier housing is used to ensure that *Pasteurella*-free rabbits do not become infected. The rabbits should be maintained using barrier facilities and procedures, the specifics of which range from semi-isolation to strict isolation, depending on the needs of the facility and availability of equipment and personnel (Scharf et al., 1981).

Pasteurella-free rabbits are readily available commercially, but other methods can also be used to obtain a *Pasteurella*-free colony. The simplest approach is to cull infected animals. All rabbits with clinical signs of pasteurellosis or positive nasal cultures should be removed (Webster and Burn, 1927). The prospect of success is improved if rabbits that are either related to infected animals or housed nearby are also culled (Griffin, 1952).

Since some rabbits harboring *P. multocida* may be consistently negative by nasal culture (Holmes et al., 1986), culling may be more effective if culture is combined with other screening tests, such as PCR or serologic assays.

Methods besides culling have also been used to select *Pasteurella*-free stock. These methods include cesarian derivation (Baba et al., 1987; Scher et al., 1969; Weisbroth and Scher, 1969), and transfer of fertilized ova to *Pasteurella*-free does (Ward, 1973). Another method is addition of either furazolidone or sulfaquinoxaline to the diet of does prior to kindling until weaning (Hagen, 1966). Treatment of infected does with enrofloxacin prior to and up to a week after kindling may be sufficient to derive *Pasteurella*-free offspring, although the treatment is unlikely to eliminate the organism from the does. Intramuscular and oral routes of administration appear equally effective (Suckow et al., 1996b).

It is possible that early weaning, even without use of antimicrobials, helps select for *Pasteurella*-free stock. Preweanling rabbits have a relatively low rate of infection, possibly because of resistance conveyed by passively acquired maternal antibodies (DiGiacomo et al., 1983a, 1987a; Nakagawa et al., 1986).

VACCINATION

At present there is no commercially available vaccine that is fully effective against pasteurellosis. Many approaches to vaccination have been evaluated, including the use of killed whole bacteria, live avirulent streptomycin-dependent strains, potassium thiocyanate bacterial extracts, and inactivated exotoxin. Strategies have also been employed to enhance the immune response, such as administering antigens encapsulated in alginate microspheres or delivered in conjunction with cholera toxin. Other novel approaches to vaccination include preparations containing iron-regulated outer membrane proteins and preparations designed to evoke antibodies to core antigens of Gram-negative bacteria. In general, regardless of the route of administration, these strategies provide incomplete protection against disease and do not prevent colonization of the upper respiratory tract. While immunity may be enhanced in comparison with more traditional approaches, colonization still occurs.

Early attempts to control pasteurellosis by vaccination used killed whole bacteria (Alexander et al., 1952; Bapat and Sawhney, 1972; Okerman and Spanoghe, 1981; Torres-Anjel and Cotrino-Badillo, 1977). These vaccines provided protection against mortality, particularly when rabbits were challenged with homologous organisms, but they did not prevent clinical illness or colonization. To overcome this, live vaccines using avirulent streptomycin-dependent *P. multocida* were tried

(Chengappa et al., 1980; Deeb et al., 1989; DiGiacomo et al., 1987b; Lu and Pakes, 1981; Percy et al., 1985; Wei and Carter, 1978). These vaccines proved relatively effective against clinical disease and colonization caused by homologous strains, but they were less effective against heterologous strains (DiGiacomo et al., 1987b; Percy et al., 1985). In addition, they provided no control of pasteurellosis under field conditions (Deeb et al., 1989).

Bacterial derivatives, including potassium thiocyanate extracts, purified outer membranes, and inactivated toxin have also been evaluated for their ability to protect against pasteurellosis (Lu et al., 1987b, 1991c; Ringler et al., 1985; Suckow, 2000; Suckow et al., 1995). Several of these trials involved intranasal administration in an attempt to stimulate mucosal immunity and inhibit bacterial colonization. In most cases, intranasal administration provided only partial protection against disease and colonization, even when animals were challenged with homologous organisms. Interestingly, a field trial of potassium thiocyanate extract vaccine compared intranasal and subcutaneous routes of administration and found that subcutaneous administration provided better protection (Suckow et al., 2008). Intranasal administration can be more effective when antigens are combined or administered with adjuvants such as cholera toxin. For example, intranasal administration of potassium thiocyanate extract combined with inactivated toxin conveys better protection than potassium thiocyanate extract or toxin alone (Jarvinen et al., 1998). The level of protection provided by potassium thiocyanate extract or inactivated toxin vaccines may be improved by including cholera toxin, which strengthens the mucosal immune response, or by alginate encapsulation, which may enhance antigen presentation to macrophages (Jarvinen et al., 2000; Suckow et al., 1996, 1996a, 2002). However, cholera toxin combined with alginate encapsulation offers little advantage over alginate alone (Suckow et al., 1996a).

Another approach to vaccination was designed to elicit antibody against antigens that are non-serospecific, and thereby shared by most, if not all, serotypes. A commercially available *E. coli* J5 bacterin was evaluated, in the hope that antibodies elicited Gram-negative core antigens would be cross protective, thereby reducing the severity of pasteurellosis in infected rabbits. This strategy was not successful, however (Ruble et al., 1999a).

Iron-regulated outer membrane proteins expressed by bacteria may be important in virulence and tend to induce a strong immune response (Gu and Lu, 2006). Therefore, outer membrane proteins containing iron-regulated proteins might be good vaccine candidates. In fact, such a preparation, administered intranasally and combined with cholera toxin, provided protection

against experimental challenge and reduced the number of *P. multocida* organisms that could be recovered from the nasal cavity (Confer et al., 2001).

Future directions in *P. multocida* vaccine research will attempt to identify individual candidate vaccine antigens by cloning various surface proteins and assessing their role in virulence. Some promising candidates include the outer membrane protein designated Oma87, the fimbrial subunit protein designated PtfA, iron-regulated transferrin binding protein, and several enzymes involved in capsule biosynthesis (Adler et al., 1999). A different approach, which attempts to immunize with a broad cross-section of antigens, involves production of bacterial “ghosts” by phage-mediated bacterial lysis. The bacterial ghosts are devoid of cytoplasmic and genomic material, yet retain all surface antigens in their native conformation (Marchart et al., 2003).

PROPHYLAXIS WITH ANTIMICROBIALS

Antimicrobials have been used prophylactically to prevent pasteurellosis, primarily by adding them to the feed or drinking water of pregnant or lactating does. Although antimicrobials may reduce the incidence of pasteurellosis in does and their offspring, they are probably not capable of eliminating the infection. Antimicrobial agents that have been used include furazolidone, sulfaquinoxaline, sulfamethazine, and chlor-tetracycline (Hagen, 1966, 1967; Plant, 1974). Treatment of does with enrofloxacin may be more effective than other antibiotics in producing *Pasteurella*-free offspring (Suckow et al., 1996a). Does remain infected, however, perhaps because the organism colonizes sites where effective concentrations of antibiotics cannot be achieved (Mähler et al., 1995).

Treatment

Treatment of pasteurellosis is difficult. Clinical signs may subside, but the organism is rarely eliminated from the host and recurrences of disease are common (Jaslow et al., 1981). The clinical forms of pasteurellosis most likely to respond to therapy are rhinitis, conjunctivitis, and subcutaneous abscesses. Rhinitis can be treated with systemic antibiotics alone, whereas conjunctivitis should be treated with antibiotic ophthalmic ointments as well as systemic antibiotics. Abscesses should be drained, flushed, and antibiotics should be administered. Other forms of pasteurellosis respond poorly to treatment, probably because the diagnosis is only made when the disease is far advanced.

Historically, penicillin has been the most widely used antibiotic for treatment of pasteurellosis. Nearly all *P. multocida* isolates are sensitive to penicillin. One problem with penicillin therapy, which may partially explain why treatment of pasteurellosis is often unsuccessful, is that it is difficult to maintain therapeutic

blood levels. Procaine penicillin must be administered at 8-hour intervals to ensure adequate levels in blood and nasal secretions (Welch et al., 1987). If once-a-day dosage is desired, combined preparations of procaine and benzathine penicillin should be used (Gaertner, 1991). Aerosol preparations of penicillin have been suggested for treatment of rhinitis, but they offer no advantage over systemic administration (Jaslow et al., 1981).

Pasteurella multocida is sensitive to many antibiotics, and a variety have been used for treatment, including tetracyclines, chloramphenicol, gentamicin, tilmicosin, and fluoroquinolones, including enrofloxacin and related compounds (Hanan et al., 2000; Marangos et al., 1997; McKay et al., 1996; Okewole and Olubunmi, 2008; Rougier et al., 2006). Most of these antibiotics offer few advantages over penicillin; for example, one study showed that penicillin is preferable to gentamicin for treatment of rhinitis (Gaertner, 1991). Enrofloxacin and other fluoroquinones, however, may sometimes be more effective than penicillin. Enrofloxacin has been suggested as the drug of choice for treatment of *Pasteurella*-associated rhinitis or conjunctivitis, since there is a high rate of response when the drug is administered either orally or by injection (Broome and Brooks, 1991). Enrofloxacin is also effective in an experimental model of septicemic pasteurellosis (Okerman et al., 1990). A pharmacokinetic evaluation of this drug showed that two or three times a day dosing is required, depending on the route of administration (Broome et al., 1991). As is the case with most antibiotics, although enrofloxacin treatment can result in improvement of clinical signs, it does not appear to eliminate the carrier state (Mähler et al., 1995; Suckow et al., 1996a).

Regardless of which antibiotic is used for treatment of pasteurellosis, treatment should be discontinued in the event of anorexia, reduced fecal production, or diarrhea. Antibiotic therapy can cause gastrointestinal disturbances and has been associated with fatal enterocolitis (Rehg and Lu, 1981).

ENTEROTOXEMIA

Background

Enteric diseases are second only to pasteurellosis as a health problem in domestic rabbits. The primary clinical sign of these diseases is diarrhea, which is sometimes accompanied by sudden death. The etiologic agents and mechanisms responsible for enteric diseases of rabbits are varied and poorly understood. In some instances a specific etiologic agent can be identified, but frequently the cause of diarrhea is obscure. Many infectious agents have been associated with diarrhea, including coccidia (*Eimeria* sp.), rotavirus, and bacteria such as

clostridia, coliforms, and *Salmonella* (Percy et al., 1993). Therefore, enteric diseases are often grouped together under the designation “rabbit enteropathy complex.”

The term enterotoxemia is used to refer to enteropathy caused by toxigenic micro-organisms of the genus *Clostridium*. The first association of *Clostridium* spp. with rabbit enteric disease was made by Morcos (1932), who isolated an organism that he named *Bacillus* (*Clostridium*) *tympani-cuniculi*. Several years later, Lesbouyries and Berthelon (1936) observed spiral-shaped organisms in the feces of rabbits with diarrhea. Subsequent reports on rabbit enteropathy did not establish a definite link with *Clostridium* spp. until Patton et al. (1978) reported the presence of clostridial iota toxin in the cecal contents of rabbits which died in an outbreak of enteritis. At about the same time, iota toxin was found in cecal contents of rabbits that were challenged with clindamycin or lincomycin in an attempt to model *C. difficile*-mediated antibiotic associated colitis of humans (Katz et al., 1978; La Mont et al., 1979; Reh and Pakes, 1982).

When iota toxin was first identified in rabbits with diarrhea, the source of the toxin was thought to be *C. perfringens* type E (Baskerville et al., 1980; Eaton and Fernie, 1980; Patton et al., 1978). This was because *C. perfringens* type E antitoxin had the ability to neutralize the toxin, but *C. perfringens* was not isolated from the affected rabbits. Later studies implicated *C. spiroforme* as the source of the iota toxin, because it was consistently isolated from toxin-positive cecal contents (Carman and Borriello, 1982). The initial confusion about the causative organism was probably due in part to the fact that the two species produce toxins that are almost identical (Wilkins et al., 1985).

C. spiroforme is clearly an important cause of rabbit enterotoxemia. Therefore, the disease has been referred to as *C. spiroforme*-mediated diarrhea (SMD) (Carman and Evans, 1984). The importance of *C. spiroforme* in rabbits is in contrast to most animal species, in which gastrointestinal infections are associated primarily with *C. perfringens* (Borriello, 1995). In rabbits, *C. perfringens* and other clostridial species such as *C. difficile* are involved infrequently (Dubos et al., 1984; Kunstyr et al., 1975; Reh and Lu, 1981). *C. difficile* has usually been associated with prior antibiotic administration, but in at least one case it has been confirmed as the cause of a small outbreak in which there was no antibiotic exposure (Perkins et al., 1995). This outbreak was different from most cases of *C. spiroforme* enterotoxemia, in that *C. difficile* was found primarily in the ileum rather than the cecum.

Etiologic Agent

C. spiroforme is a Gram-positive spore-forming non-motile anaerobe that can be isolated from the feces of healthy humans (Cato et al., 1986). It is distinguished

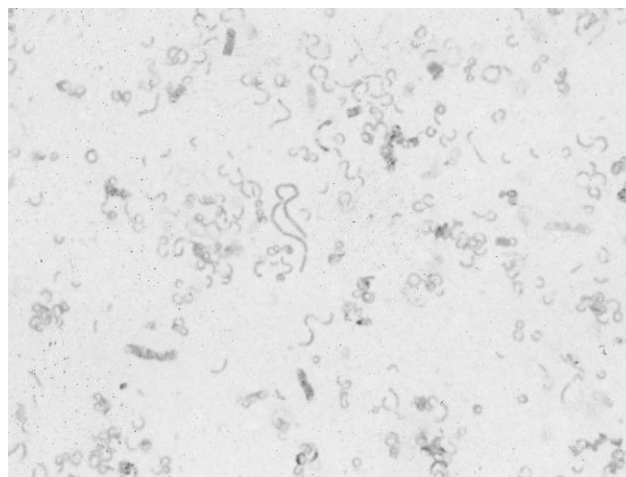


FIGURE 13.9 Enterotoxemia: etiologic agent *Clostridium spiroforme*. (Courtesy of Dr. Roland Gunther)

from most other species of *Clostridium* by its curved or coiled shape, the coils being produced by long chains of linked curved organisms (Figure 13.9). *C. spiroforme* can be differentiated from *C. cocleatum*, a similar species which also has been reported in rabbits (Lelkes and Chang, 1987), by the inability of the former organism to ferment galactose (Kaneuchi et al., 1979). *C. spiroforme* organisms are able to agglutinate human erythrocytes, which implies the presence of adhesins on the bacterial surface (Baldassarri et al., 1989).

Rabbit isolates of *C. spiroforme* produce a toxin that is neutralized by antiserum to *C. perfringens* iota toxin. The toxin is cytotoxic, lethal to mice, and causes dermonecrosis in guinea pigs. Production of toxin in vitro requires the use of chopped meat medium supplemented with glucose. Non-toxigenic variants of *C. spiroforme* exist, but virtually all rabbit isolates are toxigenic (Borriello and Carman, 1983).

C. spiroforme produces a binary protein toxin consisting of two components, A and B, which are physically separate in solution. The mechanism of action involves activation of the B component by host serine proteases, either in solution or on the target cell surface. Upon activation, the B component, which is responsible for host cell binding and translocation, forms a complex with A component, which has enzymatic activity. The complex then gains access to the cytosol via receptor-mediated endocytosis. Cell damage occurs primarily as a result of disruption of the cytoskeleton by ADP ribosylation of actin (Barth 2008; Barth and Stiles, 2004; Perelle et al., 1997; Popoff et al., 1989).

Epizootiology

Recently weaned rabbits are most susceptible to enterotoxemia. The disease may occur sporadically,

but epizootics are common. One report documents the death of 47% of weanling animals over a period of 1 year in a barrier-maintained rabbit colony (Yonushonis et al., 1987). The relatively high prevalence of enterotoxemia in young rabbits is probably the result of an unstable and incompletely developed gastrointestinal flora, which allows proliferation of *C. spiroforme* in exposed animals (Carman and Borriello, 1984; Lelkes and Chang, 1987).

Adult rabbits may develop enterotoxemia, but they are more resistant than young animals. Disease in adults depends on disruption of the normal gastrointestinal flora by a mechanism such as antibiotic administration or coinfection with other pathogens (Carman and Borriello, 1984). One study showed that 86% of rabbits with enterotoxemia were coinfecting with other potentially pathogenic gastrointestinal micro-organisms such as enteropathogenic *E. coli*, *Clostridium piliforme*, rotaviruses, and *Eimeria* spp. (Peeters et al., 1986a).

Clinical Manifestations

Enterotoxemia is most likely to follow an abrupt change in the animal's life, such as weaning, lactation, change of feed, illness, or antibiotic administration. Rabbits with enterotoxemia have an acute onset of brown watery diarrhea that soils the perineal area. This is sometimes preceded by a 6–12-hour period of anorexia, during which *C. spiroforme* can be recovered from cecal contents. After the onset of diarrhea, rabbits rapidly become hypothermic and moribund, with death following in 24–48 hours. Occasionally, rabbits may survive for several days, and in rare cases they may recover. Enterotoxemia sometimes occurs without concomitant diarrhea, either as a peracute fatal illness, or as a chronic disease manifested by anorexia and weight loss (Carman and Evans, 1984; Harris and Portas, 1985).

Pathology

The pathologic changes seen in enterotoxemia have been described (Carman and Evans, 1984; Eaton and Fernie, 1980; Patton et al., 1978). Most cases have fecal soiling of the perineum, abdomen, and rear legs. The animals are usually in good condition with adequate body fat, but dehydration may be evident. The most characteristic gross finding is the presence of petechial and ecchymotic hemorrhages on the serosal surface of the cecum. The appendix, proximal colon, and distal ileum also may be involved. The cecum may be distended with gas and contain fluid to mucoid dark brown feces. Hemorrhages are evident on the mucosa, accompanied in some cases by ulcers or pseudomembranes. On cut section the submucosa of the cecum is thickened and edematous (Figure 13.10).



FIGURE 13.10 Enterotoxemia: mucosal hemorrhage and submucosal edema of cecum.

Microscopically, the most consistent changes are serosal hemorrhages and edema of the cecal submucosa. Numerous inflammatory cells, mostly neutrophils, are present in the submucosa and lamina propria, but in some cases inflammatory cells are rare. Mucosal lesions consist principally of swollen, vacuolated, and necrotic epithelium with focal erosions and ulcers. The ulcers are often overlaid by a layer of exudate composed of proteinaceous fluid, inflammatory cells, and cell debris. In some cases, areas of intact mucosa have evidence of epithelial cell proliferation, such as increased numbers of mitoses and lengthening of crypts. In others, mucosal attenuation is a prominent lesion.

Pathogenesis

Enterotoxemia is generally preceded by a change in the normal flora of the gastrointestinal tract that permits colonization and proliferation of *C. spiroforme*. Coliforms proliferate as well, but this is probably incidental and not critical for the development of enterotoxemia (Fesce et al., 1977; Sinkovics, 1976, 1978). Enteropathogenic and enterohemorrhagic strains of *E. coli* can be primary pathogens of rabbits, but the role of these strains in enterotoxemia is unclear.

Healthy rabbits have a diverse population of micro-organisms in the cecum, including ciliated protozoa, large anaerobic metachromatically staining bacilli, and small Gram-positive rods, but *C. spiroforme* is usually absent (Carman and Evans, 1984; Lelkes and Chang, 1987). Several conditions can alter the normal flora and favor colonization and proliferation of *C. spiroforme*. These include weaning, change in diet, parturition and lactation, illness, and antibiotic administration. One of the most important predisposing factors is the feeding of high-energy, low-fiber diets to weanlings.

This practice favors disruption of cecal flora by inducing cecal hypomotility and increasing the availability of simple carbohydrate substrates, such as glucose, required by pathogenic micro-organisms. *C. spiroforme* is known to require substantial amounts of glucose for toxin production (Borriello and Carman, 1983; Cheeke and Patton, 1980; Cheeke et al., 1986; Patton et al., 1978; Whitney, 1976).

Since *C. spiroforme* is not normally present in the intestinal tract of healthy rabbits, induction of enterotoxemia requires exposure to the organism. In weanlings, exposure alone is sufficient to induce disease, probably because of the unstable cecal flora characteristic of this age. In adults, exposure to *C. spiroforme* does not produce disease unless the normal intestinal flora is first disrupted. Experimentally, this has been accomplished by administration of clindamycin and lincomycin (Carman and Borriello, 1984; Fesce et al., 1977).

Diagnosis

A definitive diagnosis of *C. spiroforme*-mediated enterotoxemia requires isolation of toxigenic micro-organisms and neutralization of toxic cecal contents with specific antisera. A presumptive diagnosis can be made by identifying typical coiled Gram-positive organisms in the feces or cecal contents (Carman and Borriello, 1983).

Isolation of *C. spiroforme* is facilitated by centrifuging cecal contents for 15 minutes at 20000g (Holmes et al., 1988). This procedure concentrates the organism at the interface of the pellet and supernatant. The surface of the pellet is washed and an aliquot is taken for Gram staining. Another aliquot is plated on sheep blood agar and incubated anaerobically for 24–48 hours at 37°C. Rifampicin or tylosin can be added to the media to promote selection of the organism (Agnoletti et al., 2009; Carman and Wilkins, 1991). *C. spiroforme* can be identified by its characteristic coiled appearance, which is evident both in fresh samples and after growth in vitro.

The supernatant fluid from centrifuged cecal contents can be analyzed for the presence of toxin. Various assays have been recommended, including the guinea pig dermonecrosis assay (Yonushonis et al., 1987), mouse lethality assay (Harris and Portas, 1985; Patton et al., 1978), and an in vitro cytotoxicity assay using Vero cells (Butt et al., 1994). The supernatant is injected intradermally for the dermonecrosis assay and intraperitoneally for the lethality assay. Identification of toxin depends on neutralization of the supernatant's toxic effects by specific antiserum. Immunologic assays have also been proposed for detection of *C. spiroforme* toxin (Carman and Evans, 1984).

Polymerase chain reaction protocols have been developed for detection of *C. spiroforme* and its toxin (Drigo et al., 2008). Primers specific for detection of

the organism are based on 16S rDNA sequences; primers specific for detection of toxin are based on the sequences of the toxin genes *sas* and *sbs*. One disadvantage of this approach to diagnosis is that cecal contents typically contain polymerase inhibitors that limit somewhat the sensitivity of the assay.

Prevention and Therapy

As with all infectious diseases of rabbits, prevention of enterotoxemia depends on maintaining optimal husbandry and minimizing stress. Feeding practices are especially important (Whitney, 1976). A high-quality feed with up to 20% fiber should be fed and sudden changes in diet avoided. If a change in diet must be made, a gradual introduction of the new feed over 7 days is recommended. Infant rabbits should have feed available at 2–3 weeks of age, and early or forced weaning should be avoided.

Dietary supplementation may be helpful in reducing the incidence of enterotoxemia in rabbits. Copper sulfate, when added to the diet at 400ppm, appears to reduce the mortality from enterotoxemia in rabbit colonies (Patton et al., 1982). This may be due to inhibition of toxin production by *C. spiroforme*; levels over 250ppm have been shown to inhibit toxin production in vitro (Grobner et al., 1986). Probiotics, which are orally administered preparations of lactobacilli, yeasts, and other micro-organisms, also appear to reduce mortality from enterotoxemia when given to weanlings. Probiotics are commercially available and may be mixed with either food or water (Hollister et al., 1989, 1990).

In the event of a large outbreak, it is possible to protect against enterotoxemia by vaccinating susceptible animals with a toxoid prepared from supernatant of *C. spiroforme* cultures. Optimal protection requires two injections administered 14 days apart (Ellis et al., 1991).

Antimicrobial administration in feed or water has been advocated to reduce the prevalence of rabbit enteric diseases, but with the possible exception of coccidiosis the value of this practice is not well established (Whitney, 1976). In general, antibiotics should be used cautiously in rabbits, since they may precipitate enteric disease in some animals (Hara-Kudo et al., 1996; Rehag and Lu, 1981). This is especially true when penicillin or one of its derivatives, such as ampicillin, amoxicillin, lincomycin, clindamycin, and erythromycin, is given orally (Carman and Evans, 1984).

Antimicrobials have limited value in treating enterotoxemia. Although isolates of *C. spiroforme* are often sensitive to amoxicillin, ampicillin, chloramphenicol, sulfaquinoxaline, and erythromycin, there is little evidence that these antibiotics are effective in treating individual cases or controlling outbreaks (Agnoletti et al., 2009; Carman and Evans, 1984). This may be in

part due to the rapid progression of the clinical disease. On the other hand, imidazole derivatives, which have a good anaerobic spectrum, may be of value in controlling an outbreak. In vitro, metronidazole is uniformly active against *C. spiroforme* (Carman and Wilkins, 1991). Iprnidazole, dimetridazole, and metronidazole have been reported to reduce the number of deaths from enterotoxemia when added to the drinking water or the food (Carman and Evans, 1984; Eaton and Fernie, 1980). The efficacy of these agents is, however, variable and influenced by the stage and extent of the outbreak.

A novel approach to prevention of enterotoxemia is the use of cholestyramine, an ion exchange resin capable of binding bacterial toxins. When administered daily by gavage at a dose of 2g/20ml of water for 18 or 21 days, cholestyramine prevented mortality in rabbits with clindamycin-induced enterotoxemia (Lipman et al., 1992).

TYZZER'S DISEASE

Background

Tyzzler's disease is an enterohepatic infection caused by *Clostridium piliforme*, a poorly characterized filamentous bacterial organism. Until 1993 the organism was known as *Bacillus piliformis* based on morphologic criteria, but it was reassigned to the genus *Clostridium* based on its 16S rRNA sequence (Duncan et al., 1993). The disease has been reported in many species, including mice, rabbits, rats, hamsters, gerbils, cats, and rhesus monkeys (Ganaway, 1971a). In addition, a case of *C. piliforme* infection has been reported in a patient infected with HIV-1 (Smith et al., 1996). Clinical signs in animals typically include profuse diarrhea and rapid death. Lesions are most common in the liver and cecum.

Tyzzler's disease was first described in 1917, when Ernst Tyzzler observed an epizootic of focal hepatitis and enteritis in Japanese waltzing mice (Tyzzler, 1917). He found the etiologic agent in hepatic parenchymal cells and in cecal epithelium, but was unsuccessful in propagating it on artificial media. In the years following Tyzzler's original description the disease was reported occasionally in mice, but it was not recognized in rabbits until 1965, when Allen et al. described two epizootics which had clinical and pathologic features similar to the mouse disease. Weanlings were most often affected, and death ensued rapidly after a period of brief diarrhea. Lesions included multifocal hepatic necrosis, necrosis of cecal mucosa, and edema of cecal submucosa. Intracytoplasmic bacilli were visible in all affected organs, but attempts to isolate them on artificial media were unsuccessful. The disease was transmitted to healthy rabbits treated with cortisone and then exposed to contaminated bedding.

Tyzzler's disease has been reported in rabbits many times since it was first recognized (Cutlip et al., 1971; Ganaway and Allen, 1981; Ononiwu and Julian, 1978; Peeters et al., 1985; Prescott, 1977; Simon, 1977; Van Kruiningen and Blodgett, 1971). Most of these reports describe clinical and pathologic features similar to those outlined by Allen et al. (1965).

Etiologic Agent

Clostridium piliforme is a large, pleomorphic, Gram-negative, spore-forming, obligate intracellular bacterium. It possesses peritrichous flagella and is therefore motile. *C. piliforme* is a unique pathogen and poorly characterized, in part because it is difficult to propagate in vitro. Although there are two reports of isolation of the organism on cell-free media (Kanazawa and Imai, 1959; Simon, 1977), these findings have not been replicated and attempts using 190 different microbiological media have been unsuccessful (Thunert, 1984). It is now generally accepted that living cells are required for propagating the organism.

C. piliforme can be isolated and serially passaged by yolk sac injection of embryonated hens' eggs (Craigie, 1966a, 1966b; Fries, 1977a; Ganaway et al., 1971b). Under these conditions the organism retains its pathogenicity for numerous passages. Cell culture can also be used for cultivation of *C. piliforme* (Ganaway et al., 1985b). Various established mammalian cell lines are suitable, including mouse and rat hepatic cells and mouse fibroblasts (Riley et al., 1990).

Studies of *C. piliforme* isolates from various species indicate a high degree of antigenic diversity, but some shared antigens do exist (Fujiwara et al., 1971, 1973, 1985; Riley et al., 1990; Toriumi et al., 1986). Most notable is a cross-reacting group of immunodominant antigens with molecular weights of approximately 56–59 kDa. These are believed to represent bacterial flagellar proteins (Motzel and Riley, 1991). The high degree of antigenic diversity is probably related to the fact that isolates of *C. piliforme* differ in host specificity; some have the ability to infect only the species from which the isolate was obtained, whereas others have a broad host range (Franklin et al., 1994).

Epizootiology

Tyzzler's disease is widespread in rabbit colonies. Infection is probably transmitted by ingestion of spores, since the vegetative form of *C. piliforme* is unstable outside the host (Ganaway and Allen, 1981). Classically, the disease occurs epizootically. At the peak of outbreaks, the mortality rate may approach 50%. Losses are particularly high in 6–12-week-old rabbits, but younger rabbits and adults may also contract the disease (Allen et al., 1965; Cutlip et al., 1971; Ononiwu and Julian,

1978; Peeters et al., 1985; Van Kruiningen and Blodgett, 1971). Tyzzer's disease may also occur as an enzootic infection, with large numbers of subclinically infected animals and only sporadic clinical cases (Fries, 1979b; Prescott, 1977). A serologic survey of rabbits from 15 breeding or research facilities in Japan revealed antibody to *C. piliforme* in nine facilities (Goto et al., 1991). In positive facilities, approximately 15% of tested rabbits had detectable antibody.

Clinical Manifestations

Most *C. piliforme* infections are subclinical, but when clinical signs occur they typically appear suddenly. Young animals are the most susceptible, with entire litters sometimes affected. The most prominent signs are profuse watery diarrhea and fecal staining of the perineum. Affected rabbits are listless, anorectic, and dehydrated. Death occurs within 1–2 days after the onset of symptoms in about 90% of the animals (Allen et al., 1965; Cutlip et al., 1971). Rabbits that survive acute infection sometimes develop chronic disease, the signs of which include progressive weight loss and cachexia (Peeters et al., 1985).

Pathology

Lesions of Tyzzer's disease in rabbits are described in detail by Allen et al. (1965). They are characterized principally by necrosis and occur in the cecum, adjacent intestine, liver, and heart. The cecum and intestine are consistently affected, but the heart is frequently spared. Lesions are usually present in the liver, but may be absent in peracute cases (Licois, 1986).

Grossly, there is fecal soiling of the perineum and dehydration may be evident. The serosal surface of the cecum has ecchymotic or petechial hemorrhages, the wall is thickened and edematous, and the mucosa contains patchy areas of necrosis. These lesions often extend to the adjacent distal ileum and proximal colon. In chronic cases there may be stenosis of the intestine due to fibrosis at the sites of necrosis. The liver contains numerous pinpoint white foci, usually 2 mm in diameter or less, throughout the parenchyma (Figure 13.11). White streaks or pale foci may be present in the myocardium, most often in the left ventricle and septum.

Microscopically, there are hemorrhages in the subserosa of the cecum. Submucosal edema and mucosal necrosis are consistent findings in the cecum and adjacent intestine. Mucosal necrosis sometimes extends into the submucosa and muscularis. The liver contains focal areas of necrosis that are often close to portal areas (Figure 13.12). Polymorphonuclear leukocytes may be present around the necrotic foci and in hepatic sinusoids. Chronic hepatic lesions may be calcified, with

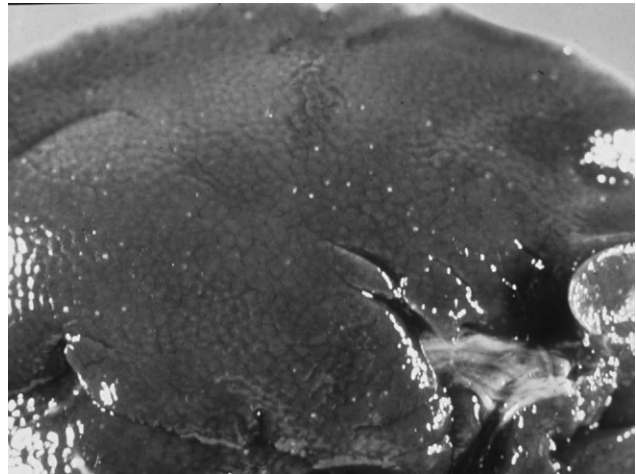


FIGURE 13.11 Tyzzer's disease: liver, small foci of necrosis. Similar lesions occur in diseases such as listeriosis and tularemia.

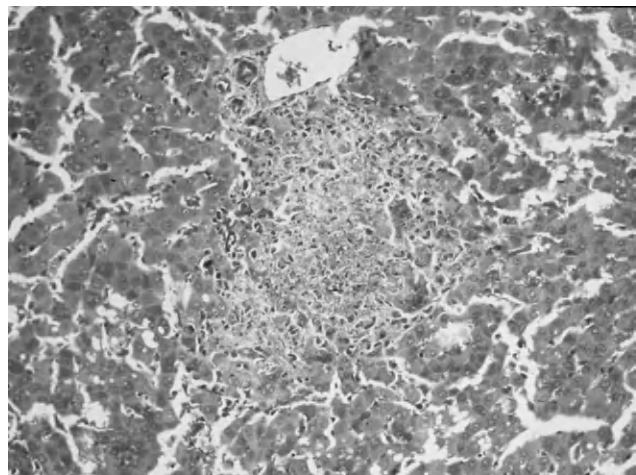


FIGURE 13.12 Tyzzer's disease: focal area of necrosis. Similar lesions occur in diseases such as listeriosis and tularemia.

surrounding areas of fibrosis and multinucleated giant cells. Myocardial lesions consist of focal or linear areas of necrosis, sometimes associated with a moderate inflammatory cell response, in close proximity to myocardial blood vessels.

C. piliforme can be demonstrated in affected tissues, especially the liver, by special stains (Figure 13.13). The Warthin-Starry silver stain is most effective but periodic acid-Schiff or Giemsa stains can also be used. With the Warthin-Starry stain the bacilli appear as light brown to black rods in the cytoplasm of viable hepatocytes surrounding areas of necrosis. In longstanding lesions the bacilli are not present.

Pathogenesis

C. piliforme is transmitted by the fecal-oral route. Spores are shed in the feces and infection occurs by

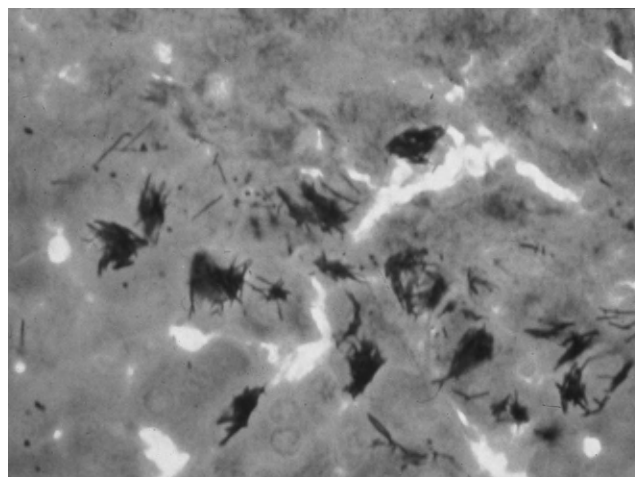


FIGURE 13.13 Tyzzer's disease: *Clostridium piliforme* in hepatocytes. Warthin-Starry stain. (courtesy of Dr. Roland Gunther)

ingestion of contaminated material (Allen et al., 1965; Tyzzer, 1917). It is not known whether other routes of transmission are important in rabbits, but transplacental transmission has been documented in rodents (Fries, 1978, 1979a). Other events in the pathogenesis of the disease are unclear. The presence of specific antibody in apparently healthy animals suggests that the organism initially resides latently in the intestinal tract (Goto et al., 1991). Subsequently, stress or immunosuppression appears to precipitate overt disease, possibly by favoring invasion of the intestinal mucosa and systemic spread of the organism. This is suggested by the need for corticosteroid administration to consistently reproduce the disease experimentally (Allen et al., 1965). After the organism penetrates the mucosa, infection of the liver occurs by way of the portal circulation, whereas infection of the heart occurs via the lymphatics (Allen et al., 1965). Studies in mice suggest that host defenses that are particularly important in determining the course of disease include neutrophils and natural killer cells, with macrophages playing a much more limited role (Van Andel et al., 1997).

Diagnosis

A presumptive diagnosis of Tyzzer's disease can be made on the basis of typical gross and microscopic lesions, but a definitive diagnosis depends on demonstration of characteristic intracellular bacteria in affected tissues. Immunofluorescence may help identify *C. piliforme* when the organisms are not evident in specially stained preparations (Fries, 1977; Savage and Lewis, 1972). Isolation of the organism by inoculation of embryonated eggs or tissue culture is desirable but not often attempted in practice. Rabbit carriers of *C. piliforme* can

sometimes be identified by housing weanling gerbils on soiled bedding. Since gerbils are particularly susceptible to *C. piliforme* infections, they can be expected to develop histologically evident Tyzzer's disease when exposed to spores present in feces (Gibson et al., 1987).

Screening for serum antibodies against *C. piliforme* can be used as a means of rabbit colony surveillance. Antibodies are produced in subclinically infected animals and persist for long periods, possibly throughout life (Fries, 1979b). They can be detected in rabbits with an indirect fluorescent antibody assay (Fries, 1977b). Enzyme-linked immunosorbent assays can also be used and have the advantage of being simpler to perform while providing comparable results (Motzel et al., 1991; Waggle et al., 1987). Antigens for either assay can be prepared from *C. piliforme* grown in primary chick embryo liver cultures or a rat hepatic cell line (BRL 3A). An alternative antigen for the enzyme-linked immunosorbent assay is a trivalent preparation of purified *C. piliforme* flagella (Motzel and Riley, 1991).

Polymerase chain reaction (PCR) protocols for detection of *C. piliforme* infection have been described. Goto and Itoh (1996) developed an avidin-biotin-based enzyme assay to detect DNA amplified by PCR from rodent tissue specimens, using a primer directed toward a conserved area of the 16S rRNA gene. Detection of *C. piliforme* in rodent fecal specimens by PCR has also been reported (Furukawa et al., 2002). Feldman et al. (2006) further refined PCR methodology and extended its application to rabbit isolates.

A problem confronting the development of reliable *C. piliforme* PCR assays is that there is significant nucleotide sequence diversity in 16S rRNA genes of individual isolates; it is also likely that some sequences are shared by closely related non-pathogenic clostridia (Feldman et al., 2006). Therefore, the sensitivity and specificity of any given assay remain uncertain. In an effort to address this problem, Niepceron and Licois (2009) developed a nested PCR designed to maximize specificity while retaining adequate sensitivity.

Regardless of which PCR assay is used, it is important to recognize that positive results not confirmed by clinical or pathologic signs of disease may represent latent *C. piliforme* infection or, alternatively, cross reaction with a similar but non-pathogenic strain. Similarly, negative PCR results in the presence of serologic evidence of infection may suggest elimination of the organism, intermittent shedding, or the presence of a *C. piliforme* strain with a different DNA sequence in the region where the PCR primers bind. A study reported by Pritt et al. (2010) demonstrated negative PCR results in a group of 20 rabbits seropositive for *C. piliforme*, underscoring the importance of not relying on a single diagnostic technique when attempting to ascertain whether the organism is present in a colony.

Prevention and Therapy

There is no treatment known to be of value for clinically ill rabbits. Antibiotic therapy yields poor results, perhaps because the intracellular location of the organism limits efficacy. This is substantiated by a study in which six antibiotics were tested for their ability to inhibit the growth of *C. piliforme* in embryonated eggs (Ganaway et al., 1971b). None was effective: chlortetracycline, penicillin, streptomycin, and erythromycin were only partially inhibitory, whereas chloramphenicol and sulfamethazine completely lacked inhibitory properties. Although antibiotics are ineffective in clinical cases, anecdotal evidence suggests they may be of value in limiting epizootics. In one outbreak, oxytetracycline was added to the drinking water and the epizootic ended in 36 hours (Van Kruiningen and Blodgett, 1971). In another instance, clinical disease was eliminated from a colony of mice after administration of tetracycline in the drinking water (Hunter, 1971).

No vaccine is available for Tyzzer's disease, so prevention depends mostly on good husbandry. Practices such as frequent cage cleaning, prevention of crowding, and dust control should be of value in reducing exposure of animals to spores. Spores can be inactivated by sodium hypochlorite solution (0.3%) or by heating at 80°C for 30 minutes (Ganaway, 1980). Removal of sick animals and minimization of stress are of obvious value in reducing the likelihood of disease outbreaks. Sulfonamides should be administered cautiously to rabbits, since sulfaquinoxaline administration has been associated with losses from Tyzzer's disease (Ganaway et al., 1971a).

Elimination of Tyzzer's disease from an infected colony might be possible by serologically screening the colony and removing animals testing positive. Cesarean derivation might be possible, but transplacental transmission has been documented in rodents and may occur in rabbits as well (Fries, 1979a).

COLIBACILLOSIS

Background

Escherichia coli is recognized as a cause of enteric disease in many species, but as recently as the late 1970s the prevailing view was that *E. coli* is not a primary pathogen in rabbits (Holmes, 1984). Conclusive evidence to the contrary has been presented since that time. The earlier view stemmed from the fact that *E. coli* proliferates as a secondary response to an altered intestinal environment (Prohaszka, 1980). *E. coli* is frequently present in rabbits with diarrhea but is uncommon in the intestinal tract of healthy animals (Gouet and Fonty, 1979; Peeters et al., 1984b; Vetesi and Kutas, 1973). This

pattern would seem to implicate *E. coli* as an etiologic agent of enteric disease, but it was difficult to confirm that *E. coli* is a primary cause of gastrointestinal disease until Koch's postulates were fulfilled.

This was accomplished in 1977, when Cantey and Blake reproduced diarrhea in rabbits by oral inoculation of *E. coli* strain RDEC-1 (Rabbit Diarrhea *E. coli*-1), which had been isolated from diarrheic rabbits. As few as 150 organisms were capable of producing severe disease, yet they failed to invade the mucosa or produce classic *E. coli* enterotoxins (Cantey and Blake, 1977). This was one of the first reports documenting a role for non-invasive, non-toxicogenic *E. coli* in enteric disease. RDEC-1 is now referred to as an "enteropathogenic" strain of *E. coli*. Enteropathogenic strains are the most common causes of rabbit colibacillosis, although other pathotypes, such as enterohemorrhagic and necrotoxicogenic strains, have been reported as well (see below).

Since Cantey and Blake's work with RDEC-1, many investigators have isolated similar strains of *E. coli* from rabbits and successfully reproduced diarrhea (Camguilhem, 1985; Camguilhem and Milon, 1989; Okerman and Devriese, 1985; Peeters et al., 1984a, 1984c; Prescott, 1978; Varga and Pesti, 1982). The interest of these investigators stems from frequent epizootics of fatal enteropathy in the commercial rabbitries of Europe, many of which are associated with enteropathogenic *E. coli* (Camguilhem and Milon, 1989; Milon et al., 1990; Peeters et al., 1984c). These epizootics remain a problem, particularly in European rabbitries. This, coupled with the fact that the rabbit disease is useful as a model for human *E. coli* infection, has generated much research interest.

Etiologic Agent

E. coli is a facultatively anaerobic Gram-negative bacillus, which is either motile by peritrichous flagella or non-motile. Most strains ferment glucose and other carbohydrates; the ability to ferment lactose is an important distinguishing characteristic of *E. coli* and closely related species. In addition to flagella, many strains have pili, which function as adhesive organelles. *E. coli* are serogrouped according to the presence or absence of specific somatic (O) and flagellar (H) antigens. Capsular (K) antigens may be used as well, but they are absent from a high percentage of strains (Ørskov, 1984). Another method of grouping *E. coli* is biotyping, which is based on carbohydrate fermentation patterns (Camguilhem and Milon, 1989; Okerman and Devriese, 1985; Peeters et al., 1988).

Most isolates of *E. coli* do not cause disease, but some strains possess plasmid- or bacteriophage-acquired virulence factors that render them pathogenic. At least five types of pathogenic *E. coli* can produce diarrheal

disease: enteropathogenic, enterotoxigenic, enterohemorrhagic, enteroinvasive, and enteroaggregative. A necrotoxic group has also been proposed (DebRoy and Maddox, 2001). In rabbits, enteropathogenic strains are by far the most important cause of *E. coli*-associated diarrhea (Okerman, 1987; Peeters et al., 1988). Enteropathogenic *E. coli* produce neither classical toxins nor invasion factors, depending instead on microvillus attachment and effacement for expression of pathogenicity (Moon et al., 1983). Other types of pathogenic *E. coli* do not typically cause disease, although an outbreak of bloody diarrhea in Dutch Belted rabbits was attributed to enterohemorrhagic *E. coli* (Garcia et al., 2002) (Figures 13.14 and 13.15). Further investigation found that 25% of Dutch Belted rabbits from a

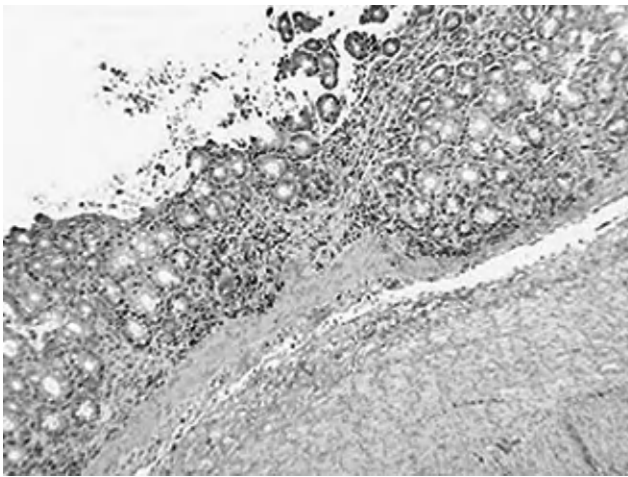


FIGURE 13.14 Enterohemorrhagic *E. coli* infection. Locally extensive erosion of cecal mucosa. (Courtesy of Dr. James G. Fox and Infectious Diseases Society of America)

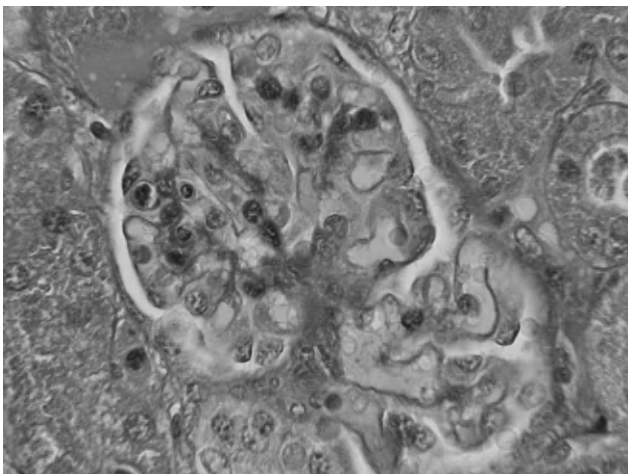


FIGURE 13.15 Enterohemorrhagic *E. coli* infection. Thrombi in glomerular capillaries exhibit positive staining for fibrin. Carstairs's stain. (Courtesy of Dr. James G. Fox and Infectious Diseases Society of America)

commercial vendor carried the organism (Garcia and Fox, 2003). The authors experimentally reproduced intestinal and renal lesions in Dutch Belted rabbits following oral inoculation with enterohemorrhagic *E. coli* or intravenous infusion with Shiga toxin (Garcia et al., 2006, 2008). Necrotoxic *E. coli* have also been reported in rabbits, but this group appears to be an infrequent cause of disease (Ansuini et al., 1994).

Many serotypes of enteropathogenic *E. coli* have been associated with rabbit diarrhea. Serotype O109:H2 is mainly isolated from suckling rabbits, whereas other serotypes (O15:H-, O103:H2, O20:H7, O109:H2; O128:H2, O132:H2, O153, and others) are isolated from weanlings (Cantey and Blake, 1977; Peeters, 1984c; Penteado et al., 2002; Prescott, 1978; Varga and Pesti, 1982). The most pathogenic, and perhaps the most common, serotypes from weanlings are O15:H- and O103:H2; small numbers of these can induce diarrhea and result in over 50% mortality (Peeters et al., 1988). Serotype O103:H2 is particularly prevalent in European rabbits, with animals of 4–5 weeks of age most susceptible to infection (Fiederling et al., 1997; Licois et al., 1992).

Biotyping can be used as an alternative to serotyping for characterizing rabbit isolates of enteropathogenic *E. coli*. Biotyping is based on carbohydrate fermentation patterns, so it can readily be performed by most laboratories. A method for biotyping has been described by Okerman and Devriese (1985), with modifications proposed by Peeters et al. (1988) and Camguilhem and Milon (1989). There is good correlation between biotype and pathogenicity, so biotyping is a potentially useful pathologic and epidemiologic tool. For example, isolates from weanling rabbits with severe clinical signs are usually rhamnose-negative, whereas isolates from weanlings with mild signs are rhamnose-positive (Camguilhem and Milon, 1989; Fiederling et al., 1997). An important exception to this rule is serotype O15:H- (RDEC-1-like), which causes high mortality in weanlings yet is rhamnose-positive.

Studies of the genetics of enteropathogenic *E. coli* have contributed enormously to an understanding of disease pathogenesis. The genome sequence of enteropathogenic *E. coli* has been reported and will facilitate future work to identify and characterize unique virulence determinants (Iguchi et al., 2009; Rasko et al., 2008). Some of the main genes and protein gene products important for pathogenicity are described below.

Enteropathogenic *E. coli* has developed a sophisticated mechanism for initial attachment to epithelial cells. In the case of human isolates, attachment is mediated, at least in part, by bundle-forming pilus proteins (BFP), which are encoded by 14 genes located on a plasmid. Other proteins promoting colonization, including fimbriae or flagella, play a role as well (Chen and Frankel, 2005; Erdem et al., 2007). Fimbriae are

particularly important in rabbit isolates, which have not been shown to express BFP (Newton et al., 2004).

At least three different plasmid-encoded fimbrial adhesins have been reported in rabbit strains of enteropathogenic *E. coli*. These have been termed AF/R1, AF/R2, and Ral fimbriae and appear to play a role in host species specificity (Fiederling et al., 1997; Hart et al., 2009; Krejany et al., 2000; Pillien et al., 1996; Robins-Browne et al., 1994, 1994a). Any given isolate does not typically contain all three types of adhesins; the prevalence of each adhesin varies depending on geographical region and serotype (Dow et al., 2005; Penteado et al., 2002). AF/R1 and Ral are usually expressed on serotype O15:H-, whereas AF/R2 is expressed on serotype O103:H2.

In addition to plasmid-encoded fimbriae, long polar fimbriae that are encoded by O-islands on the bacterial chromosome have been identified in a rabbit strain designated 83/39 (Newton et al., 2004). Recently, in the same strain, a gene coding for yet another possible adhesive structure has been identified. The gene has been designated as *rpeA* (rabbit enteropathogenic *E. coli* plasmid encoded autotransporter) and appears to be related to serine protease adhesins from other Gram-negative pathogens (Leyton et al., 2007). Experiments with mutant strains lacking the ability to express the various types of fimbriae mentioned above suggest that fimbriae enhance virulence but are not essential for colonization and disease to occur; other virulence factors appear to be involved as well.

Once bacterial attachment occurs, enteropathogenic *E. coli* relies on effector proteins to disrupt host cellular functions (Dean and Kenny, 2009). The most well-known effector proteins are encoded by a segment of the genome called the locus of enterocyte effacement (LEE), although some are encoded outside this region. Those encoded by LEE include Tir, Map, EspA, EspB, EspF, EspG, EspH, and EspZ. The Esp designation refers to 'enteropathogenic *E. coli* secreted protein'. These proteins have multifunctional, cooperative, and redundant properties. They act in concert to interfere with phagocytosis, disrupt microvilli and tight junctions, induce pedestal formation by the host cell, and modulate inflammatory pathways. A study of the role of EspF from a rabbit isolate suggests that it plays a role in tight junction disruption and pedestal formation (Peralta-Ramirez et al., 2008). Regulation of LEE gene expression occurs via the LuxS quorum sensing (QS) signaling system (Zhu et al., 2007).

Proteins encoded by the LEE region appear to be important for pathogenicity in rabbits. For example, in a prototype rabbit isolate of serotype O103, insertional mutagenesis studies have shown that several proteins encoded by the LEE region are important for cytopathic effects in vitro and virulence in vivo (De Rycke et al.,

1997). More specific analyses of individual proteins have shown that EspA and EspB proteins are essential for virulence of rabbit isolates; mutations constructed in the genes coding for these proteins inhibit the formation of typical lesions in vivo (Abe et al., 1997, 1998).

Effector proteins are delivered directly into host epithelial cells by a type three secretion system (T3SS) that is encoded by 12 genes on the LEE segment of the genome. This type of system resembles a needle and syringe on the molecular level and is used by many enteric pathogens to inject the effector proteins into the host cell cytosol. A major constituent of the system is EspA protein, which forms hollow tubular structures through which effector proteins are translocated (Chen and Frankel, 2005).

Enteropathogenic *E. coli* uses an outer membrane protein known as intimin to induce enterocyte effacement and promote intimate bacterial attachment to the host cell. Intimin is encoded by a gene named *eae* (for *E. coli* attaching and effacing) located on the LEE region of the genome. Intimin is necessary for full virulence. The *eae* gene is present in most highly pathogenic strains isolated from rabbits (Blanco et al., 1996, 1997; Penteado et al., 2002; Pohl et al., 1993). The *eae* gene from rabbit strain RDEC-1 has been sequenced and shown to have a high degree of homology with other pathogenic bacteria that produce enterocyte effacement (Agin and Wolf, 1997; Agin et al., 1996).

Intimin promotes close attachment with the host cell by a unique mechanism, whereby the bacterium actually inserts a receptor for intimin into the host cell membrane via the type III secretion system. This receptor is known as Tir (translocated intimin receptor). The binding of intimin to Tir allows initiation of pathogenic effects while keeping the organism on the extracellular surface. Tir seems to have a particularly important role in initiation of host cell pedestal formation at the site of attachment (Chen and Frankel, 2005; DeVinney et al., 1999; Nougayrede et al., 2003; Oliver-Gonzalez et al., 2008). Both Tir and intimin have been shown to be essential for full pathogenicity in rabbit isolates belonging to serotype O103; *eae* and *tir* mutants survive in the rabbit intestinal tract but do not cause clinical disease (Marches et al., 2000).

Epizootiology

Rabbit colibacillosis has attracted more attention from European than American investigators. The disease may be more prevalent in Europe than in North America, but there are no specific data to support this view. Indeed, the disease may be present but largely unrecognized in American rabbit colonies.

Several different syndromes of colibacillosis have been identified, each of which is associated with

specific serotypes and biotypes of *E. coli* (Camguilhem and Milon, 1989; Okerman and Devriese, 1985; Peeters et al., 1988). These syndromes include: (1) neonatal diarrhea with high mortality, (2) weanling diarrhea with high mortality, and (3) weanling diarrhea with low mortality. Neonatal diarrhea is associated with serotype O109:H2. Weanling diarrhea with high mortality is usually caused by serotype O103:H2, which is rhamnose-negative, or by serotype O15:H- (RDEC-1-like), which is rhamnose-positive. Weanling diarrhea with low mortality is caused by serotypes O123, O128, O132, and others, most of which are rhamnose-positive.

The prevalence of the various serotypes of enteropathogenic *E. coli* depends on geographic location. In the United States serotype O15:H- predominates (Cantey and Blake, 1977). In France, serotype O103:H2 is isolated most often (Camguilhem and Milon, 1989; Fiederling et al., 1997), whereas in Belgium serotypes O15:H-, O128:H2, and O132:H2 are the most common (Peeters et al., 1988).

Clinical Manifestations

The clinical signs of colibacillosis are similar to those of other diarrheal diseases of rabbits. Colibacillosis can occur in two distinct age groups: 1–2-week-old sucklings and 4–6-week-old weanlings, the latter being most commonly affected. In suckling rabbits, clinical signs include severe yellowish diarrhea and high mortality, sometimes encompassing an entire litter. In weanlings, two types of enteropathy occur with quite different clinical signs. The first is characterized by profuse liquid diarrhea, dehydration, weight loss, and high mortality within 5–14 days. This type is caused by highly virulent strains such as O15:H- and O103:H2. The second type of enteropathy is characterized by mild diarrhea without dehydration, transient growth retardation, and minimal mortality. This type is caused by less virulent strains such as O123, O128, and O132 (Camguilhem and Milon, 1989; Okerman and Devriese, 1985; Peeters et al., 1988).

Pathology

Gross necropsy findings in colibacillosis include thickening of the wall of the ileum, cecum, and colon. Paintbrush hemorrhages are sometimes evident on the serosal surface, and mesenteric lymph nodes may be swollen. Cecal contents are watery and brown. Microscopically, “attaching and effacing” lesions of the intestine are characteristic. There is atrophy and fusion of microvilli in the affected portions of intestine. Epithelial cells are flattened and disorganized (effaced), and ulcers may be present, but the basement membrane is usually intact. Infiltration of the lamina propria by polymorphonuclear leukocytes and submucosal edema are common

findings. An important feature is the presence of large numbers of coliform bacilli intimately attached to the epithelium (Cantey and Blake, 1977; Coussement et al., 1984; Peeters et al., 1984b, 1988). There may be prominent adherence of organisms to the epithelial cells overlying Peyer’s patches, especially with the O15:H- serotype (Inman and Cantey, 1983, 1984).

Electron microscopic studies of experimental infections with serotype O103 reveal classic attaching and effacing lesions. Bacterial fimbriae can be identified within 24 hours of infection. At 48 hours, bacteria are adhered to microvilli overlying Peyer’s patches, the microvilli are disorganized, and intestinal mucus secretion appears increased. Interestingly, at this time fimbriae are no longer evident. At 72 hours, cuplike projections indicative of pedestal formation are apparent in the epithelial cells underlying attached bacteria (Heczko et al., 2000). The extent of early bacterial association with epithelium overlying Peyer’s patches may vary depending on the particular isolate and conditions of infection. For example, another electron microscopic study found that bacterial association with Peyer’s patches was less apparent with the O103 serotype than with the O15 serotype (Licois et al., 1991).

Pathogenesis

E. coli is present in low numbers in the intestine of healthy rabbits, yet it proliferates in diarrheic rabbits regardless of the primary etiologic agent. This may be due in part to an increase in cecal pH that accompanies most cases of diarrhea. In the normal rabbit cecum, at pH below 6.8, fatty acids exert an inhibitory effect on the growth of *E. coli* (Skrivanová and Marounek, 2007). This is particularly true prior to weaning, during which time medium-chain fatty acids (capric and caprylic acid) present in does’ milk appear to inhibit bacterial growth (Gallois et al., 2008; Skrivanová et al., 2009). This effect is evidently independent of specific antibody directed against *E. coli* (Gallois et al., 2007; Skrivanová et al., 2009). The loss of a source of these fatty acids at weaning, coupled with an increase in pH that occurs at the same time, means that the inhibitory effect of the remaining fatty acids is reduced and conditions become favorable for *E. coli* proliferation (Prohaszka, 1980; Prohaszka and Baron, 1981; Vetesi and Kutas, 1973).

The changes in intestinal environment that occur at weaning are necessary but not sufficient for disease to occur. In colibacillosis the key event in pathogenesis, without which disease does not occur, is attachment of enteropathogenic *E. coli* to the mucosal surface and subsequent effacement of epithelial cells. The ability to attach to and efface rabbit intestinal epithelium is possessed only by enteropathogenic serotypes of *E. coli*; isolates from healthy rabbits do not have this ability.

There are two groups of enteropathogenic serotypes, with tropisms for different age groups and intestinal compartments. Isolates from suckling rabbits attach to the entire length of the small and large intestine, whereas isolates from weanling rabbits attach only to the distal small intestine and large intestine. Cross-infection experiments have shown that weanling isolates are only weakly pathogenic for sucklings and vice versa (Peeters et al., 1984c, 1984d).

Differences in bacterial attachment that vary with animal age and intestinal location may be mediated in part by characteristics of intestinal mucins, some of which have been shown to inhibit bacterial attachment (Mack and Sherman, 1991; Mack and Blain-Nelson, 1995). Other factors that influence colonization include the presence of other bacterial species in the intestine. For example, segmented filamentous bacteria, a poorly characterized family of bacteria that colonize ileal Peyer's patches in a variety of species, appear to inhibit the colonization ability of enteropathogenic *E. coli*, which attach preferentially to the same site (Heczko et al., 2000a).

Mucosal adhesion of enteropathogenic *E. coli* has been studied extensively, especially for serotype O15:H- (strain RDEC-1), and the mechanism is similar for other serotypes. Serotype O15:H- adheres in a two-step process. Initial adhesion occurs at the tips of the microvilli overlying Peyer's patches (Von Moll and Cantey, 1997). This is mediated by fimbriae, designated AF/R1 (attaching factor/rabbit 1), which consist of subunits of molecular weight of about 19000 (Berendson et al., 1983). Ral fimbriae are probably involved as well (Krejany et al., 2000). Expression of AF/R1 is mediated by an 86-MDa plasmid (Wolf et al., 1988), and the gene has been cloned (Wolf and Boedeker, 1990). The second step in adhesion involves attachment of the organism to the cell membrane after effacement of microvilli. This event is independent of fimbriae expression, and can occur even when not preceded by the initial step. Therefore, fimbriae are not essential for pathogenicity; rather, they serve as an accessory virulence factor that promotes initial interaction of the organism with normal epithelial cells (Cantey et al., 1989; Wolf et al., 1988). This view is supported by the observation that there is a weak correlation between the ability of an isolate to adhere to epithelial cells in vitro and its ability to induce disease in vivo (Robins-Browne et al., 1994, 1994a).

Adhesion of highly pathogenic O103 strains has also been studied, and is mediated by the fimbrial protein designated AF/R2. AF/R2 is important for pathogenicity, but for strain RDEC-1 there are probably other factors involved as well (Milon et al., 1990; Okerman, 1987).

From the bacterial standpoint, the pathogenesis of infection following initial adhesion is governed by

the secretion of effector proteins via a type 3 secretion system, the details of which are described above (see Etiologic Agent, above). Binding occurs between intimin and the translocated bacterial receptor, Tir, and this produces the intimate attachment required for the effacement of microvilli and the formation of actin-rich epithelial cell pedestals. Comprehensive reviews of bacterial factors important in pathogenesis have been prepared by Chen and Frankel (2005) and Dean and Kenny (2009).

The development of diarrhea after intimate bacterial attachment is likely due to multiple perturbations of host cell function. The exact mechanisms are poorly characterized, but may include: (1) loss of microvilli with subsequent malabsorption; (2) alterations in electrolyte transport; (3) alteration in epithelial cell permeability by disruption of tight junction integrity; (4) production of proinflammatory cytokines (Chen and Frankel, 2005; Ramirez et al., 2005). Increased apoptosis is one recognized mechanism by which intestinal pathogens produce diarrheal disease, but conflicting results about the role of apoptosis have been reported in rabbits. Tsalie et al. (2006) reported an increase in epithelial cell apoptosis in the ileum, whereas Heczko et al. (2001) concluded that there was little evidence that rabbit isolates caused increased apoptosis.

Resistance of rabbits to strain RDEC-1 is probably mediated by antibodies to fimbriae or O antigen (Cantey et al., 1987; McQueen et al., 1992). High levels of anti-fimbrial IgA are produced by lymphoid cells in the intestinal mucosa, then transported to the liver and secreted into the bile. Surprisingly, antigen-specific lymphoid cells are most numerous in the duodenum and jejunum, whereas colonization is densest in the ileum, cecum, and colon. This may be due to migration of B lymphocytes from the distal intestine to more proximal sites (McQueen et al., 1992).

Diagnosis

A presumptive diagnosis of rabbit colibacillosis can be made by demonstration of bacterial attachment and effacement of intestinal epithelium. Although other lesions are often present as well, they are less specific and easily confused with lesions of enterotoxemia. A definitive diagnosis requires isolation of enteropathogenic *E. coli*. Enteropathogenic strains can be differentiated from non-pathogenic ones by serotyping, biotyping, or by PCR detection of the *eae* gene that codes for intimin. It is important to recognize that not all isolates of a given serotype or biotype are equally pathogenic. For example, there are non-pathogenic O103 strains as well as highly pathogenic ones.

A capture enzyme-linked immunosorbent assay (cELISA) has been developed for screening rabbitries for

the presence of enteropathogenic *E. coli* (Vandekerckhove et al., 2002). This assay uses monoclonal antibodies to intimin to “capture” antigen from a crude *E. coli* outer membrane protein preparation. The cELISA detects serum antibody specific for intimin.

Prevention and Therapy

Therapy of colibacillosis consists of supportive care and antibiotic administration. In severe cases treatment is likely to be unrewarding, but in cases of weanlings with mild diarrhea therapy is sometimes worthwhile. Rabbit isolates of *E. coli* are susceptible to a variety of antibiotics, including ampicillin, amoxicillin, gentamicin, and colistin (Milon et al., 1990). Empirically, it would seem prudent to select an antibiotic with minimal activity against Gram-positive bacteria, so as to interfere as little as possible with re-establishment of normal intestinal flora. In colibacillosis caused by serotype O15H-, chloramphenicol and neomycin have each been used successfully (Peeters et al., 1986b). Regardless of which antibiotic is chosen, severely affected animals should be culled and facilities thoroughly sanitized. In addition, high-fiber diets should be offered, both as a therapeutic measure and a preventive one.

Attempts have been made to use dietary administration of medium-chain fatty acids to protect weanling rabbits against *E. coli* infection. The rationale behind this strategy was the recognition that fatty acids present in does' milk appear to offer protection to suckling rabbits. Although these compounds are bacteriostatic in vitro, dietary administration of caprylic acid or triglycerides of medium-chain fatty acids did not affect the course of disease in rabbits that were challenged experimentally. Treated groups did, however, have fewer coliforms present in the intestine (Gallois et al., 2008; Skrivanová et al., 2008).

Several novel approaches have been evaluated for their ability to prevent, or reduce the severity of, enteropathogenic *E. coli* infections. These include dietary administration of vitamin E and orogastric administration of epidermal growth factor.

Vitamin E has been shown to have beneficial effects on the outcome of *E. coli* infections in some animal species, possibly by modulating the inflammatory response. In experimentally challenged rabbits, however, administration of vitamin E did not prevent colonization or clinical signs of disease, although it ameliorated somewhat the disruption of mucosal architecture and enhanced the inflammatory response (Tsalie et al., 2006).

Epidermal growth factor (EGF) causes diffuse lengthening of microvilli, so administration of it might be expected to protect against enteropathogenic *E. coli* infection. Indeed, daily orogastric administration

beginning 3 days prior to challenge was associated with inhibition of colonization, reduced microvillous injury, and absence of diarrhea (Buret et al., 1998).

There is no commercially available vaccine for rabbit colibacillosis, but several studies demonstrate that immunization can prevent the disease. In one study, an orally administered vaccine made from formalin-killed serotype O103 organisms protected weanlings against challenge with homologous live organisms (Camguilhem and Milon, 1990). At least four daily doses, however, were required to induce protection. In another study, oral administration of live non-pathogenic bacteria protected weanlings against challenge with heterologous pathogenic O103 organisms (Milon et al., 1992). The bacteria used for vaccination expressed one of the two factors important for the pathogenicity of O103 strains: either lipopolysaccharide or fimbriae. This approach to vaccination had the advantage of requiring only one dose.

Recent approaches to developing enteropathogenic *E. coli* vaccines have focused on the construction of live, but avirulent, mutants. Mutations have been generated in various genes known to be important in virulence, including *tir*, *espB*, and *ler* (Boullier et al., 2003; Stakenborg et al., 2006; Zhu et al., 2006, 2007). Vaccination with all of these attenuated mutants offers strong protection against homologous challenge, but protection against challenge with organisms of a different serotype is typically weak or absent.

SALMONELLOSIS

Background

Salmonellosis is an uncommon disease of rabbits, but it can produce epizootics of high morbidity and mortality. It is primarily a septicemic disease with a brief clinical course, but diarrhea and abortion may be observed in some animals. In rabbits, the disease is most often caused by *S. enterica* serotypes Typhimurium or Enteritidis, but other serotypes have been reported. Typhimurium and Enteritidis are also the most common serotypes associated with non-typhoidal salmonellosis in humans (Velge et al., 2005).

Although uncommon now, salmonellosis was prevalent in the early 1900s. Several authors reported explosive outbreaks of the disease that resulted in the deaths of large numbers of rabbits (Duthie and Mitchell, 1931; Grasovsky, 1939; Olney, 1928). In most cases there was rapid death from septicemia, but diarrhea was occasionally seen as well. An outbreak of salmonellosis affecting pregnant does was reported by Garofolo (1936). *S. enteritidis* was isolated from this outbreak, which was characterized by abortion and subsequent death.

In recent years salmonellosis has been reported infrequently, but it is not entirely a disease of the distant past. *S. typhimurium* was the cause of an outbreak of salmonellosis which began in breeding does and spread to young stock, eventually resulting in 50% mortality (Harwood, 1989). In another instance, *S. mbandaka* was isolated from rabbits that died peracutely after extensive experimental manipulation (Newcomer et al., 1984).

Etiologic Agent

Bacteria of the genus *Salmonella* are Gram-negative, facultatively anaerobic bacilli that are members of the family *Enterobacteriaceae*. Most isolates are motile by peritrichous flagella, produce gas from glucose, and can use citrate as a sole carbon source. Lactose is ordinarily not fermented; this is an important distinguishing characteristic of the genus.

Salmonellae possess three major antigens: H or flagellar antigen, O or somatic antigen, and Vi or capsular antigen. These antigens are used to classify members of the genus into over 2000 serologically distinct types, each of which is designated by a name, as if it were a separate species. This is done for diagnostic and epidemiologic purposes and does not reflect the true taxonomic relationships among the organisms.

The genus *Salmonella* was at one time viewed as encompassing many different species. With the advent of DNA hybridization analysis, however, it became apparent that there was a high degree of homology among the species (Le Minor, 1984). Therefore, in the late 1990s salmonella taxonomy was revised, such that the members of the genus are assigned to only two species: *S. enterica* and *S. bongori*. *S. enterica* is responsible for most infections of warm-blooded vertebrates and is divided into six subspecies: *enterica*, *salamae*, *arizonae*, *diarizonae*, *indica*, and *houtenae*. Most commonly isolated human and animal pathogens belong to subspecies *enterica*, and this is true of the organisms formerly known as *S. typhimurium* and *S. enteritidis*. They are now commonly known as *S. enterica* serotype Typhimurium and *S. enterica* serotype Enteritidis. Subspecies other than *enterica* are usually isolated from the environment or from cold-blooded animals (Uzzau et al., 2000).

Epizootiology

Salmonellosis is usually transmitted by ingestion of contaminated food. Transmission can also occur by way of contaminated water, bedding, and cages, as well as by direct contact with infected animals. Contamination occurs when organisms are shed in the feces of carriers or clinically ill animals. There is a wide animal host range for salmonellae, but poultry and wild rodents are especially likely to harbor the organism and transmit it

to susceptible animals. One outbreak of salmonellosis in rabbits was attributed to feeding of contaminated chicken eggs (Olney, 1928). Another occurred in a rabbitry in close proximity to an area where chickens were housed, and the outbreak was eventually attributed to feeding contaminated hay (Harwood, 1989).

Rabbits are not common reservoirs of *Salmonella*, but they sometimes carry the organism. One survey conducted on a group of *Pasteurella* and *Eimeria*-free rabbits demonstrated that 38% were carriers of *S. mbandaka* (Newcomer et al., 1983). It is therefore important to consider rabbits as a potential source of salmonellae in an outbreak.

Martin et al. (2000) proposed that rabbits exhibit seasonal variation in susceptibility to *S. enterica* serotype Typhimurium infection, with the nadir of susceptibility occurring in the mid-summer. This conclusion was based on experimental studies that demonstrated a decrease in invasiveness for rabbit terminal ileum during the months of June and July and a maximum in the winter months.

Clinical Manifestations

Salmonellosis is frequently a peracute disease, and clinical signs are not observed. Sometimes fever, anorexia, and depression are noticed prior to death. This pattern, typical of septicemia, was observed in a study in which rabbits were experimentally infected with wild-type *S. enterica* serotype Typhimurium (Boedeker et al., 1995). In another experimental infection study, however, the same serotype produced primarily diarrheal disease (Hanes et al., 2001). The difference may be due to factors such as inoculum size, host susceptibility, and bacterial expression of virulence factors under the conditions of the experiment.

In spontaneous salmonellosis diarrhea has been reported, but it is an inconsistent finding (Ghosh and Chatterjee, 1960; Grasovsky, 1939; Iyer and Uppal, 1956). If the respiratory tract is involved, dyspnea and cyanosis may be observed (Newcomer et al., 1984). In pregnant does, abortion and endometritis are important clinical signs and may be the first indication of an outbreak (Garofolo, 1936; Harwood, 1989).

Pathology

Peracute salmonellosis is characterized by lesions compatible with septicemia, which include vascular congestion of many organs and diffusely distributed petechial hemorrhages. In acute cases, foci of necrosis surrounded by polymorphonuclear leukocytes are sometimes present in liver, kidney, spleen, and myocardium. Lymph nodes and gut-associated lymphoid tissue may be edematous and contain similar

foci of necrosis. Lesions of the respiratory system are prominent in some cases of salmonellosis (Duthie and Mitchell, 1931; Newcomer et al., 1984; Onyekaba, 1985). Congestion, edema, focal hemorrhages, and atelectasis occur early in the disease and progress rapidly to terminal suppurative bronchopneumonia. In pregnant does, especially in late gestation, there may be evidence of abortion or fetal death, together with suppurative necrotizing metritis (Harwood, 1989).

Pathogenesis

A great deal is known about the pathogenesis of *Salmonella* infections, but because the disease is uncommon in rabbits only a brief overview will be presented here. For a detailed discussion, refer to the excellent review articles by McGhie et al. (2009) and Haraga et al. (2008).

Salmonellae enter the host either by oral ingestion of contaminated food or water, or by direct contact with a carrier. Salmonellae can survive the low pH of the stomach and readily gain access to the small intestine, where they traverse the intestinal mucous layer and attach to the apical surface of epithelial cells. Salmonellae have multiple mechanisms for crossing the intestinal barrier, but they preferentially enter antigen-sampling M cells overlying Peyer's patches and are transported to the underlying lymphoid cells. They may also be taken up by CD18-positive phagocytes that penetrate the epithelial cell layer or, alternatively, gain direct entry into ordinary intestinal epithelial cells by bacterial-mediated endocytosis. Once the bacteria are intracellular, they proliferate within phagosomes termed *Salmonella*-containing vacuoles. The ability to survive within these vacuoles is critical for virulence and is mediated by over 30 specific bacterial effector proteins, which essentially hijack the host cell to create a milieu conducive to bacterial replication. Major functions include modulation of host cell signal transduction and membrane trafficking, as well as production of pro-inflammatory cytokines. Effector proteins are delivered to the host cell cytoplasm by two type 3 secretion systems that resemble those found in *E. coli* and other Gram-negative pathogens. Other bacterial virulence factors include toxins and lipopolysaccharide (Chitra et al., 2002; Giannella et al., 1973; Martin et al., 2000).

Salmonellae often persist in the gastrointestinal tract for long periods, producing either gastroenteritis or a chronic asymptomatic carrier state. Organisms may spread to regional lymph nodes and other organs, especially if they gain initial entry via CD18-positive phagocytes, but in most hosts they are cleared by the monocyte-macrophage system before disease develops. If the host is compromised or if the organism is especially virulent, clearance mechanisms fail and systemic

disease occurs (Giannella, 1991). The importance of stress and immunosuppression in salmonellosis is demonstrated by a study in which prednisone, either alone or together with minor surgery, precipitated peracute disease in carrier animals (Newcomer et al., 1984).

Diagnosis

Diagnosis of salmonellosis in rabbits depends on isolation and identification of the etiologic agent. Special procedures are usually required for isolation of *Salmonella*, especially when samples contain a mixed bacterial flora. Samples should be inoculated into liquid enrichment media, such as selenite or tetrathionate broth. After incubation, the enrichment culture can be plated on moderately selective media such as MacConkey's or desoxycholate citrate agar, or on highly selective media such as SS agar, brilliant green agar, or Hektoen medium. These media contain lactose and a pH indicator. Since a distinguishing feature of *Salmonella* is its inability to ferment lactose, suspect colonies can be selected by color. These can then be differentiated from related species by biochemical tests (LeMinor, 1984).

Prevention and Therapy

Treatment of active salmonellosis has been attempted rarely in rabbits, and is not recommended due to the zoonotic potential of the disease and the possibility of survivors becoming inapparent carriers. In the event that the disease is diagnosed, animals in contact with infected rabbits should be bacteriologically monitored to detect carriers. Control of salmonellosis depends on eliminating potential sources of exposure, such as poor-quality feed and wild rodents.

If treatment is required, chloramphenicol is ordinarily the drug of choice and the animals should be housed in biocontainment facilities. This approach is complicated, however, by the emergence in the 1990s of multiple-antibiotic-resistant strains, which are especially common in serotype Typhimurium. These strains are resistant to chloramphenicol, ampicillin, streptomycin, sulfonamides, and tetracycline (Velge et al., 2005).

STAPHYLOCOCCOSIS

Background

Staphylococcosis is a common disease of rabbits caused by *Staphylococcus aureus*. It is characterized by suppurative inflammation of any organ, but the skin and subcutaneous tissues are most frequently involved. It can also be manifested as fatal septicemia. The

susceptibility of rabbits to staphylococci has made the species valuable as an experimental model for orthopedic and ophthalmic infections, but this review will concentrate on spontaneous rather than experimentally induced disease.

Several investigators recognized rabbit staphylococcosis in the early 1900s. In 1945, Lesbouyries (Lesbouyries, 1945) reviewed what was known about the disease and described subcutaneous and mammary gland abscesses, as well as septicemia. Hagen (1963) described similar lesions associated with disseminated disease in 3–5-day-old rabbits. Affected animals had widespread subcutaneous abscesses, as well as abscesses in the lungs and heart. Disseminated disease in adult animals was reported by Snyder et al. (1976), who observed not only subcutaneous abscesses and visceral abscesses, but also conjunctivitis, rhinitis, and fibrinous pneumonia.

Staphylococcosis is a major problem in some European rabbitries, but appears to be less common in the United States. The reason for this is unknown, but it may be that the most virulent strains have a geographic distribution concentrated in Europe.

Etiologic Agent

There are at least 20 species of *Staphylococcus*, most of which are non-pathogenic. They are common inhabitants of skin and mucous membranes and many species prefer certain habitats, such as the head, auditory canal, axilla, or perineum. Even pathogenic staphylococci can reside on epithelial surfaces without causing disease; *S. aureus* is a common inhabitant of the upper respiratory tract of humans and other species, including rabbits (Renquist and Soave, 1969).

Staphylococci are unencapsulated Gram-positive cocci that occur singly, in short chains, or in irregular grape-like clusters. They are non-motile, non-sporeforming, and catalase-positive. *S. aureus* is the primary pathogenic species and the only one which has been recovered from rabbits. It can be distinguished from non-pathogenic staphylococci by a positive coagulase test, which measures ability to coagulate plasma. Most isolates of *S. aureus* have hemolytic activity as well. A few isolates have the ability to produce capsules; these tend to be more virulent than ordinary strains.

S. aureus can be categorized into biotypes based on biochemical properties and phage types based on bacteriophage susceptibility. This is done primarily for epizootiologic purposes. Most animal species, including rabbits, tend to carry unique bio- and phage types of *S. aureus* that are distinct from those of other species. Although biotypes and phage types tend to be host-species specific, it is not always the case.

Both low- and high-virulence strains of *S. aureus* can be isolated from rabbits, and bio- and phage typing can

be used to characterize each type of isolate. The low- vs. high-virulence distinction is particularly significant in European rabbitries, where low-virulence strains cause relatively minor lesions in small numbers of animals in some colonies, and high-virulence strains cause epizootic disease and widespread losses in others (Hermans et al., 2003).

Low-virulence strains belong to biotypes usually associated with humans or poultry. High-virulence strains belong to a biotype designated “mixed CV-C”, which differs from the low-virulence biotypes by the ability to produce beta hemolysin. Not all mixed CV-C isolates are equally virulent, however, so within the mixed CV-C biotype it is necessary to distinguish among strains of differing virulence. Phage susceptibility typing can do this. Virulent isolates are usually phage type 3A/3C/55/71, so the complete biotype-phage type designation is mixed CV-C 3A/3C/55/71 (Hermans et al., 2003).

Genotype analysis has also been used to characterize low- and high-virulence strains. One useful technique is randomly amplified polymorphic DNA (RAPD) analysis, which involves performing polymerase chain reaction with a single short oligonucleotide primer of random sequence. RAPD analysis reveals specific banding patterns that can be used to distinguish between high- and low-virulence strains (Hermans et al., 2000a, 2001a).

Some genotypic studies have focused on the presence of specific virulence-associated genes in high- vs. low-virulence strains. Of particular interest are genes that code for bacterial adhesins and extracellular toxins. Regarding adhesins, one study showed that virulent, but not avirulent, strains typically harbor the *bbp* gene, which encodes for bone sialoprotein binding protein and appears to mediate bacterial adhesion to host cells (Vancraeynest et al., 2004). Regarding toxins, another study demonstrated that virulent strains almost always possess the enterotoxin gene cluster (*egc*), which mediates production of an extracellular toxin (Vancraeynest et al., 2006a).

Epizootiology

In rabbit colonies, staphylococcosis typically presents in one of two ways – either as a sporadic syndrome affecting only small numbers of animals, or as a widespread epizootic associated with large losses. Sporadic cases may be caused by several avirulent biotypes, but when an epizootic of staphylococcosis occurs, one virulent biotype-phage type is usually responsible (mixed CV-C, 3A/3C/55/71). This type is common in Europe, and genotype analysis of many different isolates suggests a clonal origin with subsequent international dissemination (Vancraeynest et al., 2006).

Biotypes associated with clinical disease can often be isolated from the nasopharynx of asymptomatic animals, suggesting that carriers can serve as a reservoir of infection (Lenz et al., 1978). In European rabbitries suffering epizootics of staphylococcosis, as many as 90% of the animals may be carriers. In carriers, staphylococci can be recovered from many anatomical sites, but ear and perineum appear to be colonized most intensely (Hermans et al., 1999).

Even though distinct biotypes of *S. aureus* predominate in different animal species, it is still possible for interspecies transmission to occur. Isolates from humans, dogs, and cattle can infect rabbits under experimental conditions (Adekeye, 1980). The potential for transmission between humans and rabbits is suggested by studies that identified the same biotype in infected animals and apparently healthy personnel (Lenz et al., 1978; Renquist and Soave, 1969). Meticillin-resistant *S. aureus* strains are being identified with increasing frequency worldwide, and there is evidence that these strains can be transmitted among humans and various animal species, including rabbits (Morgan, 2008; Walther et al., 2008).

Clinical Manifestations

Healthy rabbits frequently carry *S. aureus* as a part of the flora of the nasopharynx, conjunctiva, and skin. When disease is present, the signs may be suggestive of pasteurellosis and include pneumonia, rhinitis, conjunctivitis, and otitis media. But more commonly, subcutaneous abscesses, pododermatitis, or mastitis are observed (Hermans et al., 2003). A common clinical presentation of staphylococcosis is infection of the mammary glands, which can occur either as localized abscesses or disseminated, gangrenous mastitis (Adlam et al., 1976; Carolan, 1986; Millichamp and Collins, 1986; Okerman et al., 1984; Snyder et al., 1976). Septicemia can also occur, sometimes accompanied by disseminated abscesses in parenchymatous organs. The milk of does with mastitis may act as a vehicle for transmission of *S. aureus* to nursing offspring, resulting in septicemia and death of the nurslings (Krishna and Kaushal, 1988).

Pathology

Staphylococcosis is characterized by suppurative inflammation, which is indistinguishable from that caused by other pyogenic bacteria, including *P. multocida*. The skin, subcutis, mammary gland, and respiratory system are most commonly involved, but if septicemia occurs, lesions may develop in any internal organ. In acute lesions, there is a diffuse infiltrate of neutrophils accompanied by edema, hemorrhage, and fibrin deposition. In subacute or chronic lesions,

inflammation is more localized and abscesses develop. Abscesses contain viscous, creamy exudate composed mostly of neutrophils and necrotic cell debris. In long-standing cases they may be surrounded by a fibrous capsule.

Pathogenesis

Because *S. aureus* is part of the resident flora of healthy laboratory rabbits, it is sometimes considered an opportunistic pathogen. Disease is likely to occur when an appropriate portal of entry, such as a skin wound, is available. Host resistance and bacterial virulence govern the severity of disease. Regarding host resistance, rabbits as a species are unusually susceptible to systemic infection with *S. aureus*, and stress increases the susceptibility of individual animals (Snyder et al., 1976).

The virulence of various *S. aureus* strains depends on their ability to adhere to host cells, produce biofilms, and elaborate extracellular protein toxins. In rabbits, the role of biofilm production is unclear, but the ability of organisms to adhere to host cells is clearly important. Bacterial colonization, which is critical for virulence, is facilitated by production of adhesion proteins such as bone sialoprotein binding protein, the product of the *bbp* gene. The *bbp* gene is produced by most high-virulence strains (Hermans et al., 2000, 2000a; Vancraeynest et al., 2004).

Extracellular toxins are also important for virulence, and *S. aureus* produces several types, including exfoliative toxins, leukotoxins (such as hemolysins and leukocidins), and superantigens (enterotoxins and similar molecules with immunomodulatory capability). Leukotoxins and superantigens appear to be more important in rabbit infections than exfoliative toxins (Vancraeynest et al., 2006a). At least three leukotoxins are lethal for rabbit neutrophils: alpha toxin, delta toxin, and Panton-Valentine leukocidin (Adlam et al., 1980). Virulent strains secrete superantigens as well, typically possessing the enterotoxin gene cluster (*egc*), which mediates production of an extracellular toxin with superantigen properties (Vancraeynest et al., 2006a).

In addition to toxins, a variety of enzymes such as coagulase, lipase, nuclease, and hyaluronidase may be produced, as well as protein A, which non-specifically binds the Fc portion of IgG. IgG binding inhibits opsonization and phagocytosis and blocks complement-mediated bactericidal mechanisms (Cohen, 1991). A study of *S. aureus* isolates from Spanish rabbitries showed that strains associated with lesions typically carry genes for coagulase and protein A, as well as clumping factor B (Viana et al., 2007). Another study compared the presence of extracellular secreted antigens between virulent and avirulent strains. Virulent

strains produced two specific proteins, of approximately 29 and 27 kDa, that were not produced by avirulent strains (Hermans et al., 2001, 2001a). Whether these proteins represented some of the enzymes or extracellular toxins mentioned above is uncertain.

An in vivo rabbit model that could be useful in further studies of the pathogenesis of high- and low-virulence infections has been described by Meulemans et al. (2007). The model involves scarification and inoculation of the flank, with subsequent development of abscesses, the severity of which is proportional to the virulence of the isolate. This model could also be useful in evaluating potential vaccine candidates.

Diagnosis

Diagnosis of staphylococcosis depends on isolation and identification of the organism. Culture is essential for reliable differentiation of staphylococcosis from pasteurellosis. Gram-positive cocci that ferment mannitol and produce coagulase can be presumptively identified as *S. aureus* (Snyder et al., 1976).

A multiplex polymerase chain reaction (PCR) assay for rapid identification of high-virulence isolates has been described (Vancraeynest et al., 2007). The test relies on detection of two genes: the *bbp* gene, which is important for adhesion, and the *selm* gene, which is part of the *egc* gene cluster responsible for production of enterotoxin genes with superantigen activity.

Prevention and Therapy

Staphylococcosis should be treated locally and systemically with antibiotics. In vitro antibiotic sensitivity testing should be performed to help select the most effective antibiotic. Penicillin is the antibiotic of choice for penicillin-sensitive isolates. Alternatively, enrofloxacin is almost always effective (Vancraeynest et al., 2004a). Other choices for penicillin-resistant organisms include cephalosporins, aminoglycosides, or chloramphenicol. Antibiotic resistance is rare in rabbit isolates, but aminoglycoside- and meticillin-resistant strains have been reported and may have originated in, or be a threat to, human populations (Goni et al., 2004).

Despite the susceptibility of rabbit isolates to many antibiotics, antimicrobial therapy appears incapable of eliminating infections from rabbit colonies (Hermans et al., 2003; Vancraeynest et al., 2004a). This is a particular problem for colonies infected with a high-virulence strain. Administration of tetracycline in the food, accompanied by cleaning and disinfection of cages and equipment, may reduce the severity of disease temporarily but will not eliminate it. Probably the only way to eliminate infection is to depopulate the colony, disinfect the premises, and start over.

All animals introduced into a new colony or added to a pre-existing colony should be screened to make sure they do not carry a virulent strain. This can be done by culture of multiple sites on new animals, followed by evaluation of any *S. aureus* isolates by biotyping-phage typing, or by genomic analysis. Genomic analysis is simpler, and either RAPD typing or multiplex PCR typing can be used to differentiate virulent from avirulent isolates (Hermans et al., 2003; Vancraeynest et al., 2007).

Attempts have been made at immunizing rabbits against staphylococcosis, using either killed bacteria or purified toxins. Most of the attempts have been unsuccessful. In one trial, vaccination with killed bacteria protected against intradermal challenge with living organisms, but there was no correlation between degree of protection and titer of serum antibodies (Cameron, 1971). In another trial, a killed bacterial vaccine was ineffective in preventing mastitis (Adlam et al., 1976). One report describes the use of an autogenous bacterin to treat staphylococcal conjunctivitis in rabbits (Hinton, 1977). Clinical improvement occurred, but the number of rabbits was small and some also received antibiotic therapy. As an alternative to killed bacteria, immunization with purified staphylococcal toxins has been tried. These preparations evoked significant antibody titers, but they were largely ineffective in preventing mastitis (Adlam et al., 1977, 1980). Limited studies on rabbit vaccine development have occurred since the 1980s, and it is noteworthy that successful immunization against staphylococcal infections has been problematic, regardless of the species.

TREPONEMATOSIS

Background

Treponematosis is a disease of domestic and wild rabbits caused by *Treponema paraluiscuniculi* (formerly *T. cuniculi*), a spirochete similar to *T. pallidum*, the causative agent of human syphilis. The disease is characterized by inflammatory lesions of the genitalia. The face and regional lymph nodes are sometimes involved as well. Treponematosis has many synonyms, including venereal spirochetosis, treponemiasis, and rabbit syphilis.

Treponematosis was first reported by Ross (1912), who observed spiral-shaped organisms in the blood of rabbits that had inflammatory lesions of the genitalia and mouth. Bayon (1913) isolated and described the causative organism and passed the disease successfully in rabbits. He questioned the validity of much of the syphilis research that had been done previously on rabbits, because they were not known to be free

of the naturally occurring infection. Treponematoses was studied in more detail by [Noguchi \(1921, 1922\)](#), who proposed the name *Treponema cuniculi* for the causative organism and noted its striking morphologic resemblance to *T. pallidum*. [Levaditi et al. \(1921a\)](#) documented the venereal mode of transmission of the disease and described the histologic changes in affected tissues.

Because of the resemblance between *T. paraluiscuniculi* and *T. pallidum*, some investigators thought that the two organisms might be identical. This question was resolved when attempts to infect humans with *T. paraluiscuniculi* failed. [Levaditi et al. \(1921b\)](#) believed that the organisms were distinct, so they inoculated themselves with *T. paraluiscuniculi* in the hope that an asymptomatic infection would occur that would convey protection against *T. pallidum*. Infection did not occur and antibodies were not detected, but if the outcome had been different a vaccine against syphilis might have resulted. The organisms are closely related serologically, and rabbits infected with *T. paraluiscuniculi* are substantially but not completely resistant to challenge with *T. pallidum* ([Graves, 1980](#)).

By the 1930s rabbit treponematoses had been reported in many countries, including England ([Adams et al., 1928](#)), Belgium ([Bessemans et al., 1928](#)), Russia ([Fried and Orlov, 1932](#)), and the United States ([Noguchi, 1922](#)). The disease is known to occur not only in domestic rabbits, but also in wild lagomorphs, including hares ([Horvath et al., 1980](#)). Although widespread, treponematoses is not ubiquitous. For example, the disease is virtually non-existent in some wild rabbit populations in Australia ([Graves et al., 1980](#)).

Etiologic Agent

Rabbit treponematoses is caused by *T. paraluiscuniculi*, which is a slender, spiral-shaped, microaerophilic, Gram-negative organism closely related to *T. pallidum*, the cause of human syphilis ([Figure 13.16](#)). The genus *Treponema* contains pathogenic and non-pathogenic species. The non-pathogens are part of the normal flora and are especially prominent in the oral cavity (although *T. denticola* may play a role in periodontal disease). The pathogens include *T. pallidum* (which has several subspecies), *T. carateum*, and *T. paraluiscuniculi*, none of which can be cultured continuously in vitro. Organisms must be propagated in vivo, with inoculation of rabbit testis being the most common method. It is generally agreed that the pathogenic treponemes are indistinguishable on the basis of morphology, protein content, or physiology ([Strouhal et al., 2007](#)). They all are approximately 6–15 µm long and 0.1–0.2 µm wide with tapered ends and exhibit characteristic motility, consisting of longitudinal rotation and flexion ([Fitzgerald, 1985](#)). Nevertheless, one author asserted that *T. paraluiscuniculi*



FIGURE 13.16 Treponematoses: erosions on vulva.

can be differentiated from *T. pallidum*, the former being thicker and with blunt rather than pointed ends ([Warthin et al., 1923](#)).

The high degree of relatedness among pathogenic treponemes extends to the immune responses that they generate in the host. Antibodies produced in response to infection with *T. paraluiscuniculi* cross-react with *T. pallidum* and vice versa. This makes serologic differentiation difficult, but it means that serologic tests for *T. pallidum* can be used for detection of *T. paraluiscuniculi* infection in rabbits.

Even though antibody produced in response to infection with one *Treponema* species, subspecies, or strain cross-reacts strongly with heterologous organisms, that antibody does not protect against heterologous challenge. Protection against homologous challenge is complete, however. The reason for this is unclear, but the observation suggests that protective antigens must exhibit diversity among species, subspecies, or strains. A key protective antigen was at one time thought to be a strongly immunogenic 15-kDa lipoprotein produced by all pathogenic *Treponema*. Doubt was cast upon this hypothesis when, rather than exhibiting the antigenic diversity necessary to explain lack of cross-protection, the protein proved to be virtually identical for all isolates. The gene coding for the protein also showed only minor differences among isolates, and these were limited to the flanking sequences ([Centurion-Lara et al., 1997, 1998](#)). Conclusive evidence that the protein was not protective was obtained when immunization of rabbits failed to alter the course of experimental challenge or induce production of opsonic antibody. The 15-kDa lipoprotein is still thought to have a role in pathogenesis, however, possibly mediating early inflammatory changes that occur during infection ([Centurion-Lara et al., 1997](#)).

Although traditional methods largely have failed to discriminate among pathogenic treponemes, comparative genomic methods have been used with somewhat more success. The complete genome sequence of *T. pallidum* has been elucidated and shown to be relatively small, suggesting that the organism depends on the host for its metabolic requirements. Within the genome, a family of repeat genes, the *tpr* genes, may be important in virulence by encoding proteins that mediate attachment to host tissues (Peeling and Hook, 2006). This gene family has been extensively studied and has shed light on potential virulence mechanisms and on the evolutionary relationships among members of the genus (Gray et al., 2006).

The *tpr* genes have been classified into subfamilies designated I, II, and III, all of which appear fully functional in *T. pallidum*. This is in contrast to *T. paraluiscuniculi*, in which subfamilies I and II are rendered inactive by a frameshift caused by an extra guanosine in the coding sequence. This means that only subfamily III is fully active. Within this subfamily, the *tprK* locus is of interest because it is immunogenic and appears to have an ability to undergo antigenic variation. The latter property may be relevant to successful host adaptation (Giacani et al., 2004).

Another gene that has received attention is the acid repeat protein gene (*arp*). This gene has been proposed as a component of a typing system for pathogenic treponemes. The number of base pair repeats in *arp* is variable among isolates and this is the basis for its potential for its use in a typing system. *T. paraluiscuniculi* and *T. pallidum* subspecies *pallidum* are both sexually transmitted pathogens and share a high number of repeats with diverse motifs in the *arp* region. This is in contrast with non-sexually transmitted strains. Thus, the *arp* gene can be used to help differentiate venereal from non-venereal isolates (Harper et al., 2008).

The entire genome of *T. paraluiscuniculi* has been compared to that of *T. pallidum* by DNA microarray hybridization, whole genome fingerprinting, and DNA sequencing (Strouhal et al., 2007). There was a high degree of sequence similarity, with the most significant differences in the *tpr* loci, which is consistent with the observations of Giacani et al. (2004). The differences at these loci suggest that they may represent determinants that govern host species specificity.

Epizootiology

Clinical treponematosis is uncommon in modern, well-managed rabbit colonies. When the disease was first recognized in the early 1900s, as many as 30% of animals had clinical signs (Adams et al., 1928; Fried and Orlov, 1932; Noguchi, 1922). More recently, the prevalence rate was estimated at well under 10% in

enzootically infected colonies (Cunliffe-Beamer and Fox, 1981a). Although clinical signs are infrequent, serologic evidence indicates that subclinical infections are common. Screening of apparently healthy rabbits suggests that 15–25% are infected (Clark, 1970; Pannu et al., 1967). One survey of a single colony found serological evidence of infection in virtually all experienced breeders. Clinical disease in this colony was almost non-existent (DiGiacomo et al., 1983b).

T. paraluiscuniculi is usually transmitted horizontally during breeding. Infection is unusual in virgin rabbits and prevalence increases with age and length of time in a breeding program. The incidence in males and females is roughly comparable (Cunliffe-Beamer and Fox, 1981b; DiGiacomo et al., 1983b, 1984).

Although the major mode of *T. paraluiscuniculi* transmission is venereal, the occasional presence of infection in non-breeding rabbits suggests that the organism can also be transmitted by other routes (Cunliffe-Beamer and Fox, 1981b; Fried and Orlov, 1932; Gaertner and Barthold, 1989). Vertical transmission may be responsible for some cases of treponematosis and may help perpetuate infection in a colony. Although transplacental transmission has not been documented, cross-fostering experiments have shown that infection can occur at birth or during the nursing period (Cunliffe-Beamer and Fox, 1981b). This might be expected to be an important means of transmission, but serologic data suggest that it actually happens infrequently (DiGiacomo et al., 1983b). This could be because neonatal rabbits are more resistant to infection than adults (Cunliffe-Beamer and Fox, 1981b).

Genetic factors appear to influence the susceptibility of rabbits to treponematosis. Cunliffe-Beamer and Fox (1981b) studied susceptibility and expression of the disease in several inbred strains of rabbits, and found significant differences between strains. The most severe disease occurred in X/J rabbits; lesions were common on the face as well as the genital area. Less severe disease occurred in other strains, including AC/J, ACEP/J, and III/J.

Clinical Manifestations

Lesions of treponematosis are usually restricted to vulvar or preputial epithelium and adjacent areas (Figure 13.16). The nostrils, eyelids, and mouth are sometimes involved as well, probably as a result of auto-infection (Figure 13.17). Surprisingly, facial lesions can occur without apparent genital involvement (Cunliffe-Beamer and Fox, 1981a; DiGiacomo et al., 1984; Gaertner and Barthold, 1989). Lesions begin near mucocutaneous junctions as areas of erythema and edema sometimes accompanied by minute papules or vesicles. They progress quickly to ulcers and finally to characteristic scales



FIGURE 13.17 Treponematoses: erosions adjacent to nares.

and crusts, which are the most frequently recognized evidence of infection. Lesions usually persist but sometimes resolve after several weeks (Cunliffe-Beamer and Fox, 1981a).

Pathology

Treponematoses ordinarily involves only the skin and mucous membranes. Occasionally, regional lymph nodes are involved, but systemic infections have not been described. Gross lesions of treponematoses are described above. Microscopic lesions consist of ulceration, hyperkeratosis, hyperplasia, and acanthosis of the epidermis. Ulcers are frequently overlaid by crusts composed of exudate, necrotic epithelial cells, and inflammatory cells. The dermis contains a prominent inflammatory cell infiltrate, consisting mainly of macrophages and plasma cells. In early lesions, epidermal microabscesses or vesicles are sometimes present (Adams et al., 1928; Cunliffe-Beamer and Fox, 1981a; Noguchi et al., 1922). Spirochetes can be demonstrated in the superficial dermis and epidermis by Warthin-Starry or modified Dieterle silver staining techniques (Gaertner and Barthold, 1989). Hyperplasia of regional lymph nodes has been reported to occur in some cases of treponematoses (Small and Newman, 1972).

Pathogenesis

T. paraluiscuniculi usually infects rabbits by penetration of intact or damaged mucous membranes (Cunliffe-Beamer and Fox, 1981b). Once infected, proliferation of the organism occurs locally. The incubation period is lengthy: lesions do not appear until 3–6 weeks after exposure, and another 5–6 weeks may be required for the development of a positive serologic test. Lesions can persist for long periods, over 20 weeks in many

rabbits. When lesions resolve in untreated animals, serologic tests often remain positive (Cunliffe-Beamer and Fox, 1981b). In contrast, rabbits treated with antibiotics experience a gradual decline or disappearance of detectable antibody (DiGiacomo et al., 1984).

The presence of antibody in rabbits without lesions suggests the possibility of inapparent infections. Regional lymph nodes may be involved in such infections, since they can harbor the organism for long periods in apparently healthy rabbits (Smith and Pesetsky, 1967). It is not known whether rabbits with infection restricted to the lymph nodes can subsequently develop overt cutaneous lesions.

As with other bacteria, pathogenesis depends on bacterial virulence and host response. Genes important in virulence include the *tpr* region of the genome, which is discussed in detail above. Host response has been studied with special emphasis on *T. pallidum* infections in humans, but the striking similarity between this species and *T. paraluiscuniculi* suggests that similar responses occur in rabbits.

Infection produces a strong humoral and cell-mediated immune response, but neither results in elimination of the organism from the host. Opsonizing antibody is produced against strain-specific antigens rather than antigens that are shared with other spirochetes (Shaffer et al., 1993). Of particular importance are opsonizing antibodies to the TprK protein, which is encoded by a segment of the *tpr* gene. Immunization with this protein offers partial protection against homologous challenge. Cellular immune responses involve both CD4+ and CD8+ cells, and cytokines such as IL-2 and IFN- γ have been detected in lesions (Peeling and Hook, 2006).

Diagnosis

Definitive diagnosis depends on demonstration of spirochetes in typical lesions. The direct fluorescent antibody test is the most sensitive and specific test when lesions are present (Ratnam, 2005; Wicher et al., 1999). Classically, the Warthin-Starry silver technique has been used to selectively stain the organisms in smears or tissue sections, but dark-field examination of wet mounts of lesion scrapings is a simpler method; the organism can be readily identified by its characteristic spiral morphology and corkscrew motility. Lesion scrapings can also be stained with Giemsa, but detection of the organisms is more difficult than by dark-field examination (Cunliffe-Beamer, 1981a).

A variety of serologic tests can be used to detect infected rabbits. All were developed for diagnosis of human syphilis, but the similarity between the causative organisms makes the tests applicable to rabbits. Two types of tests are available: “non-treponemal”

tests, such as the Venereal Disease Research Laboratory slide test (VDRL) and the Rapid Plasma Reagin card test (RPR), and “treponemal” tests, such as the fluorescent treponemal antibody absorption test (FTA-ABS) and the microhemagglutination *T. pallidum* test (MHA-TP). The non-treponemal tests are ordinarily used for screening, whereas the treponemal tests can be used for confirmation. Non-treponemal tests detect antilipid antibodies (traditionally referred to as reagins) produced in response to lipoidal antigens generated in treponemal disease. These tests may detect antilipid antibodies associated with other disease processes, so they are not highly specific. Treponemal tests, on the other hand, detect antibodies specific for treponemal antigens. Treponemal tests have not been used as widely as non-treponemal tests, in part because the inability to grow *T. pallidum* in vitro makes it difficult to obtain antigen (Bradford and Larsen, 1985). This problem has been circumvented by new treponemal tests that use recombinant treponemal antigens (Wicher et al., 1999).

The tests available for serodiagnosis of syphilis were compared by DiGiacomo et al. (1983). The FTA-ABS treponemal test was chosen as the benchmark test because of its recognized sensitivity and specificity. Of the two non-treponemal tests, the VDRL was more sensitive and the RPR more specific. Compared to the FTA-ABS, the overall sensitivity and specificity of the non-treponemal tests was 77% and 87% for the VDRL, and 55% and 100% for the RPR. The MHA-TP treponemal test agreed almost exactly with the FTA-ABS, with a sensitivity and specificity of 100% and 99%, respectively. Therefore, given that the MHA-TP is much simpler to perform than the FTA-ABS, the most suitable screening test for treponematosi is the microhemagglutination assay. If non-treponemal tests are selected, care must be used in the interpretation of results since false-positive reactions can occur, particularly with the VDRL (Tight et al., 1981).

A number of polymerase chain reaction-based methods have been developed for detection of *Treponema* in clinical samples. These tests are highly sensitive and specific, but are used mostly in research laboratories because of lack of standardization and commercial availability (Ratnam, 2005; Wicher et al., 1999). It is likely that standard protocols will be developed so that PCR tests will be readily available in the future.

Prevention and Therapy

Penicillin is the drug of choice for treatment of treponematosi. Three injections at 7-day intervals of a combination of benzathine and procaine penicillin should be given at doses of 42000–84000 IU/kg. Following treatment, lesions heal within 2 weeks and RPR and

VDRL titers decline markedly or disappear within several months. In contrast, FTA-ABS titers may persist for a year (Cunliffe-Beamer and Fox, 1981c; DiGiacomo et al., 1984). This treatment is not only effective for individual rabbits; it can also be used to eradicate treponematosi from a colony if all animals are treated (Cunliffe-Beamer and Fox, 1981c). In colonies that are free of infection, introduction of new breeding stock should be avoided. If it is necessary to bring in new animals, they should be examined for lesions and serologically tested before entering the colony.

NECROBACILLOSIS

Background

Necrobacillosis, also known as Schmorl’s disease, is an infection of the skin and subcutaneous tissues, usually of the head and neck, caused by *Fusobacterium necrophorum*. It occurs sporadically and is characterized by necrosis, ulceration, and abscessation.

Necrobacillosis was first described in rabbits by Schmorl in 1891, who also isolated the etiologic agent and named it *Streptothrix cuniculi*. He noted that the organism caused severe necrotizing lesions of the skin, which occasionally led to fatal septicemia and widespread necrosis of visceral organs. Schmorl reproduced the clinical disease experimentally by subcutaneous inoculation of the organism.

Although necrobacillosis is ordinarily caused by *F. necrophorum*, there is one report of a clinically and pathologically identical disease caused by *F. nucleatum* (Ward et al., 1980).

Etiologic Agent

Fusobacterium necrophorum is an anaerobic, non-spore-forming, non-motile, Gram-negative bacterium. There are two subspecies, *necrophorum* and *fundiliforme*, the former being more virulent (Tan et al., 1996). All members of the genus *Fusobacterium* produce butyrate as a major end product of amino acid utilization and are poor fermenters of carbohydrates. *F. necrophorum* morphology is variable, ranging from coccobacillary to filamentous, but filamentous organisms are most common, especially in new cultures. The key to differentiating *F. necrophorum* from similar species is its ability to form propionate from lactate (Moore et al., 1984). There are several obsolete synonyms for *F. necrophorum*, including *Sphaerophorus necrophorus*, *Fusiformis necrophorus*, and *Bacillus necrophorus*. The bacterium is a normal inhabitant of the gastrointestinal tract, but it can be pathogenic for many species, including humans. Ruminants are highly susceptible to hepatic abscesses and foot abscesses but carnivores

are resistant (Garibaldi et al., 1990a; Moore et al., 1984; Schmorl, 1891; Tan et al., 1996).

F. necrophorum produces several toxins, the most important being leukotoxin. This is a high-molecular-weight protein with specific activity against leukocytes. Leukotoxin has been sequenced and shown to encode a protein of 3241 amino acids (Narayanan et al., 2001). The gene sequence is markedly different from those of other bacterial leukotoxins. Interestingly, *F. necrophorum* leukotoxin is substantially more active against ruminant than rabbit leukocytes, which may explain the difference in prevalence of infection among these species (Tan et al., 1994).

Epizootiology

Necrobacillosis is a sporadic disease with no apparent age, sex, or breed predisposition. The organism is present in the feces of rabbits, and most infections are thought to result from fecal contamination of wounds to the skin or oral cavity. Such contamination is especially likely under conditions of poor husbandry. Schmorl (1891) observed that lesions often originated near the oral cavity and proposed that this was due to exposure of skin wounds to fresh feces during coprophagy. Factors other than skin wounds and poor husbandry may predispose rabbits to necrobacillosis. In one reported case, a retained premolar root acted as a nidus for infection (Garibaldi et al., 1990a).

Clinical Manifestations

Clinical signs of necrobacillosis include swelling, inflammation, abscessation, and necrosis of the skin and subcutaneous tissue. The head and neck are most often affected, but infections involving the plantar surfaces of the feet can also occur. Osteomyelitis of the mandible or maxilla sometimes develops by extension of infection from overlying tissue. Cases with osseous involvement tend to resist treatment and are prone to recrudescence. In animals with chronic necrobacillosis, anorexia and cachexia are prominent findings (Garibaldi et al., 1990a; Schmorl, 1891; Ward et al., 1981). Widely disseminated embolic abscesses involving lung, liver, myocardium, brain, and other organs have been reported in a rabbit in which infection appeared to originate in the oropharyngeal cavity (Seps et al., 1999).

Pathology

Lesions of necrobacillosis typically include ulceration and caseous necrosis of the skin and subcutaneous tissue. Sometimes necrosis and inflammation of the underlying bone occur as well. Caseous lesions consist

of necrotic cellular debris surrounded by a zone of suppurative inflammation. Depending on the duration of the lesions, they may be partially encapsulated by fibrous tissue. If the infection spreads hematogenously, similar lesions, accompanied by thrombophlebitis, may occur in visceral organs (Garibaldi et al., 1990a; Schmorl, 1891; Seps et al., 1999; Ward et al., 1981).

Pathogenesis

Necrobacillosis begins as an acute inflammation of the subcutaneous tissue. This is rapidly followed by necrosis and ulceration of the overlying skin, and sometimes by direct invasion of deeper tissue, including bone. Hematogenous spread can occur, leading to necrotizing lesions in visceral organs (Schmorl, 1891; Seps et al., 1999).

The pathogenesis of this infection is not well defined, but several bacterial products have been implicated. These include leukotoxin, hemolysin, endotoxin, hemagglutinin, proteases, and adhesins (Nagaraja et al., 2005). Leukotoxin appears to be particularly important and is discussed in detail under Etiologic Agent, above. Hemolysin has been shown to increase guinea pig ileal smooth muscle motility, and this has been proposed as a mechanism that facilitates colonization (Kanoë et al., 1999). A cell wall component of *F. necrophorum* causes hemorrhagic dermonecrosis in guinea pigs and rabbits (Kanoë et al., 1995). This component, which appears distinct from endotoxin, has been termed a collagenolytic cell wall component and has been shown to exhibit toxicity for rabbit granulocytes and hepatocytes (Okamoto et al., 2006). Adhesins produced by *F. necrophorum* are not well characterized, but may play a role in infection. One study demonstrated that the organism adheres strongly to rabbit cheek cells, in contrast to cells from several other species. Bacterial hemagglutinin may mediate this attachment in part, because hemagglutinin antiserum inhibits attachment (Okada et al., 1999).

Diagnosis

A tentative diagnosis can be made based on the appearance and location of lesions, but isolation of the agent should be attempted. *F. necrophorum* can be cultured on blood agar by incubating anaerobically for 2 days at 37°C. Isolation is essential for a definitive diagnosis, since similar lesions can be caused by other organisms, such as *P. multocida*, *S. aureus*, and *P. aeruginosa* (Dominguez, 1975; Garibaldi et al., 1990a; Jepsen, 1977).

An enzyme-linked immunosorbent assay (ELISA) designed to detect antibody in animal sera has been described (Evans and Berg, 1985). The ELISA used a ribosome-rich extract as an antigen preparation. The ELISA was developed using rabbit sera, but the assay

was designed primarily for use in cattle. A polymerase chain reaction assay capable of detecting *Fusobacterium* spp. in clinical specimens has also been described, but it has not been used in rabbits (Nagano et al., 2007).

Prevention and Therapy

Treatment of necrobacillosis is difficult because lesions tend to recur. If therapy is attempted, it should include drainage and debridement of the lesions, coupled with antibiotic administration. *F. necrophorum* is susceptible to several antibiotics, including penicillin, cephalosporins, chloramphenicol, and tetracycline (Moore et al., 1984). Metronidazole may be suitable as well. Penicillin is normally the antibiotic of choice, but cephalosporins may be preferable in cases of osteomyelitis because of their good distribution in osseous tissue (Ward et al., 1981). *F. necrophorum* is usually resistant to aminoglycosides (Tan et al., 1996).

Prevention of necrobacillosis should be focused on preventing cutaneous wounds and minimizing fecal contamination of wounds that do occur. Cages and accessories should be kept clean and free of sharp edges. As for most bacterial diseases, good management practices are imperative for prevention of necrobacillosis. No vaccine is available.

LISTERIOSIS

Background

Listeriosis, which is caused by *Listeria monocytogenes*, is a septicemic disease of rabbits characterized by sudden death, chronic cachexia, or abortion. The disease was first described by Murray et al. (1926) in young rabbits and guinea pigs. The authors isolated a small Gram-positive organism and named it *Bacterium monocytogenes*, to reflect the high number of monocytes in the peripheral blood of affected animals. The name of the genus was changed to *Listeria* by Pirie (1940), who studied the disease in African rodents and reproduced it experimentally in rabbits and other species. At about the same time, the first case of listeriosis in a pregnant rabbit was described by Paterson (1940). Pregnant does have since been shown to be more susceptible to the disease (Gray et al., 1955a, 1955b; Watson and Evans, 1985).

Etiologic Agent

Listeria monocytogenes is a short, non-sporeforming, motile, Gram-positive rod with rounded ends. It is facultatively anaerobic and grows over a wide temperature range. In contrast to several other non-pathogenic members of the genus, it is hemolytic on blood

agar. Isolation of the organism from clinical cases can be difficult and special enrichment or selective procedures are often required. A cold enrichment technique has been described, and chemicals such as potassium thiocyanate, thallos acetate, and nalidixic acid can be added to media to help facilitate isolation (Seeliger and Jones, 1986). *L. monocytogenes* is pathogenic for many animal species, especially ruminants. In humans, meningitis and materno-fetal infections are common manifestations. Gastroenteritis can also occur in response to ingestion of the organism via contaminated food. In other species, similar syndromes have been reported, including abortion, encephalitis, and septicemia.

Listeria is normally a saprophyte that undergoes a transition to an intracellular parasite after ingestion by a susceptible host. Many bacterial products facilitate this process; some of the most important include internalin A and B (InlA and InlB), which bind to host cell receptors and facilitate entry; listeriolysin O (LLO), which breaks down the host cell phagosome once the organism is internalized; Hpt (hexose phosphate transporter), which helps obtain hexose sugars for bacterial metabolism; ActA, which polymerizes actin as a means to induce motility; and phosphatidylcholine phospholipase C, which acts in concert with LLO to help release organisms that are incorporated into vacuoles during cell-to-cell spread. Expression of these genes is controlled by PrfA (Positive regulatory factor A), which is in turn regulated in part by environmental conditions (Cossart and Toledo-Arana, 2008; Freitag et al., 2009). For example, temperatures and carbon sources found in animal hosts appear to enhance expression of this protein, thereby causing production of the various virulence-associated proteins that facilitate entry and survival in the host cell.

Epizootiology

Listeriosis is an uncommon disease of rabbits, usually occurring only in animals that are immunocompromised or have experienced stress or pregnancy. Stress may have been a factor in the outbreak reported by Murray et al. (1926), because the rabbits were young and received inadequate amounts of a poor-quality diet. The importance of pregnancy was demonstrated by Gray et al. (1955a, 1955b), who observed that pregnant does challenged with the organism developed severe disease, whereas non-pregnant females and males did not. The increased susceptibility of pregnant animals may be partly a result of physiologic stress, but it is also true that the pregnant uterus is a favorable environment for replication of the organism. Colonization of the female reproductive tract occurs readily; the organism can be isolated from the uterus and vagina even when exposure occurs by another route (Gray, 1955a, 1955b).

Transmission of listeriosis occurs by ingestion of the organism, which is a frequent contaminant of food. Another potential means of exposure is by inhalation. Evidence for this is that the organism can be recovered from the nasal cavity of ruminants and humans (Gray and Killinger, 1966). Once exposed, animals may become asymptomatic carriers and shed bacteria in the feces. The prevalence of carriers is unknown, but one study conducted in Spain suggests that there may be significant numbers in some rabbit colonies (Rodríguez-Calleja et al., 2006). Rabbit carcasses tested for presence of bacterial foodborne pathogens revealed that 13.7% were positive for *Listeria*. In contrast, neither *Salmonella* nor *E. coli* O157:H7 was detected.

Clinical Manifestations

In males and non-pregnant females, the clinical signs of listeriosis are non-specific. In acute cases sudden death may occur, whereas in chronic cases there may be anorexia, depression, and weight loss. In pregnant females, listeriosis should be suspected in cases of abortion or vaginal discharge.

Pathology

The most consistent lesions in listeriosis are pinpoint pale gray foci throughout the parenchyma of the liver (Figure 13.13). Similar foci are sometimes present in the spleen and adrenal glands. Microscopically, these are areas of necrosis surrounded by varying numbers of inflammatory cells, mostly neutrophils (Figure 13.14). In addition to focal to multifocal hepatic necrosis, in pregnant does there is necrotizing acute suppurative metritis, and dead fetuses may be present. Gram-positive organisms are readily identifiable within and adjacent to the necrotic lesions (Murray et al., 1926; Paterson, 1940; Watson and Evans, 1985).

Pathogenesis

After ingestion by the host, *L. monocytogenes* penetrates epithelial cells of the gastrointestinal tract. This is mediated by interaction of internalin (InlA and InlB) with host cell receptors, including cadherin. It is then taken up by macrophages and enters the systemic circulation, eventually being found in the liver and spleen (Ireton, 2007; Watson and Evans, 1985).

Species susceptibility to infection may be influenced by the ability of internalin to bind host cell receptors and thus gain entry into cells. For example, InlB binds to a cell surface receptor called Met (hepatocyte growth factor receptor) in humans but not in rabbits. Similarly, InlA binds to cadherin in humans but not in mice; rabbits were not studied (Khelef et al., 2006). These

observations may help explain not only species susceptibility to infection, but also tissue tropisms that differ from one species to another. For example, *Listeria* infections in humans are frequently associated with central nervous system involvement, but CNS involvement is uncommon in rabbits.

Because *L. monocytogenes* is a facultative intracellular pathogen, the cell-mediated immune response is generally considered more important for protection than the humoral response (Hof, 1991). Nevertheless, a humoral response occurs in rabbits, and is particularly strong against internalin, as well as five other proteins of unknown function, designated IspA-E (Yu et al., 2007). There is evidence that antibody produced in response to *Listeria* is opsonizing and may enhance bacterial killing by neutrophils and macrophages (Suga et al., 1996; Vahidy and Jehan, 1996).

Diagnosis

Diagnosis of listeriosis depends on isolation of the organism, but a presumptive diagnosis can be made upon identification of Gram-positive organisms in typical lesions. It is important to consider other possible causes of focal hepatocellular necrosis, such as Tyzzer's disease, salmonellosis, and tularemia.

Several serologic screening techniques are available for listeriosis. The most popular are a microagglutination test using heated and trypsinized antigen and an immunofluorescent assay using lyophilized antigen. An immunoperoxidase technique performed on histologic sections of liver from intraperitoneally challenged mice has also been described. Regardless of the method, a high percentage of normal animals have titers, indicating either a carrier state or prior exposure to the organism (Briones et al., 1989).

Polymerase chain reaction techniques can also be used for diagnosis. Various assays have been described; some can identify species and allow differentiation of serotypes, whereas another uses primers specific for internalin genes to facilitate simultaneous confirmation of species identity and virulence (Borucki and Call, 2003; Doumith et al., 2004; Liu et al., 2007).

Prevention and Therapy

There are no reports that describe treatment of rabbits with listeriosis. In vitro, most antibiotics except cephalosporins are active against *L. monocytogenes*, but a combination of ampicillin and aminoglycoside is probably the most effective (Hof, 1991). In a rabbit model of experimentally induced meningitis, treatment with a combination of ampicillin and gentamicin was compared to moxifloxacin. Both regimens were similarly effective (Sipahi et al., 2008). Whatever antibiotic is

used, treatment efficacy is likely to be hindered by the intracellular location of the bacterium.

Since listeriosis is an uncommon disease of rabbits, prevention has received little attention. Good husbandry practices should be employed to minimize stress, and since food is a common source of the organism, high-quality feed should be used. This is especially important in breeding colonies. Vaccination is not practiced in rabbits, but killed vaccines have been used in ruminants and appear to be partially effective (Burdarov and Savova-Burdarova, 1985).

TUBERCULOSIS

Background

Tuberculosis is a rare disease of domestic rabbits. As in other species, it is characterized by granulomatous inflammation of the lungs, digestive tract, liver, kidneys, and lymph nodes. It is almost always caused by *Mycobacterium bovis*, but *M. avium* and *M. tuberculosis* have been isolated from some cases. This review will concentrate on spontaneously occurring tuberculosis, although the rabbit has been used extensively as a model for study of the pathogenesis of the disease.

Robert Koch recognized rabbit tuberculosis in 1884, only 2 years after he first isolated and cultivated mycobacteria. His description was followed by a similar report by Guérin, in 1908. These papers, along with many others published in the following decades, were reviewed in detail by Flatt (1974). Most of the reports described nodular lesions with caseous centers which primarily involved the lungs. Other organs, including the kidneys, liver, lymph nodes, gastrointestinal tract, and bones, were sometimes affected as well. Most cases were attributed to the bovine variety of mycobacteria, now known as *M. bovis*, but the avian variety was also described.

Rabbits are also susceptible to paratuberculosis, which is caused by *M. avium* subspecies *paratuberculosis*. Rabbits challenged experimentally with the organism develop intermittent diarrhea and multifocal granulomas in the sacculus rotundus and cecum (Mokresh and Butler, 1990). Spontaneous paratuberculosis was first reported in a wild rabbit with typical lesions. The etiologic agent was not cultured, but the rabbit had contact with a deer herd that experienced an epizootic of the disease (Angus, 1990). Since then the presence of paratuberculosis in wild European rabbits (*Oryctolagus cuniculus*) has been well documented (Beard et al., 2001; Greig et al., 1997). Rabbits are known to be an important reservoir of the organism, thereby posing a potential risk to grazing livestock (Daniels et al., 2003a, 2003b).

Etiologic Agent

Tuberculosis is caused by members of the genus *Mycobacterium*, all of which are slender, rod-shaped, aerobic, slow-growing, non-motile, Gram-positive, and acid-fast. The cell wall contains complex waxes and glycolipids that stimulate the granulomatous inflammatory response seen in tuberculosis. Species of *Mycobacterium* that have been associated with tuberculosis in rabbits include, in order of frequency, *M. bovis*, *M. avium*, and *M. tuberculosis*. These can be differentiated based on biochemical reactions, molecular diagnostic techniques such as PCR, agglutination by specific antisera, or pathogenicity for various species of experimental animals. For animal species other than non-human primates, *M. bovis* and *M. avium* are typically more pathogenic than *M. tuberculosis* (Himes et al., 1989; Wayne and Kubica, 1984).

M. bovis, the most important cause of spontaneous tuberculosis in rabbits, is a member of the *M. tuberculosis* complex, which is a group of very closely related species with over 99.9% similarity at the DNA level. Other members of the group include *M. africanum*, *M. caprae*, *M. microti*, and *M. pinnipedii*. *M. tuberculosis* and *M. africanum* affect humans almost exclusively, whereas the latter three species affect goats, voles, and pinnipeds, respectively. *M. bovis* has the largest host range. From an evolutionary standpoint, the traditional view is that *M. tuberculosis* originated as a zoonotic pathogen derived from *M. bovis*, but recent genomic analyses of members of the complex suggest that the reverse is true; evidently, successive loss of DNA segments from *M. tuberculosis* gave rise to *M. bovis* and other members of the complex (Gordon et al., 2009).

M. bovis and other members of the *M. tuberculosis* complex produce various gene products that are critical for host-pathogen interactions. These include enzymes that are important in the synthesis and transport of several lipids and glycolipids that are present on the cell surface and appear to be necessary for persistence and multiplication in the host. Secreted proteins are also important, including ESAT-6 (early secreted antigenic target), which is recognized by T cells and induces production of interferon gamma (Gordon et al., 2009).

Epizootiology

Rabbit tuberculosis is a rare disease in laboratory rabbits, but the large number of reports in the early 1900s suggests that it was once more prevalent than it is today. Many of the early cases may have been acquired from cattle, since it was a common practice to feed cow's milk to rabbits as a growth promoter (Harkins and Saleeby, 1928). Discontinuation of this practice, together with control programs for bovine tuberculosis,

are probably responsible for the fewer number of cases reported in recent years. Interestingly, current trends indicate that the tables have turned: rather than cattle serving as a source of *M. bovis* for rabbits, rabbits now appear to be a source of *M. avium* subspecies *paratuberculosis* for cattle, at least in the United Kingdom (Daniels et al., 2003a, 2003b).

As in other species, transmission of tuberculosis in rabbits occurs by inhalation or ingestion. Inhaled organisms colonize the lungs and bronchial lymph nodes, whereas ingested organisms penetrate the gastrointestinal epithelium and colonize the mesenteric lymph nodes or intestinal wall. In either case, organisms sometimes gain access to the systemic circulation and disseminate throughout the body. Following exposure by inhalation, organisms may be coughed up and swallowed, resulting in secondary exposure via the gastrointestinal route.

Wild rabbits in the United Kingdom have a high prevalence of infection with *M. avium* subspecies *paratuberculosis*, ranging from 17–40%. Organisms can be isolated from reproductive tissues and from feces. Gross lesions are not typically present, but histopathologic lesions can occur in almost 20% of infected animals. Transmission is thought to occur horizontally via ingestion of contaminated feces or vertically via the placenta or by ingestion of contaminated milk (Beard et al., 2001; Daniels et al., 2003a, 2003b; Judge et al., 2006).

Clinical Manifestations

Clinical signs of tuberculosis are non-specific and include findings such as anorexia, weight loss, and pallor. Occasionally there are signs referable to a particular organ system. Diarrhea, suggesting gastrointestinal involvement, has been reported; this may be more likely in cases of *M. avium* infection. Swollen joints and ocular lesions have also been observed (Flatt, 1974).

Pathology

Gross lesions of tuberculosis consist of tan to gray caseous nodules in various organs, especially the lungs, bronchial lymph nodes, kidneys, and liver. Cavitory lesions in the lungs can occur in cases of *M. bovis* infection, but such lesions are rarely associated with other mycobacterial species, except under carefully controlled experimental challenge conditions (Manabe et al., 2003; Nedeltchev et al. 2009).

The gastrointestinal tract may contain caseous nodules, especially at sites of lymphoid tissue, and the mucosa overlying the nodules may be ulcerated. With skeletal involvement there is caseous necrosis of bone, accompanied by areas of osseous proliferation. Widespread central nervous system involvement can occur, with caseous nodules in the cerebrum, medulla,

and leptomeninges. Ocular lesions such as uveitis also have been observed (Collins et al., 1983; Flatt, 1974; Himes et al., 1989).

Microscopic lesions consist of foci of caseous necrosis surrounded by epithelioid macrophages, lymphocytes, and fibrous tissue. Acid-fast organisms can be found in impression smears or histologic sections of the lesions. Mineralization of the lesions is uncommon, and multinucleated giant cells are usually scarce (Himes et al., 1989).

Lesions associated with *M. avium* subspecies *paratuberculosis* involve the intestine and associated lymph nodes. Granulomas characterized by macrophages, giant cells, and intracellular acid-fast bacteria are present in the small intestine (Beard et al., 2001; Greig et al., 1997).

Pathogenesis

Mycobacteria are facultative intracellular pathogens, accumulating in large numbers in macrophages at the site of a lesion. The organisms provoke a granulomatous inflammatory response, in part because of the high lipid content of their cell walls. Infection also stimulates an intense cell-mediated immune response, which ordinarily inhibits dissemination of organisms and blocks progression of disease. In some cases, however, there is an aberrant immune response characterized by hypersensitivity. This accelerates the development of lesions and induces clinical disease. The nature of the immune response and the consequent level of resistance are influenced by host genotype (McMurray, 1991). A relationship between genotype and resistance has been seen in experimental infection studies performed in rabbits (Dorman et al., 2004; Lurie, 1963; Mendez et al., 2008). Susceptible rabbits have impaired delayed-type hypersensitivity responses and impaired TNF- α production, underscoring their importance in the pathogenesis of the disease (Dorman et al., 2004).

Diagnosis

Because the clinical signs are non-specific, diagnosis of tuberculosis is ordinarily made at necropsy. Demonstration of typical lesions containing acid-fast organisms is highly suggestive, but culture and identification of the organism are required for definitive diagnosis. Molecular diagnostic techniques such as polymerase chain reaction allow rapid presumptive diagnosis and species identification from clinical specimens (Balasingham et al., 2009; Gopinath and Singh, 2009).

Prevention and Therapy

There have been no reports of treatment of naturally occurring tuberculosis in rabbits. Theoretically, drugs

such as isoniazid and rifampin should be effective. Rifampin is helpful in treating experimentally induced *M. avium* infection in rabbits, but it is not curative (Singh et al., 1981). Because of the rarity of the disease, prevention of tuberculosis by vaccination or other strategies has received little attention and need not be a major concern.

TULAREMIA

Background

Tularemia is an acute septicemic disease caused by *Francisella tularensis*. It can occur in many vertebrate species and is common in wild rabbits and hares, but in domestic rabbits the disease is rare.

Tularemia was first described as a plague-like disease occurring in ground squirrels (*Citellus beecheyi*) from California (McCoy, 1911). The etiologic agent was isolated and named *Bacterium tularense* after Tulare County, California (McCoy and Chapin, 1912). The first human cases were described in 1914, and were characterized by ulcerative conjunctivitis, fever, weakness, and lymphadenopathy (Wherry and Lamb, 1914a, 1914b). These and most subsequently reported human cases were associated with exposure to rabbits. Francis (1925) recognized the importance of this association and also studied the etiologic agent and the pathogenesis of the disease. *Francisella* was named in his honor, in recognition of this early work on tularemia.

Etiologic Agent

Tularemia is caused by *Francisella tularensis*, a Gram-negative, aerobic, non-motile, pleomorphic bacterium that is distantly related to the genera *Brucella*, *Yersinia*, and *Pasteurella*. It cannot be cultured on standard laboratory media, but glucose cysteine agar or cysteine heart agar can be used successfully provided they are first enriched with blood. Classically, two biovars of *F. tularensis* are recognized: *tularensis* and *palaeartica*. Biovar *tularensis* is highly virulent and found almost exclusively in North America, whereas biovar *palaeartica* is less virulent and found in Eurasia as well as the Americas. Lagomorphs are especially likely to harbor biovar *tularensis*, thus explaining the strong association between rabbit exposure and human cases (Eigelsbach and McGann, 1984; Morner et al., 1988).

Recent DNA-sequence-based classifications of the species *Francisella tularensis* reflect four subspecies: *tularensis*, *holarctica*, *mediasiatica*, and *novicida* (Foley and Nieto, 2010; Keim et al., 2007). Subspecies *holarctica* and *mediasiatica* were previously included within biovar *palaeartica*; subspecies *novicida* was previously regarded as a separate species.

Epizootiology

F. tularensis is widely distributed in nature and has been isolated from over 200 species of wildlife, including mammals, birds, reptiles, fish, and arthropods, including ticks and biting insects. Clinically, the most important subspecies are *tularensis* and *holarctica*; the other subspecies have relatively low virulence. *F. tularensis* subspecies *tularensis* is restricted almost exclusively to North America, whereas *F. tularensis* subspecies *holarctica* exists in both North America and Europe. This means that subspecies *holarctica* is the only significant cause of tularemia in Europe, whereas either subspecies can cause tularemia in North America (Foley and Nieto, 2010).

Several studies attest to the prevalence of *F. tularensis* in wild lagomorphs, both in Europe and North America. In a survey of wild cottontail rabbits from Illinois, 16% had detectable antibodies to *F. tularensis* (Lepitzki et al., 1990). Another study, conducted in Sweden, found that 7% of wild hares submitted for necropsy died from tularemia (Morner et al., 1988). Interestingly, tularemia occurred in the varying hare (*L. timidus*) but not in the brown hare (*L. europaeus*). Cases were most common in the autumn and may have been transmitted by blood-sucking arthropods, most likely ticks. Ticks are important vectors of tularemia, and the genera *Dermacentor*, *Amblyomma*, and *Ixodes* have all been incriminated. In North America, *Dermacentor andersoni* have been shown capable of transmitting tularemia to rabbits (Gordon et al., 1983).

Many species that harbor *F. tularensis* may transmit the infection to humans, but rabbits are most often incriminated. Up to 90% of human cases have been linked to lagomorph exposure, with rabbits of the genus *Sylvilagus* presenting the greatest risk in North America (Hoff et al., 1975; Jellison, 1974). In Europe, hares of the genus *Lepus* present the greatest risk; there have been no reported cases of tularemia acquired from *Oryctolagus cuniculus*.

The incidence of tularemia in humans declined from a peak of 2291 cases in the United States in 1944 to around 10% of that level in the 1980s (JAMA, 1987; Jellison, 1974; MMWR, 1986). During the period from 2000 to 2008, an average of 126 cases per year were reported (http://www.cdc.gov/tularemia/Surveillance/Tul_CasesbyState.html). Rabbits still represent the primary source of exposure, but transmission from arthropods to humans has been recognized more frequently (Boyce, 1975; Foley and Nieto, 2010).

Transmission of tularemia can occur by several routes. The organism usually enters the host through the skin or conjunctiva, either via a wound or by penetrating intact mucosal epithelium. This can be mediated by direct contact with infected material or by an

arthropod bite. Transmission may also occur by ingestion of infected meat or contaminated water, or by inhalation of infectious aerosols (JAMA, 1987).

Clinical Manifestations

Tularemia usually causes sudden death in affected animals, but depression, anorexia, and ataxia may be observed. In humans, tularemia is an acute, febrile disease. A primary lesion consisting of a skin ulcer occurs, and inflammation of regional lymph nodes follows. This is termed the ulceroglandular form of the disease and represents the most common syndrome seen in humans. In severe cases, there may be progression to septicemia and pneumonia (Eigelsbach and McGann, 1984).

Pathology

The main lesions of tularemia in lagomorphs include focal coagulative necrosis and congestion of the liver, spleen, and bone marrow (Figure 13.18). The extent of these lesions depends on the duration of the disease, so they may be absent or minimal in peracute cases. Sometimes hemorrhagic enteritis and typhlitis are observed in addition to the classic lesions, particularly in hares that develop the disease in winter months (Morner et al., 1988).

Pathogenesis

Asymptomatic infection with *F. tularensis* probably occurs frequently; this is suggested by the high prevalence of antibody in wild rabbits and the occasional isolation of *F. tularensis* from apparently healthy animals (Lepitzki et al., 1990). Factors that determine whether

disease occurs could be expected to include the immunologic status of the host, the route of exposure, and the dose and virulence of organisms. There is a substantial difference in virulence between subspecies of *F. tularensis*, subspecies *tularensis* being more virulent than the others (Morner et al., 1988). The reason for this difference in virulence is not known, but early studies suggested that virulent organisms have higher catalase activity and higher levels of cytochrome *b1* than non-virulent ones (Eigelsbach and McGann, 1984). The presence of a capsule is also associated with virulence.

Recent experimental studies of tularemia suggest that the ability of organisms to survive and replicate within macrophages is critical for disease pathogenesis. This ability depends on the bacterial gene *mgla*, which regulates transcription of genes located within a segment of the genome referred to as the *Francisella* Pathogenicity Island (Barker and Close, 2007). Other bacterial components, such as lipopolysaccharide and pili, may be important as well.

Diagnosis

Tularemia is usually diagnosed at necropsy. Typical lesions are suggestive of the disease, but definitive diagnosis requires identification or isolation of *F. tularensis*. Classically, isolation of the organism is performed by first inoculating guinea pigs intraperitoneally with suspect material. The guinea pigs die within 10 days, at which time the organism can be readily isolated from the tissues. This technique has the disadvantage of being hazardous to personnel, and is usually not necessary if appropriate culture techniques are used. Blood-enriched glucose cysteine agar or cystine heart agar can be inoculated directly with clinical or necropsy specimens and incubated at 37°C aerobically for 48–96 hours. Isolation is facilitated by incorporation of antibiotics in the media to suppress normal flora (Eigelsbach and McGann, 1984).

As an alternative to isolation of the organism, a fluorescent antibody technique can be used for identification of the organism in fixed tissue; this is probably the safest method to arrive at a definitive diagnosis in a suspected clinical case (Morner, 1981).

Serologic screening procedures for *F. tularensis* infection have been developed, including a rapid slide test for agglutinating antibodies and several enzyme-linked immunosorbent assays (Lepitzki et al., 1990; Schmitt et al., 2005).

Methods for detection of organisms in biological specimens have been described. These include a capture enzyme-linked immunosorbent assay that is specific for bacterial lipopolysaccharide and several polymerase chain reaction assays (Grunow et al., 2000; Molins et al., 2009).



FIGURE 13.18 Tularemia: multiple foci of hepatic necrosis. Similar lesions occur in diseases such as listeriosis and Tyzzer's disease. (Courtesy of Dr. James G. Fox)

Prevention and Therapy

Because tularemia exists almost exclusively in wild animal populations, treatment is rarely attempted. If an opportunity for treatment arises, fluoroquinolones or aminoglycoside antibiotics should be effective. Alternatively, tetracyclines can be used and have the advantage of oral administration, but recrudescence of the disease may occur when therapy is stopped. *Francisella* is resistant to penicillin and first-generation cephalosporins (Foley and Nieto, 2010).

Control of tularemia in wild animal populations has not been attempted. To prevent spread to humans or domestic animals, is it important to minimize contact with animal carcasses and biting arthropods when in enzootically infected areas. In addition, meat from wild-caught lagomorphs should be thoroughly cooked. For exceptionally high-risk individuals, vaccination may be attempted. Several different candidates have been produced, including killed bacterins and live attenuated strains. The best-studied vaccine candidate is an attenuated strain designated LVS (live vaccine strain). LVS was derived from *F. tularensis* subspecies *holarctica* after multiple passages and laboratory manipulations that gave way to various mutations in parts of the genome thought to be important for virulence (Barker and Close, 2007). Progress in vaccine development will depend on further characterization of bacterial genes associated with virulence, followed by induction of attenuating mutations in virulence-associated genes.

PROLIFERATIVE ENTEROPATHY

Background

Proliferative enteropathy is caused by *Lawsonia intracellularis*, an obligate intracellular bacterium that causes disease in a wide range of hosts including pigs, hamsters, rabbits, ferrets, dogs, horses, and macaques. The disease is characterized by thickening of the ileum, mucosal hyperplasia, and intracellular organisms in crypt enterocytes. Early reports in rabbits described the intracellular bacteria as morphologically similar to *Campylobacter*, but immunologic and molecular evidence suggested that the organisms were not closely related to known *Campylobacter* species (Moon et al., 1974; Schoeb and Fox, 1990; Umemura et al., 1982). Eventually, it became evident that the causative organism was a member of a newly recognized genus named *Lawsonia*, which caused a nearly identical disease in pigs, ferrets, and hamsters (Fox and Lawson, 1988; Fox et al., 1994; Hotchkiss et al., 1996).

Etiologic Agent

Lawsonia intracellularis is a Gram-negative, non-spore-forming, curved intracellular pathogen that has

not been cultivated in cell-free media. Sequencing of DNA encoding 16S rRNA suggests that it is closely related to the genus *Desulfovibrio* (Hotchkiss et al., 1996). *L. intracellularis* has been cultured in a rat small intestinal cell line designated IEC-18 and in a mouse fibroblast line known as McCoy cells (Lawson et al., 1993; Watarai et al., 2008). The mouse fibroblast line may offer better passage efficiency.

The extent to which *L. intracellularis* isolates vary depending on the host species is uncertain, but there is evidence that isolates from rabbits and pigs are antigenically distinct and have different biological properties. Rabbit and pig isolates cultured in mouse fibroblasts have distinct antigenic profiles that could be useful in serologic typing or epizootologic analysis (Watarai et al., 2008). Furthermore, rabbit and pig isolates demonstrate different levels of pathogenicity in BALB/c mice (Murakata et al., 2008).

Epizootiology

Little is known about the prevalence of *Lawsonia intracellularis* infection in rabbits, especially with respect to whether animals can be asymptomatic carriers. Most reports describe outbreaks involving multiple animals during a short time frame, which could imply that a carrier state is unlikely. On the other hand, there is at least one report of rabbits that had typical lesions at necropsy but no prior clinical signs (Duhamel et al., 1998). In this case it is possible that overt disease simply did not have enough time to develop.

Clinical Manifestations

Proliferative enteropathy is typically characterized by diarrhea, weight loss, dehydration, and depression, although rabbits infected with *L. intracellularis* may show no clinical signs. The disease is most common in weanlings, although adults can be affected. Mortality is typically low, but morbidity can approach 30% (Duhamel et al., 1998; Horiuchi et al., 2008; Hotchkiss et al., 1996).

Although mortality is ordinarily low, coinfection with other enteric pathogens might be expected to exacerbate clinical signs. In fact, an outbreak of enterocolitis characterized by unusually high mortality was observed in rabbits coinfecting with *L. intracellularis* and enteropathogenic *E. coli* (Schauer et al., 1998).

Pathology

Gross necropsy findings in cases of proliferative enteropathy involve thickening of the small intestine, cecum, and colon, with the ileum most prominently affected. Mesenteric lymph nodes are often enlarged.

Microscopic lesions consist of marked thickening of the mucosa accompanied by hyperplasia and distortion of villous and crypt epithelium. Inflammation is typically modest, but cell inflammatory cell infiltrates may be observed in the lamina propria, consisting primarily of histiocytes, plasma cells, lymphocytes, and a few neutrophils. Warthin-Starry staining reveals curved silver-staining bacteria in the apical cytoplasm of crypt enterocytes (Duhamel et al., 1998; Horiuchi et al., 2008; Hotchkiss et al., 1996).

Pathogenesis

Studies of the pathogenesis of proliferative enteropathy are limited, perhaps because of challenges presented by the obligate intracellular nature of the causative organism. In contrast to other intestinal pathogens, factors influencing colonization, host cell attachment, and cell entry are not well defined at the molecular level. Organisms enter via a mechanism that resembles uptake by clathrin-coated pits, and reside for a brief time in an intracellular vacuole. The bacteria quickly escape from the vacuole and live freely in the cytoplasm. *L. intracellularis* has a strong tropism for crypt cells rather than mature intestinal epithelial cells. Only infected cells proliferate, but the mechanism that mediates proliferation is not known (Smith and Lawson, 2001).

Host immune response is surprisingly limited, considering the dramatic proliferative lesions that are observed; this may facilitate persistence of the organism within epithelial cells. Nevertheless, animals often recover from the disease and appear to clear organisms from the epithelium. There is evidence from experimental studies in mice that this may be mediated, at least in part, by gamma interferon production (Smith et al., 2000).

Diagnosis

L. intracellularis can be identified in tissue sections by classical Warthin-Starry staining or by immunohistochemical techniques that rely on monoclonal antibodies or polyclonal antiserum against *Lawsonia* surface antigens. Alternatively, polymerase chain reaction can be used to amplify DNA extracted from fixed tissue specimens (Duhamel et al., 1998; Horiuchi et al., 2008; Schauer et al., 1998; Watarai et al., 2004).

For clinical specimens, an enzyme-linked immunosorbent assay using synthetic *Lawsonia* surface antigens can be used to detect the presence of serum antibody. Another approach, which allows detection of *Lawsonia* in fecal samples, uses magnetic beads coated with anti-*Lawsonia* antibodies. Bacteria captured by the beads are then detected by ATP bioluminescence (Watarai et al., 2005).

Prevention and Therapy

Treatment of rabbits suspected of having proliferative enteropathy should be symptomatic. Ill animals should be isolated from others in the colony, and fluid support should be provided as needed. Antibiotics should be used with caution because they may exacerbate diarrhea. There are no reports of the use of antibiotics in rabbits with the disease, but in ferrets, chloramphenicol was shown to be effective at a dose of 50 mg/kg body weight, given every 12 hours for up to 21 days (Krueger et al., 1989).

MISCELLANEOUS BACTERIAL DISEASES

Actinobacillus

Several cases of soft tissue swelling and inflammation around the joints of the extremities have been attributed to *Actinobacillus* spp. (Arseculeratne, 1962). Histologically, the affected tissues were characterized by granulomatous inflammation. The lesions contained colonies of Gram-negative organisms surrounded by a zone of eosinophilic radially arranged club-shaped structures.

A. capsulatus has been isolated from visceral organs of North American snowshoe hare (*Lepus americanus*) (Zarnke and Schlater, 1988). This organism appears to be prevalent in wild lagomorphs. A serological survey showed that over 40% of snowshoe hares from Alaska or Alberta have titers to the organism (Zarnke et al., 1990).

Isolated cases of *Actinobacillus* infections have been reported in pet rabbits, most commonly as opportunistic infections. In one case, a rabbit with Tyzzer's disease was found to have concurrent infection with *A. equuli*, which was isolated from lungs and liver (Moyaert et al., 2007). In another, a rabbit with gastric stasis syndrome developed a febrile illness and died shortly thereafter. *A. capsulatus* was isolated in pure culture from the lungs and fibrin thrombi were present in blood vessels (Meyerholz and Haynes, 2005). *Actinobacillus* may be confused with *Pasteurella multocida* in standard microbiological testing, so it is important to use care in differentiating between the two organisms. Both are members of the family *Pasteurellaceae* and therefore non-motile, facultatively anaerobic, oxidase-positive, and catalase-positive; carbohydrate fermentation patterns may be required for conclusive identification.

Actinomyces

Osteitis involving the bones of the head, vertebral column, and distal extremities has been attributed to *Actinomyces* spp. (Sorenson and Saliba, 1961). Lesions in affected rabbits included granulomatous inflammation

with “sulfur granules,” as well as bone lysis and proliferation. The inflammatory process extended into the soft tissue adjacent to bone.

Actinomyces israelii has been identified as an occasional cause of rabbit mandibular or maxillary abscesses (Tyrell et al., 2002). Interestingly, this study identified a variety of organisms associated with these abscesses (see also *Fusobacterium nucleatum*, below), but *Pasteurella* was not isolated.

Bordetella

Bordetella bronchiseptica is a common inhabitant of the upper respiratory tract of laboratory rabbits. Other species of laboratory animals, such as dogs, cats, and guinea pigs, also harbor the organism. Colonization of weanling rabbits is common, and the number of infected animals increases as the animals mature (Deeb et al., 1990; Glass and Beasley, 1989). Rabbits naturally infected with *B. bronchiseptica* ordinarily remain free of respiratory disease unless concurrently infected with another pathogen such as *P. multocida* (Deeb et al., 1990). Under experimental conditions, however, the organism can be pathogenic. For example, intranasal challenge with *B. bronchiseptica* results in acute serous rhinitis and bronchopneumonia in some animals, especially sucklings (Glavits and Magyar, 1990; Maeda and Shimuzu, 1975; Watson et al., 1975; Yoda et al., 1982).

Colonization of the respiratory tract depends on bacterial attachment to ciliated epithelium; this, in turn, requires expression of multiple adhesins, including filamentous hemagglutinin, pertactin, fimbriae, adenylate cyclase-hemolysin toxin, and dermonecrotic toxin. Host mechanisms for defense against colonization include both humoral and cell-mediated responses, but at the earliest stages of infection non-specific surfactant proteins predominate (Edwards et al., 2005).

Although *B. bronchiseptica* is primarily an opportunistic pathogen, colonized animals have abnormal immune responses. Alveolar macrophages demonstrate decreased adherence, phagocytic uptake, and bactericidal activity compared to macrophages from non-colonized animals (Zeligs et al., 1986). This suggests that *B. bronchiseptica* can act as a copathogen, particularly in rabbits infected with *P. multocida* or other respiratory pathogens.

Because of its high prevalence and limited pathogenicity, control of *B. bronchiseptica* infection is not usually attempted. If elimination of the organism from a rabbitry is desired, embryo transfer or cesarian rederivation can be used (Suzuki et al., 1990). Subsequently, animals should be housed under barrier conditions to prevent reintroduction of the organism. Monitoring for the presence of *B. bronchiseptica* can be done with standard microbiologic techniques, an enzyme-linked

immunosorbent assay, or by molecular diagnostic techniques (Boot et al., 1993; Friedman et al., 2006).

Borrelia

Borrelia burgdorferi, the spirochete that causes Lyme disease, has been isolated from several species of mammals, most notably mice, which are considered the primary reservoir. Spontaneous infection of *O. cuniculus* has not been reported, however. In fact, the species is generally considered to be a non-permissive host for long-term infection, although some evidence to the contrary has emerged (Embers et al., 2007).

B. burgdorferi has been cultured from eastern cottontail rabbits (*Sylvilagus floridanus*) and the cottontail rabbit tick *Ixodes dentatus* (Anderson et al., 1989). *B. burgdorferi* has also been found in another widely distributed lagomorph, the black-tailed jackrabbit (*Lepus californicus*). In areas where Lyme disease is endemic, jackrabbits are especially likely to be infected. Therefore, serologic monitoring of this species can be used as a surveillance procedure for the presence of borreliosis in various geographic areas (Lane and Burgdorfer, 1988).

Brucella

Brucellosis is almost unknown in domestic rabbits, but it is common in wild lagomorphs, particularly members of the genus *Lepus*. The disease is widespread in the European hare (*L. europaeus*) and is usually caused by *Brucella suis*. In fact, hares, along with wild boars, are thought to be the natural reservoir of *B. suis*.

Hare brucellosis is characterized by multifocal chronic granulomatous inflammation of the reproductive organs, liver, and spleen. The lesions are similar to those of tuberculosis, containing a central area of caseous necrosis surrounded by histiocytes and sometimes Langerhans giant cells. Although *B. suis* is usually isolated from the lesions, *B. melitensis* and *B. abortus* have been recovered as well (Sterba, 1982, 1983; Vitovec et al., 1976; Witter and O'Meara, 1970). Brucellosis has also been reported in black-tailed jackrabbits (*L. californicus*) from Utah (Thorpe et al., 1965).

Serologic methods of screening for hare brucellosis include traditional methods such as the complement fixation test, as well as an enzyme-linked immunosorbent assay (Szulowski et al., 1999). Molecular diagnostic techniques such as polymerase chain reaction have been reported, but identification to the species level is difficult due to high interspecies homology of the genus (Mayer-Scholl et al., 2010).

Cilia-associated Respiratory (CAR) Bacillus

Cilia-associated respiratory (CAR) bacillus is a Gram-negative, filamentous, rod-shaped bacterium responsible

for some cases of chronic respiratory disease in rats and mice. The bacillus colonizes the ciliated epithelial cells of the respiratory tract, resulting in peribronchial infiltration and hyperplasia of mononuclear cells in the lamina propria (Ganaway et al., 1985a).

Spontaneous infection with CAR bacillus also occurs in rabbits, but rabbit isolates are substantially different from rodent isolates, with the two sharing less than 50% sequence homology. This suggests that the strains of CAR bacillus isolated from rodents and rabbits are host-specific and may actually represent different bacterial genera (Cundiff et al., 1994, 1995; Schoeb et al., 1993).

CAR bacillus appears to be prevalent in some rabbit colonies (Caniatti et al., 1998). In contrast to rodents, however, clinical disease has not been observed and lesions are mild. Lesions are restricted to the bronchi and trachea and are characterized by slight hypertrophy and hyperplasia of ciliated epithelium, coupled with infiltration of the lamina propria with inflammatory cells. In sections stained with Grocott's methenamine silver, argentophilic filamentous bacteria can be seen aligned perpendicular to the epithelial surface, situated among the cilia (Caniatti et al., 1998; Kurisu et al., 1990; Oros et al., 1997).

Interestingly, two experimental studies reported no lesions at all following inoculation of CAR bacillus, although seroconversion did occur (Matsushita et al., 1989; Shoji-Darkye et al., 1991). This may be because of the strain used for inoculation; in both cases rodent isolates were administered to rabbits; subsequent work by other investigators revealed that rodent isolates are generally non-pathogenic for rabbits and vice versa (Cundiff et al., 1994, 1995).

Chlamydophila

According to current taxonomy, the family Chlamydiaceae is divided into the genera *Chlamydophila* (Cp.) and *Chlamydia* (C.). *Chlamydophila* includes the species *Cp. psittaci*, *Cp. pneumoniae*, *Cp. abortus*, *Cp. pecorum*, *Cp. felis*, and *Cp. caviae*. *Chlamydia* includes the species *C. suis*, *C. trachomatis*, and *C. muridanum* (Everett, 2000).

Rabbits are often used as an experimental model for the study of atherosclerosis associated with *Cp. pneumoniae*, but there are few reports of spontaneous disease caused by this organism or other members of the genus. A specific strain of *Cp. psittaci*, designated M56, causes epizootic chlamydiosis with high mortality in snowshoe hares (*L. americanus*). The organism proliferates in the monocyte-macrophage system, producing congestion and focal necrosis of the liver and spleen (Spalatin et al., 1966). Strain M56 is the only *Cp. psittaci* strain known to be pathogenic for the snowshoe hare, and other lagomorphs are resistant to infection (Iversen

et al., 1976). *S. floridanus* and *O. cuniculus* ordinarily survive experimental challenge with few lesions, although anterior uveitis can occur in *O. cuniculus* after intravenous inoculation (Iversen et al., 1974).

There are several other reports of isolation of chlamydiae from rabbits, but their significance is unclear. Krishna and Kulshrestha (1985) reported spontaneous conjunctivitis in six rabbits and attributed the condition to a chlamydial organism. The diagnosis was based on indirect fluorescent antibody tests of conjunctival smears, and treatment with tetracycline was followed by remission of clinical signs. Flatt and Dungworth (1971b) isolated a chlamydial agent from the lungs of a domestic rabbit with pneumonia. They reproduced pneumonia experimentally by injecting the agent intratracheally. The disease was mild and self-limiting. Lesions included mild exudation of heterophils and a pronounced accumulation of macrophages in alveoli and alveolar septae. A similar case was reported by Krishna and Gupta (1989). The predominant lesions were interstitial pneumonia and proliferation of lymphoid tissue around vessels and airways. Eosinophilic inclusions and elementary bodies were demonstrated by special stains. Finally, Zumpt (1976) claimed that chlamydiae were responsible for a high percentage of gastrointestinal disease in the rabbitries of South Africa, but this report was incomplete in its description of lesions and isolation procedures for the organism.

Polymerase chain reaction assays have been developed to detect *Chlamydophila* and *Chlamydia* species of veterinary interest (Pantchev et al., 2009). Four rabbits screened because of unspecified clinical signs of chlamydial infection were infected with *Cp. caviae*. The significance of this finding is unclear, however, because the rabbits were in close contact with guinea pigs, which are considered to be the natural host for the organism.

Corynebacterium

There is one reported case of spontaneous disease due to *Corynebacterium bovis* (Arseculeratne and Navaratnam, 1975). The organism was isolated from a testicular abscess and lung of a rabbit. A similar disease was produced by intravenous injection of *C. bovis* in an experimental rabbit. Both animals developed agglutinating antibody to the *C. bovis* isolate.

Dermatophilus

Dermatophilus congolensis is an actinomycete that infects the skin of many species, producing a superficial, purulent dermatitis with abundant crust formation. Rabbits are susceptible to infection with *D. congolensis* and have been used as experimental

models to study the disease (Abu-Samra and Imbabi, 1976; Abu-Samra and Walton, 1981; Bucek et al., 1992; How and Lloyd, 1990). Only one spontaneous case has been reported, and that was in a cottontail rabbit (*S. floridanus*). The rabbit had typical lesions, consisting of scabs and heavy encrustations overlying a layer of purulent material. The foot pads, rear legs, and perineum were most severely affected (Shotts and Kistner, 1970). If dermatophilosis is suspected, direct smears of purulent material should be made. *D. congolensis* can be identified as two or more long parallel rows of Gram-positive cocci.

Fusobacterium nucleatum

Mandibular and maxillary abscesses are common in rabbits, and in the absence of culture it is tempting to assume that they are caused by *Pasteurella multocida*. A study by Tyrrell et al. (2002) demonstrates that *Pasteurella multocida* is actually seldom involved; rather, *Fusobacterium nucleatum* is the most common cause. *F. nucleatum* is a Gram-negative non-spore-forming rod that shares at least two antigens with *F. necrophorum* (Kaur and Falkier, 1992). Other bacterial species associated with odontogenic abscesses include *Actinomyces* spp. and Gram-positive cocci of the *Streptococcus milleri* group. This pattern is similar to that observed in humans.

Antibiotic treatment of mandibular or maxillary abscesses should be based on culture and sensitivity. The organisms responsible are typically sensitive to clindamycin, chloramphenicol, penicillin, cefazolin, and ceftriaxone (Tyrrell, 2002). Clindamycin is generally contraindicated in lagomorphs; care should be taken with any antibiotic because of the risk of serious gastrointestinal side effects.

Haemophilus

There are few reports of rabbit infections caused by bacteria of the genus *Haemophilus*, but the organism can be cultured from the respiratory tract in clinically normal rabbits (Boot et al., 1999). An organism identified as *Haemophilus* sp. was described by Gibbons (1929) as a common cause of subcutaneous abscesses, but it is likely that this organism was actually *Pasteurella multocida*. There is one reported case of conjunctivitis in a rabbit caused by *Haemophilus* sp. (Srivastava et al., 1986). When the organism was inoculated into the eyes of experimental rabbits, transient blepharitis was observed and the organism was reisolated. An organism named *H. paracuniculus* has been recovered from the gastrointestinal tracts of rabbits with mucoid enteropathy, but it is not known whether it contributes to the pathogenesis of the disease (Targowski and Targowski, 1979b).

Helicobacter

Helicobacter species have been reported to occur occasionally in laboratory rabbits, with one study detecting the organism in the stomach of four out of 65 rabbits tested by polymerase chain reaction (Van den Bulck et al., 2006). The organisms were identified as enterohepatic helicobacters and were not associated with clinical signs or significant gastric inflammation.

Leptospira

Leptospirosis has not been described in domestic rabbits, but wild lagomorphs are susceptible to the disease and may be an important reservoir. Serologic surveys indicate that it is prevalent in some populations, including cottontail and swamp rabbits from the southeastern United States (Shotts et al., 1971). Up to 40% of European hares from local populations in the Netherlands have antibodies to *L. interrogans* serovar *grippotyphosa*; the infection is probably acquired from contact with field mice (Hartman and Broekhuizen, 1980).

Moraxella

Moraxella spp. are frequent inhabitants of the conjunctiva and nasolacrimal duct apparatus of rabbits, but disease is rare (Marini et al., 1996). There is one reported case of *Moraxella bovis*-induced metritis and septicemia in a rabbit (Soave et al., 1977). The rabbit was presented with a copious purulent vaginal discharge, and on necropsy was found to have necrotizing suppurative metritis. There was also serofibrinous pneumonia and multifocal areas of necrosis in the liver. *M. bovis* was cultured from the uterus, lung, liver, and spleen. Cattle may have been the source of infection, since they were housed in close proximity to the affected rabbit.

Mycoplasma

Two strains of *Mycoplasma pulmonis* have been isolated from rabbits and characterized immunologically (Deeb and Kenny, 1967a, 1967b). These isolates were recovered from the upper respiratory tract, but were not associated with clinical disease.

***Pasteurella* spp.**

Pasteurella species other than *P. multocida* are occasionally isolated from rabbits. *P. pneumotropica*, which is a major cause of clinical pasteurellosis in laboratory rodents, sometimes colonizes the nasopharynx of rabbits (Kirchner et al., 1983). It is ordinarily not pathogenic in this species, but there is one report of a rabbit with *P. pneumotropica*-associated rhinitis (Okuda and

Campbell, 1974). *P. aerogenes* has been isolated from the uterus of a rabbit which aborted due to suppurative metritis (Thigpen et al., 1978). Mouse pathogenicity tests indicated that the organism was of low virulence and therefore probably an opportunistic pathogen.

Pseudomonas

Pseudomonas aeruginosa is difficult to culture from clinically normal rabbits, but up to 75% have IgG antibodies to outer membrane proteins or lipopolysaccharide, suggesting that many animals are exposed to the organism and either clear or retain it as part of their resident flora (Weisner et al., 2005).

Occasionally, *P. aeruginosa* causes an exudative, moist dermatitis which involves the dewlap or other areas of the skin that have contact with moisture (Garibaldi et al., 1990b; O'Donoghue and Whatley, 1971; Schoenbaum, 1981; Williams and Gibson, 1975). A blue-green discoloration is sometimes evident in affected areas due to the presence of pyocyanin pigment produced by the organism. Treatment of the condition involves clipping the hair and cleansing the lesions, followed by application of a topical antibiotic preparation. Depending on severity, systemic antibiotics may be indicated as well. Since water is a frequent source of *P. aeruginosa*, the water supply should also be evaluated for the presence of the organism.

In addition to moist dermatitis, *P. aeruginosa* can cause abscesses and septicemia. It has been isolated from a rabbit with a mandibular abscess (Dominguez et al., 1975), and from rabbits involved in an outbreak of fulminating pneumonia and diarrhea (McDonald and Pinheiro, 1967).

Streptococcus

Streptococcus is an infrequent pathogen of laboratory rabbits. Prior to 1950, there were several reports of *Streptococcus* sp. as the cause of an acute septicemic syndrome that usually involved young rabbits. These reports, all in the European literature, are reviewed by Flatt (1974). In recent decades no similar reports have appeared, but there is one description of *Streptococcus*-associated osteomyelitis secondary to a subcutaneous abscess (Yanoff, 1983).

Yersinia

Pseudotuberculosis is caused by *Yersinia pseudotuberculosis*. The disease is rare in domestic rabbits, yet quite common in rodents, especially guinea pigs. Pseudotuberculosis can occur in wild lagomorphs, and in Europe, the hare is an important reservoir of the causative agent, which can be isolated from the oral

cavity as well as many visceral organs (Nikolova et al., 2001). There are also several reports of isolation of *Y. pseudotuberculosis* from feces or cecal contents (Borg, 1968; Mollaret, 1968; Schneider, 1968). In Japan, similar attempts to isolate the organism from hares have been mostly unsuccessful, suggesting that a different species harbors the organism in that country (Tsubokura et al., 1984). Lesions of pseudotuberculosis usually include large areas of caseous necrosis in the mesenteric lymph nodes, liver, and spleen. Necrosis is also common in Peyer's patches of the small intestine and in the cecum. Other organs, including the lungs and kidneys, may be involved as well (Flatt, 1974).

Acknowledgments

The author is Veterinary Medical Officer, Research Service, Minneapolis Veterans Affairs Health Care System. This material is the result of work supported with resources and facilities provided by the Minneapolis Veterans Affairs Health Care System, Minneapolis, Minnesota.

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Viral Diseases

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INTRODUCTION

The major focus of this chapter is the naturally occurring viral diseases of rabbits and hares although asymptomatic infections are also discussed (Tables 14.1 and 14.2). Some viral infections of rabbits have provided fundamental information on basic mechanisms of agent–host interrelationships, e.g., *Myxoma virus*, and others have been useful as models for human diseases,

e.g., *Rotavirus*. Although the principal emphasis is on virus infections of domestic rabbits of the genus *Oryctolagus*, naturally occurring and experimental infections of other rabbits and hares are also discussed.

In indoor facilities, it is unlikely that rabbits will encounter any of these viruses. Incoming rabbits should be obtained from sources free of these infections, however appropriate quarantine and screening measures should be standard operating procedure. With outdoor

TABLE 14.1 DNA Virus Infections of Rabbits

Family/subfamily	Genus	Species/strain	Host	Geographic Distribution
Poxviridae	Leporipoxvirus	Myxoma virus	<i>Sylvilagus brasiliensis</i>	South America
			<i>Sylvilagus bachmani</i>	North America
			<i>Oryctolagus cuniculus</i> (wild and domesticated)	South America, North America, Europe, Australia, New Zealand
		Rabbit (Shope) fibroma virus	<i>Sylvilagus floridanus</i>	North America
		Hare fibroma virus	<i>Lepus europaeus</i>	Europe
	Orthopoxvirus	Rabbitpox	<i>Oryctolagus cuniculus</i> (domesticated) ^a	United States (laboratory colonies), Holland
Herpesviridae/ Gammaherpesvirinae	Rhadinovirus	Leporid herpesvirus 1	<i>Sylvilagus floridanus</i>	United States
		Leporid herpesvirus 2	<i>Oryctolagus cuniculus</i> (domesticated)	England, United States (laboratory colonies)
Herpesviridae/ Alphaherpesvirinae	Simplexvirus	Leporid herpesvirus 4	<i>Oryctolagus cuniculus</i> (domesticated)	North America
		Human herpesvirus 1	<i>Oryctolagus cuniculus</i> (domesticated) ^a	United States
Papillomaviridae	Kappa-papillomavirus	Cottontail rabbit papillomavirus	<i>Sylvilagus floridanus</i>	United States
			<i>Oryctolagus cuniculus</i> (domesticated)	United States
		Rabbit oral papillomavirus	<i>Oryctolagus cuniculus</i> (domesticated)	United States, Mexico, New Zealand
Polyomaviridae	Polyomavirus	Rabbit kidney vacuolating virus	<i>Sylvilagus floridanus</i>	United States
			<i>Oryctolagus cuniculus</i> (domesticated)	United States
Adenoviridae	Mastadenovirus	Adenovirus	<i>Oryctolagus cuniculus</i> (domesticated)	Hungary, Quebec ^b
Parvoviridae	Parvovirus	Lapine parvovirus	<i>Oryctolagus cuniculus</i> (domesticated)	Japan, United States, Switzerland ^b

^a Aberrant host.^b Serologic evidence only.**TABLE 14.2** RNA Virus Infections of Rabbits

Family	Genus	Species/strain	Host	Geographic Distribution
Reoviridae	Rotavirus	Rotavirus	<i>Oryctolagus cuniculus</i> (domesticated)	North America, Japan, Europe,
			<i>Sylvilagus floridanus</i>	Canada ^a
			<i>Lepus americanus</i> ^a	Canada ^a
Coronaviridae	Coronavirus	Pleura effusion disease	<i>Oryctolagus cuniculus</i> (experimental)	Europe, United States, Japan
		Rabbit enteric coronavirus	<i>Oryctolagus cuniculus</i> (domesticated)	North America, Europe
Caliciviridae	Lagovirus	Rabbit hemorrhagic disease virus	<i>Oryctolagus cuniculus</i> (wild and domesticated)	Europe, Asia, North Africa, Middle East, Australia, New Zealand, Mexico, United States
			<i>Lepus</i> sp.	China
		European brown hare syndrome virus	<i>Lepus europaeus</i>	Europe, Argentina

(Continued)

TABLE 14.2 RNA Virus Infections of Rabbits (Continued)

Family	Genus	Species/strain	Host	Geographic Distribution
Paramyxoviridae	Vesivirus		<i>Lepus timidus</i>	Europe
		Rabbit Calicivirus	<i>Oryctolagus cuniculus</i> (wild and domesticated)	Europe, Australia
		Michigan Rabbit Calicivirus	<i>Oryctolagus cuniculus</i> (domesticated)	United States
		Not assigned	<i>Oryctolagus cuniculus</i> (domesticated)	United States
		Rabbit syncytium virus	<i>Sylvilagus floridanus</i>	United States
Bunyaviridae	Orthobunyavirus	California encephalitis virus	<i>Sylvilagus floridanus</i> ^a	North America
			<i>Lepus californicus</i> ^a	North America
		Snowshoe hare	<i>Lepus americanus</i>	North America,
		Tahyna	<i>Lepus europeus</i> ^a	Europe
			<i>Oryctolagus cuniculus</i> (wild) ^a	Europe
		Inkoo	<i>Lepus timidus</i> ^a	Finland
	Bunyamvera	Cache valley	<i>Sylvilagus auduboni</i> ^a	North America
			<i>Sylvilagus floridanus</i>	North America
			<i>Lepus californicus</i> ^a	North America
		Tenshaw	<i>Sylvilagus aquaticus</i> ^a	United States
			<i>Sylvilagus palustris</i> ^a	United States
			<i>Sylvilagus transitionalis</i>	United States
		Northway	<i>Lepus americanus</i>	Alaska, California
	Not assigned	Silverwater	<i>Lepus americanus</i>	Canada
Togaviridae	Alphavirus	Western equine encephalitis virus	<i>Sylvilagus</i> sp. ^a <i>Lepus californicus</i> ^a	North America North America
			<i>Lepus americanus</i> ^a	North America
			<i>Oryctolagus cuniculus</i> (domesticated)	North America
		Eastern equine encephalitis virus	<i>Lepus americanus</i> ^a	North America
		Venezuelan (VEE) equine encephalitis virus	<i>Lepus americanus</i> ^a	North America
			<i>Sylvilagus floridanus</i> ^a	North America
Flaviviridae	Flavivirus	St. Louis encephalitis virus	<i>Lepus americanus</i> ^a	North America
		West Nile virus	<i>Sylvilagus floridanus</i> (experimental)	North America
		Powassan virus	<i>Lepus americanus</i>	North America, Russia
Picobirnaviridae	Picobirnavirus	Not assigned	<i>Oryctolagus cuniculus</i> (domesticated)	United States
Rhabdoviridae	Lysavirus	Rabies virus	<i>Sylvilagus floridanus</i>	United States
			<i>Oryctolagus cuniculus</i> (domesticated)	United States

^aSerologic evidence only.

facilities, appropriate measures should be undertaken, as described in the "Control" section for each of the viral diseases, to prevent spread of viruses from wild rabbits in the surrounding environment. None of the viral infections of rabbits are known to be of public health importance as there are no reports of the definitive spread of viruses from rabbits to humans.

The viral diseases of rabbits are discussed in an order based on the taxonomic groups to which the viruses belong and are independent of the order of importance of the various diseases. The material is presented under uniform subject headings, including history, etiology, epidemiology, clinical signs, pathology, diagnosis, and control. Control is interpreted broadly and includes both prevention and eradication. This review uses the most widely accepted viral terminology. The recommendations of the VIII International Committee on Taxonomy of Viruses (Fauquet et al., 2005) are followed, but well-established common names for viruses are used when appropriate. Rabbits and hares are referenced by common names as indicated in *Mammals of the World, A Checklist* (Duff and Lawson, 2004).

DNA VIRUS INFECTIONS

Poxvirus Infections

Poxviruses cause several important diseases in domestic and wild mammals and birds. Infection with poxviruses usually results in relatively mild disease involving the skin of infected animals, but generalized and often fatal disease may also occur, as, for example, in myxomatosis in rabbits. Close antigenic relationships exist among many poxviruses derived from different animal species. In spite of close antigenic relationships, the poxviruses of rabbits which produce distinct disease syndromes are discussed as separate entities.

Myxoma Virus

HISTORY

The disease myxomatosis, caused by *Myxoma virus*, was first recognized by Sanarelli (1898) in Uruguay in 1896. European rabbits of the genus *Oryctolagus*, acquired for antiserum production, developed a highly fatal disease characterized by numerous mucinous skin tumors. Sanarelli (1898) named the disease "infectious myxomatosis of rabbits" and, since no microbial agents were detected, proposed that the disease was caused by a newly recognized group of infectious agents known as "filterable viruses." The virus which caused the first known outbreak of myxomatosis is believed to have originated from the Forest rabbit (*Sylvilagus brasiliensis*) in which the virus causes relatively mild disease. Transmission from wild to domestic rabbits probably

occurred by mosquitoes of the genus *Aedes* (Aragao, 1943; Fenner and Ratcliffe, 1965).

Myxomatosis spread to other countries of South America where it occasionally causes sporadic outbreaks in domesticated rabbits. In Chile, the disease is considered endemic in the wild European rabbit population (Fenner and Ratcliffe, 1965). The disease was first recognized in North America in 1928 when natural outbreaks of a fatal disease of rabbits occurred in several rabbit colonies near San Diego, California (Kessel et al., 1931). The virus which caused the first outbreaks in southern California may have been introduced into the United States from Mexico by importation of infected domestic rabbits (Vail and McKenney, 1943). The disease is endemic in the western United States, where the Brush rabbit (*Sylvilagus bachmani*) is the natural reservoir (Marshall and Regnery, 1960; Regnery and Miller, 1972).

Myxomatosis was introduced intentionally into Australia in an effort to control what had become Australia's major animal pest, the European rabbit (*Oryctolagus cuniculus*). The virus was first introduced into Australia in 1926 and used only in experimental studies aimed at determining its feasibility as a control measure for rabbits. In 1950, the virus was released into the wild rabbit population where it decimated the rabbit population of the continent by 1953. The disease is now endemic in the wild rabbit population of Australia, where it occasionally assumes epidemic proportions when climatic conditions favor vector activity. Within a decade following release of *Myxoma virus* into the rabbit population, it became evident that through natural selection genetically resistant strains of rabbits had emerged. In these rabbits, a virulent strain of *Myxoma virus* caused only 25% mortality compared to 90% mortality in non-resistant strains of rabbits (Fenner and Ratcliffe, 1965). This selective pressure continues with regional increases in resistance of Australian rabbits (Williams et al., 1990).

Genetic modification of *Myxoma virus* was recognized soon after its release into the rabbit population, and by the fourth year markedly attenuated strains of virus had replaced virulent virus as the dominant strains. The naturally attenuated viruses caused a milder disease of longer duration, which favored vector transmission and thus persistence of the virus (Fenner and Woodroffe, 1965; Fenner et al., 1957). The evolution of myxomatosis in Australia is a classic example of natural modification of both a virus and host until a state of equilibrium is reached, permitting the continued existence of both.

The introduction of myxomatosis into Europe followed the early successes of the Australian campaign. In 1952, while French officials were considering the desirability of introducing the disease, a private individual acquired the virus and released it on his own estate in an effort to control the rabbit population. The

virus spread rapidly through the countryside, and by the end of 1953 myxomatosis had been diagnosed in Belgium, the Netherlands, Germany, Luxembourg, Spain, and England (Armour and Thompson, 1955; Fenner and Ratcliffe, 1965; Lubke, 1968). Myxomatosis is now endemic in rabbits of the genus *Sylvilagus* in both North and South America and in wild European rabbits (*Oryctolagus cuniculus*) in South America, Europe, Australia, and New Zealand.

ETIOLOGY

Myxomatosis is caused by one of several strains of the species, of which *Myxoma virus* is the type species of the genus *Leporipoxvirus* in the *Chordopoxvirinae* subfamily of the family *Poxviridae* (Fauquet et al., 2005). Antigenic differences, demonstrated among different strains of the virus (Fenner, 1965; Reisner et al., 1963), have prompted some to consider the California strains of virus as distinct from *Myxoma virus*, and the designation "California rabbit fibroma virus" has been used to describe this virus. However, the demonstrable antigenic differences are insufficient to justify this distinction, and California strains of virus are considered strains of *Myxoma virus* (Fauquet et al., 2005).

Myxoma virus is antigenically closely related to the *Rabbit fibroma virus* as demonstrated by agar-gel diffusion microprecipitation techniques (Fenner, 1965). Heat-inactivated *Myxoma virus* has been reactivated by fibroma virus (Berry and Dedrick, 1936; Fenner, 1962), further demonstrating the close relationship between these two viruses. The Berry-Dedrick phenomenon of poxvirus reactivation was confirmed by Smith (1952), who demonstrated a spectrum of virulence for strains of myxoma and fibroma viruses. Fenner and Marshall (1957), in a study involving 92 strains of virus, established a virulence spectrum ranging from strains causing over 99% mortality in European rabbits to others causing less than 30% mortality. The most virulent strains were the Standard Laboratory, Lausanne, and California strains, whereas the least virulent were the neuromyxoma and Nottingham strains. Ecological pressures such as those previously described in Australia could have been responsible for the emergence of many of these strains of viruses. In many instances, however, viruses have been manipulated in the laboratory to the point of permanent modification (Kilham, 1957, 1958). The entire genome of the Lausanne strain has been sequenced (Cameron et al., 1999). Infection of *Oryctolagus cuniculus* with *Myxoma virus* has provided a model system for elucidating the immunopathogenesis of poxvirus in the host (Stanford et al., 2007).

The chemical and physical characteristics of *Myxoma virus* have been described (Andrewes and Porterfield, 1989; Fenner, 1953; Fenner and Ratcliffe, 1965). *Myxoma virus* is readily propagated at 35°C on the chorioallantoic

membrane of embryonated hens' eggs, forming distinct pocks (Fenner and McIntyre, 1956). Different strains of virus cause pocks of various sizes, the variation being sufficiently distinct to allow tentative strain identification. The South American strains cause large pocks, whereas the California strains produce small focal lesions on the membrane (Fenner and Marshall, 1957). The virus can also be propagated in cell cultures derived from rabbits and other species, including chicken, squirrel, rat, hamster, guinea pig, and human (Andrewes and Porterfield, 1989; Woodroffe and Fenner, 1965). Distinct differences in plaque size on rabbit kidney cell cultures can be demonstrated between the South American and California strains, the former causing much larger plaques (Woodroffe and Fenner, 1965). The most sensitive method for isolation of *Myxoma virus* under laboratory conditions is inoculation of the skin of European rabbits (Fenner and McIntyre, 1956).

EPIDEMIOLOGY

Myxomatosis is endemic on four continents: Australia, Europe, North America, and South America. In Brazil and Uruguay, the virus is endemic in wild rabbits (*Sylvilagus*), particularly *Sylvilagus brasiliensis* (Aragao, 1943). A similar situation may exist in Panama and Colombia, where the strains of virus are similar in virulence to South American strains, but antigenically more closely related to the California strains (Fenner, 1965). In the forested area of Argentina, the virus is also endemic in *Sylvilagus* rabbits, but in the southern part of the country and in Chile the principal reservoir is the wild European rabbit (*Oryctolagus cuniculus*) (Fenner and Ratcliffe, 1965). The California strains of *Myxoma virus*, also known as the California rabbit fibroma virus (Andrewes and Porterfield, 1989), are endemic in wild rabbits (*Sylvilagus*), especially *Sylvilagus bachmani*, which serves as the principal source of infection for domestic rabbits (Marshall et al., 1963). A similar situation exists in Mexico, where *Sylvilagus bachmani* has been shown to be the reservoir in Baja California (Licón Luna, 2000). In Australia, the *Myxoma virus* has been endemic in wild European rabbits (*Oryctolagus cuniculus*) since its introduction into the rabbit population in 1950. Following the introduction of the virus into France in 1952, myxomatosis has become established in most countries of Europe, the wild European rabbit serving as the predominant host species. In Europe, *Myxoma virus* and *Rabbit hemorrhagic disease virus* occur endemically in the same free-living populations of *Oryctolagus cuniculus* (Calvete et al., 2002) and seropositivity to both viruses occurs significantly suggesting the possibility of predisposition (Marchandeau et al., 2004). In rabbit farms in Belgium, the Netherlands, and Germany, the seroprevalence of *Myxoma virus* was

higher in herds with recurrent respiratory or digestive diseases (Marlier et al., 2001).

The naturally susceptible species are the European rabbit (*Oryctolagus cuniculus*), the European hare (*Lepus europaeus*), the Mountain hare (*Lepus timidus*), the Forest rabbit (*Sylvilagus brasiliensis*), the Brush rabbit (*Sylvilagus bachmani*), and the Eastern Cottontail rabbit (*Sylvilagus floridanus*). Experimentally, several additional species of *Sylvilagus* can be infected (Fenner and Ratcliffe, 1965; Regnery and Marshall, 1971).

The principal mode of transmission of the virus is mechanical transport of virus on mouth parts by arthropod vectors, mosquitoes and fleas being most often incriminated (Grodhaus et al., 1963), but biting flies, gnats, mites, and lice may serve as vectors (Fenner and Woodroffe, 1953; Mykytowycz, 1957). The source of virus is usually the superficial layers of the skin, especially of the eyelids and at the base of the ears (Fenner and Woodroffe, 1953), where surface-feeding arthropods obtain the virus and serve as mechanical vectors. Experimentally, virus can spread by contact in the absence of arthropod vectors, and such transmission may occur under natural conditions in rabbit warrens (Mykytowycz, 1958, 1961). An outbreak in Hungary at a rabbit farm was attributed to airborne transmission as the disease occurred in winter when mosquitoes and fleas were uncommon (Farsang et al., 2003). Windborne spread was suspected in France (Arthur and Louzis, 1988). Transmission of *Myxoma virus* by contaminated spines of thistles (*Cirsium vulgare*) has been described (Dyce, 1961; Mykytowycz, 1961). The claws of predatory birds and carrion feeders, such as buzzards and crows, may be contaminated with virus, and such birds may play a role in dissemination of the virus (Borg and Bakos, 1963).

In an attempt to improve the usefulness of myxomatosis in rabbit control, the European rabbit flea, *Spilopsyllus cuniculi*, was introduced into Australia in 1966 (Sobey and Menzies, 1969). The flea reproduced in wild rabbit populations and transmitted both introduced and field strains of *Myxoma virus* (Shepherd and Edmonds, 1977; Sobey and Conolly, 1971). As a result of flea introduction, myxomatosis has become more prevalent in drier tableland areas (Parer and Korn, 1989), and outbreaks of myxomatosis have shifted from summer to spring (Shepherd and Edmonds, 1978; Shepherd et al., 1978). In France, there is evidence that mosquitoes of the genus *Anopheles* are the principal vectors of summer epidemics. The rabbit flea, *Spilopsyllus cuniculi*, is probably a major vector, especially during winter months when mosquito activity is low (Fenner and Ratcliffe, 1965).

Myxomatosis in Britain is characterized by milder seasonal fluctuations in disease incidence than in Australia, California, and France (Ross and Tittensor, 1986). Mosquitoes play a minor role as vectors,

whereas the rabbit flea, *Spilopsyllus cuniculi*, which is less influenced by seasonal changes, is the major vector (Andrewes et al., 1959; Armour and Thompson, 1955; Lockley, 1954; Mead-Briggs, 1964). The *Myxoma virus* in Britain has not undergone the rapid loss of virulence observed with the Australian and French viruses. The increase in resistance to myxomatosis in wild rabbit populations has resulted in the appearance of more virulent strains of *Myxoma virus* (Ross and Sanders, 1987). While mildly virulent strains have emerged in Britain, the predominant strains are moderately virulent (Chapple and Bowen, 1963; Chapple and Lewis, 1964; Fenner and Chapple, 1965), with recent estimates of between 47% and 69% mortality in infected rabbits (Ross et al., 1989). The different evolution of *Myxoma virus* in Britain has been attributed to the fact that the virus is predominantly flea-transmitted. The flea is less seasonal and less mobile than the mosquito. That fleas move in large numbers from dead animals while moving only occasionally from live ones would seem to favor transmission of virulent virus strains (Fenner and Marshall, 1957). The proportion of infective fleas produced is inversely related to the survival time of rabbits following infection (Mead-Briggs and Vaughan, 1975). The flea is also an effective reservoir of virus, possessing a longer life span than mosquitoes. The life span of active female mosquitoes is usually 2–3 weeks, whereas fleas have been known to feed actively for over 1 year. The *Myxoma virus* can persist for 105 days in rabbit fleas with no rabbit contact in artificial burrows (Chapple and Lewis, 1965).

In experimental studies of viral pathogenesis and host resistance, a virulent strain of *Myxoma virus* (SLS) inoculated into laboratory European rabbits (*Oryctolagus cuniculus*) resulted in fatal infections whereas wild European rabbits with naturally acquired innate resistance recovered (Best and Kerr, 2000). Conversely, inoculation of laboratory European rabbits with an attenuated, naturally derived field strain of *Myxoma virus* resulted in recovery from infection and rabbits with innate resistance experienced only mild disease. A similar study examined the pathogenesis of two Californian strains of *Myxoma viruses* (MSW and MSD) in European rabbits and found that both susceptible rabbits and rabbits that had naturally acquired innate resistance, experienced acute fatal infections with the MSW strain, whereas, the MSD strain caused classical signs of myxomatosis in both strains of rabbits (Silvers et al., 2006).

CLINICAL SIGNS

Considerable differences in the virulence of *Myxoma virus* strains complicate discussion of the clinical disease, as does the fact that different species and strains of rabbits vary considerably in susceptibility to *Myxoma virus*. Major emphasis is given to discussion of disease

in *Oryctolagus* by the major strains of virus found in Australia, California, Europe, and South America.

SIGNS IN SYLVILAGUS SPECIES Rabbits of the genus *Sylvilagus*, the natural host of the virus, are relatively resistant to infection and generally develop skin tumors and lesions, but rarely systemic disease, except in young rabbits (Aragao, 1943). Generally, the nodules occur at the site of infection, persist for a variable amount of time, and then regress. The size of the skin nodule and its persistence vary with the strain of rabbit and the strain of virus (Fenner and Ratcliffe, 1965; Marshall and Regnery, 1963; Regnery and Miller, 1972; Silvers et al., 2009).

SIGNS IN LEPUS SPECIES The European hare (*Lepus europaeus*) is resistant to *Myxoma virus* under experimental conditions, and field experience supports this observation. Occasionally, however, individual hares (*Lepus europaeus* and *Lepus timidus*) with mild to severe generalized myxomatosis have been encountered (Fenner and Ratcliffe, 1965).

SIGNS IN ORYCTOLAGUS CUNICULUS *Myxoma virus* infection in the European rabbit usually results in severe disease with high mortality (Marshall et al., 1963; Patton and Holmes, 1977). The severity and variety of clinical disease is largely determined by the strain of virus as well as the strain of rabbit (Sobey, 1969; Sobey et al., 1970) and have been described in detail (Fenner and Marshall, 1957; Fenner and Ratcliffe, 1965). The discussion which follows is a summation of the findings of these workers and others (Chapple and Bowen, 1963; Kessel et al., 1931).

Signs which develop following infection with California strains of the virus in susceptible rabbits can be categorized as peracute, acute, or chronic. Rabbits with the peracute form of disease die within 1 week after exposure to the virus, exhibiting only edema of the eyelids and lethargy prior to death. In the acute form of disease, in which rabbits survive for 1–2 weeks, usually edema of the eyelids, resulting in a “droopy” appearance of the eyes, appears at 6–7 days. Inflammation and edema around the anal, genital, oral, and nasal orifices are also observed. Skin hemorrhages and convulsions precede death on the ninth or tenth day. The few rabbits which survive beyond 10 days and have a chronic form may develop purulent blepharoconjunctivitis and edema at the base of the ears, signs more often associated with other myxoma strains (Figure 14.1). The nodule which develops at the site of inoculation is not a clearly defined tumor, and under natural conditions the development of myxomas is not characteristic. Although nodules on the ears, head, and legs have been reported (Kessel et al., 1934), other workers have

been unable to induce nodule development (Fenner and Marshall, 1957).

The original South American isolate of Moses (1911), results in an acute disease with a mean survival time of 11 days. From 3–4 days following inoculation or natural infection with virus, a primary tumor may become evident, and generalized tumors usually appear by the sixth or seventh day. Edema of the eyelids occurs followed by mucopurulent blepharoconjunctivitis, often resulting in complete closure of the eyes. Mucopurulent nasal discharge and pronounced edema of the base of the ears, the perineal region, the external genitalia, and lips are frequently seen. By the tenth day, hard convex lumps may cover the body, head, and ears and occasionally the legs. The lumps are not sharply demarcated but may become markedly congested and ultimately necrotic in rabbits surviving for 2 weeks. Dyspnea is often seen in protracted cases, but appetite may be maintained until shortly before death. Terminal convulsions frequently precede death, which usually occurs 8–15 days after infection. Infection with less virulent South American and Australian strains results in milder disease with less edema and nasal and ocular discharge, more clearly demarcated nodules, and lower mortality. The laboratory-attenuated neuromyxoma virus induces a mild disease with little or no mortality (Hurst, 1937b).

The predominant myxoma strains in Europe are the virulent Lausanne strain and its naturally attenuated derivatives originating from the virus introduced into France from Brazil in 1952. The more virulent European viruses cause severe disease in rabbits, resulting in mortality of up to 100%, but modified strains which have emerged are of lower morbidity and mortality (Arthur and Louzis, 1988). With some of the naturally attenuated British viruses, mortality is also decreased, and tumors are flat rather than convex, resembling some of the attenuated field strains in Australia (Chapple and Bowen, 1963). In France and Belgium, two forms of the disease are recognized, nodular and amyxomatous forms (Marlier et al., 1999). The former is characterized by florid skin lesions and severe immunosuppression, accompanied by bacterial infections of the respiratory tract, whereas in the latter, skin nodules are few and small, with acute respiratory distress and copious nasal discharge.

PATHOLOGY

The gross and microscopic pathology of myxomatosis has been comprehensively reviewed (Fenner and Ratcliffe, 1965; Hurst, 1937a; Rivers, 1930). In adult *Sylvilagus*, *Myxoma virus* usually causes localized skin tumors. The tumors resemble the fibromas in European rabbits produced by the *rabbit fibroma virus* (see later). Hares or young *Sylvilagus* usually develop a mild localized infection, although disseminated cutaneous



FIGURE 14.1 Rabbit with myxomatosis displaying facial edema with mucopurulent conjunctivitis. Courtesy of Dr. G. Van Hoosier.

fibromatous to myxomatous nodules similar to those in acute myxomatosis may be found. Prominent gross lesions in European rabbits with myxomatosis are skin tumors (not characteristic of the California disease) and pronounced cutaneous and subcutaneous edema, especially of the face and around body orifices. Hemorrhages of the skin, heart, and subserosa of the gastrointestinal tract may be observed, especially following infection with the California virus.

Lesions in the skin involve epithelial cells, fibroblasts, and endothelial cells and range from proliferative to degenerative, depending on the strain of virus. The skin tumors result from proliferation of undifferentiated mesenchymal cells, which become the characteristic large stellate (myxoma) cells surrounded by a homogeneous matrix of mucinous material interspersed with capillaries and inflammatory cells (Figure 14.2). There is typically vascular endothelial proliferation and hypertrophy with narrowing of the lumen (Hurst, 1937a; Percy and Barthold, 2007). Central necrosis of myxomas may be attributed to occlusion of blood vessels by endothelial proliferation. Epithelial cells overlying the tumor may appear normal in early tumors, or show hyperplasia or degeneration. Intracytoplasmic inclusions, in various cells types, are especially prominent in the epidermis (Patton and Holmes, 1977; Rivers and Ward, 1937).

Lesions in other organs, although not consistently present, reflect the potential generalized nature of myxomatosis. Cellular proliferation, invariably present in the skin, has also been described in pulmonary alveolar epithelium and in reticulum cells of lymph nodes and spleen (Hurst, 1937a). Focal hemorrhages may be observed in skin, kidneys, lymph nodes, testes, heart, stomach, and intestines. Degeneration and necrosis occur frequently in lymph nodes, pulmonary alveoli, spleen, and central veins of hepatic lobules. Stellate cells may occur in lymph nodes, bone marrow, uterus, ovaries, testes, and lungs (Marcato and Simoni, 1977).

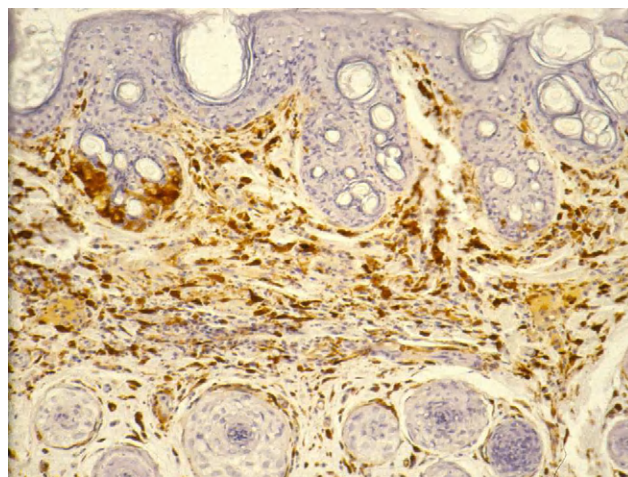


FIGURE 14.2 Myxoma virus expressing lacZ-infected primary skin lesion immunostained with antibody to β -galactosidase demonstrates the wide distribution of the virus within the dermis and subcutis. Hematoxylin and eosin counterstain. Courtesy of Dr. G. McFadden.

In a survey of 66 rabbits, presumably *Oryctolagus cuniculus*, that died of respiratory disease, *Myxoma virus* was isolated from seven (10%), mainly from rabbits with acute hemorrhagic pneumonia (Marlier et al., 2000). The mechanisms by which the virus manifests the development of disease in rabbits have been described (Stanford et al., 2007).

DIAGNOSIS

Myxomatosis in European rabbits can usually be diagnosed by the clinicopathological features, particularly the nodular form, due to the characteristic skin lesions. Infection with the California viruses may be harder to diagnose, however, owing to the frequent absence of skin nodules and other signs of disease. Diagnosis should be confirmed by virus isolation. Intracutaneous inoculation of young susceptible rabbits with fresh tissue collected from lesions free of bacterial contamination results in lesions at the site of inoculation within 1 week. The virus can be isolated by chorioallantoic membrane inoculation of 11–13-day-old embryonated chicken eggs followed by incubation at 35°C for 4–6 days. Distinct focal pocks develop if the virus is present. The South American viruses cause large pocks, the California virus intermediate-sized pocks, and the fibroma virus minute pocks. Virus isolation on chicken embryo fibroblast, Vero or RK13 cells can also be accomplished. A cytopathic effect typical of poxviruses usually develops in 1–2 days but may take up to 7 days with some strains. The virus isolated can be identified as *Myxoma virus* by direct fluorescent antibody test (Takahashi et al., 1958, 1959), the plaque-neutralization test (Woodroffe and Fenner, 1965), the agar-gel diffusion microprecipitation test (Fenner, 1965), or the

indirect immunoperoxidase test (Marlier et al., 1999). Myxoma virus DNA in tissue samples can be detected by polymerase chain reaction (PCR) (Barcena et al., 2000; Kritas et al. 2008; Pérez de Rozas et al., 2008).

Rabbits that survive infection develop antibodies to the virus, which can be detected by an enzyme-linked immunosorbent assay (ELISA) that has been shown to be more sensitive and specific than agar gel immunodiffusion, complement fixation (CF), or indirect immunofluorescence (Gelfi et al., 1999). Serum neutralization has also been used to detect antibodies (Marlier et al., 1999). Infection of *Sylvilagus* rabbits with *Myxoma virus* clinically resembles fibromatosis and should be differentiated from the latter disease by PCR or inoculation of young susceptible rabbits of the genus *Oryctolagus*. Myxomatosis causes severe to fatal disease, whereas fibromatosis causes a localized fibroma.

CONTROL

Control of myxomatosis is of prime importance in areas where the virus is endemic in wild rabbit populations. Vector control, including adequate screening to exclude mosquitoes, serves to keep the disease under control. Newly introduced rabbits should be quarantined in an insect-proof facility for 2 weeks. To prevent spread within a colony, all sick rabbits should be isolated. Fibroma virus has been used as a live vaccine for myxomatosis, but results have been variable (Fenner and Ratcliffe, 1965). A live attenuated myxomatosis vaccine (the MSD strain) results in a mild reaction followed by immunity persisting for 9 months (McKercher and Saito, 1964). Jiran et al. (1970) found this virus to be too virulent for use as a vaccine and further attenuated it by serial passage in rabbit kidney cell cultures. The additionally modified virus, designated MSD/B, was safe and highly immunogenic.

However, efficacy of a vaccine has yet to be demonstrated in commercial rabbits. In an outbreak of atypical myxomatosis in Hungary, fibroma vaccine was not protective, whereas in experimental studies, a live myxomatosis vaccine protected rabbits (Farsang et al., 2003). An assessment of the Borgi strain vaccine of *Myxoma virus* was reported during an outbreak of myxomatosis in Greece (Kritas et al., 2008). Two-month-old vaccinated pregnant does were obtained by two commercial rabbitries, only one of which had vaccinated resident does. Within several weeks of their arrival, an explosive outbreak of myxomatosis with high morbidity and mortality occurred on both farms in both unvaccinated and vaccinated rabbits, both imported and resident, with the Lausanne strain of *Myxoma virus*. Since vaccinated rabbits were affected within 6 months of vaccination, the efficacy of the vaccine was questionable.

Field trials using various strains have been conducted in free-living rabbit populations. A field trial of a recombinant vaccine, using a naturally attenuated myxoma

field strain (6918) that expressed a *Rabbit hemorrhagic disease virus* VP60 protein, conducted on an island off the coast of Spain, revealed that the vaccine induced antibodies to both viruses in vaccinated rabbits without any adverse side effects (Torres et al., 2001). Furthermore, the vaccine exhibited limited horizontal transmission, either by direct contact or fleas, as uninoculated rabbits also exhibited seroconversion. Although the observation period was brief (32 days), the vaccine appeared to be both safe and efficacious. An attenuated strain (SG33) was used in a field trial in juvenile rabbits in France and vaccinated rabbits had a 1.8-fold greater odds of surviving than unvaccinated rabbits over 4 years of surveillance (Guitton et al., 2008). In a field trial in Zaragoza province in northeast Spain, young European rabbits (*Oryctolagus cuniculus*) born in the current year and unvaccinated, were 13.6 times more likely to die than rabbits vaccinated against both myxomatosis (POX-LAP) and *Rabbit hemorrhagic disease* (CYLAP-VHD), over a 90-day observation period after vaccination (Calvete et al., 2004).

Rabbit (Shope) Fibroma Virus

HISTORY

The transmissible tumor-inducing agent now known as rabbit or Shope fibroma virus was isolated from an Eastern Cottontail rabbit (*Sylvilagus floridanus*) in 1932 (Shope, 1932a). The virus was transmissible to Eastern Cottontail and European rabbits (*Oryctolagus cuniculus*), producing localized fibromas in both species. Shope (1932a) described the gross and microscopic lesions of both the natural and experimental disease and identified the causative agent as a virus antigenically related to *Myxoma virus* (Shope, 1932b). Similarities between *Rabbit fibroma* and *Myxoma viruses* have subsequently been confirmed by cross-immunity tests (Shope, 1936), ether sensitivity (Andrewes and Horstmann, 1949; Fenner, 1953), virus-reactivation studies (Berry and Dedrick, 1936), microprecipitation procedures (Fenner, 1965), and sequence comparison (Willer et al., 1999). The *Rabbit fibroma virus*, initially believed to cause only localized benign fibromas, was later shown to cause severe generalized disease in newborn European (Duran-Reynals, 1940; Joiner et al., 1971) and Eastern Cottontail rabbits (Yuill and Hanson, 1964). The disease is historically considered a benign endemic disease of wild *Sylvilagus* rabbits, of little economic significance to commercial rabbit producers or laboratory investigators. However, an outbreak of fibromatosis in a commercial rabbitry resulting in high morbidity and mortality in newborn rabbits was reported in 1971 (Joiner et al., 1971). Thus, the disease can be a threat to commercial rabbits in areas where it is endemic in wild rabbit populations and outdoor husbandry practices permit contact with arthropod vectors.

Another tumor-producing virus, designated malignant rabbit fibroma virus, has been recovered from *Oryctolagus cuniculus* with experimentally induced Shope fibromas (Strayer et al., 1983a). Although the virus was present in a stock of *Rabbit fibroma virus*, its origin is unclear. The virus is antigenically similar to both *Rabbit fibroma* and *Myxoma* viruses and is considered a recombinant of the two viruses (Block et al., 1985). The virus induces a syndrome of severe immunosuppression resulting in disseminated malignancy and opportunistic infections (Corbeil et al., 1983; Skaletsky et al., 1984; Strayer and Sell, 1983; Strayer et al., 1983a, 1983b, 1983c). The disease may be a useful model for the study of virus-induced immunologic impairment (Strayer et al., 1985).

ETIOLOGY

Rabbit fibroma virus is a *Leporipoxvirus* of the family *Poxviridae* (Fauquet et al., 2005) and is closely related to *Myxoma virus* (Fenner, 1953) and the hare and squirrel fibroma viruses (Fenner, 1965). The complete genome of *Rabbit fibroma virus* has been sequenced (Willer et al., 1999). The chemical and physical characteristics of fibroma virus have been summarized by Gross (1983). The virus can be propagated on the chorioallantoic membrane of chicken embryos, but characteristic lesions as observed with *Myxoma virus* are not produced (Gross, 1983). The virus has been propagated in cell cultures derived from rats, guinea pigs, and humans (Chaproniere and Andrewes, 1957) and also in rabbit cell cultures derived from Eastern Cottontail and domestic rabbits (Constantin et al., 1956; Hinze and Walker, 1964; Kasza, 1974; Kilham, 1956; Padgett et al., 1962). Fibroma virus in cultured rabbit kidney cells induces pronounced changes in cell growth and morphology, and inoculation of virus-transformed cells into the cheek pouch of hamsters results in tumor formation (Hinze and Walker, 1964).

EPIDEMIOLOGY

Since *Rabbit fibroma virus* was first isolated from an Eastern Cottontail rabbit in New Jersey (Shope, 1932a), the disease has been recognized in several other states, as well as in Canada. Herman et al. (1956) found that more than 50% of wild *Sylvilagus* rabbits trapped in the Patuxent Wildlife Refuge in Maryland had *Rabbit fibroma virus* or antibodies to *Rabbit fibroma virus*. The virus has also been isolated from *Sylvilagus* rabbits in Wisconsin (Yuill and Hanson, 1964), Michigan (Herman et al., 1956), and Ohio (Kasza, 1974). Recognition of the disease in Texas (Joiner et al., 1971) indicates that it may be more widespread in the U.S. than was formerly believed.

The natural transmission cycle of *Rabbit fibroma virus* has not been completely elucidated. The virus may persist in the epidermis of experimentally infected *Sylvilagus* rabbits for 5–10 months (Kilham and Dalmat,

1955; Kilham and Fisher, 1954), which would enhance the likelihood of mechanical arthropod transmission. Experimentally, several species of mosquitoes as well as triatomines, fleas, and bedbugs can serve as vectors (Dalmat, 1959; Kilham and Dalmat, 1955; Kilham and Woke, 1953). Infected mosquitoes are capable of infecting wild *Sylvilagus* rabbits (Kilham and Dalmat, 1955). Experimental and circumstantial evidence suggests that the principal mode of transmission for fibroma virus is biting arthropods, a situation similar to that described for myxomatosis. Infection may also occur by direct contact with skin injuries being a predisposing factor (Grilli et al., 2003).

The natural host of fibroma virus is the Eastern Cottontail rabbit (*Sylvilagus floridanus*). Three other species of *Sylvilagus* (*Sylvilagus bachmani*, *Sylvilagus nuttalli*, and *Sylvilagus auduboni*) are refractory to fibroma virus (Fenner and Ratcliffe, 1965). A closely related virus, isolated from fibromas in *Sylvilagus bachmani*, failed to induce lesions in other *Sylvilagus* species (Marshall and Regnery, 1960). This virus, considered a strain of *Myxoma virus*, is a related virus named California *Rabbit fibroma virus* (Andrewes and Porterfield, 1989). The European rabbit (*Oryctolagus cuniculus*) is susceptible to fibroma virus (Shope, 1932a) as is the Snowshoe hare, *Lepus americanus* (Yuill, 1981), but the European hare, *Lepus europaeus*, is refractory to the virus (Fenner and Ratcliffe, 1965). Whereas the European rabbit is readily infected, it does not serve as a good source of infection for mosquitoes because of low virus concentrations in the skin (Fenner and Ratcliffe, 1965).

CLINICAL SIGNS

The clinical signs are largely those described by Shope (1932a, 1932b, 1936). The tumors observed in natural fibromatosis of Eastern Cottontail rabbits are almost invariably on the legs or feet, usually one to three tumors occurring on an infected rabbit. Tumors may occur on the muzzle and around the eyes, and measure up to 7 cm in diameter and 1–2 cm in thickness. The tumors are subcutaneous, unattached to underlying tissues, and may persist for several months, and in some instances for nearly a year (Kilham and Dalmat, 1955). Other clinical signs are usually absent, the tumor-bearing rabbit remaining apparently normal throughout the disease. Metastases do not occur (Shope, 1932a). The clinical signs in experimentally infected European rabbits resemble those in *Sylvilagus* rabbits. However, regression of tumors is usually more rapid than in *Sylvilagus* rabbits. Inoculation of newborn European rabbits frequently results in fatal systemic infections (Duran-Reynals, 1945). Systemic infections of adult European rabbits have also been described (Hurst, 1937c), but the usual result of infection is the development of localized benign tumors. In the only reported

natural outbreak of fibromatosis of European rabbits, diffuse subcutaneous induration involving the underlying tissues occurred (Joiner et al., 1971). Subcutaneous tumors were the predominant sign in adult rabbits but suckling rabbits had lethargy and loss of condition, in addition to the skin lesions. Hyperemia and edema of the external genitalia of male and female rabbits were also observed.

A survey of tumors in pet rabbits submitted to the surgical biopsy service of a veterinary school in Pennsylvania over a 16-year period revealed that Shope fibroma was diagnosed in 19 tumors from 179 rabbits (von Bomhard et al., 2007). The species of rabbits was not provided and diagnosis was based on histopathologic findings. The limbs, head, and pinna were affected in 75% of cases. Case reports of keratitis with a perilimbal mass due to fibroma virus have been diagnosed in individual European rabbits (*Oryctolagus cuniculus*) (Keller et al., 2007; McLeod and Langlinais, 1981).

PATHOLOGY

The gross and microscopic changes associated with fibroma virus infection in naturally and experimentally infected *Sylvilagus* rabbits and in experimentally infected European rabbits have been described by several workers (Ahlgren, 1938; Andrewes, 1936; Dalmat and Stanton, 1959; Fisher, 1953; Grilli et al., 2003; Kilham and Fisher, 1954; Shope, 1932a; Yuill and Hanson, 1964). The pathological changes in the only reported natural outbreak of fibromatosis in European rabbits were described by Joiner et al. (1971). The earliest gross lesion in experimentally infected *Sylvilagus* rabbits is a slight thickening of the subcutaneous tissue, followed by development of clearly demarcated soft swellings usually evident 6 days after inoculation. The tumors enlarge, increase in density, and usually reach maximum size by 12 days. The average size of the tumors is 4–6 cm with a thickness of 2 cm. Tumors may persist for months before regressing, leaving the rabbit essentially normal. Experimentally infected newborn *Sylvilagus* rabbits may die of generalized fibromatosis. Under natural conditions, however, this form of the disease has not been observed. Gross lesions in experimentally infected European rabbits are similar to those observed in *Sylvilagus* rabbits but regression of the tumors occurs more rapidly.

The earliest microscopic lesions in *Sylvilagus* rabbits are an acute inflammation followed by localized fibroblastic proliferation, accompanied by both mononuclear and polymorphonuclear leukocyte infiltrations. Fibroblasts proliferate until a distinct tumor is formed, consisting of spindle-shaped and polygonal connective tissue cells with abundant cytoplasm. Mitotic figures are few. Many tumor cells may have large intracytoplasmic inclusions characteristic of poxvirus infections. Mononuclear leukocyte cuffing of vessels adjacent to

the tumor may be observed, and a pronounced accumulation of lymphocytes at the base of the tumor is often seen. Degeneration of the overlying epidermis may result from pressure ischemia, followed by necrosis and sloughing of the epithelium and tumor. In many instances, however, the tumors regress without epithelial sloughing. Regression is usually complete within 2 months after appearance of tumors. Andrewes (1936) described a strain of fibroma virus which caused a more inflammatory and less proliferative lesion than the fibroma virus isolated by Shope (1932a).

Histologically, typical fibromas are observed in European rabbits experimentally infected with fibroma virus. The lesions differ only slightly from those in *Sylvilagus* rabbits, the principal difference being the absence of epidermal degeneration often observed in the latter species (Shope, 1932a). However, Ahlgren (1938) described epidermal degeneration in European rabbits. Microscopic lesions in the natural outbreak of fibromatosis in European rabbits ranged from tumors resembling myxomas to typical fibromas (Joiner et al., 1971). Eosinophilic cytoplasmic inclusions were occasionally observed in tumor cells, and similar inclusions, possibly of viral origin, were seen in epithelial cells overlying the tumors. The lesions encountered in adult and newborn rabbits did not differ significantly.

DIAGNOSIS

Fibromatosis should be differentiated from papillomatosis and myxomatosis. Clinically, differentiation from papillomatosis can be readily accomplished as fibromas are essentially flat, subcutaneous, loose, rubbery tumors, whereas papillomas are tumors of the skin which are heavily keratinized and project in irregular fashion from the skin surface. Histopathological differentiation is also easily accomplished, especially when intracytoplasmic inclusion bodies are observed. Historically, subcutaneous inoculation of young European rabbits with a tumor cell suspension was used to differentiate the two viruses. Rabbit fibroma virus induces local fibromas, whereas Myxoma virus causes severe systemic and often fatal disease. Virus isolation in cell cultures or on the chorioallantoic membranes of chicken embryos should be attempted to confirm the diagnosis. The virus can be identified by serum neutralization tests (Yuill, 1981).

CONTROL

Because the disease is endemic and of little significance in *Sylvilagus* rabbits, no control measures have been developed. Fibromatosis is also not an important problem in domestic rabbits; however, since the disease has been encountered in a rabbitry in an area where the disease is apparently endemic in wild rabbits, control measures might be considered. In such areas, the

method of choice for preventing infection of rabbits held in outdoor enclosures is vector control. Careful analysis of the role of possible vectors in natural outbreaks should be made in preparation for the establishment of vector control methods.

Hare Fibroma Virus

HISTORY

Epidemics of a fibromatous disease in European hares (*Lepus europaeus*) occurred in France and Northern Italy in 1959 (Lafenetre et al., 1960; Leinati et al., 1959). The causative agent was a poxvirus related to *Myxoma virus* (Leinati et al., 1961). In retrospect, a nodular skin disease of hares in Germany designated hare sarcoma was probably hare fibromatosis (Dungern and Coca, 1909).

ETIOLOGY

The causative agent of hare fibromatosis is a *Leporipoxvirus* in the family *Poxviridae* (Fauquet et al., 2005), which has been shown by plaque-neutralization and cross-protection tests to be antigenically related to myxoma, rabbit fibroma, and squirrel fibroma viruses (Woodroffe and Fenner, 1962). Agar-gel diffusion micro-precipitation techniques reveal that hare fibroma virus shares more common antigens with *Rabbit fibroma virus* than with *Myxoma virus* (Fenner, 1965). A considerable degree of cross-protection exists between hare fibroma and myxoma viruses. European rabbits immune to *Myxoma virus* are completely refractory to hare fibroma virus, whereas rabbits immunized with hare fibroma virus develop signs when infected with *Myxoma virus* but survive, indicating some protection (Woodroffe and Fenner, 1965).

EPIDEMIOLOGY

Hare fibromatosis has been recognized only in Europe, where under natural conditions it infects the European hare. However, dermal tumors were reported on Cape hares (*Lepus capensis*) in the Laikipia District of Kenya that had gross and histopathologic similarities to hare fibromatosis (Karstad et al., 1977). In 2001, an outbreak of fibromatosis occurred in farmed game European hares (*Lepus europaeus*) in Italy (Grilli et al., 2003). The European rabbit (*Oryctolagus cuniculus*) is susceptible to the virus, but natural outbreaks of disease in rabbits have not been reported. A seasonal occurrence of disease has been reported, with the peak incidence in late summer and autumn (Lafenetre et al., 1960; Leinati et al., 1959). The mode of transmission and interepidemic survival of the virus are unknown.

CLINICAL SIGNS

In European hares, the disease is characterized by development of numerous skin nodules, up to 2.5 cm

in size, occurring especially on the face, eyelids, and around the ears. In farmed game European hares, fibromas also occurred on the legs (Grilli et al., 2003). The nodules closely resemble rabbit fibromas (Leinati et al., 1961). In adult European rabbits the virus causes formation of small fibromas, but newborn rabbits exhibit large fibromas resembling the lesions of rabbit fibromatosis (Fenner and Ratcliffe, 1965).

PATHOLOGY

The gross and microscopic appearance of the lesions of hare fibroma is similar to that of the lesions of rabbit fibroma (Lafenetre et al., 1960; Leinati et al., 1961).

DIAGNOSIS

The disease is usually diagnosed from clinicopathological features. The diagnosis can be confirmed by virus isolation in rabbit kidney cell cultures or on the chorioallantoic membrane of chicken embryos. Serological characterization of the virus can be achieved using the agar-gel diffusion technique (Fenner, 1965).

CONTROL

Because the disease is endemic and of little significance in hares, no control measures have been developed. Farmed game hares, used for stocking areas, should be free of infection to avoid dissemination of the virus (Grilli et al., 2003).

Rabbitpox

HISTORY

Rabbitpox was first diagnosed at the Rockefeller Institute in New York when a highly fatal disease occurred spontaneously in the European rabbit (*Oryctolagus cuniculus*) colony in 1932 (Greene, 1933, 1934a; Pearce et al., 1933; Rosahn and Hu, 1935). A smaller outbreak had occurred in 1930. Rabbits developed an erythematous rash followed by cutaneous eruptions closely resembling the pocks seen in human infection with variola virus (smallpox). The disease was extremely contagious and caused high mortality, especially in young rabbits and pregnant females. Belgian hares were also susceptible to the disease. The causative agent of the disease was a poxvirus (Pearce et al., 1936; Rosahn et al., 1936a).

A spontaneous outbreak of a similar disease in a laboratory rabbit colony in Holland was described by Jansen (1941). The disease was highly fatal and differed from the Rockefeller outbreak in that it was not exanthematous, giving rise to the name "pockless" rabbitpox (Jansen, 1947). This disease was also caused by a poxvirus closely related to vaccinia virus (Jansen, 1946). A second outbreak of the disease in Holland was reported by Verlinde and Wersinck (1951). The second

outbreak of rabbitpox reported in the United States occurred in New York (Christensen et al., 1967) and was also of the pockless type first observed by Jansen (1947) in Holland. Another serious outbreak of the disease, resulting in mortality of 95%, occurred following the introduction of supposedly inactivated rabbitpox virus of the Dutch (Utrecht) strain into the rabbit colony of a medical school (Christensen et al., 1967). Various aspects of the infection have been reviewed by Fenner et al. (1989). Rabbitpox virus infection in rabbits can serve as a model for human smallpox infection as a fatal systemic disease developed with a low inoculum and inoculated rabbits naturally transmitted the virus to susceptible rabbits by aerosol (Adams et al., 2007).

ETIOLOGY

Rabbitpox virus is an *Orthopoxvirus* in the family *Poxviridae* (Fauquet et al., 2005) and is antigenically related to vaccinia virus (Fenner, 1958; Hu et al., 1936; Jansen, 1946). The biological properties of the Utrecht (Jansen, 1941) and Rockefeller (Greene, 1934a) strains of rabbitpox viruses are indistinguishable from certain neurovaccinia strains (Fenner, 1958; Fenner et al., 1989). The close antigenic relationship between rabbitpox virus and vaccinia virus, taken together with the fact that all reported outbreaks of rabbitpox have occurred in laboratory colonies, suggests that rabbitpox may be a laboratory variant of vaccinia virus (Greene, 1935a; Verlinde and Wersinck, 1951). Wittek et al. (1977) used genome mapping to show that rabbitpox virus (Utrecht strain) was a strain of vaccinia virus. The viruses have been shown to exhibit over 95% sequence similarity (Li et al., 2005).

Rabbitpox virus can be propagated on the chorioallantoic membrane of chicken embryos with development of distinct pocks. The predominant pock type is hemorrhagic (Jansen, 1946), but white pock mutants have been described (Fenner and Sambrook, 1966; Fenner et al., 1989). Rabbitpox virus has been propagated in several cell lines, including HeLa, Chang Liver, L929 (mouse fibroblast), human heart, KB (human epithelial), FL (human amnion), AT (Chinese hamster epithelial), PK-2A (pig kidney), and FAF cells (Chinese hamster fibroblast) (Christensen et al., 1967). The Rockefeller strain of rabbitpox virus hemagglutinates chicken erythrocytes, but the Utrecht strain and the strain isolated from the first American outbreak of pockless rabbitpox do not (Christensen et al., 1967).

EPIDEMIOLOGY

Rabbitpox is relatively rare, and all reported outbreaks have occurred in laboratory colonies in the United States and Holland. The highest mortality occurs in rabbits and pregnant or lactating females (Greene, 1934a, 1935b). Differences in susceptibility by rabbit breed also occur

(Greene, 1935b). Within infected colonies, the spread of disease is extremely rapid, and, in the outbreak of 1932, removal and isolation of infected rabbits failed to prevent the disease from spreading throughout the colony (Greene, 1934a). The virus appears to spread by nasal discharges, which usually appear on the third day after infection. Airborne droplets may be inhaled or ingested by susceptible rabbits (Bedson and Duckworth, 1963). Recovery from infection does not appear to result in establishment of a carrier state, as recovered rabbits can be safely mated with susceptible rabbits and clean colonies derived from recovered stock (Greene, 1934a). Arthropods have not been shown to play a role in the epidemiology of rabbitpox infection. In general, the primary sources of infection resulting in outbreaks of disease have not been determined, although the origin may have been rabbits inoculated with vaccinia virus (Greene, 1935a).

CLINICAL SIGNS

The clinical disease has been described in detail (Bedson and Duckworth, 1963; Christensen et al., 1967; Greene, 1934a). The virus initially infects and multiplies in the nasal mucosa and later in lymph nodes of the respiratory tract and in the lungs and spleen. Fever and a profuse nasal discharge usually occur 2–3 days after infection. Another early sign is enlargement and induration of the lymph nodes, especially the popliteal and inguinal nodes, which usually persist throughout the course of disease. Widely distributed skin lesions usually appear about 5 days after infection, initially as an erythematous macular rash which becomes papular and may progress to nodules up to 1 cm in size. These nodules eventually form dry, superficial crusts. Macules and papules may also occur on the mucous membranes of the oral and nasal cavities. Extensive edema of the face and oral cavity is often observed, as is focal necrosis of the hard palate and the gums. Hemorrhages in the skin may occur in severe cases.

Male rabbits frequently develop severe orchitis with extensive scrotal edema, and papules in the prepuce and urethra are observed. Similar lesions may also occur in the vulvae of females. If edema is extensive, urine retention may occur in either sex. Pregnant females usually abort. The eyes are almost invariably affected and exhibit blepharitis, purulent conjunctivitis, and acute keratitis with corneal ulceration. Death usually occurs 7–10 days after infection but may occur as early as 5 days, or rabbits may survive for several weeks before dying.

The generalized disease syndrome described above represents the findings of Greene (1934a) and Bedson and Duckworth (1963) with the Rockefeller strain of virus. This strain may occasionally result in peracute disease in which death is preceded only by fever, anorexia, and occasionally blepharitis. The peracute

form is unusual with the Rockefeller strain, but natural outbreaks of rabbitpox of the pockless type (Jansen, 1941, 1946, 1947; Christensen et al., 1967), without erythema or skin lesions, have occurred. In the first Dutch outbreak, some rabbits died within 1 week after infection, with only anorexia, fever, and lethargy (Jansen, 1941). In the first American outbreak of pockless disease, rabbits developed conjunctivitis and diarrhea and died 7–9 days after experimental infection (Christensen et al., 1967). Jansen (1941, 1946, 1947) and Christensen et al. (1967) described the occasional presence of scattered papules on the lips and tongues of rabbits with pockless rabbitpox. Experimentally, both the Utrecht and Rockefeller strains of virus produce skin lesions (Bedson and Duckworth, 1963).

PATHOLOGY

The gross and microscopic pathology of rabbitpox has been described in detail by Greene (1934b) for the Rockefeller Institute outbreak and by Christensen et al. (1967) for the first outbreak of pockless rabbitpox in the United States. The most distinctive gross lesions are the skin lesions, which may range in severity from a few localized papules to severe, almost confluent skin lesions with extensive necrosis and hemorrhage. Nodules occur in the mouth, upper respiratory tract, spleen, liver, and lungs but may also occur elsewhere in the body. Subcutaneous and oral submucosal edema and edema of other body orifices are common. Only rabbits with severe lesions of the mouth are emaciated at necropsy.

The liver is enlarged, yellowish, and has numerous small, gray nodules. Focal areas of hepatic necrosis may be seen. Small nodules may also occur in the gallbladder. The spleen is usually moderately enlarged with occasional focal nodules or areas of focal necrosis. Scattered diffusely throughout the lungs may be small white nodules and, in advanced cases, focal areas of necrosis. The testicles, ovaries, and uterus frequently exhibit diffuse white nodules and marked edema. Necrosis of the testes occurs frequently, and the uterus may contain focal abscesses. Focal nodules may be present in lymph nodes, adrenal glands, thyroid glands, parathyroid glands, peritoneum, omentum, and, rarely, the heart. Specific gross lesions are seldom observed in the central nervous system or kidneys. In the pockless form of the disease, a few pocks may be found in the mouth, and occasional skin lesions may be revealed by shaving the rabbit. The prominent gross lesions at necropsy are pleuritis, multifocal hepatic necrosis, splenomegaly, and edema and hemorrhage of the testes. The small white nodules, abundant in the more typical form of the disease, occur occasionally in the lungs and adrenal glands.

The predominant histological lesion in rabbitpox is the papule or nodule which occurs in the skin and

many other organs. A typical nodule consists of a central zone of necrosis, surrounded by mononuclear cells. Adjacent tissues are frequently edematous and occasionally hemorrhagic. Diffuse lesions with massive mononuclear cell infiltration, necrosis, hemorrhage, and edema often occur. Typical variola-like vesicles and pustules are not characteristic of rabbitpox infection. Vascular occlusion from the pronounced swelling of the endothelium may result in necrotic lesions.

In the lungs, focal nodular lesions and diffuse pneumonitis, with perivascular mononuclear and polymorphonuclear cell infiltration occur. Focal to diffuse pulmonary necrosis may be found. There is severe congestion of the spleen, with marked distention of sinuses by mononuclear cells, edema of Malpighian corpuscles, and focal to diffuse necrosis. Lymph nodes are generally greatly enlarged, mainly from severe edema. Extensive necrosis of lymph nodes and other lymphoid tissues such as Peyer's patches may occur. Hemorrhage and necrosis of the bone marrow occur frequently, interspersed with areas of mononuclear cell infiltration. Degeneration and necrosis of hepatic parenchyma may be focal or diffuse and may involve the whole organ. Focal necrosis with edema is detected in the testes, as are necrotic foci in the adrenal glands, uterus, thyroid glands, thymus, and salivary glands. The characteristic cytoplasmic inclusions associated with poxvirus infections are seldom encountered in rabbitpox.

DIAGNOSIS

Rabbitpox can be diagnosed by the clinical signs and the characteristic gross and microscopic lesions. Confirmatory diagnosis can be made by detection of viral antigen in tissues by fluorescent antibody on tissue impression smears (Christensen et al., 1967) or by virus isolation and identification. A PCR assay, using four primers, followed by electrospray ionization mass spectrometry, has been described which identified and speciated orthopoxviruses (Eshoo et al., 2009). The virus can be isolated by chorioallantoic membrane inoculation of chicken embryos or by cell culture propagation of the virus on cells derived from rabbits, mice, or any of several animal species (Christensen et al., 1967). The virus may be identified by the fluorescent antibody procedure, hemagglutination inhibition (some strains), or cross-protection tests using vaccinia-immunized and susceptible rabbits. Vaccinia-immunized rabbits should be resistant, whereas severe disease with high mortality should occur in susceptible rabbits.

CONTROL

Because the natural source of virus causing outbreaks has not been determined, control measures to prevent the occurrence of disease have not been

developed. In outbreaks, preventing spread of the virus in the colony by isolation of sick rabbits has met with mixed success (Christensen et al., 1967; Greene, 1934a). Investigators using rabbitpox virus experimentally should take exceptional precautions to prevent the virus from reaching susceptible rabbit populations. In an outbreak in a large colony, vaccination with vaccinia virus can be used to protect uninfected rabbits (Appleyard and Westwood, 1964; Boulter et al., 1971; Rosahn et al., 1936b).

Herpesvirus Infections

Herpesviruses have long been recognized as the causative agents of respiratory and genital diseases in humans, cattle, horses, and swine. They are also the recognized causes of other disease syndromes in many species of animals, including neoplastic diseases in frogs, chickens, rabbits, monkeys, and humans. An important characteristic of the herpesviruses is the capacity to cause mild or subclinical disease after which viral persistence in a latent state may ensue. Stresses of various kinds may result in viral recrudescence even after prolonged periods of latency. The possible existence of latent virus infections may have an important influence on the quality of experimental animals and potentially on the validity of experimental findings. In addition, there are numerous examples of herpesviruses that cause severe disease when infecting an abnormal host as demonstrated when *Human herpesvirus 1* infects rabbits. The *Herpesviridae* of rabbits and hares can be subdivided into *Gammaherpesvirinae* and *Alphaherpesvirinae* (Fauquet et al., 2005). The *Gammaherpesvirinae* contain two tentative members of the genus *Rhadinovirus*, Leporid Herpesvirus 1 and Leporid Herpesvirus 2. Two members of the *Alphaherpesvirinae*, genus *Simplex virus*, are known to infect rabbits, Leporid Herpesvirus 4 and *Human herpesvirus 1*.

Leporid Herpesvirus 1

HISTORY

Leporid herpesvirus I (synonyms *Herpesvirus sylvilagus*, cottontail virus, Hinze herpesvirus lymphoma) was isolated from primary kidney cell cultures from apparently healthy weanling Eastern Cottontail rabbits (*Sylvilagus floridanus*) trapped in Wisconsin (Hinze, 1968, 1971a). The virus was detected when focal areas of cell destruction were observed in cell monolayers 14 days after incubation. The virus has since been propagated in kidney cells of both Eastern Cottontail and domestic rabbits. Another herpesvirus, apparently distinct from the original isolate of Hinze (1971a), was recovered from primary kidney cell cultures of *Sylvilagus floridanus* (Cebrian et al., 1989).

ETIOLOGY

The virus possessed the physical, chemical, and biological properties of a herpesvirus and was named *Herpesvirus sylvilagus* (Heine and Hinze, 1972; Hinze, 1971a). Although infectious virus could not be demonstrated in lymphocytes from infected *Sylvilagus* rabbits, it could be detected after in vitro cocultivation with rabbit kidney cells (Hinze and Wegner, 1973; Wegner and Hinze, 1974). The virus is strongly cell-associated (Ley and Burger, 1970), and both B and T lymphocytes are infected (Kramp et al., 1985). The virus can be propagated in cells of both Eastern Cottontail and domestic New Zealand White rabbits but not in cells from humans, monkeys, hamsters, mice, nor in chicken embryos. The highest concentrations of virus are obtained by the use of primary kidney cell lines established from newborn or juvenile Eastern Cottontail rabbits (Cohrs and Rouhandeh, 1982; Medveczky et al., 1984). The virus possesses no antigenic relationship to Leporid herpesvirus 2 or to four other Leporid herpesviruses (Hinze, 1971a). The genome of the virus is similar to those of *Herpesvirus saimiri* and Epstein-Barr virus, in that it contains a variable number of repetitive DNA elements at both ends (Heine and Hinze, 1972; Cohrs and Rouhandeh, 1987; Rouhandeh and Cohrs, 1987; Medveczky et al., 1989).

EPIDEMIOLOGY

A serological survey in Wisconsin revealed antibodies to the virus in six of 101 wild Eastern Cottontail rabbits (Spieker and Yuill, 1976). In experimentally infected Eastern Cottontail rabbits, herpesvirus was not recovered from feces, urine, milk, ejaculates, or conjunctival and genital secretions, but was isolated from the oral secretions of one of 15 infected rabbits (Spieker and Yuill, 1977a). Shedding of infectious virus in oral secretions, unrelated to age, sex, or season, was demonstrated in naturally infected Eastern Cottontail rabbits (Hinze and Lee, 1980). The tonsils are the likely site of persistent infection and virus is released into the oral cavity. Transplacental transmission of virus was not found (Spieker and Yuill, 1977a). Transmission by insect vectors was not detected (Spieker and Yuill, 1977b). Repeated attempts to infect domestic New Zealand White rabbits have met with failure (Hinze, 1971a). Only rabbits of the genus *Sylvilagus* appear to be susceptible.

CLINICAL SIGNS

Inoculation of Eastern Cottontail rabbits by various routes results in a chronic low-grade viremia that persists, in most instances, for the life of the rabbit (Hinze, 1971b; Hinze and Chipman, 1972; Hinze and Wegner, 1973). Persistently infected rabbits have a pronounced lymphocytosis, with differential lymphocyte counts of up to 95% compared to 50–60% in normal rabbits (Hinze, 1969).

PATHOLOGY

The lymphoproliferative lesions in Eastern Cottontail rabbits occur in the lymphoid and other organ systems (Hesselton et al., 1988; Hinze, 1969, 1971b; Hinze and Wegner, 1973). Extensive infiltration of various tissues, commonly the kidneys, liver, lungs, and myocardium, with immature, actively proliferating lymphocytes occurs 6–8 weeks after experimental inoculation. The experimentally induced lymphoproliferative disease varies from benign lymphoid hyperplasia to lesions consistent with malignant lymphoma. Juvenile rabbits are affected more frequently and severely than adult rabbits.

DIAGNOSIS

Natural infection with Leporid herpesvirus 1 in Eastern Cottontail rabbits may result in leukocytosis, splenomegaly, and lymphadenopathy. Virus can be isolated from the oral cavity and circulating lymphocytes of infected rabbits cocultured with rabbit kidney cells (Hinze and Lee, 1980). Antibodies can be detected by indirect immunofluorescence and plaque reduction assays (Spieker and Yuill, 1976; Yang et al., 1990).

Leporid Herpesvirus 2

HISTORY

Nesburn (1969) isolated Leporid herpesvirus 2 (synonyms *Herpesvirus cuniculi*, virus III of rabbits, Leporid herpesvirus 3) from primary kidney cell cultures from *Oryctolagus cuniculus* and named it *Herpesvirus cuniculi*. This may represent a reisolation of virus III of rabbits (Rivers and Tillett, 1923), but, as the original isolate was not available, a direct serological comparison could not be made. Comparison of the reported characteristics of virus III with *Herpesvirus cuniculi*, however, indicates that they are identical (Nesburn, 1969). Virus III was isolated during attempts to find the etiologic agent of varicella (chicken pox). When inoculated into rabbits, the agent induced fever, exanthema, skin vesicles, corneal lesions, and intranuclear inclusions reminiscent of varicella infection (Rivers and Tillett, 1923). Convalescent sera from human varicella patients failed to inactivate the virus (Rivers and Tillett, 1924a). A similar virus was isolated from serially passaged normal rabbit testicular tissue during investigations of the etiology of rheumatic fever (Miller et al., 1924). McCartney isolated the virus while working on scarlet fever in England (Topacio and Hyde, 1932), and the agent has also been isolated by Doerr in Switzerland (Andrewes, 1928).

ETIOLOGY

Nesburn (1969) showed that the viral isolate possesses the physical, chemical, cytopathic, histological, and electron microscopic characteristics of a herpesvirus. The virus can be propagated in primary

or established cell lines of rabbit origin as well as in African green monkey kidney cells. The virus is now designated Leporid herpesvirus 2 (Roizman, 1982).

EPIDEMIOLOGY

Rivers and Tillett (1924a) found that four of 20 rabbits possessed antibodies to the virus and that 15% of 200 rabbits were immune to infection. Andrewes (1928), in England, found 98% of 377 experimental rabbits susceptible to the virus. He concluded that the virus was probably endemic in some rabbit colonies. Topacio and Hyde (1932), in Maryland, found that 17% of 76 rabbits were immune to infection. They suggested that older bucks, resistant to infection, were carriers of the virus. Neutralizing antibodies to the virus were detected in 6% of 196 rabbits from Connecticut and Maryland (Swack and Hsiung, 1972). Nesburn (1969) was unable to reisolate the virus from over 100 batches of primary rabbit kidney cell cultures prepared since initial isolation of the virus. Experimentally, virus was recovered from the blood of rabbits more than 100 days after inoculation (Swack and Hsiung, 1972). Appreciable titers of virus were detected in leukocytes, spleen, liver, lungs, and salivary glands, whereas the concentration of virus in kidneys was variable. However, transmission of the virus by direct contact or transplacentally failed to occur.

CLINICAL SIGNS

All reported isolations of virus have been from apparently normal rabbits, and no naturally occurring disease has been attributed to infection with the virus. However, a viral agent, presumably a herpesvirus, was recovered from the nares of rabbits with respiratory disease (Renquist and Soave, 1972). Experimentally, intradermal inoculation of rabbits with virus resulted in pronounced erythema at the site of inoculation after 4–7 days, which usually disappears within 2 weeks (Rivers and Tillett, 1924a). Occasionally, generalized reactions with anorexia, diarrhea, emaciation, fever, and skin vesicles have been reported. Intradermal inoculation may result in erythematous papules, whereas corneal scarification caused swelling and vesiculation of corneal cells (Topacio and Hyde, 1932). Intratesticular inoculation of rabbits results in acute orchitis and fever within 3–4 days (Andrewes, 1928). Intramuscular and subcutaneous inoculation of rabbits failed to induce clinical signs; however, corneal scarification with virus resulted in mild punctate keratitis (Nesburn, 1969). Similarly, intraperitoneal inoculation of virus did not induce clinical disease (Swack and Hsiung, 1972).

PATHOLOGY

No gross pathological changes have been observed in internal organs of experimentally infected rabbits.

Microscopically, testes, skin, and cornea show edema and an intense mononuclear leukocyte infiltration. Large eosinophilic intranuclear inclusions, typical of herpesviruses, characteristically occur in corneal epithelium, interstitial cells of the testicles, and in endothelial leukocytes of the skin (Rivers and Tillett, 1924b). Severe myocarditis with typical herpesviral inclusions occurred in rabbits inoculated intracardially with virus (Pearce, 1950, 1960). The absence of reported cases could be attributed to the relative rarity of disease or to lack of intensive etiologic investigation of cases which do occur.

DIAGNOSIS

Pathological findings described above including herpesviral inclusions in domestic rabbits (*Oryctolagus cuniculus*) are suggestive of Leporid Herpesvirus 2. PCR, in situ hybridization, and immunohistochemical labeling would be necessary to separate Leporid Herpesvirus 2 from the *Alphaherpesvirinae* described below (Gruber et al., 2009; Jin et al., 2008a; Weissenböck et al., 1997).

CONTROL

Both herpesvirus 1 of Eastern Cottontail rabbits and herpesvirus 2 of domestic rabbits possess the capacity to persist in infected hosts as a subclinical infection. Such infections, if not recognized, could result in considerable confounding in experimental studies, especially if conditions cause recrudescence of latent infections.

Leporid Herpesvirus 4

HISTORY

Leporid herpesvirus 4 (LHV 4) was isolated from a naturally occurring outbreak with high morbidity and mortality affecting miniature and crossbred domestic European rabbits (*Oryctolagus cuniculi*) in a rabbitry in Alaska (Jin et al., 2008a). Two other outbreaks, with similar clinical signs and pathology, have been reported in North America, although the virus was not identified (Onderka et al., 1992; Swan, 1991).

ETIOLOGY

Electron microscopy of LHV 4 demonstrated capsid and core particles similar in size and structure to *Human herpesvirus 1*. The genome of LHV 4 is 120–130kbp and partial sequences compared to other *Alphaherpesvirinae* demonstrated the closest homology by nucleotide identity with *Bovine herpesvirus 2* (Jin et al., 2008b). *Bovine herpesvirus 2* was originally isolated from cattle in Africa, but has been found in a variety of domestic and wild ruminants (Borchers et al., 2002; Kálmán and Egyed, 2005). LHV 4 infects rabbit skin and Vero (monkey kidney) cells with cytopathic effects similar to *Human herpesvirus 1* (Jin et al., 2008a).

EPIDEMIOLOGY

LHV 4 was isolated from an outbreak of acute disease in an outdoor rabbitry in Alaska (Jin et al., 2008a). The mode of herpesvirus introduction in this outbreak was not determined as there had been no travel or introduction of new rabbits. However, feral domestic European rabbits (*Oryctolagus cuniculus*) and Snowshoe hares (*Lepus americanus*) were in the area. In addition, the outbreak occurred in July and August, when mosquito and biting fly activity is typically high. A second outbreak occurred in the same rabbitry the following year in the spring and summer, although it could not be determined if this was due to a re-introduction or reactivation of a chronic infection (Jin et al., 2008b). Two outbreaks of disease caused by herpes-like viruses that were not identified were reported in Canada (Onderka et al., 1992; Swan, 1991). The clinical signs and pathology of those outbreaks were similar to the Alaska outbreak and may suggest that there is an animal reservoir in northwestern North America of this Alpha herpesvirus.

CLINICAL SIGNS

Clinical signs reported in the naturally occurring outbreaks included conjunctivitis and periocular swelling with purulent ocular discharge, ulcerative dermatitis, progressive weakness, anorexia, respiratory distress, torticollis, ataxia, weight loss, diarrhea, bruxism, genital swelling, and abortion (Jin et al., 2008a). Morbidity was approximately 50%, with 29% mortality. Some rabbits died acutely with anorexia, the only sign observed prior to death. Recovered virus administered intranasally or by the cornea to New Zealand White rabbits caused severe acute disease within 4 days of inoculation with one out of four affected animals being euthanized due to severity of the disease. The remaining rabbits recovered. Acute mortality associated with unidentified herpesviruses was reported in four adult *Oryctolagus cuniculus* in two rabbitries in Canada (Onderka et al., 1992; Swan, 1991). Acute death without clinical signs occurred in some rabbits as well as conjunctivitis, nasal discharge, edematous eyes and faces. Herpesviruses were recovered on rabbit kidney cells and experimental inoculation of rabbits with the virus reproduced the disease syndrome (Onderka et al., 1992; Swan, 1991). However, it is unclear if the herpesviruses responsible for these outbreaks are Gamma herpesviruses or Alpha herpesviruses related to LHV 4.

PATHOLOGY

Lesions included severe dermal and subcutaneous hemorrhage, edema, and necrosis with vascular necrosis and thrombosis (Jin et al., 2008a, 2008b). There was a mixed infiltrate of macrophages, lymphocytes, plasma cells with fewer heterophils. Necrosis and hemorrhage

were found within the ventricular walls, spleen, lymph nodes, and lungs. Areas of fibrin deposition were present in the spleen and lung. Intranuclear inclusions were found within the affected skin, subcutis, and ventricular walls, but not in the spleen, lungs, or lymph nodes. Similar lesions were seen following experimental infection (Jin et al., 2008b).

DIAGNOSIS

Typical lesions in the skin, spleen, lymph nodes, lungs, and heart with demonstrative intranuclear inclusions are suggestive of LHV 4 infection. DNA extracted from most tissues, including appendix, sciatic nerve, kidney, adrenal gland, spleen, liver, lung, lymph node, salivary gland, tonsil, and brain from an inoculated rabbit that died in the acute phase of the disease was specific for LHV 4 ribonuclease reductase gene by PCR (Jin et al., 2008b). At 14 days after inoculation, the eye, trigeminal ganglion, and tonsil were positive for LHV 4 by PCR analysis. In the second outbreak at the rabbitry in Alaska, the eye of an affected rabbit was positive for LHV 4 by PCR.

CONTROL

As latent infection is typical of Alpha herpesviruses, control measures are difficult. The rabbitry involved in the Alaska outbreak was depopulated after the second outbreak (Jin et al., 2008).

Human Herpesvirus 1 (HHV-1)

Herpesviruses of other species, including HHV-1, can cause encephalitis in rabbits experimentally inoculated (Chowdhury et al., 2000; Seto et al., 1995) and horizontal transmission of HHV-1 between rabbits has been reported (Kaplan, 1969). Spontaneous cases of HHV-1 encephalitis presumably transmitted from humans to pet domestic European rabbits have also been reported (Grest et al., 2002; Gruber et al., 2009; Müller et al., 2009; Weissenböck, et al., 1997). In each of these reports, rabbits with HHV-1 encephalitis were in close contact with a person with herpes labialis before they developed illness. The rabbits presented with neurological signs including anorexia, tonic-clonic spasms, circling, incoordination, ataxia, seizures, and opisthotonus. One rabbit also had a history of epiphora of the left eye, conjunctivitis, bruxism, and hypersalivation (Müller et al., 2009). Pathological findings included non-suppurative meningoencephalitis and large, eosinophilic intranuclear inclusion bodies in the neurons and glial cells. Diagnosis was confirmed by immunohistochemical labeling with polyclonal antibodies specific for HHV-1 (Grest et al., 2002; Gruber et al., 2009; Weissenböck et al., 1997), in situ hybridization using a DNA probe for HHV-1 (Gruber et al., 2009; Müller et al., 2009; Weissenböck et al., 1997),

or PCR specific for the UL42 gene of HHV-1 (Grest et al., 2002; Gruber et al., 2009; Weissenböck et al., 1997). Control measures would include preventing close contact between humans with active herpetic lesions and rabbits.

Papilloma and Polyoma Viruses

The family *Papillomaviridae* contains viruses in the genus *Kappapapillomavirus* which cause papillomas (warts) in various animals and includes two species of rabbit viruses, the *Cottontail rabbit papillomavirus* and the *Rabbit oral papillomavirus* (Fauquet et al., 2005). The family *Polyomaviridae* contains one genus, *Polyomavirus*, which includes the species *Rabbit kidney vacuolating virus*.

Cottontail Rabbit Papillomavirus

HISTORY

The Cottontail rabbit or Shope papillomavirus was recognized as a transmissible agent by Shope and Hurst (1933) while they were attempting to define the etiology of wart-like tumors on Eastern Cottontail rabbits (*Sylvilagus floridanus*) in the Midwestern United States. They demonstrated the filterability of the infectious agent, transmitted it to other Eastern Cottontail rabbits as well as domestic European rabbits, and described the histopathology of the disease (Shope, 1935). Rabbit papillomatosis was initially considered a natural benign disease of *Sylvilagus* rabbits only; however, spontaneous outbreaks of papillomatosis in *Oryctolagus cuniculus* indicate that the disease has greater relevance (Hagen, 1966). Soon after discovery of the virus, it was also shown to cause malignant neoplasms histologically resembling squamous cell carcinomas (Kidd and Rous, 1940; Rous and Beard, 1934, 1935). This was the first recognition of an oncogenic virus in mammals. The disease has served as a model system to elucidate the role of viruses in the pathogenesis of cancer in humans and animals (Evans and Thomsen, 1969; Evans et al., 1962a, 1962b; Georges et al., 1984; Kreider and Bartlett, 1981, 1985; Smith and Campo, 1985) and potential therapeutic interventions (Gambhira et al., 2007).

ETIOLOGY

The virus is the type species of the genus *Kappapapillomavirus* (formerly *Papillomavirus*) of the family *Papillomaviridae* (Fauquet et al., 2005) and possesses the characteristic circular DNA genome, icosahedral structure, and other chemical and physical properties of this family (Gross, 1983; Kass and Knight, 1965; Murphy et al., 1981). The complete genome contains 7863 nucleotides (Giri et al., 1985) and consists of ten genes. All of the papilloma viruses have two capsid proteins (major L1 and minor L2) and although the C terminus of L1 is

exposed on the surface of the viron, suggesting it may have an important role in immune responses, both proteins encode virus-neutralizing epitopes (Campo, 2003). The virus is antigenically distinct from other members of the genus *Kappapapillomavirus*. Nuclear (Yoshida and Ito, 1968) and cytoplasmic (Ishimoto et al., 1970) viral antigens have been demonstrated by immunofluorescence in virus-inoculated normal skin cells and virus-induced papillomas. However, the significance of these findings is difficult to interpret since controls for rabbit kidney vacuolating virus, a passenger virus (Hartley and Rowe, 1964), were usually not included (Kreider and Bartlett, 1981). The virus can be maintained by serial propagation in the skin of *Sylvilagus* rabbits, inoculated intracutaneously or scarified with virus (Shope and Hurst, 1933). Intramuscular inoculation does not result in clinical papillomatosis. Dogs, cats, pigs, goats, rats, mice, and guinea pigs are refractory to the virus. The skin of embryonic rats is susceptible to the virus, and typical papillomas develop following inoculation (Greene, 1953b); however, few papillomas develop, and they regress unless the host is immunosuppressed (Kreider et al., 1971). Cellular proliferation or neoplastic transformation by the virus has been reported in rabbit skin cell cultures (Coman, 1946), skin cultures derived from neonatal domestic European rabbits (DeMaeyer, 1962), and explants of embryonic rabbit skin (Greene, 1953a). Transplantation of transformed skin cultures into rabbits resulted in the formation of papillomas (Coman, 1946; Kreider, 1963). (see also Chapter 16).

EPIDEMIOLOGY

Papillomatosis occurs most frequently as a natural disease of *Sylvilagus* rabbits in the Midwestern United States extending from Minnesota and North Dakota to Texas (Gross, 1983; Hagen, 1966). The virus has apparently not become established in the eastern states. The only natural outbreaks of disease in domestic European rabbits were in southern California, suggesting that the virus is present in wild *Sylvilagus* rabbits of the area, and spreads to domestic European rabbits by arthropod vectors (Hagen, 1966). The disease in *Sylvilagus* rabbits also occurs on coastal islands in Washington, in a population of rabbits introduced from Kansas (Lancaster and Olson, 1982).

The *Sylvilagus* rabbit is the natural host, but the domestic European rabbit is also susceptible to the virus (Shope, 1935). Natural outbreaks have been reported in commercial rabbitries (Hagen, 1966). The Black-tailed jackrabbit (*Lepus californicus*) is also susceptible to the virus (Beard and Rous, 1935). Infection of the skin of domestic European rabbits results in the formation of papillomas essentially devoid of infectious virus (Shope, 1935). Papillomas, primary carcinomas, and metastases from domestic European rabbits have about 10–100 copies of viral DNA, whereas papillomas from *Sylvilagus*

rabbits have 2400–8800 copies (Stevens and Wettstein, 1979). Papillomas from domestic European rabbits can be serially transferred, but only small amounts of infectious virus are typically demonstrated (Gross, 1983; Hu et al., 2007; Selbie and Robinson, 1947; Shope, 1935), indicating that domestic European rabbits are not a source of virus for arthropod vectors.

Infection of *Sylvilagus* rabbits, Black-tailed jackrabbits, and Snowshoe hares (*Lepus americanus*) induces lesions containing a high concentration of virus (Lancaster and Olson, 1982; Syverton et al., 1950). Contact transmission of papillomatosis may occur, but transmission of the virus by the rabbit tick (*Haemaphysalis leporis-palustris*) is probably the most common natural mode (Larson et al., 1936). Transmission by mosquitoes and reduviid bugs has been demonstrated experimentally (Dalmat, 1958). In commercial rabbitries, the mosquito may be the principal vector between wild *Sylvilagus* and domestic European rabbits (Hagen, 1966). This hypothesis is strengthened by the observation that in natural cases of disease in domestic European rabbits, lesions were confined to the relatively hairless parts of the body around the eyes, ears, and anus, areas where mosquitoes are more apt to feed. Nematodes may be involved in the natural transmission of virus (Rendtorff and Wilcox, 1957). Experimentally, papillomas were induced when papilloma virus and larvae of the nematode *Nippostrongylus muris* were applied to rabbit skin, but not by virus or nematode larvae alone. See Chapter 16 for further information.

CLINICAL SIGNS

Papillomatosis of wild *Sylvilagus* rabbits is characterized by the presence of horny warts, usually on the neck, shoulders, or abdomen. The warts begin as red raised areas at the site of infection, grow to become typical papillomas with rough rounded surfaces, and may later develop into large, keratinized horny growths (Shope and Hurst, 1933). The virus was initially believed to cause only transient papillomatosis, but it was later shown that in naturally infected *Sylvilagus* rabbits papillomas may become malignant squamous cell carcinomas (Syverton and Berry, 1935). This phenomenon was later shown to be a relatively frequent occurrence in both naturally and experimentally infected *Sylvilagus* rabbits, as 25% of infected rabbits developed carcinomatous lesions following infection (Syverton et al., 1950). In approximately 35% of naturally infected rabbits, papillomas regress within 6 months after infection. In natural outbreaks of papillomatosis of domestic European rabbits in southern California (Hagen, 1966), papillomas most commonly occurred on the eyelids and ears. Experimentally induced papillomas develop more slowly in domestic European rabbits than in *Sylvilagus* rabbits, reach a stationary phase, and then occasionally regress (Syverton, 1952).

Regression occurs depending on the interaction of the genotype of the virus and host immunity (Hu et al., 2002, 2005). A variant virus that contains the regressive E6 gene has a high rate of regression (Hu et al., 2002). Certain MHC haplotypes have been associated with regression or persistence in domestic European rabbits (Salmon et al., 2000). In one study, papillomas persisted when a regressive viral strain was used if rabbits were immunosuppressed with cyclosporine A treatment, but regression also varied with the strain of rabbit as more papillomas regressed in outbred New Zealand White rabbits than inbred EIII/JC rabbits (Hu et al., 2005). Once immunosuppression was removed, most papillomas regressed in outbred rabbits (67–89%), whereas only 13% regressed in inbred rabbits, highlighting the importance of multiple factors in persistence of papillomas. Rous and Beard (1934, 1935) recognized the malignant potential of rabbit papillomavirus when they demonstrated that intramuscular inoculation of papillomatous tissue into domestic European rabbits resulted in invasive squamous cell carcinomas. In experimentally infected domestic European rabbits, 75% developed carcinomas if kept longer than 6 months (Syverton, 1952). *Sylvilagus* rabbits have a three-fold lower incidence of carcinomas than European rabbits (Rous and Beard, 1935; Syverton et al., 1950).

PATHOLOGY

The warts which develop following infection in *Sylvilagus* and domestic European rabbits are typical papillomas. The malignant tumors which arise from papillomas are squamous cell carcinomas. Metastasis to regional lymph nodes, particularly the axillary nodes, is common (Kreider and Bartlett, 1981) and about 25% of rabbits that succumb have pulmonary metastases. In addition, amyloid deposition in renal glomeruli, hepatic sinusoids and splenic red pulp is present in the majority of rabbits.

DIAGNOSIS

Cottontail rabbit papillomatosis is diagnosed clinically by the characteristic skin tumors, which never occur in the mouth, and may be confirmed by histopathological examination. A survey of tumors in pet rabbits submitted to the surgical biopsy service of a veterinary school in Pennsylvania over a 16-year period revealed that Shope papilloma was diagnosed in two tumors, both on the ears, from 179 rabbits (von Bomhard et al., 2007). No cell culture system is available for routine isolation of virus (Lancaster and Olson, 1982).

CONTROL

The endemic disease of *Sylvilagus* rabbits is of little economic significance, and thus no prophylactic methods have been developed. Because natural infection occurs in domestic European rabbits (Hagen, 1966), the

adoption of control methods may become necessary. In areas where the disease is endemic in wild *Sylvilagus* rabbits, where arthropod vectors are present, and where outdoor rabbit husbandry is practiced, arthropod control would appear to be a logical approach. Rabbits can be immunized by two intraperitoneal inoculations with glycerinated rabbit papilloma suspensions (Shope, 1937). Domestic rabbits with experimentally induced papillomas resist challenge with virus derived from papillomas of cottontail rabbits (Hagen, 1966). A tumor-specific vaccine composed of allogenic tumor cells increased the regression rate of papillomas (Evans et al., 1962a).

Rabbit Oral Papillomavirus

HISTORY

Oral papillomatosis of rabbits was recognized as a distinct viral disease of domestic European rabbits (*Oryctolagus cuniculus*) by Parsons and Kidd (1936). They found a 17% prevalence of small papillomas in the mouths of rabbits of several breeds in New York City. The lesions were usually confined to the ventral surface of the tongue. They transmitted the virus to both domestic European and *Sylvilagus* rabbits and by cross-immunity tests and tissue-susceptibility studies demonstrated that it was distinct from the Cottontail rabbit (Shope) papillomavirus. They also showed that virus is frequently present in the mouths of rabbits without lesions and that tattooing or licking of tar stimulated lesion development in such carrier rabbits (Parsons and Kidd, 1943). A spontaneous outbreak in New York of rabbit oral papillomatosis, involving several breeds of domestic European rabbits, was described (Weisbroth and Scher, 1970). New Zealand White rabbits with oral papillomas were reported in Illinois (Sundberg et al., 1985) and Mexico (Dominguez et al., 1981).

ETIOLOGY

The virus was partly characterized by Parsons and Kidd (1943) and was later included in the genus *Kappapapillomavirus* of the family *Papillomaviridae* (Fauquet et al., 2005). The virus consists of 7565 nucleotides and has the greatest amino acid sequence identity to *Cottontail rabbit papillomavirus*, although the greatest area of homology was only 68% (Christensen et al., 2000). However, the virus is immunologically distinct from the *Cottontail rabbit papillomavirus* and naturally infects only leporids (Parsons and Kidd, 1936, 1943). Nuclear viral replication, characteristic of the *Papovaviridae*, has been demonstrated (Rdzok et al., 1966; Richter et al., 1964). Neonatal hamsters, inoculated subcutaneously with a tumor suspension from naturally infected rabbits, developed fibromas (Sundberg et al., 1985). The virus has not been propagated outside of susceptible host animals.

EPIDEMIOLOGY

The virus probably spreads by direct contact, and lesion development may require oral trauma for viral entry. Coarse feed, maloccluded teeth, or other oral irritants may serve as the inciting event (Parsons and Kidd, 1943; Weisbroth and Scher, 1970). Experimentally induced lesions appear 9–38 days after inoculation (Parsons and Kidd, 1936), but the incubation period of the natural disease is unknown. The disease generally occurs in rabbits 2–18 months old (Sundberg et al., 1985; Weisbroth and Scher, 1970).

CLINICAL SIGNS

Oral papillomatosis is a benign disease characterized clinically by small discrete whitish growths on the ventral surface of the tongue. The early lesions are sessile, later become rugose or pedunculated, and ultimately ulcerate (Parsons and Kidd, 1936; Sundberg et al., 1985; Weisbroth and Scher, 1970). The lesions are seldom more than 5 mm in size and 4 mm in thickness and usually substantially smaller. Papillomas have been known to persist for as long as 145 days, but usually disappear in weeks (Parsons and Kidd, 1936). Lesions rarely occur elsewhere in the mouth and never on the body. However, in a Flemish Giant pet rabbit in New Zealand, virus recovered from lesions on the nictitating membrane and lower eyelid had 99.3% homology with *Rabbit oral papillomavirus* (Munday et al., 2007).

PATHOLOGY

The lesions are microscopically typical papillomas (Parsons and Kidd, 1943; Rdzok et al., 1966; Richter et al., 1964; Sundberg et al., 1985; Weisbroth and Scher, 1970). Cells in the stratum spinosum contain basophilic intranuclear inclusions (Dominguez et al., 1981; Sundberg et al., 1985).

DIAGNOSIS

The disease is diagnosed by typical lesions occurring only in the mouth, in contrast to rabbit papillomatosis, in which lesions are observed only on the skin. The lesions are typical papillomas, and papillomavirus antigens can be detected in cells of the stratum spinosum by the peroxidase antiperoxidase technique (Sundberg et al., 1985).

CONTROL

Rabbits recovering from disease are resistant to reinfection but are susceptible to *Cottontail rabbit papillomavirus*.

Rabbit Kidney Vacuolating Virus

The *Rabbit kidney vacuolating virus* was isolated in primary rabbit kidney cell cultures from papillomas of Eastern Cottontail rabbits (*Sylvilagus floridanus*) collected in Kansas (Hartley and Rowe, 1964). The virus causes

vacuolar cytopathic effects in cell cultures. The virus resembles the *Cottontail rabbit papillomavirus* but is a distinct virus. It does not produce papillomas when inoculated into rabbits and does not immunize rabbits against *Cottontail rabbit papillomavirus*. The virus is slightly smaller than the viruses of the genus *Papillomavirus* and resembles *Polyomavirus* in size, morphology, and DNA composition (Crawford and Follett, 1967). The virus has been classified as a member of the genus *Polyomavirus* within the family *Polyomaviridae* (Fauquet et al., 2005). Ultrastructurally, the virus is a typical *Polyomavirus* in its replication pattern (Chambers et al., 1966). The virus is not pathogenic for either domestic European or Eastern Cottontail rabbits or any other animal species. Inoculation of both neonatal and adult domestic and Eastern Cottontail rabbits by several routes did not induce disease (Hartley and Rowe, 1964). Antibodies to the virus have been found in wild *Sylvilagus* rabbits in Kansas and Maryland, but not in domestic European rabbits (Hartley and Rowe, 1964). The virus thus appears to be a fairly common non-pathogenic virus of *Sylvilagus* rabbits, causing only latent infections. Intranuclear inclusions consistent with rabbit kidney vacuolating virus have been found as an incidental finding in New Zealand White rabbits (Percy and Barthold, 2007). Although a contaminant of rabbit papillomas, the virus appears to have no role in the pathogenesis of papillomatosis (Goldman et al., 1972; Ito et al., 1968; Kreider and Bartlett, 1981).

Adenovirus Infections

Adenovirus infections of rabbits, spontaneous and experimental, have been reported. While the taxonomy of the spontaneous adenoviruses found in rabbits has not been determined, the human, swine, and bovine adenoviruses described belong to the genus *Mastadenovirus*. An adenovirus was isolated in Hungary from the spleen, kidneys, lungs, and intestines of rabbits, 6–8 weeks old, with diarrhea (Bodon et al., 1979). The virus was detected in primary rabbit kidney cultures stained with acridine orange although no cytopathic effect was observed. The virus failed to replicate in pig kidney, calf kidney, calf testicle, or human embryonic lung cells. Antisera to several swine and bovine adenoviruses failed to neutralize the virus, whereas a partial antigenic relationship to human adenoviruses was demonstrated by complement fixation and immunodiffusion tests. The virus agglutinated rabbit but not human erythrocytes. A serological survey of 30 *Oryctolagus cuniculus* from four rabbitries in Quebec revealed that three rabbits from three colonies had antibodies to bovine adenovirus type 1 (Descoteaux et al., 1980).

Experimental inoculation of rabbits with human adenovirus type 5 resulted in no clinical response but induced a persistent viral infection in lymphoid tissues for as long as 1 year (Reddick and Lefkowitz, 1969). Human adenovirus type 5 infects rabbit corneas following either intrastromal

injection or topical application to damaged cornea resulting in acute blepharoconjunctivitis, iritis, and corneal edema which peaks at 13 days after infection (Gordon et al., 1992; Trousdale et al., 1995). Other serotypes of human adenovirus induce disease as well, although adenovirus type 5 is commonly used as an animal model to test therapeutic approaches to adenovirus-induced keratoconjunctivitis (Romanowski et al., 1998). Recombinant adenoviruses have been used successfully to infect rabbits in many gene transfer protocols (Kozarsky et al., 1996; Schneider et al., 1999).

Parvovirus Infections

A virus that induced cytopathic effects in primary rabbit kidney cell cultures was isolated from the feces of a rabbit (*Oryctolagus cuniculus*) inoculated with *Herpesvirus cuniculi* in a laboratory in Japan (Matsunaga et al., 1977). The virus had the morphological, physical, and chemical properties of a parvovirus. The various proteins of the virus have been characterized (Matsunaga and Matsuno, 1983). Subsequently, parvovirus was recovered from kidney cells of neonatal rabbits in the United States (Metcalf et al., 1989). These viruses are of the species *Lapine parvovirus*, genus *Parvovirus*, family *Parvoviridae* (Fauquet et al., 2005).

Serological surveys have identified positive rabbitries in Japan, the United States, and Europe. Among 90 rabbits from a commercial source in Japan, 42 (47%) had hemagglutination-inhibiting antibody to the virus (Matsunaga et al., 1977). Of 46 rabbit sera, collected from various sources in the United States, 75% had antibodies detected by immunofluorescence or hemagglutination inhibition assays (Metcalf et al., 1989). In Switzerland, over 70% of 132 rabbit sera from various commercial breeding colonies had antibodies in the hemagglutination inhibition test (Metcalf et al., 1989). Experimental inoculation, orally or intravenously, induced anorexia, listlessness, and catarrhal enteritis in 1-month-old rabbits (*Oryctolagus cuniculus*) (Matsunaga and Chino, 1981). Pathologic changes included catarrhal enteritis with hyperemia, exfoliation of the small intestinal epithelial cells, and increased secretion of intestinal mucus. Virus was detected in feces, small intestine, liver, pancreas, spleen, appendix, and mesenteric lymph node. Rabbits developed hemagglutination-inhibiting antibodies.

RNA VIRUS INFECTIONS

Rotavirus Infections

Rotavirus

HISTORY

Rotavirus was initially isolated from rabbits with diarrhea by Bryden et al. (1976) in England. Virus was

recovered from both sporadic cases and outbreaks of diarrhea in weanling rabbits as well as from healthy rabbits. Subsequently, rotavirus was isolated from young rabbits with diarrhea in Japan (Sato et al., 1982), Europe (Castrucci et al., 1985; Eaton, 1984; Peeters et al., 1982, 1984), and the United States (DiGiacomo and Thouless, 1986; Schoeb et al., 1986). Various serological surveys have since revealed that rotavirus infection is widespread in domesticated European rabbits (*Oryctolagus cuniculus*).

ETIOLOGY

Rabbit rotaviruses belong to the family *Reoviridae*, genus *Rotavirus* (Estes and Kapikian, 2007; Fauquet et al., 2005). They are 75-nm icosahedral viruses with a triple-layered protein capsid that contains 11 segments of double-stranded RNA, each of which codes for a protein. The sixth gene (VP6) codes for an inner capsid protein which contains the subgroup specificity. The fourth (VP4) and seventh genes (VP7), code for outer capsid proteins that are the viral neutralization antigens. VP4 is the viral hemagglutinin and must be cleaved by a protease, e.g., trypsin, to yield VP5 and VP8 before the virus can infect cells (Matsui et al., 1989; Ramig and Ward, 1991).

The rotaviruses are classified serologically into seven groups (A to G). Groups A to C are found in animals and humans, whereas groups D to G are found only in animals. The rabbit rotaviruses described belong to group A (Tanaka et al., 1988; Thouless et al., 1986). Within each serogroup, rotaviruses are classified into serotypes, defined by reactivity against the outer capsid proteins VP4 (P type) and VP7 (G type). Since the glycoprotein VP7 appears to be immunodominant, classification of VP7 serotypes is more advanced and within group A, 15 serotypes (1–15) have been identified. All rabbit rotaviruses recovered to date have been serotype 3 (G3) (Tanaka et al., 1988; Thouless et al., 1986). Serotype 3 contains the largest and most diverse number of rotaviruses and is common to humans and several other animals. This reflects the genetic reassortment that occurs when different viruses of the same group simultaneously infect susceptible hosts.

Some rotavirus isolates from rabbits induce a cytopathic effect in MA104 rhesus monkey kidney cells in about 3 days (Castrucci et al., 1985; Sato et al., 1982; Thouless et al., 1986), whereas other isolates demonstrate cytopathic effect only after additional passages. Physicochemical studies reveal that rotaviruses are stable from pH 3.0 to 9.0 (Fauquet et al., 2000). Virus remains infectious for months at room temperature on porous and non-porous environmental surfaces, in fecal material, or if stabilized with CaCl₂. Rotaviruses can be inactivated when heated at 50°C for 30 min and by treatment with chlorine, formalin, phenols, and

betapropiolactone. Ethanol, 95%, is the most effective disinfectant as it renders the rotavirus non-infectious by removing the outer capsid layer.

EPIDEMIOLOGY

Serological studies indicate that rotavirus infection is widespread in domesticated European rabbits (*Oryctolagus cuniculus*). Of 91 adult rabbits from two commercial rabbitries in Ontario, Canada, 98% had antibodies to rotavirus (Petrie et al., 1978). Sera collected from fryer rabbits at two abattoirs in Ontario, Canada, revealed that 60% of 200 rabbits had antibodies (Percy et al., 1993). A survey in Tokyo prefecture, Japan, of 39 adult rabbits revealed that 82% had antibodies (Takahashi et al., 1979). A more extensive survey of ten breeding and 13 laboratory colonies in metropolitan Tokyo and Ibaragi, Saitama, Kanagawa, Shizuoka, and Nagano prefectures in Japan revealed antibodies to rotavirus in 83% of the 23 colonies and in 81% of 160 sera (Iwai et al., 1986). In Hungary, 74% of 112 sera from five large-scale rabbit farms had rotavirus antibodies (Kudron et al., 1982). In the United States, 95% of 149 sera from rabbits more than 2 months old in a commercial colony in Washington had antibodies (DiGiacomo and Thouless, 1984). Rotavirus was detected in fecal samples from rabbits in three other colonies in the state (DiGiacomo and Thouless, 1986). Antibodies against rotavirus have also been detected in 29% of 17 sera from Eastern Cottontail rabbits (*Sylvilagus floridanus*) in Ontario, 52% of 27 sera from Snowshoe hares (*Lepus americanus*) in the Yukon, and 6% of 48 sera from Snowshoe hares in Nova Scotia (Petrie et al., 1978).

In colonies with endemic rotavirus infection, nearly all adult rabbits have serological evidence of rotavirus infection (DiGiacomo and Thouless, 1984, 1986). Litters in such colonies have transplacentally acquired maternal antibodies to rotavirus at birth. In the absence of rotavirus infection, the antibodies fall to undetectable levels by 60 days of age. In colonies with endemic infection, shedding of rotavirus in the feces is detected in rabbits 4–7 weeks old (Peeters et al., 1984), followed by the appearance of naturally acquired antibodies in rabbits greater than 6 weeks old (DiGiacomo and Thouless, 1986). Subsequently, infected rabbits demonstrate antibodies for long periods. Hence, in infected colonies, rabbits usually acquire infection when maternal antibodies have declined to low concentrations, which usually coincides with weaning. Both sexes appear equally susceptible. Rotavirus infection in domesticated European rabbits has been reported in New Zealand White, Dutch, and Californian rabbits, suggesting no difference in breed susceptibility (Bryden et al., 1976).

Rotavirus is shed in the feces of infected rabbits. In a survey of 187 rabbits of mixed ages, rotavirus was detected in 4% of fecal samples (Petric et al., 1978). In

another study, 11% of 18 fecal samples contained virus (Kudron et al., 1982). Rotavirus was detected in 9% of 106 fecal samples from healthy rabbits, 1–2 months old, from four rabbitries (DiGiacomo and Thouless, 1986). Hence, transmission probably occurs by fecal-oral spread. Rabbits inoculated orally with rotavirus shed virus in the feces for 6–8 days, beginning 2–5 days after inoculation (Blutt et al., 2003; Castrucci et al., 1984; Conner et al., 1988; Hambraeus et al., 1989; Petrie et al., 1978; Thouless et al., 1988). Furthermore, uninoculated control rabbits maintained in the same room with inoculated rabbits also acquired rotavirus infection (Conner et al., 1988; Thouless et al., 1988). As fomite transmission appeared unlikely because of the handling of uninoculated rabbits first, it was concluded that rabbits were infected by airborne transmission of virus. In gastrically inoculated rabbits, rotavirus RNA was also detected in sera 4 days after inoculation (Blutt et al., 2003). Experimentally, rabbits were also infected with simian rhesus rotavirus (Ciarlet et al., 2000b).

Recent reports suggest that rabbits may be a source of rotaviruses for humans. Comparison of the sequence analysis of several rotavirus genes of rabbit and human origin revealed a close relationship with clustering of strains in phylogenetic analyses (De Leener et al., 2004; Matthijnsens et al., 2006, 2009). However, the molecular similarity of strains should not infer interspecies transmission without supporting epidemiologic evidence of spread.

CLINICAL SIGNS

Rotavirus was initially detected and recovered from rabbits with diarrhea by Bryden et al. (1976). That report included both sporadic cases and outbreaks in 5-week-old rabbits. A spectrum in the severity of disease associated with rotavirus infection has been reported, which is probably influenced by a synergy among various microorganisms responsible for diarrheal diseases in rabbits. In outbreaks of diarrhea associated with rotavirus infection, rabbits 30–80 days old are usually affected (Castrucci et al., 1985; Peeters et al., 1984; Sato et al., 1982). Rabbits exhibit severe mucoid or watery diarrhea, anorexia, and dehydration, with mortality of 60–80%. In one outbreak, rabbits 8–12 days old had a greenish-yellow watery diarrhea (Peeters et al., 1982). About 20% of litters were affected, with 98% mortality within 1–2 days after onset of signs. A similar disease was reported in a specific pathogen-free colony in which litters 7–21 days old exhibited watery diarrhea and lethargy (Schoeb et al., 1986). Approximately 40% of affected litters died within 2 days after onset of signs. That preweanling rabbits were affected in both outbreaks suggests that rotavirus had been recently introduced into the colonies.

In a comprehensive study of various infectious agents (parasites, bacteria, and viruses) associated with

diarrhea in 21 rabbitries, [Peeters et al. \(1984\)](#) detected rotavirus in 35% of 130 affected rabbits. However, the clinical signs associated with rotavirus infection consisted of watery diarrhea for 2–3 days, with low mortality. Hence, in endemically infected colonies, other factors, including other infectious agents, may enhance the pathogenicity of rotavirus ([Peeters et al., 1984](#)). That rotavirus may be only mildly pathogenic is supported by experimental studies. In general, orogastric inoculation of rabbits, 1–22 weeks old, with rotavirus did not result in diarrhea, although some rabbits developed soft or fluid feces for 2–4 days ([Conner et al., 1988](#); [Hambraeus et al., 1989](#); [Petric et al., 1978](#); [Thouless et al., 1988](#)). Whereas inoculation of one rotavirus strain induced diarrhea, depression, anorexia, and mortality in rabbits ([Castrucci et al., 1984](#)), this could not be repeated ([Hambraeus et al., 1989](#)). Inoculation of rabbits of various ages revealed that partially formed to liquid stools developed in 1-week-old rabbits but not in rabbits ≥ 2 weeks old ([Ciarlet et al., 1998](#)). Thus, in non-endemically infected rabbits, rotaviral disease may be age-restricted, occurring only in neonatal rabbits.

PATHOLOGY

In weanling rabbits infected with rotavirus, the intestines are markedly congested and distended, and petechiae are found in the colon ([Sato et al., 1982](#)). In addition to congestion, there are mucosal hemorrhages in the small intestine and distention of the cecum with fluid ([Castrucci et al., 1985](#)). However, in both of the reports, the presence of other infectious agents was not examined. [Peeters et al. \(1984\)](#) reported that, in pure rotavirus infection, gross lesions are limited to fluid cecal contents and swollen mesenteric lymph nodes. Histologically, the small intestine shows moderate to severe villous atrophy, more marked in the ileum. Apical enterocytes on the tips of villi are swollen, rounded, and desquamating; occasionally tips are denuded. The lamina propria is usually infiltrated with lymphocytes and occasional neutrophils. Lesions in the cecum are limited to focal areas of enterocyte desquamation. In outbreaks involving preweanling rabbits, gross lesions are most pronounced in the ileum ([Peeters et al., 1982](#)). Microscopically, there is villous atrophy and attenuation or desquamation of epithelial cells at the apical tips of jejunal or ileal villi ([Peeters et al., 1982](#); [Schoeb et al., 1986](#)). In some areas, the submucosa is edematous ([Schoeb et al., 1986](#)).

Experimentally, rabbits inoculated orally with rotavirus have markedly congested and distended intestines with accumulation of fluid and gas in the small intestine, cecum, and colon from 2–9 days after inoculation ([Castrucci et al., 1984](#); [Ciarlet et al., 1998](#); [Petric et al., 1978](#); [Thouless et al., 1988](#)). Microscopically, villi are shortened with villus blunting, fusion, and vacuolation, low to moderate numbers of lymphocytes and plasma

cells infiltrate the villi and lamina propria of the small intestine. There is mild lymphoid reactivity in mesenteric lymph nodes. Lesions are not observed in the cecum or colon. The pathologic changes are considered to be mediated by enterotoxin ([Ciarlet et al., 2000a](#)).

DIAGNOSIS

Diarrhea caused by rotavirus is diagnosed from clinical signs, histopathology, detection of the virus, and demonstration of antibodies. Clinical signs and pathological findings alone are not diagnostic, although villous atrophy and degeneration and desquamation of enterocytes at the tips of villi in the small intestine are characteristic features. Rotaviral diarrhea must be differentiated from other diarrheal diseases of rabbits such as coccidiosis, salmonellosis, Tyzzer disease, clostridial enterotoxemia, enteric coronaviral enteritis, and colibacillosis. In weanling rabbits with severe disease, the possibility of dual infections should be considered, since rotavirus infections are usually mild. Electron microscopy ([Ciarlet et al., 1998](#); [Peeters et al., 1984](#); [Schoeb et al., 1986](#)) or a capture enzyme-linked immunosorbent assay can be used to detect rotavirus in feces ([Blutt et al., 2003](#); [Conner et al., 1988](#); [De Leener et al., 2004](#); [Hambraeus et al., 1989](#); [Thouless et al., 1986, 1988](#)). A commercial human rotavirus detection kit, utilizing immunochromatography, tested with fecal samples from rabbits with diarrhea was shown to be applicable, as positive results were confirmed by reverse transcription-polymerase chain reaction (RT-PCR) and restriction endonuclease analysis ([Fushuku and Fukuda, 2006](#)). The electrophoretotype of rotaviruses can be determined by extraction of nucleic acid from feces and electrophoresis in polyacrylamide gel ([De Leener et al., 2004](#); [Herring et al., 1982](#)). Cytoplasmic fluorescence can be observed at the tips of ileal villi using indirect immunofluorescence ([Petric et al., 1978](#)). Reverse transcription-polymerase chain reaction (RT-PCR), immunohistochemistry, and in situ hybridization can be used to detect rotavirus in formalin-fixed tissues ([Tatti et al., 2002](#)). Serum antibodies to rotavirus can be detected by enzyme immunoassay ([Conner et al., 1991](#); [Hambraeus et al., 1989](#); [Kelkar et al., 2004](#); [Thouless et al., 1988](#)). Rabbits with rotaviral diarrhea usually have no or low concentrations of antibody to rotavirus, with a subsequent rise 2–4 weeks after onset of signs ([Bryden et al., 1976](#); [Sato et al., 1982](#)). Unaffected littermates develop high antibody concentrations, reflecting concurrent subclinical infections.

CONTROL

Rotavirus is highly infectious and is transmitted by fecal–oral spread; however, fomites cannot be excluded. Transplacental transmission has not been demonstrated. Infection appears to be acute and self-limiting. Experimentally, virus is shed in the feces for about

1 week following inoculation (Castrucci et al., 1984; Conner et al., 1988; Hambræus et al., 1989; Petric et al., 1978; Thouless et al., 1988). Recovered rabbits are refractory to challenge with homologous (Castrucci et al., 1984; Ciarlet et al., 1998; Conner et al., 1988, 1991; Hambræus et al., 1989) or heterologous rotaviruses (Hambræus et al., 1989). Hence, cessation of breeding or quarantine of the colony for 4–6 weeks, to prevent the introduction of susceptible rabbits, should permit the infection to run its course. As seropositive dams are not infectious, their offspring should remain free of infection after maternal antibodies disappear. Early weaning of rabbits with high concentrations of passively acquired maternal antibodies and removal to isolated facilities offer the possibility of rederiving rabbits free of infection. Prevention of rotavirus infection depends on barrier maintenance of rabbits.

Coronavirus Infections

Pleural Effusion Disease/Infectious Cardiomyopathy Virus

HISTORY

During the 1960s in Scandinavia, increased mortality in European rabbits (*Oryctolagus cuniculus*) inoculated with the Nichols strain of *Treponema pallidum* (Jorgensen, 1968) was attributed to a virus, named the Stockholm agent, present in rabbit testicular emulsions (Gudjonsson et al., 1970). As the principal necropsy finding was pleural effusion, the name pleural effusion disease was suggested (Fennestad et al., 1975). Later, it was shown that the major target organ was the heart, and the name infectious cardiomyopathy was suggested, since the etiologic agent appeared to be a coronavirus (Small et al., 1979). The resemblance of the clinicopathological features to feline infectious peritonitis, a systemic coronavirus infection of cats, was noted (Osterhaus et al., 1982). The failure to propagate the agent in vitro has precluded more definitive characterization. It is unclear whether the agent is a naturally occurring pathogen of rabbits or a virus from another species adapted to rabbits in contaminated treponemal stocks. However, the disease may be useful as a model for virus-induced cardiomyopathy (Alexander et al., 1992, 1993; Baric et al., 1990; Edwards et al., 1992).

ETIOLOGY

The disease, initially described in 1968, occurred in rabbits inoculated with suspensions of rabbit testes containing the Nichols strain of *T. pallidum* (Gudjonsson and Skog, 1970; Jorgensen, 1968). Rabbits developed fever, circulatory insufficiency with pulmonary edema, and pleural effusion and had high mortality (Gudjonsson and Skog, 1970; Gudjonsson et al., 1970). Subsequent inoculation of rabbits with pulmonary tissue and pleural fluid from dead rabbits, previously

inoculated with treponemes, resulted in a similar syndrome (Gudjonsson et al., 1970). Differential filtration of pleural fluid and serum from rabbits revealed an infectious particle size of 25–50 nm, which is ether-sensitive and inactivated at temperatures of 65°C or above but not at 56°C (Fennestad and MacNaughton, 1983; Gudjonsson et al., 1972; Small et al., 1979). Sera from rabbits contained pleomorphic virus particles, round or elliptic, 75–100 nm in diameter, bearing club-shaped projections 15–20 nm long (Small et al., 1979), whereas plasma from rabbits contained high concentrations of pleomorphic virus-like particles measuring 51–98 nm in diameter with projections 8–13 nm long (Osterhaus et al., 1982). Infectious sera, passed in various cell lines, produced a cytopathic effect in primary rabbit kidney and newborn human intestine cells (Small et al., 1979). However, the cellular pathogenic effect was lost after two passages. Disease was not induced in mice, hamsters, or guinea pigs (Gudjonsson et al., 1970; Small et al., 1979).

In a complement fixation test, antigens in infectious sera cross-reacted with antisera to the 229E (two-way cross) and OC43 (one-way cross) strains of human coronavirus (Small et al., 1979). Antisera to the rabbit cardiomyopathy agent cross-reacted with feline infectious peritonitis virus (FIPV), canine coronavirus (CCV), and porcine transmissible gastroenteritis virus (TGEV) by radioimmunoassay (Small and Woods, 1987). Prior incubation of the agent with antisera to CCV, FIPV, TGEV, or 229E virus reduced mortality in rabbits following inoculation. However, prior immunization of rabbits with CCV, FIV, or TGEV had little effect on survival. Immunofluorescent staining of cardiac tissue from diseased rabbits with antisera to 229E virus revealed antigen in myocardial interstitial tissue, and rabbits surviving infection developed antibodies to the virus in a complement fixation assay (Small et al., 1979). In another study, however, surviving rabbits failed to demonstrate antibodies against 229E virus in an enzyme immunoassay and neutralization test, and against OC43 virus in a complement fixation test (Fennestad and MacNaughton, 1983).

EPIDEMIOLOGY

When the disease was recognized in 1961, rabbits used in the serial propagation of *T. pallidum* had a mortality rate of 2%. By the end of the 1960s, however, mortality had increased to 35–40% owing to contamination of treponemal stocks with virus (Fennestad, 1985; Jorgensen, 1968). There is no difference in disease among breeds (Gudjonsson and Skog, 1970) or between rabbits in the United States and Sweden (Gudjonsson et al., 1970). The agent was detected in *T. pallidum*-infected rabbit tissues from Europe, the United States, and Japan (Fennestad et al., 1980). Nine isolates, obtained from treponema-infected rabbits in various countries,

exhibited a wide range in pathogenicity when inoculated into rabbits (Fennestad et al., 1986). Whereas virtually all rabbits had fever, mortality ranged from 0 to 88%, and all surviving rabbits resisted challenge with a virulent strain. Two of four dams from litters inoculated at 6–9 days of age became infected, whereas uninoculated littermates or introduced cagemates failed to develop infection after exposure for several months (Fennestad et al., 1981). Hence, transmission by direct contact occurs rarely.

CLINICAL SIGNS

Whereas clinical features vary by strain and passage of the agent, inoculated rabbits generally develop a fever in 1–4 days which persists for 5–10 days. During this early phase, acute deaths can occur without clinical signs of illness (Alexander et al., 1999). Clinical signs are often consistent with those of congestive heart failure including anorexia, weight loss, atony, tachypnea, and dyspnea. Iridocyclitis has been reported. With virulent strains, a third of infected rabbits die 3–5 days after inoculation, although deaths can occur until 14 days (Baric et al., 1990; Fennestad, 1985; Fennestad and MacNaughton, 1983; Fennestad et al., 1975, 1986; Fledelius et al., 1978; Gudjonsson and Skog, 1970; Gudjonsson et al., 1970; Small et al., 1979). Generalized or hindquarter muscular weakness was also reported in rabbits surviving the acute phase of infection (Gudjonsson et al., 1970; Small et al., 1979). During the acute phase of infection, rabbits have a transient lymphopenia, followed by heterophilia (Fennestad et al., 1975). Red cell indices were also reduced but returned to normal by 6 weeks after inoculation. There was a transient hypoalbuminemia; however γ -globulin increased significantly (Fennestad et al., 1975). Serum potassium and lactate dehydrogenase were elevated transiently. ECG abnormalities have been reported during the acute phase (days 3–5) including tachycardia in 74% of the infected rabbits, depressed R wave voltages (16%), reduced T wave voltages (45%) and QT (corrected) prolongation (32–42%) and correlated positively with mortality (Alexander et al., 1995, 1999). Ninety percent of rabbits had persistent tachycardia in the subacute phase (days 6–12 after infection), ECG changes persisted, and echocardiographic changes in the subacute phase correlated positively with mortality. Surviving rabbits usually returned to normal in 3–4 weeks. However, ECG abnormalities persisted after day 30 in some rabbits, reflecting the development of dilated cardiomyopathy (Alexander et al., 1999). Reinoculation of surviving rabbits was without effect, indicating development of resistance to the agent (Fennestad, 1985; Fennestad and MacNaughton, 1983; Fennestad et al., 1986; Gudjonsson et al., 1970).

PATHOLOGY

Rabbits dying during the acute phase of disease have pulmonary edema, pleural effusion, and dilation

of the right ventricle (Alexander et al., 1992, 1993; Baric et al., 1990; Christensen et al., 1978; Edwards et al., 1992; Fennestad et al., 1975; Small et al., 1979). The pleural cavities contain 2–50 ml of fluid, with or without fibrin, and few cells; ascites may also occur in rabbits dying after the first week. There are subepicardial and subendocardial hemorrhages. Other findings may include hepatosplenomegaly and congested lymph nodes. The heart weight is increased, and there is dilation of the right ventricle followed by dilation of the left ventricle. The cause of death appears to be congestive heart failure (Alexander et al., 1992, 1993; Baric et al., 1990; Edwards et al., 1992; Small et al., 1979). In fatal cases, there is lymphoid depletion of the splenic follicles, focal degenerative changes of the thymus and lymph nodes, and mild proliferative changes of renal glomeruli (Christensen et al., 1978; Small et al., 1979). In rabbits that survive the acute phase, there is multifocal to diffuse myocardial degeneration and necrosis, focal hepatic necrosis, and proliferative changes in the spleen, lymph nodes, interstitial pulmonary tissue, and renal glomeruli. Lesions similar to those in the heart can occur in the diaphragm (Small et al., 1979). A mild non-suppurative, non-granulomatous anterior uveitis develops during the acute phase of infection and regresses within 4 weeks (Fledelius et al., 1978). At 65 days after infection, myocarditis is evident, and about one-third of rabbits had evidence of right- and/or left-sided cardiac dilation (Baric et al. 1990). After 2 years, rabbits had pulmonary lymphoid hyperplasia, lymphoid hyperplasia of lymph nodes and spleen, siderosis in the spleen, necrosis and periportal inflammation in the liver and interstitial fibrosis of the myocardium (Fennestad et al., 1986). No evidence of circulating or tissue immune complexes was found (Fennestad et al., 1981, 1986).

DIAGNOSIS

It remains to be determined whether the agent is a naturally occurring pathogen of rabbits or perhaps a human virus adapted to rabbits in contaminated stocks of the Nichols strain of *T. pallidum*. The syndrome has only been reported in experimentally inoculated rabbits. The clinical course is characterized by onset of fever 1–3 days after inoculation. Deaths may occur from 2–17 days after inoculation, and mortality may exceed 75%. Other major signs include ocular disease, anorexia, weight loss, tachypnea, and atony. The gross and histological lesions are highly characteristic of the disease. Antibodies to the virus cross-react with the human coronavirus 229E and other members of the group I mammalian coronaviruses (FLPV, CCV, and TGEV) (Small and Woods, 1987; Small et al., 1979).

CONTROL

Because continued serial propagation of *T. pallidum* in rabbits coincided with emergence of disease associated with this agent, the possibility of contaminated stocks

should be considered (Fennestad, 1985; Fennestad et al., 1975; Gudjonsson et al., 1970). Rabbits inoculated with virus-contaminated *T. pallidum* stocks that elicited no or mild disease without mortality were protected from disease when challenged with stocks contaminated with more pathogenic virus (Fennestad, 1985; Fennestad and MacNaughton, 1983; Fennestad et al., 1980, 1986; Gudjonsson et al., 1970). This indicated that some *T. pallidum* stocks were contaminated with a non-pathogenic variant of the virus, which was able to confer protection. Furthermore, isolates decreased in pathogenicity, as a function of time, in rabbits that survived acute infection (Fennestad, 1985; Fennestad et al., 1986). Convalescent sera from rabbits that survived infection partially protected challenged rabbits against mortality but not disease (Fennestad et al., 1981; Gudjonsson et al., 1970; Small et al., 1979). Passage of contaminated *T. pallidum* stocks through hamsters removed the agent responsible for disease (Fennestad et al., 1980; Skovgaard Jensen, 1971). Subsequent reintroduction of these *T. pallidum* stocks in rabbits was without effect. Similarly, inoculation of rabbits, with popliteal lymph nodes from surviving rabbits, transferred treponemes but not the agent (Gudjonsson et al., 1970).

Rabbit Enteric Coronavirus

HISTORY

A coronavirus was detected in the feces of rabbits with diarrhea by LaPierre et al. (1980) in Canada. Rabbits were 6–10 weeks old and from several colonies. Subsequently, coronavirus was detected in young rabbits with diarrhea in several European countries (Eaton, 1984; Osterhaus et al., 1982; Peeters et al., 1984). Although the virus appears to be readily detected in rabbits by workers in Canada (Descoteaux et al., 1985; LaPierre et al., 1980), the failure to propagate the agent in vitro has hampered investigations of its prevalence in European rabbit (*Oryctolagus cuniculus*) populations.

ETIOLOGY

Feces of rabbits with diarrhea exhibit hemagglutination activity with rabbit erythrocytes, particularly with a peak of 1.18 g/cm³ from a sucrose density gradient (Descoteaux et al., 1985; LaPierre et al., 1980). Electron microscopy of fecal samples revealed pleomorphic particles with an inner diameter of 60–220 nm and surface projections of 20 nm (Eaton, 1984; LaPierre et al., 1980). Particles obtained from a Percoll gradient appeared as spherical enveloped particles 40–50 nm in size with projections of 10–12 nm (Descoteaux et al., 1985). In rabbits inoculated orally, pleomorphic particles 60–90 nm, with surface projections of 10 nm, were detected in the feces (Osterhaus et al., 1982). Electron microscopy of fecal samples using antisera to rabbit coronavirus revealed

immune aggregates of viral particles with morphological features of coronaviruses (Descoteaux et al., 1985). Analysis of structural polypeptides by immunoblotting revealed that antisera to avian infectious bronchitis virus (AIBV) and TGEV detected many of the same epitopes as antisera to the rabbit coronavirus (Descoteaux et al., 1985). Immune sera to 229E, but not AIBV nor porcine hemagglutinating encephalitis virus, inhibited the hemagglutination activity of the rabbit coronavirus. No cytopathic effect occurred following inoculation of various cell lines with fecal samples (Eaton, 1984; LaPierre et al., 1980).

EPIDEMIOLOGY

In a survey of 130 diarrheic rabbits from 21 rabbitries, coronavirus was detected in the cecal contents of one rabbit in association with *Escherichia coli* infection (Peeters et al., 1984). Rabbits with diarrheal disease associated with coronavirus infection are usually 3–10 weeks old (Eaton, 1984; La Pierre et al., 1980). Virus has also been detected in the feces of apparently healthy rabbits (Descoteaux et al., 1985). Experimentally, orally inoculated rabbits shed virus for up to 29 days (Descoteaux and Lussier, 1990). A serological survey of 238 *Oryctolagus cuniculus* from six rabbitries in Washington state revealed that 23 (10%) had antibodies to canine coronavirus (Deeb et al., 1993).

CLINICAL SIGNS

In an outbreak of enteric disease in 3–8-week-old rabbits in a barrier-maintained breeding colony, rabbits exhibited lethargy, diarrhea, and swollen abdomens (Eaton, 1984). Between 40 and 60% of rabbits were affected, and virtually all died within 24 h after onset of signs. LaPierre et al. (1980) also reported rapid death following the onset of clinical signs in the majority of affected rabbits. Experimentally, rabbits developed soft feces (Osterhaus et al., 1982) and transient watery diarrhea (Descoteaux and Lussier, 1990) after inoculation. None of the rabbits died (Descoteaux and Lussier, 1990).

PATHOLOGY

In rabbits with diarrhea, the perianal region is soiled with feces (Eaton, 1984). Rabbits appear cachectic and dehydrated. Although the stomach and intestines are unaffected, the cecum is distended with watery fluid (Eaton, 1984; Peeters et al., 1984). Histologically, in the small and large intestines there is a diffuse infiltration of inflammatory cells and mucosal edema (Eaton, 1984). In an experimental study, the small intestines were congested and the cecal contents watery (Descoteaux and Lussier, 1990). Microscopically, at 6 hours after inoculation, there was necrosis of enterocytes at the tips of intestinal villi. Villous atrophy and hypertrophic crypts

were present 2–3 days after inoculation. However, by 6 days, microscopic lesions were absent.

DIAGNOSIS

Rabbits with diarrheal disease are usually recently weaned and 3–10 weeks old (Eaton, 1984; LaPierre et al., 1980). Because the rabbit coronavirus agglutinates erythrocytes, the hemagglutination activity of the feces provides an indication of the presence of virus (LaPierre et al., 1980). Coronavirus particles can be demonstrated in the feces by electron (Eaton, 1984; LaPierre et al., 1980; Osterhaus et al., 1982; Peeters et al., 1984) and immunoelectron (Descoteaux and Lussier, 1990; Descoteaux et al., 1985) microscopy. The latter technique is more sensitive in detecting virus particles (Descoteaux et al., 1985). At necropsy, the cecal contents are fluid (Descoteaux and Lussier, 1990; Eaton, 1984); histologically, there is intestinal villous atrophy (Descoteaux and Lussier, 1990). Adult rabbits may have antibodies in the hemagglutination inhibition assay (LaPierre et al., 1980). As experimental inoculation of rabbits with coronavirus failed to mimic the field disease with its high morbidity and mortality, consideration should be given to the existence of co-pathogens (Descoteaux and Lussier, 1990; Osterhaus et al., 1982). In two studies where other agents were considered, coronavirus was associated with *Escherichia coli* and *Clostridium perfringens* infections (Eaton, 1984; Peeters, et al., 1984).

CONTROL

Because only one naturally occurring outbreak of diarrheal disease associated with coronavirus has been reported (Eaton, 1984), there is scant information on control of the disease. The feeding of hay, delay in weaning, and administration of coccidiostats and antibiotics were ineffective in preventing mortality.

Calicivirus Infections

The etiologic agents of necrotic hepatitis of leporids have profoundly affected world rabbit populations since 1984. They are classified as members of the family, *Caliciviridae*, and the genus, *Lagovirus*. This genus contains two distinct species which cause clinical disease denoted as *Rabbit hemorrhagic disease virus* (RHDV) and *European brown hare syndrome virus* (EBHSV) (Green et al., 2000). These diseases were first recognized in the mid-1980s. RHD was reported in China in 1984 and subsequently in other countries. EBHS was observed in Europe several years before RHD was diagnosed in domestic European rabbits. Antibodies to RHDV were in archived serum from 1961 in the Czech Republic and Austria (Nowotny et al., 1997) and viral RNA in serum samples from Britain stored in 1955 (Moss et al.,

2002). Antibodies to EBHSV were found as early as 1962 (Frölich and Lavazza, 2008) in serum in England and viral RNA in tissues collected in the 1970s in Sweden (Bascunana et al., 1997).

A third member of the genus *Lagovirus* was identified in domestic rabbits in Italy in 1996 and was named rabbit calicivirus (RCV) (Capucci et al., 1996). No clinical signs were associated with RCV infection although seroconversion resulted in protection from RHDV (Capucci et al., 1997). A closely related strain, denoted rabbit calicivirus, Australia 1 (RCV-A1) was recently isolated from wild European rabbits in Australia and partially sequenced (Strive et al., 2009). Whether antibodies which recognize RHDV epitopes in retrospective studies are associated with RCV and its variants rather than RHDV is unknown. Another closely related virus, denoted Michigan Rabbit Calicivirus (MRCV) was isolated from an outbreak of hemorrhagic disease in the United States in 2001 (Bergin et al., 2009). One member of the genus *Vesivirus*, family *Caliciviridae* has been described as a potential intestinal pathogen of rabbits (Martín-Alonso et al., 2005).

Rabbit Hemorrhagic Disease Virus (RHDV)

HISTORY

In early 1984, an acute fatal disease of rabbits was reported from many regions of China (Xu and Chen, 1989; Xu et al., 1988). The disease was unlike any previously reported syndrome and may have originated in rabbits imported from Europe. Tests of rabbit sera stored in Czechoslovakia in 1978 revealed antibodies to the virus (Rodak et al., 1990a). Although the syndrome was initially variously named, it came to be known as RHD. Subsequently, the disease was reported in several European countries in 1987 and 1988 (Gregg and House, 1989; Parra and Prieto, 1990; Patton, 1989). The negative impact of this disease on wild rabbit populations in Europe has been significant (Calvete et al., 2002; Marchandeu et al., 1998). Currently, it is endemic in most of Europe, Asia, Africa, Australia, and New Zealand.

By late 1988, the virus reached North America and was reported from many locations in Mexico (Gregg and House, 1989; Gutierrez, 1990; Patton, 1989). Mexico mounted an eradication campaign and has since remained free of the virus (McIntosh et al., 2007). Four outbreaks of RHDV were reported in the United States between 2000 and 2007 (Anonymous, 2000; Campagnolo et al., 2003; U.S.D.A., 2005; McIntosh et al., 2007). Each time the virus was contained, but how it was introduced was not determined (McIntosh et al., 2007).

In 1995, a laboratory strain of RHDV was being tested in field experiments under quarantine conditions at Wardang Island, Australia, to evaluate the virulence,

transmissibility, and persistence of RHDV (McColl et al., 2002). It was also being considered as a potential biological control agent for rabbits. The virus escaped and spread, first to other rabbits on Wardang Island, and then to the mainland. It resulted in significant mortality immediately, reducing rabbit populations in some areas by 95% (Mutze et al., 1998). It is currently endemic throughout Australia. In 1996, the New Zealand government was asked to consider the use of RHDV as a biological control agent (Thompson and Clark, 1997). The government denied these requests; however, RHDV was introduced to New Zealand illegally by farmers hoping to reduce local rabbit populations and is now widely distributed in both the North and South Islands of New Zealand (O'Keefe et al., 1999; Thompson and Clark, 1997).

ETIOLOGY

A virus has been consistently purified from the liver and spleen of affected rabbits, the characteristics of which have been described by several investigators (Du, 1990, 1991; Du et al., 1986; Liebermann et al., 1992; Ohlinger and Theil, 1991; Sihid et al., 1989; Smid et al., 1989; Xu, 1991; Xu and Chen, 1989; Xu et al., 1988). The virus is non-enveloped, spherical, and 28–34 nm in diameter and has a buoyant density of 1.32–1.38 g/cm³. The icosahedral capsid has 32 cylindrical capsomeres, 5–6 nm in diameter, comprised of four structural polypeptides. The virus is a member of the family, *Caliciviridae*, and is the type virus of the genus, *Lagovirus* (Green et al., 2000). RHDV was completely sequenced and has a positive strand RNA genome of 7437 kb (Meyers et al., 1991a). The genomic organization is similar to EBHSV with two open reading frames (ORF) rather than the three ORFs found in other caliciviruses (Wirblich et al., 1996). The first ORF encodes non-structural proteins as well as the large capsid protein and the second ORF encodes an additional capsid protein. One subgenomic RNA (2.2 kb) is present (Ohlinger et al., 1990; Parra and Prieto, 1990). Amino acid identity between RHDV and EBHSV is approximately 76% (Wirblich et al., 1994). The virus agglutinates erythrocytes of humans and guinea pigs, but not rabbits. Non-hemagglutinating strains of RHDV have been identified (Tian et al., 2007). While the majority of strains of RHDV have a high level of amino acid identity, antigenic variants have been described (Capucci et al., 1998; Asgari et al., 1999; Shirmmaier et al., 1999).

The virus is highly stable in the environment and sufficient infectious RNA is present to infect rabbits from liver samples stored for 20 days at 22°C (McColl et al., 2002). In a separate study, infectious virus was injected into bovine liver and then the liver was exposed outdoors to ambient temperatures (2–34°C) (Henning et al., 2005). Sufficient viral particles were

present at the longest time point studied, 91 days, to infect rabbits when inoculated undiluted. Viral infectivity is unaffected by treatment with ether, chloroform, exposure to pH 3, or heating at 50°C. However, the virus is inactivated by 1% sodium hydroxide or 0.4% formaldehyde at ambient temperature, 4°C or 37°C. The virus fails to grow in many primary and established cell lines but has been adapted to a transformed rabbit kidney cell line (Ji et al., 1991; Xu, 1991). Rabbits may be the only species susceptible to infection, as inoculation of guinea pigs, hamsters, rats, or mice failed to induce disease. Experimentally, inoculation of European hares (*Lepus europaeus*) failed to induce disease (Lavazza et al., 1996; Smid et al., 1991).

EPIDEMIOLOGY

Since the report of the first outbreak of RHDV in China in 1984, RHDV has rapidly spread throughout the world. The disease has been reported from China, Korea, most European countries, Asia, Africa, North America, Australia, and New Zealand (Chasey et al., 1997; McIntosh et al., 2007; Morisse et al., 1991). Countries experiencing epidemics with high morbidity and mortality include China (Xu and Chen, 1989), Korea (Lee and Park, 1987), Italy (Cancellotti and Renzi, 1991; Patton, 1989), Spain (Parra and Prieto, 1990; Villafuerte et al., 1994), France (Morisse et al., 1991), Germany (Loliger and Eskens, 1991), Mexico (Gregg and House, 1989; Gutierrez, 1990), Taiwan (Shien et al., 1998), Cuba (Farnós et al., 2007), Saudi Arabia (Abu Elzein and Al-Afaleqand, 1999), Bahrain (Forrester et al., 2006), Tunisia (Bouslama et al., 1996), the United Kingdom (White et al., 2004), and the United States (McIntosh et al., 2007). The disease has also been reported from India (Sundaram et al., 1991) and the Middle East (Kuttin et al., 1991). Viruses from China, Korea, and Europe appear to be similar (Du, 1990; Gregg et al., 1991), although evolution of viruses in restricted geographic areas has occurred (Muller et al., 2009). Recently, a virus isolated in the Netherlands was demonstrated to be 99% identical to RHDV from France (van de Bildt et al., 2006).

There have been four outbreaks of RHDV in the US; in Iowa in 2000 (Anonymous, 2000), in Utah in 2001, which was transferred to Illinois by shipment of infected rabbits (Campagnolo et al., 2003), in a zoo in New York in 2001 (McIntosh et al., 2007), and in Indiana in 2005 (McIntosh et al., 2007; U.S.D.A., 2005). Isolates from these outbreaks were shown by comparative genomic analysis to be closely related to isolates from China, but to have separate origins (McIntosh et al., 2007).

There is evidence that RHDV was present in wild and domesticated populations of rabbits in Europe (Moss et al., 2002; Rodak et al., 1990a), Australia (Cooke et al., 2000; Nagesha et al., 2000), and New Zealand (O'Keefe et al., 1999) prior to the recognition of RHDV. Studies in

England demonstrated serological evidence as well as partial RNA sequences recovered from tissues or serum of apparently healthy rabbits (Moss et al., 2002; White et al., 2004). These partial sequences were more closely related to RHDV than RCV, suggesting that non-pathogenic RHDV viruses have circulated in parts of the world for some time.

No apparent difference in susceptibility to infection among breeds of *Oryctolagus cuniculus* has been reported (Xu and Chen, 1989; Xu et al., 1988). Outbreaks of disease have occurred mainly in rabbits 2 months of age and older, whereas younger rabbits were clinically unaffected. Of the rabbits that survived the infection, viral RNA has been detected in various organs including the spleen, liver, mesenteric lymph node, and bile for 15 weeks after infection suggesting that a chronic carrier state can occur (Gall et al., 2007).

Transmission of virus is horizontal, primarily by direct contact with secretions and excretions of infected rabbits and fecal-oral spread may be the major mode of transmission. Contaminated fomites, such as feed, water, utensils, and animal attendants, may be important in transmission as well. Transmission by insects has been suggested as an explanation for the spread of RHDV from Wardang Island to the mainland. Laboratory experiments demonstrated that RHDV can be transmitted by blowflies (*Phormia* sp.), bushflies (*Musca vetustissima*) and mosquitoes (Gould et al., 1997; McColl et al., 2002). Transmission by the lesser brown blowfly, *Calliphora dubia* was suggested by evidence that RHDV RNA was detected in fly feces after flies were fed a meal from a RHDV-infected liver (Asgari et al., 1998). There is no evidence of vertical transmission. Fecal shedding by carnivores after ingestion of RHDV-infected carcasses has been suggested as a potential mode of spread. Serological evidence of infection in foxes has been demonstrated in several studies, although no infectious particles have been demonstrated (Frölich et al., 1998; Leighton et al., 1995; Philbey et al., 2005). Experimentally, the routes of entry, in order of importance, are oral, conjunctival, nasal, and skin trauma. In China and Europe, epidemics of disease usually begin in November and end in March. Although *Oryctolagus cuniculus* appears to be the only species susceptible to disease, hares in China also appear to be infected and capable of transmitting the virus to rabbits experimentally (Xu, 1991).

A serological survey of 1461 rabbits from 43 farms, apparently free of viral hemorrhagic disease in Czechoslovakia revealed that 283 rabbits (19%) from 33 farms had anti-RHDV antibodies (Rodak et al., 1990a). Using another set of sera, collected between 1975 and 1987 from laboratory-maintained rabbits, antibodies were detected in 32 of 42 sera (76%). This suggests that rabbit colonies in Czechoslovakia harbored a viral agent with characteristics similar to those of Rabbit hemorrhagic disease virus, but with lower pathogenicity,

several years before the syndrome was recognized in China.

RHDV has been shown to bind to histo-blood group antigens expressed on the surface of respiratory and digestive tract epithelial cells (Ruvoën-Clouet et al., 2000). This binding may partially explain the differential susceptibility of young versus older rabbits (Rademacher et al., 2008; Ruvoën-Clouet et al., 2000). In addition, polymorphisms of these antigens in wild rabbits in France have been linked to differential survival from RHDV infection (Guillon et al., 2009).

CLINICAL SIGNS

In general, the disease is acute and highly infectious, with high morbidity and mortality (Marcato et al., 1991; Xu and Chen, 1989; Xu et al., 1988). The incubation period ranges from 1–2 days. Morbidity is 70–80%, and mortality approaches 100%. During outbreaks, the number of rabbits affected usually peaks in 2–3 days and lasts 7–13 days. After viral introduction, rabbits die suddenly with few clinical signs. Rabbits become febrile and exhibit depression, lethargy, and anorexia. Other clinical signs include tachypnea, cyanosis, abdominal distention, and constipation or diarrhea. Because the disease is acute, clinical signs may be brief and often are unnoticed. In the terminal stage, rabbits become hypothermic, recumbent, and have convulsions and epistaxis. Surviving rabbits exhibit depression, anorexia, and fever which usually abates in 2–3 days. In endemic areas, the form of disease observed in surviving rabbits is more common. Hematologic evaluation usually reveals a lymphopenia, a gradual decline in thrombocytes, and prolonged prothrombin and thrombin times. A paracoagulation test with protamine sulfate gives a strong positive reaction. Fibrin degradation products can be detected in most moribund rabbits.

In young rabbits, clinical signs of infection are typically absent (Ferreira et al., 2004, 2006; Mikami et al., 1999). However, liver inflammation as evidenced by increase in hepatic transaminases and increase in heterophil liver infiltrate occurs, although there is little hepatocyte damage (Ferreira et al., 2004, 2006; Mikami et al., 1999; Prieto et al., 2000) and less replication of the virus within hepatocytes than adults as evidenced by immunohistochemical staining (Preito et al., 2000). Elevation of transaminases persists for at least 3 weeks after infection (Ferreira et al., 2004). Young rabbits infected with RHDV develop a protective antibody response that persists and protects them from infection as adults (Ferreira et al., 2008). However, these rabbits may serve as healthy carriers of the virus (Ferreira et al., 2004, 2008).

PATHOLOGY

The pathological changes apparently result from viremia, with death attributable to an acute disseminated

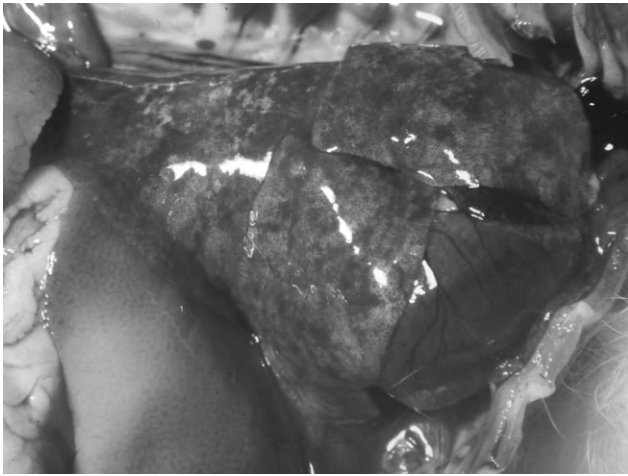


FIGURE 14.3 Diffusely congested lungs from rabbit found dead with RHD. Courtesy of Dr. D. Gregg.

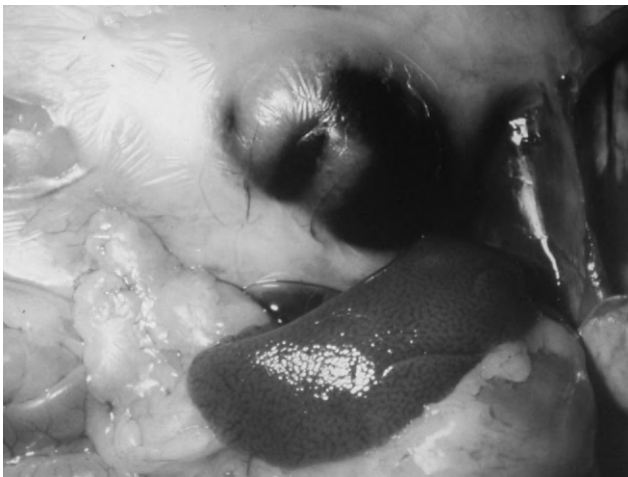


FIGURE 14.4 Reticular pattern of liver (bottom, center) consistent with periportal necrosis in a rabbit found dead with RHD. The kidney (top, center) is diffusely congested. Courtesy of Dr. D. Gregg.

coagulopathy with deep venous thrombosis (Gregg and House, 1989; Gregg et al., 1991; Marcato et al., 1991; Xu and Chen, 1989; Xu et al., 1986, 1988). Grossly, congestion and hemorrhage occur in most organs but are most pronounced in the lungs (Figure 14.3). The liver is pale and has a fine reticular pattern of periportal necrosis, the most consistent finding in the disease (Figure 14.4). The spleen and kidneys may be dark and swollen owing to acute infarction (Figure 14.4). Lymph nodes are edematous and may contain petechial hemorrhages. There is often segmental catarrhal enteritis.

In most fatal cases, rabbits die from a severe and massive intravascular coagulopathy (Gregg et al., 1991). The disease consistently causes acute hepatic necrosis, which may be the only lesion found. Hepatic necrosis

is periportal and diffuse, and when severe, may bridge acini and cause dissociation of hepatic cords (Gregg et al., 1991; Percy and Barthold, 2007). Small single or multiple intranuclear inclusion bodies can be found in degenerate hepatocytes. There is often little inflammation in necrotic areas. Many tissues, especially the lungs, spleen, and kidneys, may have varying degrees of congestion and hemorrhage due to microinfarction or major venous thrombi. Acute coagulative necrosis due to microinfarction may be found in any organ. Microinfarcts in the brain account for the terminal neurological signs. Pulmonary venous thrombosis accounts for the frothy serosanguinous discharge from the nares seen terminally. A segmental necrotizing enteritis with severe crypt necrosis and villous atrophy can be found (Gregg et al., 1991). Lymphoid tissues may have varying degrees of degeneration and karyorrhexis of lymphocytes. The spleen and thymus are more often affected than lymph nodes.

DIAGNOSIS

A presumptive diagnosis can be made on the basis of the epidemiological features, clinical signs, and pathological findings (Xu and Chen, 1989). Virus can be detected with RT-PCR using multiple tissues including blood (Gall et al., 2007; Guittre et al., 1995; Shien et al., 2000; Vende et al., 1995; Wang et al., 2008; Yang et al., 2008). No satisfactory in vitro isolation methods have been described, and rabbit inoculation is the only method available to isolate or propagate a new strain of virus (O. I. E. 2008). Immunocapture RT-PCR was developed to simplify the tissue processing required to analyze numerous samples (Le Gall-Reculé et al., 2001). By electron microscopy, viral particles, 28–34nm in diameter, are frequently found in hepatocytes (Valicek et al., 1990; Xu and Chen, 1989). The hemagglutination test, with type O human erythrocytes, is useful for detection of virus in suspensions of liver, lungs, spleen, and kidneys, from infected rabbits (Shien et al., 2000; Xu and Chen, 1989). Antisera or monoclonal antibodies to the virus can detect virus in hepatocytes in infected rabbits by immunoenzyme and immunofluorescence tests (Berninger and House, 1995; Capucci et al., 1991; Carrasco et al., 1991; Gregg and House, 1989; Gregg et al., 1991; Park and Itakura, 1992; Rodak et al., 1990b, 1991; Stoerckle-Berger et al., 1992). Serological tests include the hemagglutination inhibition test and the enzyme-linked immunosorbent assay, the latter being preferable because of increased sensitivity and specificity (Chasey et al., 1995; Cooke et al., 2000; Rodak et al., 1990b). Both indirect sandwich ELISAs and competitive-ELISAs have been developed for detection of antibodies (Guittre et al., 1995). Seroconversion to RHDV without clinical signs of disease is suggestive of RCV infection and should be interpreted with caution (Capucci et al., 1997).

CONTROL

Measures to prevent introduction of the virus include restricted access and disinfection of all equipment entering or leaving a facility (Xu and Chen, 1989). Cages and equipment can be disinfected with 0.5% sodium hypochlorite or 1% formalin. Rabbits from endemic areas should not be introduced directly into the colony, but quarantined for at least 1 month and tested serologically for anti-RHDV antibodies. Similarly, colonies with disease should be quarantined and depopulated, since surviving rabbits shed virus for at least a month and possibly longer (Gregg and House, 1989; Gregg et al., 1991). Antiserum has been shown to be protective (Du, 1990; Huang, 1991). Tissue-derived vaccines, inactivated with formaldehyde (Du, 1990; Du et al., 1986; Huang, 1991; Xu and Chen, 1989) or β -propiolactone (Arguello Villares, 1991; Smid et al., 1991), have been shown to be safe and efficacious in preventing disease. Resistance develops within 1–2 weeks after vaccination and lasts for 5–15 months. Although disease may be prevented in vaccinated rabbits, persistent infection may develop on exposure to the virus (House et al., 1990). Thus, vaccinated rabbits infected with virus should be considered infectious and should not be introduced into previously unexposed rabbitries. Additional vaccines have been developed, including those combining protection against both RHDV and myxomatosis (Bertagnoli et al., 1996; Calvete et al., 2004; Fernández-Fernández et al., 2001; Torres et al., 2001). Since the decline of rabbit populations in southern France and the Iberian peninsula has affected the survival of rabbit-prey species (Marchandeau et al., 2000), vaccination of free-living rabbits has been undertaken in a field trial on an island off Spain and subsequently on the mainland (Calvete et al., 2004; Torres et al., 2001).

European Brown Hare Syndrome Virus (EBHSV)

HISTORY

A disease, characterized by hemorrhages in the trachea and lungs, pulmonary edema, and necrotic hepatitis, with high mortality, has been observed since 1980–1985 in European hares (*Lepus europaeus*) and Mountain hares (*Lepus timidus*) in many European countries (Billinis et al., 2005; Duff et al., 1994; Frölich 1996; Frölich et al., 2001, 2003a, 2007; Gavier-Widen and Morner, 1991; Le Gall-Reculé et al., 2006; Morisse et al., 1990, 1991; Syrjälä et al., 2005). The virus has also been detected in European hares translocated to Argentina (Frölich et al., 2003b). The disease, named European brown hare syndrome (EBHS), is similar to RHD, but affects hares.

ETIOLOGY

Electron microscopy of hepatocytes from affected hares reveals non-enveloped, icosahedral particles about 30 nm in diameter (Chasey and Duff, 1990; Marcato et al.,

1991). Immunoblotting of the virus reveals a major structural protein of about 60 kDa, similar to RHDV; however, other proteins of the latter virus are absent, suggesting that the viruses are similar but not identical (Capucci et al., 1991; Ohlinger and Thiel, 1991). Sequencing of the genome (Gould et al., 1997; Le Gall et al., 1996; Meyers et al., 1991b; Nowotny et al., 1997; Parra et al., 1993; Rasschaert et al., 1995; Wirblich et al., 1994) revealed that RHDV and EBHSV have similar genomic organization and are both members of the family, *Caliciviridae*, and the genus, *Lagovirus*, however they represent two distinct species (Green et al., 2000). EBHSV and RHDV have been shown to have approximately 76% amino acid identity (Wirblich et al., 1994).

EBHSV fails to grow in primary hare and rabbit cell lines (Gavier-Widen and Morner, 1991; Henriksen et al., 1989), and inoculation of mice and guinea pigs failed to induce disease (Henriksen et al., 1989). In general, inoculation of rabbits (*Oryctolagus cuniculus*) with EBHSV, or tissues from infected hares, has not resulted in clinical disease in rabbits (Capucci et al., 1991; Chasey et al., 1992; Eskens and Volmer, 1989; Nauwynck et al., 1993) or cross-protective immunity (Nauwynck et al., 1993), although clinical disease similar to RHD was induced by Morrisset al. (1990) following inoculation of liver homogenates from affected hares in France.

EPIDEMIOLOGY

The disease has been reported in wild hares from several European countries, including England (Chasey and Duff, 1990), the Czech Republic (Nowotny et al., 1997), Greece (Billinis et al., 2005), Finland (Syrjälä et al., 2005), Slovakia (Frölich et al., 2007), Switzerland (Frölich et al., 2001), France (Le Gall-Reculé et al., 2006), Poland (Frölich et al., 1996, 2003a), as well as South America (Frölich et al., 2003b). EBHSV has also been reported from breeding farms for hares in Denmark (Henriksen et al., 1989) and Sweden (Gavier-Widen and Morner, 1991). In France, Germany, and Italy, the geographic distribution of EBHS coincides with RHD in wild and domesticated rabbits (Cancellotti and Renzi, 1991; Loliger and Eskens, 1991; Morisse et al., 1991). Seroprevalence has been determined in many countries. Thirty-eight percent of hares tested in Poland were positive for EBHSV antibodies (Frölich et al., 1996), 29% in Germany (Frölich et al., 2003) while 73% of hares shot by hunters in Slovakia had anti-EBHSV antibodies, suggesting some variation in the prevalence of infection.

Similar to RHDV, young hares are resistant to the virus (Morisse et al., 1991). The major mode of transmission appears to be fecal-oral (Morisse et al., 1991). Contamination of feed and water with excreta from infected hares is probably a common mode of transmission (Gavier-Widen and Morner, 1991). Among wild

and farmed hares in Denmark and Sweden, mortality is seasonal, beginning in October and continuing through March (Gavier-Widen and Morner, 1991; Henriksen et al., 1989), presumably as there are more young susceptible hares at the end of the breeding season. Recent evidence from Finland indicates that the majority of the cases occurred in the spring and summer, with a minor peak in October and November (Syrjälä et al., 2005). The hare population in Finland was lowest in winter and this may influence transmission. In England, excess mortality in wild hares was reported in September through November (Chasey and Duff, 1990).

CLINICAL SIGNS

In general, the disease is acute and highly infectious, with high morbidity and mortality (Henriksen et al., 1989; Marcato et al., 1991). Clinical signs include depression, anorexia, muscular tremors, incoordination, paralysis, convulsions, and occasionally epistaxis. Death occurs 5–24h after onset of signs, and affected hares rarely recover. The reported morbidity is 75%, and mortality approaches 100% (Gavier-Widen and Morner, 1991; Henriksen et al., 1989). In Argentina (Frölich et al., 2003b) and Slovakia (Frölich et al., 2007) evidence of a chronic, less pathogenic form of EBHSV has been demonstrated. In Argentina, no clinical signs suggestive of EBHSV were reported; however 11% of 80 spleen samples were positive for EBHSV antigen (Frölich et al., 2003b) whereas antibodies to EBHSV were present in only one sample. In Slovakia, antibodies against EBHSV were found in 73% of 86 sera tested although hare populations were reported to be stable with no increase in mortality noted before or during the sampling period (Frölich et al., 2007).

PATHOLOGY

Death is attributable to multiple organ failure resulting in pulmonary edema and hemorrhage, adrenocortical necrosis, renal circulatory disorders, and hepatic necrosis (Henriksen et al., 1989; Marcato et al., 1991). Gross examination of hares reveals marked pulmonary congestion and edema, as well as hepatic congestion and hemorrhages. Moderate splenomegaly and gastric ulceration are detected in some hares. A catarrhal to necrotizing conjunctivitis may be present. Microscopically, there is diffuse acute coagulation necrosis of hepatic periportal and midzonal areas, accompanied by formation of acidophilic bodies (Gavier-Widen and Morner, 1991; Henriksen et al., 1989). In many hares, there is basophilic stippling in the cytoplasm of hepatocytes in periportal areas, representing granular calcification. Many livers have microvacuolar fatty degeneration. About one-fourth of affected hares have splenic cellular depletion and hyaline-like changes in the sinuses and cords, and about one-third have renal tubular necrosis and calcification. In the brain, cerebral neurons and cerebellar Purkinje cells exhibit

granulovacuolar degeneration. In apparently healthy hares with antibodies to the virus, hepatic vacuolar degeneration, tracheitis, and hyperplasia of splenic follicles are observed (Marcato et al., 1991).

DIAGNOSIS

Diagnosis is similar to that for RHD. RT-PCR has been used to detect virus in tissues (Bascunana et al., 1997; Gould et al., 1997). The hemagglutination test for detection of virus in tissue specimens is less sensitive (Capucci et al., 1991). Use of two ELISAs, in series, employing monoclonal antibodies, in which the first test detects group antigen and the second virus-specific antigen, has also been used to detect virus (Capucci et al., 1991). The ELISA is preferred for detection of antibodies (Capucci et al., 1991).

CONTROL

Methods of control in breeding farms for hares are similar to those for rabbit hemorrhagic disease. Preventive measures include restricted access, disinfection of equipment, and quarantine of newly acquired hares. As subclinically infected hares may shed virus, serological screening of quarantined hares may be advisable. Only seronegative hares should be permitted entry into the colony. Colonies with the disease should be quarantined and depopulated. No vaccine has been developed for use in hares.

Rabbit Calicivirus (RCV)

HISTORY

Non-pathogenic rabbit caliciviruses were first isolated from rabbits (*Oryctolagus cuniculus*) in a rabbitry in Italy that seroconverted to RHDV without exhibiting clinical signs of disease (Capucci et al., 1996). Serological studies revealed rabbit populations in Europe with serological evidence of RHDV, but no clinical evidence of disease (Capucci et al., 1996, 1997; Forrester et al., 1997; Rodak, 1990). In addition, retrospective studies demonstrated serological evidence of anti-RHDV antibodies prior to the spread of RHDV virus in Europe (Moss et al., 2002; Rodak, 1990a), Australia, and New Zealand (Bruce and Twigg, 2004; Nagesha et al., 2000; Robinson et al., 2002). Investigators in Italy isolated a non-pathogenic calicivirus, rabbit calicivirus (RCV), from domestic rabbits (Capucci et al., 1996). Partial sequences of apparently non-pathogenic caliciviruses were detected in rabbits (*Oryctolagus cuniculus*) from Lambay Island, Eire in 2007 (Forrester et al., 2007). In 2009, another apparently non-pathogenic calicivirus, RCV-A1, was isolated from wild rabbits (*Oryctolagus cuniculus*) in Australia (Strive et al., 2009).

ETIOLOGY

RCV is considered a strain of RHDV (Fauquet et al., 2005). Currently, similar viruses have been isolated from

Italy (RCV), Eire (Lambay Island strain), and Australia (RCV-A1) (Capucci et al., 1996; Forrester et al., 2007; Strive et al., 2009). Genome organization is similar to RHDV and EBHSV in that there are two open reading frames as opposed to three which are found in other caliciviruses (Capucci et al., 1996; Strive et al., 2009). RCV is most closely related to RHDV with an amino acid identity of 91% of the capsid protein, VP60. The amino acid identity to EBHSV was 75% in the same study (Capucci et al., 1996). The average identity of the full-length sequence of RCV-A1 when compared to RHDV was 87%. Phylogenetic analysis of RCV, the Lambay Island strain, and the Ashington strain (England) suggests that these isolates are more closely related to each other than to other strains of RHDV (Capucci et al., 1996; Forrester et al., 2007; Strive et al., 2009). Interestingly, the Ashington strain was isolated from a rabbit (*Oryctolagus cuniculus*) with typical signs of RHDV. Antigenically, sera from rabbits infected with RCV cross-reacted with anti-RHDV antibodies, but not anti-EBHSV antibodies (Capucci et al., 1996). Sera from ten of 11 healthy rabbits on Lambay Island, Eire, also cross-reacted with antibody to RHDV (Forrester et al., 2007) and RT-PCR detected virus sequences shown to be closely related to RCV from two serum samples.

EPIDEMIOLOGY

RCV-A1 RNA was recovered from the small and large intestines of wild European rabbits (*Oryctolagus cuniculus*) as well as the fecal pellets from the distal colon, suggesting a fecal–oral method of transmission similar to RHDV (Strive et al., 2009). Viral RNA was detected in Peyer's patches, spleen, and less frequently in liver. Experimental inoculation of *Oryctolagus cuniculus* with RCV by oronasal route resulted in seroconversion by day 6 after inoculation (Capucci et al., 1996). Uninoculated cagemates of infected rabbits were seropositive 1–2 days later, presumably from contact. Rabbits housed in the same room that had no contact with the infected rabbits did not seroconvert. RT-PCR detected viral antigens from the intestine of infected rabbits 3, 5, 6, and 7 days after inoculation but not from the liver or spleen. Western blot analyses of intestinal extracts were also positive. Infection with RCV provided complete protection from challenge with RHDV. Inoculation of hares with RCV resulted in no seroconversion and 5 days after inoculation all organs were negative for RCV by RT-PCR. Hares previously inoculated with RCV and then challenged with EBHSV were not protected from disease.

CLINICAL SIGNS

Experimental inoculation of *Oryctolagus cuniculus* with RCV resulted in no clinical signs of disease (Capucci et al., 1996) and no clinical signs of disease were observed in rabbitries with positive serology (Capucci et al., 1997). No clinical signs were observed in wild rabbits (*Oryctolagus*

cuniculus) positive for the Lambay Island strain (Forrester et al., 2007) or RCV-A1 (Strive et al., 2009).

PATHOLOGY

Gross or histopathologic lesions have not been described.

DIAGNOSIS

Seroconversion to RHDV without clinical signs of disease is suggestive of RCV infection (Capucci et al., 1997). The virus can be detected by RT-PCR of intestinal contents or feces (Capucci et al., 1996; Forrester et al., 2007; Strive et al., 2009).

CONTROL

Serologically, RCV is difficult to definitively distinguish from RHDV. RCV may represent an older non-pathogenic lagovirus that provides protection from RHDV and thus explains why in some areas seroreactivity to RHDV occurs without evidence of disease.

Michigan Rabbit Calicivirus (MRCV)

In January, 2001, an outbreak of hemorrhagic disease with a 32.5% case fatality rate occurred in a private New Zealand White rabbitry in Michigan (Bergin et al., 2009). Clinical signs consisted of acute fatality, inappetance, vulvar hemorrhage, conjunctival congestion, opisthotonus, and cyanosis of the lips and ear tips. On gross necropsy, evidence of icterus, gastric petechiae and ecchymoses, colonic serosal hemorrhage, and multifocal hemorrhage in the caudal lung lobes was observed. Histopathology revealed multifocal random or periportal hepatocellular necrosis and inflammation, pulmonary and uterine hemorrhages with fibrin clots, bile duct proliferation, and periductal fibrosis. RT-PCR of tissue samples ruled out RHDV, however, positive hepatocytes were detected by immunohistochemistry utilizing RHDV-specific antibodies.

Further amplification of sequences from pooled liver samples demonstrated a calicivirus with capsid sequences that most closely aligned to RCV (91.7% similarity) but were also closely related to RHDV (89.8–91.3% similarity). When ORF-1 polypeptide genomic sequence was aligned, excluding the capsid sequences, MRCV was 77.9–78.5% identical to RHDV, although other strains of RHDV share similarities of 87.9–98.1% in these same comparisons. These sequences of RCV have not been determined (Bergin et al., 2009).

Inoculation of homogenized liver from infected rabbits into naïve, SPF (free of *Pasteurella* sp.) rabbits did not induce disease after 7 days, although inoculated rabbits had viral RNA in the liver detected by RT-PCR and in situ hybridization. This short time course did not allow for determination of seroconversion (Bergin et al., 2009).

Clinical signs of disease resolved and rabbits were depopulated approximately 2 months after the initial outbreak. Antibody against MRCV was detected in depopulated rabbits and 20% had gross and non-specific histopathological changes in their livers consisting of biliary hyperplasia and periductal to bridging portal to portal fibrosis. How the virus was introduced into the colony was not determined (Bergin et al., 2009).

Rabbit Vesivirus

A novel member of the family Caliciviridae was isolated from pooled intestinal contents of five European rabbits (*Oryctolagus cuniculus*) that died with intestinal disease in Oregon (Martín-Alonso et al., 2005). Coccidia were detected in two rabbits and *Escherichia coli* was cultured from the pooled intestinal contents. Calicivirus particles were observed by electron microscopy and a calicivirus was isolated on porcine kidney cell lines and partially sequenced. The virus was not neutralized with anti-calicivirus antibodies (from 40 different antisera) and sequence analysis indicated it is a novel member of the Vesivirus genus. The genome consists of a single-stranded positive sense RNA of 8295 nucleotides and contains a 2.6-kb subgenomic RNA. Its genomic organization is unlike lagoviruses, as it contains three ORFs, rather than two. Phylogenetic analysis grouped it in a clade containing marine caliciviruses and non-human primate calicivirus, Pan 1.

Paramyxovirus Infections

Rabbit syncytium virus was isolated in chicken embryos inoculated with extracts of liver and spleen from a wild Eastern Cottontail rabbit (*Sylvilagus floridanus*) in Virginia (Morris et al., 1965). The agent causes a cytopathic effect and syncytia in monkey and hamster kidney cell cultures. Experimentally, suckling mice are susceptible to the virus, but weaned mice, guinea pigs, domestic European and Eastern Cottontail rabbits failed to develop signs or lesions, although antibodies developed, following inoculation. Sera from eight of 25 (32%) Eastern Cottontail rabbits trapped in the same area as the original rabbit had antibodies to the virus. Antibodies to the virus were not detected in sera from seven other species trapped in the same area, *Oryctolagus cuniculus*, or humans. The virus resembles the paramyxoviruses in size, nucleic acid type, and in ether and heat sensitivity. Neither hemagglutination nor hemadsorption were observed, and the ultrastructure of the virus has not been described. The virus is serologically distinct from the known paramyxoviruses.

Evidence of another paramyxovirus, *Sendai virus*, has been found in domestic European rabbits (*Oryctolagus cuniculus*). *Sendai virus* is the type species of the genus *Respirovirus*, family *Paramyxoviridae* (Fauquet et al., 2005)

and a primary pathogen of mice (Percy and Barthold, 2007). A serological survey of 23 breeding and laboratory colonies, in metropolitan Tokyo and Ibaragi, Chiba, Saitama, Kanagawa, Shizuoka, and Nagano prefectures, revealed that 85 of 160 (53%) Japanese White or New Zealand White rabbits had antibodies to *Sendai virus* (Iwai et al., 1986). Ito et al. (1987) also detected antibodies to *Sendai virus* in rabbit sera in studies comparing antigenic relationships among paramyxoviruses. Intranasal inoculation of *Oryctolagus cuniculus* with *Sendai virus* resulted in infection as rabbits shed virus for 3–7 days after inoculation, and viral antigen was detected by immunofluorescence in the nasal cavities (Machii et al., 1989). Although rabbits showed no clinical signs and had only a moderate increase of goblet cells in the nasal epithelium, antibodies to the virus developed. One of three uninoculated rabbits exposed to inoculated rabbits acquired infection. These studies suggest that *Oryctolagus cuniculus* is susceptible to *Sendai virus* and that this or a related virus may be endemic in rabbit colonies. Experimentally, genetically modified Sendai viruses have been used in gene therapy in rabbits (Nakamura et al., 1998).

Bunyavirus Infections

Evidence of infection with several viruses of the genus *Orthobunyavirus* in the family *Bunyaviridae* has been detected in leporids. Strains of *California encephalitis virus* found to infect rabbits or hares include California encephalitis, Snowshoe hare, Tahyna, and Inkoo viruses (Fauquet et al., 2005). Similar evidence of infection has been found with viruses of the genus *Bunyamwera virus* including cache valley virus, Tensaw virus, and Northway virus. One other member of the *Bunyaviridae* that infects rabbits and is not assigned to a genus is the Silverwater virus.

Antibodies to California encephalitis virus were initially detected in Eastern Cottontail rabbits (*Sylvilagus floridanus*) and Black-tailed jackrabbits (*Lepus californicus*) in California (Hammon and Reeves, 1952). The first virus of the group to be recovered from leporids was the Snowshoe hare virus, isolated in 1959 from the blood of a sick Snowshoe hare (*Lepus americanus*) in western Montana (Burgdorfer et al., 1961). The virus is widespread in Snowshoe hare populations of North America, as serological surveys revealed prevalences of 40–97% in adult hares (Hoff et al., 1969; McLean et al., 1975; Mean, 1983; Newhouse et al., 1963; Yuill et al., 1969). The virus has been isolated from seven species of boreal forest mosquitoes, including 0.04% of *Aedes communis* (McLean, 1983), and also from the rabbit tick, *Haemaphysalis leporis-palustris* (Newhouse et al., 1963).

The European hare (*Lepus europeus*) and wild European rabbit (*Oryctolagus cuniculus*) appear to be the major reservoirs of Tahyna virus in Europe (Bardos,

1965, 1975; Danielova et al., 1969; Hannoun et al., 1969; Simkova, 1963). Clinical disease in infected hares has not been reported. Experimental infection of European rabbits results in viremia and antibody formation without clinical disease (Hammon and Sather, 1966; Simkova, 1962). Thus, domestic European rabbits are useful as sentinels of viral activity since they develop antibodies and can serve as a source for recovery of virus (Kolman et al., 1966; McKiel et al., 1966). A serological survey in Finland revealed that 5% of Mountain hares (*Lepus timidus*) and none of the *Lepus europeus* tested had antibodies against Inkoo virus (Brummer-Korvenkontio, 1973). Similar viruses have been isolated from mosquitoes and humans in Russia (Vanlandingham et al., 2002).

Cache valley virus, Tensaw virus, and Northway virus are mosquito-borne viruses of North America. Cache valley virus has a wide geographic distribution in North America (Blackmore and Grimstad, 2008), whereas Tensaw virus is found predominantly in the Southeastern United States (Bigler et al., 1975) and Northway virus in Alaska and California (Campbell et al., 1990, 1991; Walters et al., 1999; Zarnke et al., 1983). Recent evidence suggests these host ranges may not be as restrictive as earlier thought (Sahu et al., 2002). The amplifying hosts for all of these viruses are unclear, however antibodies recognizing Cache valley virus have been found in Desert cottontails (*Sylvilagus audubonii*), Eastern cottontails (*Sylvilagus floridanus*), and Black-tailed jackrabbits (*Lepus californicus*) (Blackmore and Grimstad, 2008). Antibodies to Tensaw virus have been found in the Swamp rabbit (*Sylvilagus aquaticus*) (Calisher et al., 1986), Marsh rabbit (*Sylvilagus palustris*) (Bigler et al., 1975), and New England cottontail rabbit (*Sylvilagus transitionalis*) (Sudia et al., 1969) and those to Northway virus in the Snowshoe hare (*Lepus americanus*) (Zarnke et al., 1983). Experimental infection of Eastern cottontail rabbits with Cache valley virus resulted in transient viremia of insufficient magnitude to infect mosquitoes (*Coquillettidia perturbans*) suggesting that this species may not be an important vertebrate host for this virus (Blackmore and Grimstad, 2008). In contrast, rabbits and hares may be important hosts for Tensaw and Northway viruses as experimental infection of New England cottontail rabbits (*Sylvilagus transitionalis*) with Tensaw virus resulted in viremia of sufficient magnitude to infect mosquitoes (*Anopheles quadrimaculatus*) (Sudia et al., 1969). Living near Snowshoe hares (*Lepus americanus*) was found to be a significant risk factor for human serologic reactivity to Northway virus (Walters et al., 1999).

Silverwater virus has been isolated from Snowshoe hares (*Lepus americanus*) and from rabbit ticks (*H. leporis palustris*) from Snowshoe hares in Ontario and Alberta, Canada (McLean and Larke, 1963; Yuill et al., 1969). Although no disease has been reported in infected hares, they appear to play a central role in the natural cycle of the virus.

Togavirus Infections

Serological evidence of infection with Western (WEE), Eastern (EEE), and Venezuelan (VEE) equine encephalitis viruses, members of the genus *Alphavirus*, in the family *Togaviridae* (Fauquet et al., 2005) has been found in rabbits and hares. Wild *Sylvilagus* as well as hares (*Lepus californicus* and *Lepus americanus*) have antibodies to WEE (Bowers et al., 1969; Yuill et al., 1969). Infection studies of *Oryctolagus cuniculus* with strains of WEE isolated from South America and North America demonstrated virus strain variation in clinical signs and mortality (Bianchi et al., 1997). A South American strain (AG80-646) resulted in early onset of anorexia and rear limb paralysis with rabbits dying 6 days after inoculation. The North American strain caused no detectable clinical signs, but the rabbits seroconverted. Antibodies to EEE (Yuill et al., 1969) and VEE (Hoff et al., 1970) have been detected in Snowshoe hares (*Lepus americanus*) and antibodies to VEE have been detected in Eastern cottontail rabbits (*Sylvilagus floridanus*) (Smart and Trainer, 1975).

Complement fixation, hemagglutination-inhibition, ELISA, and plaque reduction neutralization tests are commonly used for diagnosis of these infections in humans and animals, although cross-reactivity between different viruses is problematic with all of these tests (O. I. E., 2008). An epitope-blocking assay was developed and tested on rabbit sera to better separate the individual virus groups (Pässler and Pfeffer, 2003). The infectious agent can be identified by viral isolation or PCR (O. I. E., 2008; Vodkin et al., 1993).

Flavivirus Infections

Two members of the Japanese encephalitis group in the family *Flaviviridae*, genus *Flavivirus* have been shown to infect rabbits or hares, *St. Louis encephalitis virus* and *West Nile virus* (WNV) and one member of the mammalian tick-borne virus group of the genus *Flavivirus* has been shown to infect hares, *Powassan virus* (Fauquet et al., 2005). Antibodies to *St. Louis encephalitis virus* have been detected in Snowshoe hares (*Lepus americanus*) (Yuill et al., 1969). Experimentally, Eastern cottontail rabbits (*Sylvilagus floridanus*) have been shown to be susceptible to WNV infection (Tiawsirisup et al., 2005). WNV is a mosquito-transmitted pathogen of significant public health importance (Hayes et al., 2005). The major route of infection of humans is by the bite of a mosquito infected by ingesting a blood meal from an infected bird. In general, mammalian hosts of WNV are dead-end hosts and do not develop viremia of sufficient magnitude to infect mosquitoes (Bowen and Nemeth, 2007). However, certain species, including Eastern cottontail rabbits (*Sylvilagus floridanus*), develop viremia which infects mosquitoes (*Culex pipiens* and

Culex salinarius) and therefore, may contribute to the endemic cycle of WNV in North America (Tiawsirisup et al., 2005). Infection in rabbits occurs without clinical signs. *Powassan virus* is a tick-borne virus of emerging public health concern in the United States, Canada, and Russia (Ebel, 2010). Antibodies to *Powassan virus* have been found in Snowshoe hares (*Lepus americanus*) (McLean et al., 1961; Zarnke and Yuill, 1981). Snowshoe hares experimentally infected with the virus develop no clinical symptoms, but become viremic for 1–3 days. Other vertebrates, including red squirrels (*Tamiasciurus hudsonicus*), chipmunks (*Tamias amoenus*), groundhogs (*Marmota monax*), and white-footed mice (*Peromyscus leucopus*) may be more important in perpetuating the virus than Snowshoe hares (Ebel, 2010).

Picobirnavirus Infections

A rabbit *Picobirnavirus*, a member of the *Picobirnaviridae* family, was detected in rabbit feces in a study in which rabbits were inoculated with human *Picobirnavirus* (Fregolente et al., 2009; Gallimore et al., 1993). Picobirnaviruses are non-enveloped bisegmented, double-stranded RNA viruses of 35–40nm in diameter. Members of this family of viruses have been found in the feces of many different hosts, including humans, pigs, rabbits, dogs, rats, snakes, and birds (Fregolente et al., 2009). They are thought to be opportunistic gastrointestinal pathogens associated with clinical disease in humans (Giordano et al., 1999). These viruses have been associated with gastrointestinal disease in HIV-infected humans (Giordano et al., 1999; Grohmann et al., 1993) and may be important pathogens or co-pathogens in immunosuppressed rabbits. The *picobirnavirus* genome detected in rabbits consisted of two segments; 2.3kbp and 1.85kbp (Gallimore et al., 1993). The first segment contains one major ORF and two smaller ORFs and has been partially sequenced (Green et al., 1999). This segment is thought to encode a capsid protein while the second segment appears to encode an RNA-dependent RNA polymerase (Rosen et al., 2000). Picobirnaviruses do not replicate in tissue culture, although they were successfully detected in the feces of rabbits inoculated orally with rabbit *Picobirnavirus* (Gallimore et al., 1993; Ludert et al., 1995). No clinical signs of disease resulted, however, anti-*Picobirnavirus* antibodies developed (Gallimore et al., 1993).

Rabies Virus Infections

Rabies virus, the type species of the genus *Lyssavirus* and family *Rhabdoviridae* (Fauquet et al., 2005), is a neurotropic virus that infects mammals resulting in a generally fatal, progressive encephalomyelitis (Manning et al., 2008). It is found in high titers in the saliva

of clinically ill animals and is typically transmitted through a bite. Rabies virus infections in rabbits and hares are rare, as these species often do not survive encounters with rabid animals. From 1971 to 1984, seven rabid rabbits were reported to the CDC, including four Eastern cottontails (*Sylvilagus floridanus*) (Fishbein et al., 1986), while in the next 9 years, 17 cases of rabies in rabbits were reported to the CDC (Childs et al., 1997). Of these 17, 11 were domestic European rabbits (*Oryctolagus cuniculus*) and the others were unknown. Four viruses were typed and shown to be either raccoon or skunk variants of rabies virus. In 1995, raccoon-variant rabies was reported in seven domestic European rabbits in New York State (Eidson et al., 2005). Rabies is typically diagnosed by fluorescent antibody test on brain tissue, however, strains may be further identified using monoclonal antibodies or genetic means such as nucleic acid probes, PCR, and DNA sequencing (O. I. E., 2008).

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Parasitic Diseases

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INTRODUCTION

Rabbits serve not only as an important animal species for biomedical research but also as a common food source for people throughout the globe. Over the past

several decades, great attention has been paid to controlling and eliminating the parasitic diseases of rabbits with the goal of making them more suitable animals for the laboratory environment as well as to increase their meat production capacity. Maintaining excellent levels

of hygiene is especially critical with treating, controlling, and/or preventing parasitic infections in rabbits. The use of non-solid flooring, along with proper sanitation, has been noted to decrease the level of fecal-borne parasites in rabbits today compared to other housing systems (Patton et al., 2008).

Given the increase of the rabbit in popularity as a pet in the United States, laboratory animal practitioners should be cognizant of pet rabbits that facility staff may have at home or visit at other locations (e.g. pet stores, fairs) that may not be free of common rabbit parasites. Items supplied from pet stores or other areas such as treats, hay, and toys, may also be potentially contaminated with parasites. Inattentive personnel and contaminated items can introduce parasites into specific pathogen-free (SPF) colonies of laboratory rabbits.

Commercial vendors of SPF, research-exclusive rabbits in North America are mostly located in the eastern United States and Canada. Therefore, many laboratory animal facilities in other parts of North America use non-SPF rabbits alongside SPF rabbits. Parasites would be among some of the first organisms that could be transmitted from non-SPF to SPF rabbits. Great control needs to be exercised in such facilities to prevent such pathogen spread.

PROTOZOA

Apicomplexa (Sporozoa)

Eimeria

Coccidiosis is considered to be one of the most important diseases of rabbits worldwide, affecting wild rabbits and domestic rabbits used for research, meat production, and companionship. The genus *Eimeria* contains 65 species which are considered to be host-specific (Kvicerova et al., 2008). Eleven *Eimeria* species make up the coccidia species known to infect *Oryctolagus cuniculus* under natural conditions. Other *Eimeria* species may be found in related non-*Oryctolagus* species such as cottontails (*Sylvilagus*), hares (*Lepus*), and pygmy rabbits (*Brachylagus*) (Duszynski et al., 2005). Research is ongoing for evaluating diagnostic methodologies, treatment regimens, identification methods, preventative measures, and characterization of new variants. Out of several rabbit coccidia species, one infects the liver (hepatic coccidiosis) while the remaining ten infect different areas of the intestinal tract with varying pathogenicity (intestinal coccidiosis). All follow the fecal-oral route of transmission (Figure 15.1).

Due to the severity of some coccidial infections, many countries require that imported rabbits are coccidia free. While this is targeted more towards hepatic coccidia, importation restrictions may extend to the more pathogenic species of intestinal coccidia as well.

HEPATIC COCCIDIA

BACKGROUND Hepatic coccidiosis, caused by *Eimeria stiedae*, was first identified over 100 years ago and is considered to be a devastating disease for rabbit colonies resulting in high morbidity and mortality (Kvicerova et al., 2008). Its pathogenicity is considered to be mild to severe (Kvicerova et al., 2008). The agent is still found in rabbitries throughout the world but is rare in laboratory animal facilities (Baker, 1998; Pakes and Gerrity, 1994).

ETIOLOGY/ETIOLOGIC AGENT *Eimeria stiedae* oocyst size is approximately $30\text{--}41 \times 15\text{--}24\text{mm}$ (average $36.9 \times 19.9\text{mm}$) in size with an ellipsoid shape (Kvicerova et al., 2008). Younger animals (around weaning age) are more susceptible to infection than older ones (Gomez-Bautista et al., 1987).

EPIZOOTIOLOGY Transmission is by the fecal-oral route. Experimental infection by other routes (intravenous, intraperitoneal, and intramuscular) can also result in disease but in a milder form compared to oral transmission (Pakes and Gerrity, 1994). Contaminated feed and water are primary sources of infection (Patton et al., 2008). The non-infective (un-sporulated) oocysts are shed in the feces of infected animals and become infective (sporulate) in the environment usually within about 3 days (Baker, 1998; Patton et al., 2008). Prepatency is 14–18 days while patency is 21–37 days (Schoeb et al., 2007). *E. stiedae* migrates from the intestinal tract, through the intestinal wall, either hematogenously or through the lymphatic system, into the bile ducts where its reproductive cycle takes place (Baker, 1998; Patton et al., 2008). Prevalence in populations can reach 100% with carrier adult animals serving as a source of infection for young animals although these adults may have lower body masses and fecundity when compared to non-infected individuals (Lello, 2005; Wang and Tsai, 1991). Experimental infection in mice resulting in a similar disease course as that of rabbits has been reported following immunosuppression with dexamethasone (Aly, 1993). Co-infection with intestinal coccidia is common. Proper hygiene is important in the control and elimination of this disease.

CLINICAL MANIFESTATIONS Clinical pathological findings have been reported with active hepatic coccidiosis. Gomez-Bautista et al. (1987) reported alanine aminotransferase (ALT) increases with no other enzymatic elevations. No enzymatic elevations were reported by Abdel-Ghaffar et al. (1990). Decreased fibrinogen and increased bilirubin and alkaline phosphatase (AP) have also been observed (Cam et al., 2006; Sherkov et al., 1986).

Anorexia, wasting, a “pot belly” appearance from ascites and/or hepatomegaly, icterus, diarrhea, constipation,

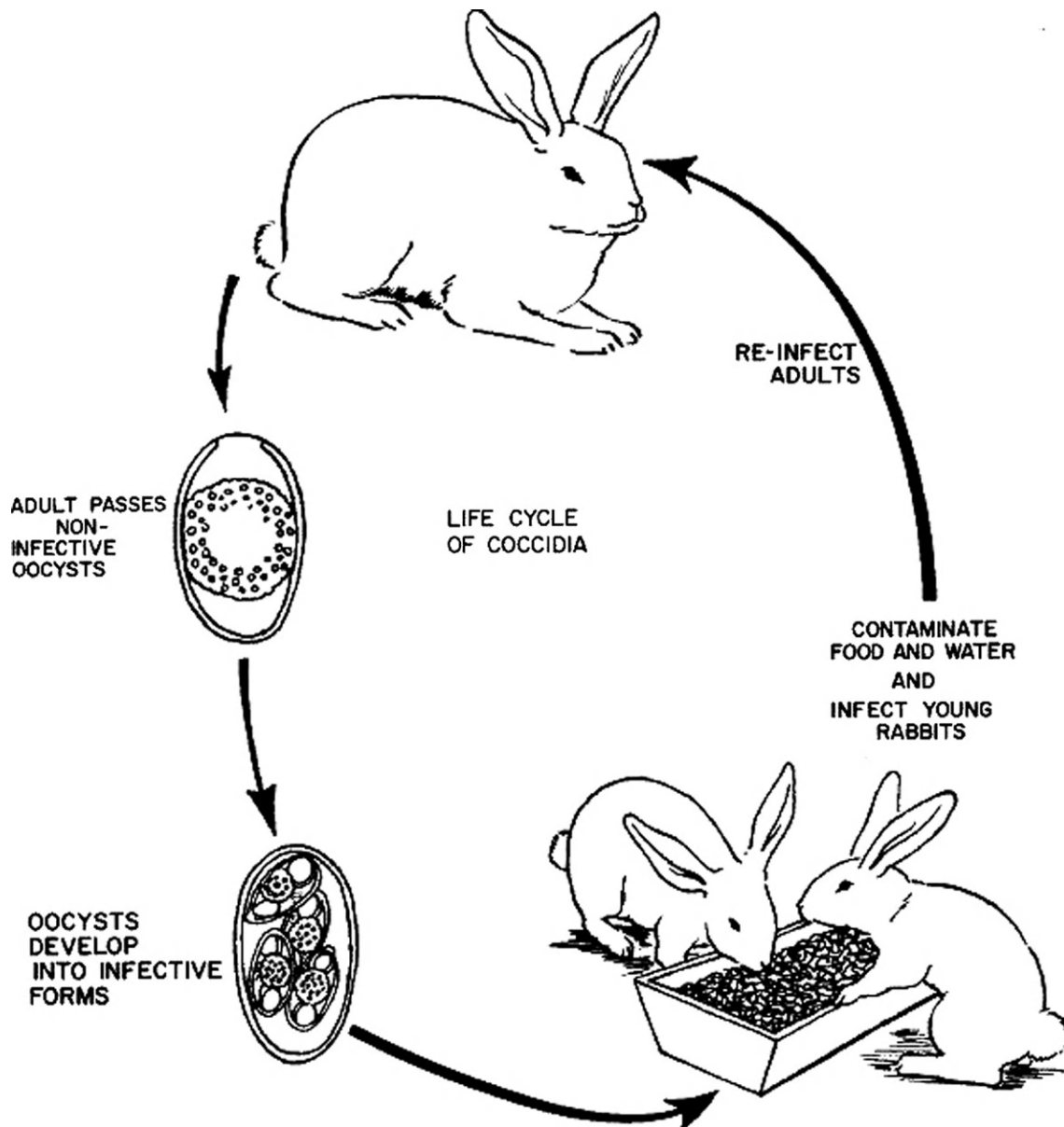


FIGURE 15.1 Life cycle of *Coccidia* in rabbits. (from Patton, N.M., K.W. Hagen, J.R. Gorham, and R.E. Flatt. 2008. *Domestic Rabbits: Diseases and Parasites*. PNW 310. Corvallis, OR: Oregon State University Extension Service. © 1986, 1998 Oregon State University. Used with permission)

death, and other clinical signs consistent with liver failure can be seen (Baker, 2008; Pakes and Gerrity, 1994; Patton et al., 2008; Schoeb et al., 2007). Death is more commonly seen in younger animals, in particular those around 2–3 months of age. Many animals may exhibit no clinical signs (Pakes and Gerrity, 1994).

An enlarged liver and gall bladder may be noted on radiographs (van Praag, 2009).

PATHOLOGY Both gross and microscopic lesions are well-defined. The liver is the major organ affected with diffuse, multi-focal white nodules on the surface

that range in size from 0.1–2 cm (Schoeb et al., 2007; Wang and Tsai, 1991) (Figure 15.2). Enlargement of the liver, gall bladder, and bile duct may be seen at necropsy (van Praag, 2009). A dark, green mucoid exudate has been described in the intestinal lumen (Wang and Tsai, 1991). Hyperplasia and cystic enlargement of the bile duct epithelium (Figure 15.3) results in the white nodules seen on the hepatic surface (Pakes and Gerrity, 1994; Wang and Tsai, 1991). Various life cycle stages of the organism may be seen histopathologically with oocysts in bile duct lumina (Figure 15.4) and inflammatory cell infiltration (lymphocytes and plasma cells

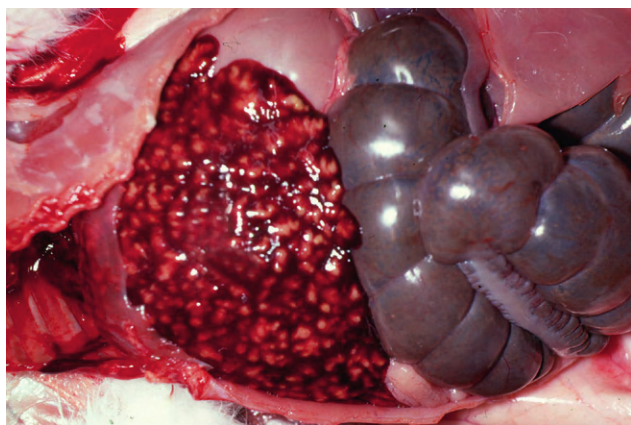


FIGURE 15.2 Liver of rabbit infected with *Eimeria stiedae*. Note the diffuse, multi-focal white nodules on the hepatic surface which result from biliary hyperplasia. (photo courtesy of James G. Fox)

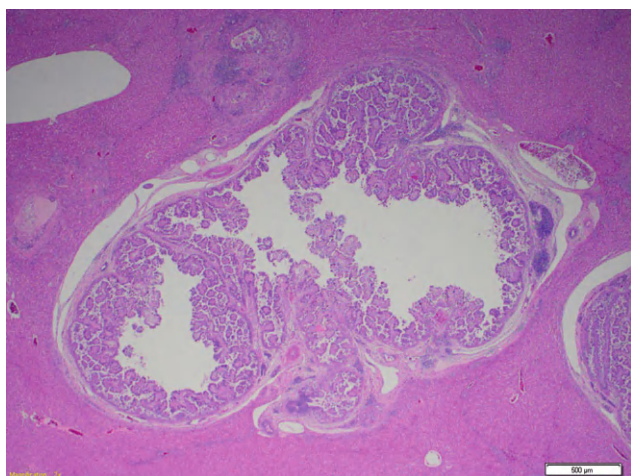


FIGURE 15.3 Hyperplasia of biliary duct epithelium in a rabbit infected with *Eimeria stiedae*. (photo courtesy of James G. Fox)

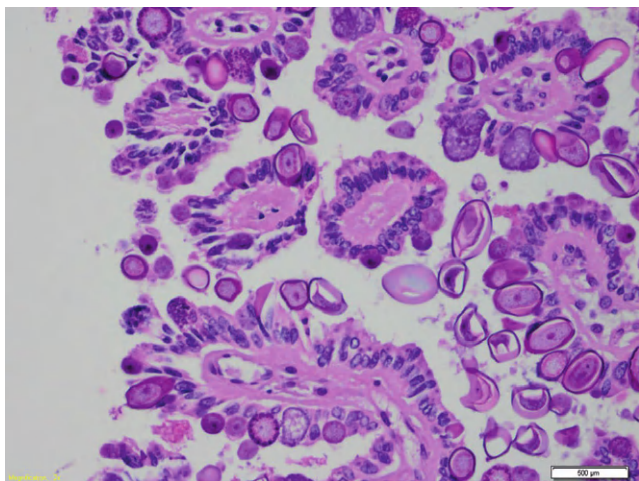


FIGURE 15.4 Oocysts of *Eimeria stiedae* among biliary epithelium within the lumen of a bile duct. (photo courtesy of James G. Fox)

and potentially neutrophils and macrophages) around the ducts (Pakes and Gerrity, 1994; Schoeb et al., 2007; Wang and Tsai, 1991). Alternatively, organisms may be seen with an impression smear of the liver at the time of necropsy (van Praag, 2009). Bile duct enlargement may lead to rupture, granulomatous reactions, and fibrosis (Pakes and Gerrity, 1994). Fibrosis will increase with the chronicity of active disease (Schoeb et al., 2007). Other organs are not affected by *E. stiedae*, however, secondary bacterial infections may result (van Praag, 2009; Wang and Tsai, 1991).

PATHOGENESIS Excysting oocysts within the duodenum travel to the liver via the bloodstream or lymphatics and infect the epithelial cells of the bile ducts. The resulting inflammatory reactions along with an “obstructive jaundice due to oocyst shedding” in the bile ducts result in disease signs (Omata et al., 2001a).

DIAGNOSIS Diagnosis can be made by identification of the oocyst in the feces through fecal direct smear, fecal flotation or, more preferably, centrifugation (Pakes and Gerrity, 1984). Oocysts may also be found in bile duct aspirates. *Eimeria stiedae* can be differentiated from other *Eimeria* species through size and morphology.

Gross and microscopic lesions, previously described, can provide a diagnosis of active disease.

Other diagnostic methods described in the literature include antibody detection in serum and complement fixation (Omata et al., 2001b; Pakes and Gerrity, 1994).

PREVENTION AND THERAPY Several anti-hepatic coccidial treatments have been described, especially within the past 30 years. Due to rabbits serving as popular meat animals in many parts of the world, certain medications in the feed and/or water may be illegal depending on local and federal legislation. Some feed companies will not mix medication into feed due to these regulations or the adverse effects the medications may have on a species that could occur through contamination at the feed mill (e.g. ionophore toxicosis in horses). Additionally, not all medications can be found in the United States as approved medications for rabbits are more common in Europe and other parts of the world. Treatments reported in the literature include:

1. Robenidine, a non-ionophore synthetic drug (guanidine), has been used as a coccidiostat for several decades. The compound can be mixed into feed and dosages of 100 parts per million (PPM) have been shown to be effective (Peeters and Halen, 1980). Robenidine wafers are also available. It is approved for use against hepatic coccidia in rabbits in some countries where the dosage recommendation is 50–66 mg/kg in the feed even though robenidine

- is less effective against *E. stiedae* than the intestinal coccidia species (EFSA, 2008; Peeters et al., 1988; Polozowski, 1993).
2. Several sulfa-based antibiotics have been reported to be efficacious in preventing and treating coccidiosis.
 - a. Sulfamerazine at 0.02% in the drinking water (Pakes and Gerrity, 1994).
 - b. Sulfadimethoxine and pyrimethamine (10:3) have been used as prophylaxis against *Eimeria stiedae* infection (Gomez-Bautista and Rojo-Vazques, 1986).
 - c. Sulfamethoxine in the feed for 7 days at 75 mg/kg of body weight (Pakes and Gerrity, 1994). Sulfaquinoxaline, 0.025% in the feed for 3 weeks (Patton et al., 2008) or 0.03% (Pakes and Gerrity, 1994). In drinking water, the dose is 0.05% (Pakes and Gerrity, 1994).
 3. Toltrazuril, a coccidiostat and triazinetrione derivative, decreases oocyst shedding and prevents clinical signs when used at 10–15 ppm in water and up to 25 ppm during parasite reproduction (Peeters and Geeroms, 1986). Ivermectin combined with toltrazuril has been shown to be highly effective in reducing oocyst shedding and keeping liver lesions to minimal or non-existent levels in experimentally infected animals (Cam et al., 2008).
 4. Diclazuril can be used as both a prophylactic or therapeutic agent. Prophylactic doses are 0.5, 1, and 2 mg/kg and the treatment dose is 4 mg/kg. These doses are associated with decreased oocyst shedding and organism numbers in liver in experimental infections (Pan et al., 2008).
 5. Lasalocid is a coccidiostat and is used at levels of 90 or 125 ppm in the feed (Polozowski, 1993).
 6. Monensin, an ionophore, may be used at 20 ppm in feed (Polozowski, 1993).
 7. Narasin, another ionophore and coccidiostat, reduces oocyst output and prevents clinical signs when dosed at 12–24 ppm in the feed (Peeters et al., 1981). This drug is also known as salinomycin.
 8. Garlic has been used as a non-traditional treatment which resulted in decreased oocyst shedding and near-normal histopathology of hepatic tissue (Toula and Al-Rawi, 2007).

Practical measures for laboratory animal veterinarians focus on ensuring that rabbits supplied to research facilities are free of hepatic coccidia. Good hygiene practices of individuals handling animals, vermin control, and separation of sick young from older animals is important (Pakes and Gerrity, 1994).

Positive results have been preliminarily obtained from research into vaccination of rabbits with *E. stiedae* antigens. Organism shedding and inflammatory reactions have been decreased, resulting in little to no

disease after experimental vaccination (Hanada et al., 2003; Abdel Mageed et al., 2005).

INTESTINAL COCCIDIA

BACKGROUND As previously described, coccidia in rabbits are described as either hepatic or intestinal. Some authors have attempted to portray intestinal coccidia of rabbits as a singular group of pathogens responsible for diarrhea in weanling rabbits without discussion of the individual species involved. However, intestinal coccidia vary greatly in terms of pathogenicity from highly pathogenic resulting in high morbidity/mortality to non-pathogenic. Over two dozen intestinal coccidia species have at one time or the other been described to inhabit the gastrointestinal tract in rabbits, in many cases due to one species being given multiple names. There are ten accepted, well-characterized species of intestinal coccidia that will be described here that affect *Oryctolagus cuniculus*. They have different pre-patent periods, pathogenicity levels, and target areas within the intestines for causing disease. Co-infection is seen (Baker, 1998; de Almeida et al., 2006; Li and Ooi, 2008). *Eimeria perforans* has generally been considered the most common and least pathogenic intestinal coccidia species for laboratory rabbits (Pakes and Gerrity, 1994). Intestinal coccidia can still be common among SPF laboratory rabbit breeders. The three most common species encountered are *E. perforans*, *media*, and *magna*. Rabbits, including SPF laboratory rabbits, are commonly co-infected with these three species of intestinal coccidia.

ETIOLOGY/ETIOLOGIC AGENT Several species of *Eimeria* are considered to make up the intestinal coccidia infecting *O. cuniculus*. Speciation is done primarily through morphological criteria of oocysts identified in fecal or intestinal content preparations, and it is reported that the sporulated oocysts provide the best stage for identification (Licois, 2004). Other mechanisms for identification include the area of intestines affected as well as genomic profiles (Cere et al., 1996, 1997; Licois, 2004). Table 15.1 summarizes information from several sources (Kvicerova et al., 2008; Licois, 2004; Saxonet, 2005; Schoeb et al., 2007; van Praag, 2009) with respect to oocyst size and shape, pre-patent period, pathogenicity, and intestinal areas affected, listed in order of species pathogenicity.

EPIZOOTIOLOGY The *Eimeria* species described here are considered species-specific for the rabbit. Transmission is fecal-oral. Contaminated food, water, caging, as well as other fomites all serve as common means of spreading intestinal coccidia. Adult carriers often serve as a source of infection for newborns with rabbits aged 2–3 months being most susceptible to intestinal coccidiosis (Licois, 2004; Sherkov et al., 1986). Since *Eimeria* are monoxenes (having only one host),

TABLE 15.1 Summary of Common Rabbit *Eimeria* Species

Species	Oocyst Shape	Oocyst Size (μm)	Intestinal Areas Affected	Pathogenicity	Pre-Patent Period (days)
<i>E. intestinalis</i>	Ellipsoid, piriform, or ovoid	21–36 × 15–21 average 26 × 18	Jejunum and/or ileum	High	5–10
<i>E. flavescens</i>	Ovoid or broad ellipsoid	25–37 × 14–24 average 30 × 21	Cecum and/or proximal colon	High	9
<i>E. media</i>	Ellipsoid to ovoid	19–37 × 13–22 average 31 × 17	Jejunum and/or ileum	Mild	4–5
<i>E. magna</i>	Ovoid to ellipsoid	27–41 × 17–29 average 31 × 18	Jejunum and/or ileum	Mild	5–6
<i>E. irresidua</i>	Ovoid to ellipsoid	31–44 × 19–28 average 38 × 23	Jejunum and/or ileum	Mild	9
<i>E. piriformis</i>	Piriform	25–33 × 15–21 average 29 × 18	Colon	Mild	9
<i>E. vejdoskyi</i>	Ovoid	25–38 × 16–22 average 31 × 19	Ileum	Slight	10
<i>E. perforans</i>	Ellipsoid	15–30 × 11–20 average 21 × 13	Duodenum and/or proximal jejunum	Slight	5–6
<i>E. exigua</i>	Ovoid to spherical	10–21 × 10–18 average 15 × 13	Ileum	Slight	7
<i>E. coecicola</i>	Ovoid to ellipsoid	23–40 × 15–22 average 29 × 18	Cecum (GALT/vermiform appendix)	None	9

there are no other hosts involved with the life cycle (Licois, 2004). The number of young animals affected in colonies can vary greatly, but has been documented at 100% in some instances (Nosal et al., 2009).

CLINICAL MANIFESTATIONS The severity of clinical disease is dependent on the amount of infective agent present, the susceptibility of the animal, and the pathogenicity of the agent. Subclinical infections can exist with no clinical signs (Schoeb et al., 2007). Diarrhea is the main clinical sign of disease associated with all of the intestinal coccidia species. Undigested blood may be seen in the diarrhea and positive fecal occult blood test results may be obtained, depending on the species infecting the rabbit (Baker, 1998; Li and Ooi, 2009; van Praag, 2009). Due to inhibition of cellular function, impaired intestinal motility, and villous atrophy clinical signs including malabsorption, electrolyte imbalances, anemia, hypoproteinemia, dehydration, and even death (usually within just a few days after clinical onset) can be seen (Baker, 1998; Licois, 2004; van Praag, 2009). Additional clinical signs include a rough coat, anorexia, and weight loss (van Praag, 2009). A 20% weight loss has been associated with death within 24 hours (van Praag, 2009).

PATHOLOGY The diarrhea observed is usually dark and watery, possibly with mucus, accompanied

by a foul odor (Baker, 1998; Pakes and Gerrity, 1994). Intestinal bleeding and ulceration plus intussusceptions may be seen (Baker, 1998; van Praag, 2009). Epithelial sloughing has also been reported (Patton et al., 2008). Microscopically, villous atrophy, cellular congestion and edema, epithelial necrosis, leukocytic exudates, along with the different stages of the reproductive cycle may be visualized (Baker, 1998; Licois et al., 1992). Affected cells can become hypertrophied (van Praag, 2009).

PATHOGENESIS Once excreted in the feces, the oocyst undergoes sporogony and sporulates, becoming infective. After ingestion, the oocyst wall is destroyed in the stomach and spores are released (van Praag, 2009). Spores infect the epithelial cells of the intestinal walls, having a specificity for certain areas of the intestinal tract as expressed in the above table. Reproduction of *Eimeria* occurs in these cells (schizogony and gamogony), devastating them and causing disease. Oocysts are then shed in the feces to infect other rabbits. Surviving rabbits develop activity immunity, going on to become asymptomatic carriers (Licois, 2004). Concurrent microbial infections (e.g. *E. coli*) can exacerbate clinical signs, delay the formation of immunity, and lead to higher mortality and morbidity (Licois, 2004; Pakes and Gerrity, 1994). There is no cross-immunity between the intestinal

coccidia species and immunity is cell-mediated, meaning that passive immunity from dam to offspring does not occur (Baker, 1998; Licois, 2004).

DIAGNOSIS Diagnosis is customarily made based on the identification of oocysts in fecal preparations (direct smears, flotations, and centrifugations). Samples from intestinal preparations (e.g. smears, histopathology) may also be used (Pakes and Gerrity, 1994). It is recommended that veterinarians ask for the identified coccidia to be speciated in order to determine the significance of the finding to the individual rabbit as well as the colony (Licois, 2004). Finding oocysts in the feces, but not in histopathological samples from the intestines, or vice versa, is not uncommon given the reproductive cycle of the parasite or a “very heavy primary infection” (Pakes and Gerrity, 1994). Therefore, it is preferable to examine both to determine the presence of intestinal coccidia. Multiple animals in a colony may also need to be scrutinized to ascertain all *Eimeria* species involved (since co-infection can frequently occur) and level of infection in the colony.

PREVENTION AND THERAPY Prevention is primarily through acquiring only coccidia-free animals and maintaining good sanitation. Treatment can be carried out at the individual and colony level.

1. Sulfa drugs (e.g. sulfadimethoxine (0.8%), sulphaquinoxaline (0.025–3%)) have historically reported good results (Licois, 2004). Sulphaquinoxaline at 0.025% fed in the feed for 2–3 weeks will reduce parasite load (Patton et al., 2008).
2. As with *E. steidae*, robenidine has also been used successfully as a prophylaxis (at 66 ppm) and treatment for decades (Licois, 2004; Peeters and Halen, 1980; Peeters et al., 1983). However, reports of robenidine resistance exist in the literature (Licois, 2004; Peeters et al., 1987).
3. Salinomycin (narasin), an ionophore, is also used as a food additive to prevent intestinal coccidiosis at 20 ppm (Licois, 2004) although lower levels have been reported at 8–12 ppm (Peeters et al., 1981).
4. Diclazuril can be used in both prophylactic or therapeutic regimens. Prophylaxis can be achieved at 0.5, 1, or 2 mg/kg while the therapeutic dose is 4 mg/kg. Decreased oocyst shedding and organism numbers in the liver in experimental infections have resulted from these treatments (Pan et al., 2008; Vanparijs et al., 1989).
5. Toltrazuril is a coccidiostat and triazinetrione derivative which decreases oocyst shedding and prevents clinical signs when dosed 10–15 ppm in water. Up to 25 ppm may be used during parasite reproduction (Peeters and Geeroms, 1986).
6. Research is active in the area of creating a vaccination for rabbits against intestinal coccidia but all work is currently in the experimental stages (Drouet-Viard et al., 1997; Licois, 2004).

CRYPTOSPORIDIUM

BACKGROUND Many parasitologists consider *Cryptosporidium* more closely related to a group of Apicomplexa known as the gregarines than to coccidia (Carreno et al., 1999). Replicating stages of *Cryptosporidium* are located within the microvillus border of the host cell and protrude above the surface which is different than the typical coccidia location in the cytoplasm beneath the cell surface (Schoeb et al., 2007). Cryptosporidia infections have been reported in laboratory rabbits and a cottontail rabbit in the United States though the prevalence in laboratory rabbits is presumed to be low (Schoeb et al., 2007).

ETIOLOGY/ETIOLOGIC AGENT. Cryptosporidia were first described in the rabbit by Tyzzer in 1929, however the species found in lagomorphs remains unnamed and minimally described (Schoeb et al., 2007). In some reports, the agent is referred to as *Cryptosporidium cuniculus* (Pakes and Gerrity, 1994). Most research and investigation has been conducted with the species *C. parvum* (Schoeb et al., 2007).

EPIZOOTIOLOGY

The life cycle of the *Cryptosporidium* species in rabbits has not been described. It is thought, however, that it is similar to that of *C. parvum* which has been extensively studied and will be described here (Schoeb et al., 2007). Oocysts are ingested and release sporozoites which invade intestinal epithelial cells in the lower half of the small intestine (Bowman, 2009; Schoeb et al., 2007). Once in the intestinal epithelial cells, sporozoites undergo two generations of merogony. Second-generation merozoites develop into sexual stages which fuse to produce zygotes and mature into unsporulated oocysts. Oocysts undergo two internal divisions to form four sporozoites each. The oocyst wall develops while still in the parasitophorous vacuole. About 20% of the oocysts are thin-walled and are capable of autoinfection which is believed to allow the organism to persist in the host. The remaining thick-walled environmentally resistant oocysts are shed in the feces and are infectious for new hosts when ingested (Schoeb et al., 2007). Oocysts remain viable in the environment for months unless exposed to extreme temperature (below 0°C, above 65°C), desiccation, or concentrated disinfectants (Bowman, 2009). Sporozoites of another species, *Cryptosporidium muris*, have a similar life cycle, however they invade the microvillous border of the gastric glands instead of the small intestine (Bowman, 2009).

Cryptosporidium is considered a zoonotic concern and there is a report from the United Kingdom of transmission of the rabbit *Cryptosporidium* to humans (Chalmers et al., 2009).

CLINICAL MANIFESTATIONS Inapparent infections of cryptosporidia in rabbits are common. Infection has also been found in rabbits with diarrhea but other etiologic agents were also present leaving the role of *Cryptosporidium* questionable (Schoeb et al., 2007). Immunocompromised animals may develop a life-threatening hyperinfective form of cryptosporidiosis (Bowman, 2009).

PATHOLOGY

Intestinal lesions in most host species follow a pattern which includes loss of enterocytes and villus atrophy and fusion accompanied by lamina propria edema and infiltration by inflammatory cells which can be mixed or primarily mononuclear (Schoeb et al., 2007). In rabbits, changes in the intestinal mucosa are minimal and include a slightly decreased villus to crypt ratio, loss of microvilli from enterocytes where the organisms attached, and slight lamina propria edema.

PATHOGENESIS

Most infections result in no clinical signs. Diarrhea may be caused due to damage to the intestinal epithelium.

DIAGNOSIS

Cryptosporidium oocysts are difficult to find on fecal slides because they are colorless, transparent and small, but they may be found in wet mounts when numerous. Concentrated sucrose solution is the flotation medium of choice for concentrating oocysts. Special stains may enhance visibility of oocysts. Immunofluorescent techniques, enzyme-linked immunosorbent assay (ELISA), and polymerase chain reaction (PCR) methods have been developed to assess exposure to *Cryptosporidium*. The diagnosis in laboratory rabbits is usually based on histologic examination of the intestine (Bowman, 2009; Schoeb et al., 2007).

PREVENTION AND THERAPY

Prevention is achieved by adhering to strict sanitary practices to interrupt fecal-oral transmission. Waterborne transmission is a significant risk since only a small number of oocysts are necessary to establish infection (Schoeb et al., 2007). Currently no known treatment exists. However, since most cases are subclinical, treatment is generally not warranted. Supportive care should be offered in the face of clinical disease to maintain hydration and electrolyte balance (Schoeb et al., 2007).

Hepatozoon cuniculi

Very little is written about *Hepatozoon cuniculi*, also known as *Leukocytoegregarina cuniculi*. It was described by Sangiorgi in 1914 in domestic rabbits in Italy. Sangiorgi described gametocytes in leukocytes and schizonts in the spleen. The intermediate host was not identified although fleas were suspected (Pakes and Gerrity, 1994). Another source reports that asexual stages occur in the liver or other visceral organs of a vertebrate host, gametes develop in erythrocytes or leukocytes, and fertilization and sporogony occur in hematophagous arthropods (Levine, 1985a, 1985b).

Toxoplasma gondii

BACKGROUND

Toxoplasma gondii has been identified in rabbits worldwide, but its ability to cause disease as well as its significance is inconsistent (Cox et al., 1981; Schoeb et al., 2007). Its discovery in rabbits in 1908 coincided with its discovery in rodents (de Souza et al., 2009; Pakes and Gerrity, 1994). In the United States, some laboratory rabbit vendors include *T. gondii* on their pathogen exclusion list while others do not, thus its prevalence in laboratory rabbits would be unknown and variable, although likely quite low. Even though there are only rare reports of *T. gondii* causing natural disease in *Oryctolagus cuniculus*, serological prevalence in some domestic colonies can approach 50% while it can be higher in wild *O. cuniculus* (Dubey et al., 1992; Hughes et al., 2008; Ishikawa et al., 1990; Pakes and Gerrity, 1994; Patton et al., 2008).

Rabbits serve as a model of ocular toxoplasmosis characterized by a retinochoroiditis, a disease seen in immunodeficient humans (Garweg et al., 1998; Rollins et al., 1982).

ETIOLOGY/ETIOLOGIC AGENT

This protozoal agent is typically associated with cats who act as the definitive host.

EPIZOOTIOLOGY

Transmission is by ingestion of the sporulated oocyst. It is thought that exposure to the agent comes from animal handlers, the environment, feed, water, or fomites being contaminated with cat feces. Cats are the definitive host species for the agent while rabbits can serve as an intermediate host (Schoeb et al., 2007). Placental transmission from pregnant does to their offspring is another route of infection (Patton et al., 2008). Breeding facilities may consider limiting animal handler exposure to cats to decrease the potential for introduction into the colony. Conversely, infected rabbits may serve as a source of infection for cats (Pakes and Gerrity, 1994). Recent reports have postulated that a sexual route of transmission could exist since experimentally infected bucks can shed *T. gondii* in their sperm

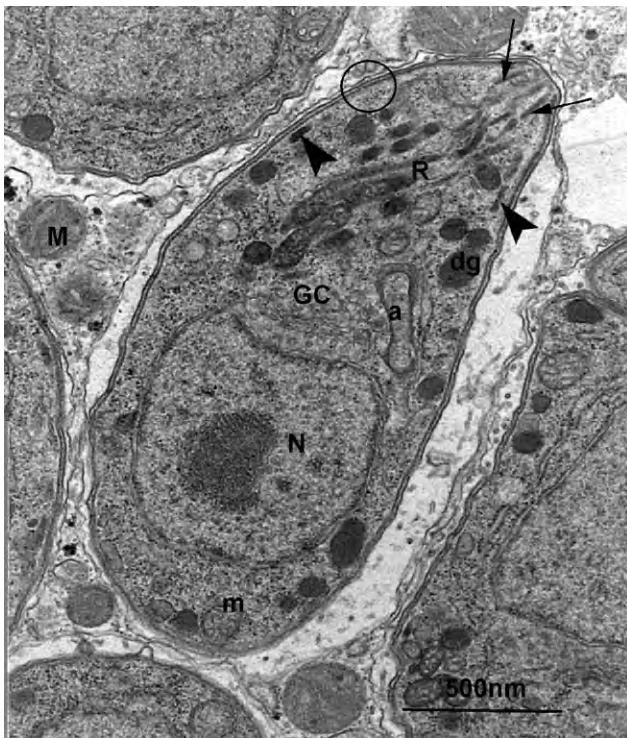


FIGURE 15.5 Tachyzoite form of *Toxoplasma gondii* inside a host cell as seen by transmission electron microscopy. In this longitudinal section some of the main features of its ultrastructure can be observed, such as the apical complex composed by the conoid (arrows), the club-shaped rhoptries (R) converging to it, and profiles of the micronemes (arrowheads). Dense granules (dg) are also secretory structures that are distributed without specific location in the host cell. The Golgi complex (GC) lies above the nucleus (N) and next to it, the apicoplast (a). Profiles of the single mitochondrion (m) are also seen. Notice the three-layered pellicle (circle). Several other parasitophorous vacuoles with other parasites are also seen. Bar: 500 nm. (photo courtesy of Paredes-Santos T.C., Attias M., DeSouza, W.)

within a week of inoculation (Liu et al., 2006a, 2006b). Trophozoites may also be found in the blood of experimentally infected animals (Liu et al., 2006a). Anatomical structures of *T. gondii* can be seen in the transmission electron micrograph shown in Figure 15.5.

T. gondii is a recognized zoonotic pathogen and animals carrying the agent can represent a human health hazard. Rabbits can be considered a major source of infection for humans when dealing with intensive breeding operations (Sroka et al., 2003). Several documented cases of direct transmission between rabbits and humans exist (Sroka et al., 2003).

CLINICAL MANIFESTATIONS

When clinical signs appear, they are considered to take place in two phases (Patton et al., 2008):

1. Acute: Anorexia, fever (greater than 104°F), lethargy, increased respirations, ocular and nasal discharges,

and central nervous signs are seen (ataxia, paralysis, seizures) (Percy and Barthold, 2007; Pakes and Gerrity, 1994). Death occurs a week after the onset of clinical signs. Because of these acute signs, *T. gondii* is considered a differential diagnosis for rabbits with neurological clinical signs (Müller et al., 2009; Sroka et al., 2003).

2. Chronic to latent: Asymptomatic, intermittent signs that appear with stress (such as from research procedures), or progressive clinical signs including emaciation that result in paralysis and/or death (Pakes and Gerrity, 1994).

Cervicofacial lumps in infected pet rabbits have been observed (Ishikawa et al., 1990). Death from experimental infection may occur (Garweg et al., 1998; Liu et al., 2006b).

Recovery from active infection has been reported (Schoeb, 2007).

PATHOLOGY

Lesions in the acute form usually include necrosis of lymph nodes and the liver, spleen, heart, and lung (Pakes and Gerrity, 1994; Patton et al., 2008; Schoeb, 2007). The organs may be enlarged with small white foci (Patton et al., 2008). Organisms may be found in microscopic sections of the various organs plus skeletal muscle (Gustafsson et al., 1997; Patton et al., 2008). Gross lesions are less likely in the chronic form and the organism may only be found in the CNS at that time (Schoeb et al., 2007; Patton et al., 2008). Reticuloendothelial hyperplasia may be seen in the lymph nodes, spleen, liver, and the CNS (Pakes and Gerrity, 1994). Generally, the chronic form is usually seen in older rabbits (Pakes and Gerrity, 1994; Schoeb, 2007).

PATHOGENESIS

After ingestion, the parasite will take up residence in organs and tissues previously noted. The organism reproduces (multiplies) in these locations, which causes disease. Organismal replication will continue until the infected animal dies or develops immunity (Schoeb et al., 2007).

DIAGNOSIS

Diagnosis is through serology (antibody detection tests). ELISA, immunofluorescent antibody (IFA), and Multiplexed Fluorometric ImmunAssay™ (MFIA) methods are all utilized, depending on the diagnostic laboratory (Ise et al., 1985). Histopathological and gross necropsy findings can also be used (Waller and Bergquist, 1982). Identification of the organism can also be accomplished through immunohistochemistry and PCR (Schoeb, 2007).

PREVENTION AND THERAPY

Prevention is achieved through good sanitation as well as eliminating the possibility of contamination of the rabbit area with cat feces. Rabbits should be physically separated from cats in laboratory animal facilities (Pakes and Gerrity, 1994). Sulfadiazine, pyrimethamine, sulfones, tetracyclines (e.g. minocycline), spiramycin, and 1,2-dihydrotriazones have all been used to treat toxoplasmosis in laboratory animals (Pakes and Gerrity, 1994; Rollins et al., 1982). Treatment of infected rabbits is generally not considered warranted (Schoeb, 2007).

Sarcocystis cuniculi

BACKGROUND

Sarcocystis was first described in rabbits by Manz in 1867 in Germany. Brumpt identified the protozoan in 1913 in the European Hare and named it *Sarcocystis cuniculi*. A year later *Sarcocystis leporum* was described by Crawley in the cottontail rabbit in the United States (Pakes and Gerrity, 1994).

ETIOLOGY/ETIOLOGIC AGENT

Sarcocystis cuniculi infections are common in wild rabbits worldwide but have not been reported in laboratory rabbits (Schoeb et al., 2007). The cyst may be septate and the wall contains radial spines, cytophaneres (Pates and Gerrity, 1994). Mature cysts are curved and fusiform in shape and contain bradyzoites which are 11–16 µm by 4–6 µm. Immature cysts contain round metrocytes which are 4–6 µm in diameter.

EPIZOOTIOLOGY

Cats are the definitive hosts for *S. cuniculi*. Rabbits become infected by consuming the feces of cats which contain infective oocysts. Within 3 months, infective sarcocysts develop within the striated muscle of the intermediate host (Schoeb et al., 2007). Transmission to the definitive host is by consumption of infected muscle tissue of rabbits.

CLINICAL MANIFESTATIONS

Sarcocystosis infections in the rabbit are generally asymptomatic, however heavy infestations may result in lameness due to the lesions and inflammation described below.

PATHOLOGY

Infections result in the formation of cysts that can be 5 mm long and visible within the muscle tissue. Gross lesions are generally seen in cardiac and skeletal muscle of the loins, flank, and hind limbs (Levine, 1973). Heavily infected tissue may appear grossly as white streaks oriented in the direction of the myofibers. Microscopically, intact cysts may be visible within muscle tissue with no inflammatory component.

PATHOGENESIS

Ruptured cysts can cause an inflammatory response resulting in severe focal myocarditis and myositis characterized by an infiltration of lymphocytes, plasma cells, macrophages, and eosinophils with subsequent mineralization of myofibers (Pakes and Gerrity, 1994). Healed lesions result in scarring.

DIAGNOSIS

Diagnosis is generally made by histopathological examination, however visualization of white streaks paralleling the muscle fibers at gross exam can be diagnostic in heavily infected animals (Pakes and Gerrity, 1994). Fresh muscle tissue smears can be examined for *Sarcocystis* using phase microscopy or Giemsa stain. The sarcocysts are usually septate and have radial spines in the wall.

PREVENTION AND THERAPY

There are no effective methods for treatment of *Sarcocystis*. Good sanitation can serve to prevent disease by minimizing exposure to cat feces.

Microsporidia

Encephalitozoon cuniculi

BACKGROUND

Microsporidia are intracellular, obligate, spore-forming eukaryotic organisms to which all mammalian species are susceptible to infection. *Encephalitozoon cuniculi* was first described in young rabbits with granulomatous encephalitis resulting in motor paralysis in 1922 (Wright and Craighead, 1922). It was later named by Levatiditi et al. in 1923, whose group identified the protozoal agent from the tissue of rabbits with the same pathology (Pakes and Gerrity, 1994). The organism is currently considered a highly derived fungus (Keeling and Fast, 2002), but is included here due to its historical classification as a parasite.

Based on the variation in ribosomal RNA and karyotypes, *E. cuniculi* has been divided into three strains (Schoeb et al., 2007). Each strain has a distinct host distribution. Strain I (karyotypes A, B, and C) is considered the “rabbit” strain and infects humans, mice, and rabbits. The “mouse strain”, strain II (karyotype F), infects mice and blue foxes. Strain III (karyotypes D and E) infects both humans and dogs and is considered the “dog” strain.

ETIOLOGY/ETIOLOGIC AGENT

The mature spore is oval, measures 1.5 × 2.5 µm and is encased by a thick cell wall. Each spore contains an eccentric nuclei and a polar filament coiled within the inner wall which is characteristic of Microsporidia. This

polar filament is extruded by the spore when it comes in contact with the host cell membrane and inserts a sporoplast into the cell. The sporoplast proliferates within a vacuole giving rise to meronts which become sporonts and ultimately mature spores. This vacuole may rupture the host cell releasing mature spores into the vascular system which may further disseminate. Mature spores are shed via excreta.

EPIZOOTIOLOGY

Natural infections in rabbits occur primarily through the ingestion of mature spores in urine. Experimental modes of transmission include intranasal, tracheal, and parenteral routes (Pakes and Gerrity, 1994). Intrauterine transmission has also been reported though it is not considered a major mode of transmission (Csokai et al., 2009).

Encephalitozoon cuniculi is considered a life-threatening opportunistic pathogen in immunocompromised human patients (Weber et al., 1997). Though no known cases of direct zoonotic transmission exist between rabbits and humans, one should still consider *E. cuniculi* as a potential zoonotic organism.

CLINICAL MANIFESTATIONS

In rabbits, most *E. cuniculi* infections are subclinical though acute disease does occur sporadically. Neurological signs prevail in acute disease and include torticollis, ataxia, paresis, opisthotonos, and convulsions. Chronic renal failure and phacoclastic uveitis may also result from chronic infection (Kunzel et al., 2007). *E. cuniculi* is generally considered to be a top differential diagnosis of neurological clinical signs in all rabbits. *T. gondii*, space-occupying masses, and other infectious agents such as *Pasteurella multocida* are also common differential diagnoses.

PATHOLOGY

The classic pathological lesion of encephalitozoonosis in rabbits is pyogranulomatous encephalitis (Figure 15.6) with perivascular and leptomeningeal lymphoplasmocytic cuffing. The cerebrum is the most frequent site of infection with less frequent infection in the cerebellum and vestibular cores (Csokai et al., 2009). Gross lesions of the kidneys are common and present as multiple, white pinpoint, slightly depressed foci (often called “pitting”) randomly scattered on the capsular surface (Figure 15.7). Microscopically, evidence of necrosis exists with pyogranulomatous, lymphoplasmocytic inflammation. Interstitial nephritis was identified in 52% of pet rabbits evaluated for histopathological changes in one study (Csokai et al., 2009).

PATHOGENESIS

The clinical signs and granulomatous lesions seen in *E. cuniculi*-infected rabbits are the result of

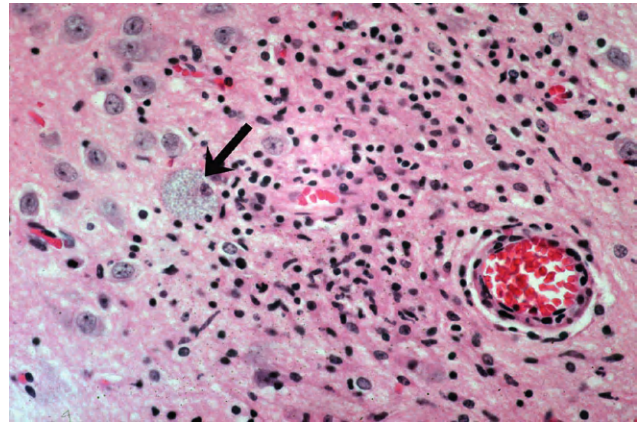


FIGURE 15.6 Photomicrograph of granulomatous encephalitis with perivascular accumulation of lymphocytes. The arrow points to *E. cuniculi* organism. (photo courtesy of James G. Fox)



FIGURE 15.7 Pitting of the kidney surface (right side) resulting from infection with *E. cuniculi*, demonstrated on two halves of a rabbit kidney. (from Patton, N.M., K.W. Hagen, J.R. Gorham, and R.E. Flatt. 2008. *Domestic Rabbits: Diseases and Parasites*. PNW 310. Corvallis, OR: Oregon State University Extension Service. © 1986, 1998 Oregon State University. Used with permission)

inflammation caused by spore release from ruptured cells. Seroconversion occurs within 30 days of infection and precedes renal shedding (Cox et al., 2007). It has not been clearly elucidated which organs the spores infect first once ingested. However in the acute stage of experimental infection of rabbits with *E. cuniculi*, spores were identified in the lung, kidney, and liver but not the brain or gut. During chronic infection however the heart, brain, and kidney were the only organs involved (Cox et al., 2007).

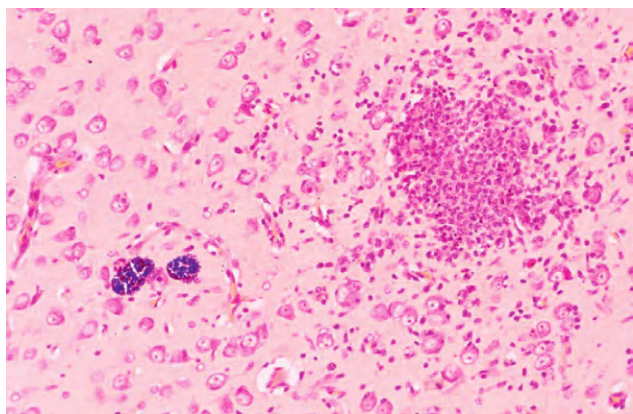


FIGURE 15.8 Photomicrograph of Gram-positive (purple) spores of *E. cuniculi* in the brain.

DIAGNOSIS

Current serologic testing modalities include ELISA, IFA, and MFIA. Results using MFIA are generally more sensitive than those based on ELISA. PCR can be conducted on fresh urine, fecal, fresh or fixed tissue or cultured cells though this method is not routinely used for screening. However, a recent study reported that PCR failed to detect parasite DNA in urine or cerebrospinal fluid from pet rabbits displaying clinical signs of encephalitozoonosis (Kunzel et al., 2007).

Gross pathological signs can lead to a presumptive diagnosis. A definitive diagnosis is readily made post-mortem when organisms are identified in tissue using special staining techniques. *E. cuniculi* spores are “strongly” Gram-positive, stain dark purple with Goodpasture’s carbol fuschin, and are acid fast trichrome positive (Figure 15.8) (Harcourt-Brown, 2008).

PREVENTION AND THERAPY

Commercial SPF rabbit colonies exist to provide *E. cuniculi*-free animals for research purposes. Screening panels are also available through commercial laboratories and should be routinely performed to monitor SPF status. Maintaining adequate husbandry techniques will help to minimize exposure from contaminated excreta. Spores are resistant to standard environmental conditions, however they are readily susceptible to routine disinfectants and autoclaving. It should be noted that anecdotally the presence of histopathological lesions (both in the CNS and kidney) can result in misinterpretation of findings in toxicological studies utilizing rabbits, causing concern. Therefore, it is recommended that rabbits from *E. cuniculi*-free sources be used for toxicity testing.

Treatment success depends on whether an infection is chronic or acute. Acute infections can be treated with fenbendazole at 20mg/kg orally every 24h for 28 days

with some success (Suter et al., 2001). Acute neurological signs can be treated with steroids (0.5–1 mg/kg dexamethasone with further doses at 0.2 mg/kg if needed) (Harcourt-Brown, 2008). Chronic cases generally present with neurologic symptoms and if cell damage is severe treatment is usually not successful (Meredith, 2008). Preventive regimens of fenbendazole are used in areas of the world where both wild and domestic rabbits are of the species *O. cuniculus* (e.g. United Kingdom and Europe) (Harcourt-Brown, 2008).

Flagellates and Amoebae

Chilomastix cuniculi

Chilomastix cuniculi is a non-pathogenic organism observed in the cecum of the rabbit. The trophozoite is pyriform with three anterior flagella, a large cytosomal groove near the anterior end and an anterior nucleus. The trophozoite ranges in length from 3–20 μm (Pakes and Gerrity, 1994). Diagnosis is by microscopic evaluation of cecal contents or fecal smear.

Giardia duodenalis

Giardia is a bilaterally symmetrical flagellate organism with two axostyles, two anterior nuclei and four pairs of flagella. Trophozoites of *G. duodenalis* measure approximately $16 \times 9 \mu\text{m}$ (Hegner, 1922) and contain cysts with two to four nuclei which are shed via feces. *G. duodenalis* is occasionally found in the anterior intestinal tract of laboratory rabbits, however infection is generally not known to be pathogenic (Levine 1957, 1985a). Several cases of diarrheal disease have been attributed to this flagellate, some with successful treatment using metronidazole in drinking water (Schoeb et al., 2007). This organism is zoonotic, however there is no known evidence of rabbit-to-human transmission. Rabbits have been used to cultivate *Giardia* spp. for experimental purposes (Pakes and Gerrity, 1994).

Monocercomonas cuniculi

Monocercomonas cuniculi (Tanabe, 1926) (synonyms *Eutrichomastix cuniculi*, *Trichomastix cuniculi*) is a flagellate organism found within the cecum of the rabbit that is apparently non-pathogenic. Trophozoites range in length from 5–14 μm and are pyriform with three anterior and one posterior flagellum. A slender axostyle protrudes from the posterior of the body (Kulda and Nohynkova, 1978; Morgan and Hawkins, 1952).

Retortamonas cuniculi

Retortamonas cuniculi (Collier and Boeck, 1926) is a relatively uncommon, non-pathogenic flagellate organism found in the cecum. Flagellates measure approximately 7–13 by 5–10 μm (Pates and Gerrity, 1994). Cysts are oval

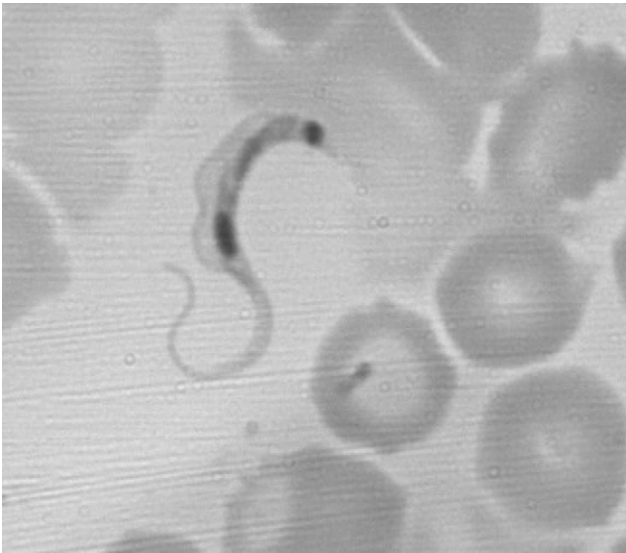


FIGURE 15.9 Light microscope image of *Trypanosoma nabiasi* in rabbit blood. (photo courtesy of Hamilton, PB)

and measure 5–7 by 3–4 μm (Collier and Boeck, 1926; Kreir, 1978).

Trypanosoma nabiasi

Trypanosoma nabiasi is a hemoflagellate organism that has been identified occasionally in wild *Oryctolagus cuniculi* in Europe and domestic rabbits outside Europe (Hamilton et al., 2005). Recently, the first identification of the organism in wild *O. cuniculi* was made in Australia (Hamilton et al., 2005) (Figure 15.9). There are no published reports of *T. nabiasi* in laboratory rabbits. The rabbit flea, *Spilopsyllus cuniculi*, is the vector for *T. nabiasi* (Hoare, 1959). The hemoflagellate measures 24–28 μm long and develops in the gut of the intermediate host *Spilopsyllus cuniculi* (Pakes and Gerrity, 1994). Hemoflagellates appear in the bloodstream within 5–12 days after infection (Grenwal, 1956). Infection of the rabbit likely occurs during grooming via consumption of fecal material from the flea (Hamilton et al., 2005). Rabbits are subsequently immune to reinfection (Grenwal, 1956). Furthermore, experimental evidence suggests that *T. nabiasi* is host-specific (Hamilton et al., 2005).

Entamoeba cuniculi

Entamoeba cuniculi is a non-pathogenic amoeba found in the cecum and colon of rabbits. The trophozoites measure 10–30 μm in diameter and the cysts are oval with eight nuclei that measure 7–21 μm in diameter (Hoare, 1959). *Entamoeba cuniculi* is morphologically similar to the rodent amoeba, *Entamoeba muris* (Baker, 2007).

Other

Pneumocystis

BACKGROUND

Pneumocystis oryctolagi is an extracellular non-mycelian fungal organism specific to the lagamorph host. However, because it was previously believed to be a parasite, information on the organism is included here as well as in Chapter 17. Previously this organism was unclassified and named *Pneumocystis carinii*. To date, five *Pneumocystis* species have been identified: *P. wakefieldiae* and *carinii* in the rat, *P. murina* in the mouse, *P. oryctolagi* in the rabbit, and *P. jirovecii* in the human (Beck and Cushion, 2009).

ETIOLOGY/ETIOLOGIC AGENT

The organism exists in two forms, a trophic and cystic form. The trophic form can range in size from small, ellipsoid, and 1–2 μm in length to rather large, irregular and 4–8 μm in length with filopodia. The cystic form is round, 5 μm in diameter, and possesses a thick wall (Dei-Cas et al., 2006). Filopodia are rare. Each cyst contains a maximum of eight spores.

EPIZOOTIOLOGY

Immunocompromised animals are most susceptible. Transmission is generally horizontal from other animals harboring the fungal organism.

CLINICAL MANIFESTATIONS

Disease is rare as infection is often subclinical. Pneumonia was reported in a group of weanling rabbits that resolved in most animals within 2–3 weeks (Soulez et al., 1989).

PATHOLOGY

Lesions include pneumonitis, vascular congestions, edema, and an inflammatory infiltrate of the alveolar walls. Infiltrate is made up of macrophages and lymphocytes with occasional plasma cells (Pakes and Gerrity, 1994).

PATHOGENESIS

All identified *P. oryctolagi* life cycle phases occur in the lungs (Dei-Cas et al., 2006), specifically the type I alveolar cells that line the air sacs. Inflammation caused by the fungal organisms within the lungs results in pneumonitis or pneumonia.

DIAGNOSIS

Diagnosis is often made by the presence of organisms in tissue at necropsy. Lung tissue can be stained with silver stains or toluidine blue O (TBO) to visualize the organism histologically. PCR can be used to identify

DNA from rabbit nasal and lung wash samples (Dei-Cas et al., 2006).

PREVENTION AND THERAPY

No definitive treatments have been described. Rederivation may be an option, however vertical transmission has not been ruled out.

ARTHROPODS

Arachnids

Psoroptes cuniculi

BACKGROUND

This mite affects domestic rabbits worldwide but is rare in wild rabbits (Schoeb et al., 2007). The parasite used to be very prevalent in laboratory rabbit colonies but improved husbandry and use of specific pathogen-free rabbits has greatly reduced the prevalence over the years (Schoeb et al., 2007).

ETIOLOGY

The mite (Figure 15.10) has also been called by different names throughout history including *Dermatodectes cuniculi*, *Psoroptes longirostris* var. *cuniculi*, *Psoroptes*

communis var. *cuniculi* and *Psoroptes equi* var. *cuniculi* (Hofing and Kraus, 1994). Within this genus, mites identified from various hosts (including goat, horse, mule, domestic sheep, white-tailed deer, mule deer, and black buck antelope) are morphologically and behaviorally similar and cross-infection of these mites to rabbits is possible (Hofing and Kraus, 1994).

Psoroptes may be distinguished from other mites such as *Chorioptes* by the jointed pedicels of the tarsal suckers (Schoeb et al., 2007).

EPIZOOTIOLOGY

Psoroptes cuniculi is a non-burrowing parasite which primarily lives on the inner surface of the pinna. Some sources report lesions caused by the parasite on other areas of the body (Hofing and Kraus, 1994). All life cycle stages can be found on the host where they feed on tissue fluids exuding from inflamed surfaces or obtained by piercing the epidermis. Epidermis and sebaceous secretions may also be ingested (Schoeb et al., 2007). The life cycle requires about 3 weeks and eggs hatch about 4 days after being laid. Mites can survive off the host for up to 3 weeks at low temperature and high humidity, but survive a much shorter time at high temperature and low humidity (Smith et al., 1999).

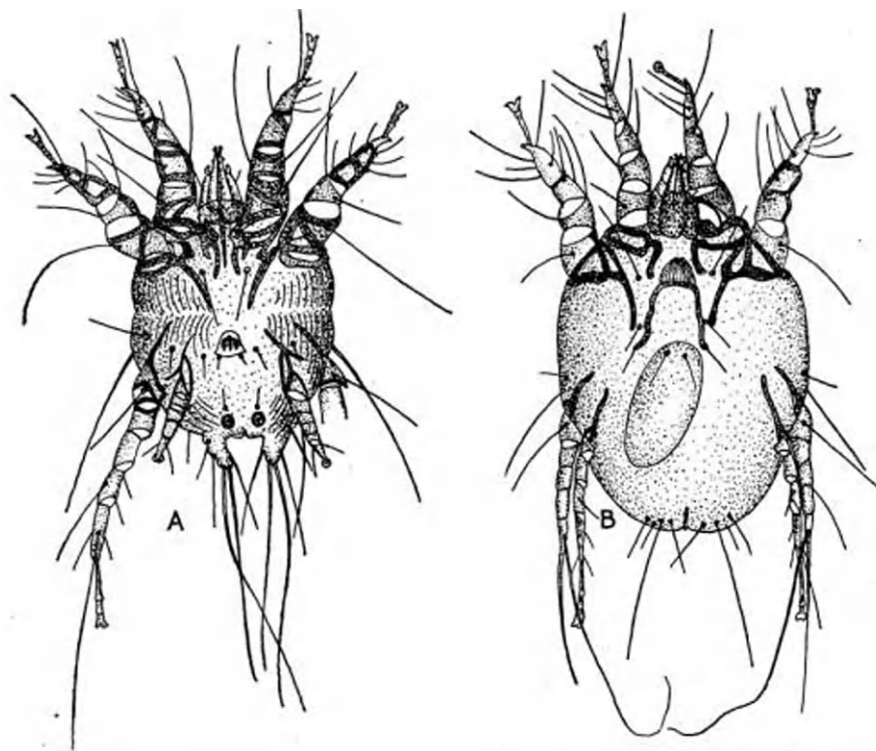


FIGURE 15.10 *Psoroptes cuniculi*. A is male B is female. Viewed from the underside. Farmers Bulletin 1568, revised 1931. (from Canestrini 1894. Used with permission from the University of North Texas Libraries, Digital Collection)

CLINICAL MANIFESTATIONS

Infestation with this mite results in otocariasis, also known as psoroptic mange, ear mange, ear canker, or psoroptic scabies (Hofing and Kraus, 1994). A copious, brown exudate builds up on the inner aspect of the ear. Exudate covering the entire inner surface of the ear may be seen and the underlying tissue may be red and inflamed. Alternatively, infested animals can be clinically normal until placed under stress (Bowman, 2009).

PATHOLOGY

The main microscopic lesion is chronic proliferative dermatitis. Early lesions have a dry whitish-gray to tan crusty exudate which thickens with disease progression to 2 cm or more. Exudate consists of desquamated epithelial cells, serum, inflammatory cells, mite feces, and adult mites. The skin surface is moist and red, may be odoriferous and very painful when touched (Hofing and Kraus, 1994). Histologically the epidermis is hyperplastic and parakeratotic and the dermis and epidermis are infiltrated with lymphocytes, eosinophils, and heterophils (Schoeb et al., 2007).

PATHOGENESIS

Due to intense pruritus, affected rabbits may shake or scratch at their ears. Pruritus may lead to excoriation and self-mutilation and secondary bacterial infections (Hofing and Kraus, 1994; Schoeb et al., 2007). An allergic response to the mites may play an important role in pathogenesis (Schoeb et al., 2007).

DIAGNOSIS

Adult mites may be seen upon visual or otoscopic examination of the ear. Observing the exudate, even from afar, can be considered pathognomonic. Samples of exudate from affected ears examined underneath a microscope usually reveal numerous mites (Hofing and Kraus, 1994).

PREVENTION AND THERAPY

Careful selection of vendors when acquiring laboratory rabbits is necessary to prevent ear mite infestations. Quarantine and prophylactic treatment of animals whose history is unknown is recommended. Historically, topical application of mineral oil in the ears was used as a preventive measure (Hofing and Kraus, 1994).

Treatments include subcutaneous administration of ivermectin, topical selamectin, oral moxidectin, and topical imidacloprid plus moxidectin (Beck, 2007; Schoeb et al., 2007; McTier et al., 2003; Wagner and Wendlberger, 2000). Many treat, either prophylactically or therapeutically, the ears with one drop each of concentrated ivermectin solutions with success.

Experts are torn when it comes to the removal of the exudate. Older texts recommend the removal of the

exudate in order to decrease the parasite load while more recent advice indicates that the removal of the exudate may be traumatic to the rabbit and could potentially result in necrosis of the thin and delicate structures of the pinna. Bleeding from the fragile ear veins and arteries could also result. In this case, treatment would proceed in the face of exudate and as the mite population decreases, the exudate would naturally clear or be removed with mineral oil when sufficiently diminished.

Notoedres and Sarcoptes scabiei

BACKGROUND

Notoedres cati is primarily a parasite of cats but can infect rabbits (Soulsby, 1982). Both *N. cati* and *S. scabiei* are found throughout the world but are not frequently reported in rabbits (Hofing and Kraus, 1994).

ETIOLOGY

Notoedres cati is referred to in older literature sources as *Notoedres minor cuniculi*. *Sarcoptes scabiei* (Figure 15.11) is also known as the itch or scab mite (Hofing and Kraus, 1994). It is thought that *S. scabiei* from different host species are separate varieties, however this is still being debated (Schoeb et al., 2007). It is also thought that host specificity has arisen in the parasite so that, for example, *S. scabiei* infecting a human would spread more readily to another human. Interspecies transmission does still occur, however resulting dermatitis may be atypical and transient (Bowman, 2009).

EPIZOOTIOLOGY

The life cycle is described from human infestations, however, the life cycle on the rabbit is presumed to be similar (Soulsby, 1982). The adult female mites burrow in the epidermis and lay eggs which hatch in 3–5 days. Six-legged larvae develop into eight-legged protonymphs then deutonymphs (Schoeb et al., 2007). Females, eggs, and young larvae are found in burrows in the epidermis. Older larvae, nymphs, and adult males are found on the surface (Hofing and Kraus, 1994). Mites develop into mature males and females about 17 days after hatching. All forms feed on epithelial cells and tissue fluid. Transmission is by contact with organisms on the skin surface. Adult mites are very susceptible to drying and only live a short time off the host (Hofing and Kraus, 1994).

CLINICAL MANIFESTATIONS

Disease caused by *Notoedres cati* is clinically and pathologically indistinguishable from that caused by *Sarcoptes scabiei*, although it is usually less severe (Schoeb et al., 2007). Early lesions consist of partial alopecia, hyperemia, and oozing of serous fluid which progresses to a yellow-white or gray-white crust of epidermal cells and dried serum. Lesions are very pruritic and rubbing and

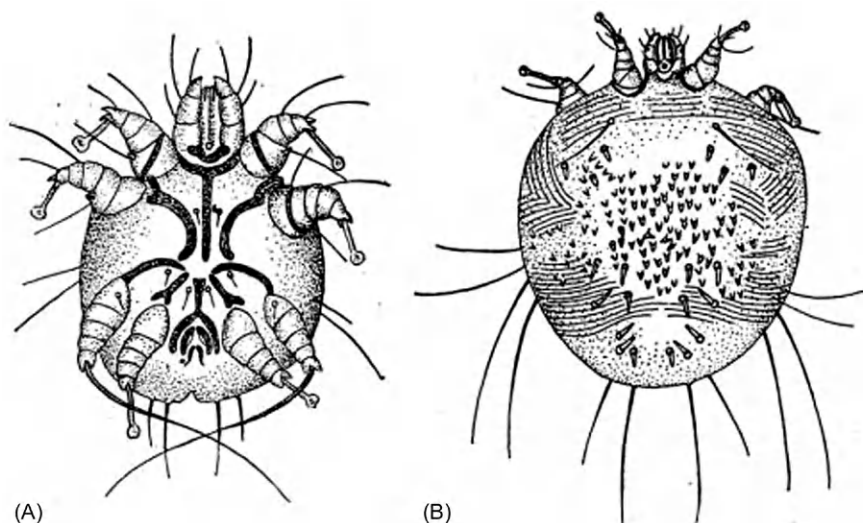


FIGURE 15.11 *Sarcoptes scabiei*. A is male and B is female. Viewed from the underside (from *Farmers Bulletin* 1568, revised 1931; from *Canestrini* 1894. Used with permission from the University of North Texas Libraries, Digital Collection)

scratching by the rabbit may increase severity and allow for secondary bacterial infections to develop. Lesions usually first appear on the nose and lips and extend to the eyes, forehead, face, and occasionally other body areas such as the external genitalia (Hofing and Kraus, 1994). Extensive lesions on individual animals can be severe, leading to general debilitation, emaciation, anemia, leukopenia, and death within weeks (Hofing and Kraus, 1994).

PATHOLOGY

Lesions are characterized microscopically by epidermal hyperplasia with orthokeratotic and parakeratotic hyperkeratosis and an overlying layer of keratinic and proteinaceous debris (Schoeb et al., 2007). Mite and mite tunnels can be found in the debris and outer epidermis. The dermis contains inflammatory cell infiltrate, primarily lymphocytes, and eosinophils. A suppurative response may also be seen with secondary bacterial infections (Schoeb et al., 2007).

PATHOGENESIS

An immunologic hypersensitivity reaction to the mites is thought to be the major pathogenic mechanism of the dermatitis and pruritus (Schoeb et al., 2007). Pruritus may lead to excessive scratching and rubbing leading to secondary traumatic injuries.

DIAGNOSIS

Identification of mites in deep skin scrapings is the main diagnostic method for identification. *Notoedres cati* is slightly smaller than *Sarcoptes scabiei* and the position of the anus is dorsal in *N. cati* versus terminal in *S. scabiei* (Schoeb et al., 2007). Additionally, the dorsal dentate spines of *Sarcoptes* are significantly larger than *Notoedres* (Hofing and Kraus, 1994).

PREVENTION AND THERAPY

Limiting exposure to *Sarcoptes*- and *Notoedres*-affected individuals is necessary to prevent transmission. This includes the education of animal handlers with regards to the potential of disease transmission from humans to rabbits. Various sources recommend ivermectin, doramectin, or selamectin to treat infections (Kurtdele et al., 2007; Schoeb et al., 2007; Voyvoda et al., 2005).

Cheyletiella parasitovorax

BACKGROUND

There are several species of the fur mite *Cheyletiella* that occur with wide geographic distribution. Several species affect rabbits, with *C. parasitovorax* being the most commonly identified and described and thus classically associated with rabbits. However, the mite also infests dogs and cats and has been labeled one of the most common dermatological zoonosis of those species even though it is also considered under-diagnosed (Jeromin, 2006; Moriello, 2003). Laboratory animal veterinarians should take this into account when planning for biosecurity measures in the vivarium.

ETIOLOGY/ETIOLOGIC AGENT

C. parasitovorax is described as having a “distinctive” appearance with the adults having “large, curved palpal claws” (Schoeb et al., 2007). Other aspects of the mite include a semicircular dorsal shield, legs that reach out well beyond the body that have feathered bristles and terminal tarsal combs. As adults, males are up to 320µm long and 160µm wide and the females are larger measuring up to 500µm long and 200µm wide (Schoeb et al., 2007). Some authors have suggested that

the prevalence of *C. parasitovorax* in laboratory rabbit populations is now “rare” (Schoeb et al., 2007). In the experience of one author of this chapter, multiple research rabbit populations have been identified with the pathogen within a short time period. Obtaining rabbits from non-SPF sources and contact with potentially infested dogs and cats would be considered risk factors for introducing or maintaining mite infestations in rabbit colonies.

EPIZOOTIOLOGY

This fur mite does not burrow. Staying on the skin surface, it feeds on “tissue fluid” (Schoeb et al., 2007). Eggs will be attached to hair shafts approximately 2–3 mm above the skin surface (Hofing and Kraus, 1994; Schoeb et al., 2007). No other hosts are involved in the life cycle and transmission is by direct contact. Fomite transmission may also occur as adult female mites have been known to live for up to 10 days off the host (Bowman, 2003; Paterson, 2006). *C. parasitovorax* can also temporarily infest humans (Cohen, 1980).

Co-infections with other mites and lice have been reported (Flatt and Wiemers, 1976; Kim et al., 2008b).

CLINICAL MANIFESTATIONS

Clinical manifestations are variable, with some animals displaying no clinical signs with subclinical infections. The main clinical sign is excessive flakes and scales on the skin, especially in the scapular region, but this has been also reported on the face and ventrally (Cloyd and Moorhead, 1976; Richardson, 2000; Schoeb et al., 2007). Some cases result in layers of dead skin flakes building up on the skin surface. Areas of alopecia may be seen. Inflammation and pruritis are usually not observed. Skin flakes may move around on the skin surface due to mite movement with *C. parasitovorax* infestation or the mite might be visualized with the naked eye, giving the infestation the name “walking dander” (Bowman, 2003; Hess, 2004). Caution should be taken to ensure that cheyletiellosis is not misdiagnosed as ringworm. At least one author has suggested that clinical signs may be more pronounced in immunocompromised animals (Paterson, 2006).

PATHOLOGY

Gross findings include the excessive amount of skin scales and flakes when present. Mild epidermal hyperkeratosis along with dermal inflammatory cell infiltrate with neutrophils, lymphocytes, plasma cells, and eosinophils may be seen microscopically (Hofing and Kraus, 1994; Schoeb et al., 2007).

PATHOGENESIS

The skin exfoliates in response to the fur mite residing on the skin surface and penetrating the skin with its

“needle-like mouth parts” resulting in the clinical signs (Paterson, 2006).

DIAGNOSIS

Multiple methods exist for diagnosing and identifying *C. parasitovorax*. Perhaps the simplest method is to apply clear adhesive tape to the affected areas, or scapular region, remove it and then examine the tape on a slide with a microscope (Richardson, 2000). Performing a skin scrape and brushing the epidermis with a stiff bristled brush can also be used to find organisms (Hofing and Kraus, 1994; Patton et al., 2008). Debris gathered by these methods can be examined using a dissecting scope. Identification of the mite is considered easy given its characteristic structures. Eggs can also be detected using these diagnostic methods.

PREVENTION AND THERAPY

Prevention is best accomplished by acquiring rabbits from a source known to be free from *C. parasitovorax*. Good biosecurity control is also needed so that potentially infested dogs, cats, and fomites do not serve as a source of infection. Preventive treatments may be considered for incoming rabbits from non-SPF vendors.

Ivermectin has been described as an efficacious treatment for *C. parasitovorax* infestations in rabbits, dogs, and cats and is easy to administer. Because the mites have a life cycle of 14–21 days, multiple treatments are needed. The basic dose is 400 µg/kg delivered subcutaneously once every 10–14 days for three consecutive treatments (Paterson, 2006).

Topical selamectin, permethrin, and carbaryl products may also be used as treatments (Fisher and Shanks, 2008; Hess, 2004; Mellgren and Bergvall, 2008; Paterson, 2006). Selamectin in particular has been noted to be efficacious and safe, even at a single dose of 12 mg/kg (Kim et al., 2008b; Mellgren and Bergvall, 2008).

At the time of the first treatment, the animal’s caging, room environment, and items that could serve as fomites should be sanitized and/or treated with flea products such as 5% carbaryl dust (Hess, 2004; Jeromin, 2006; Paterson, 2006). All animals with potential contact with infested animals should be treated (Paterson, 2006).

Listrophorus gibbus (Also Known as *Leporacarus gibbus*)

BACKGROUND

Listrophorus gibbus, also called *Leporacarus gibbus* in the veterinary literature, is considered to be a clinically silent disease in rabbits. This mite has been reported in both wild and domestic rabbits in North America, Europe, Australia, and New Zealand (Hofing and Kraus, 1994; Schoeb, 2007).

ETIOLOGY/ETIOLOGIC AGENT

Since the mite does not generally reside on the skin, it is found on hair shafts. Its dark brown appearance comes from dense chitin (Hofing and Kraus, 1994). Adult males measure $240\mu\text{m} \times 440\mu\text{m}$ and females measure $310\mu\text{m} \times 560\mu\text{m}$ (Hofing and Kraus, 1994). In addition to having short legs, the body is “compressed laterally” or oval and there is a projection that extends out over the mouthparts (Hofing and Kraus, 1994).

EPIZOOTIOLOGY

The mite does not burrow and is described as feeding on sebaceous secretions, epithelial scales, and hair debris (Hofing and Kraus, 1994; Paterson, 2006). Rabbits are the mite’s necessary host and all life stages are completed on the animal.

Co-infections with *Cheyletiella* and other ectoparasites have been reported (Kim et al., 2008a; Pinter, 1999). Rare reports of human cases exist (Paterson, 2006; Schoeb et al., 2007).

CLINICAL MANIFESTATIONS

No clinical signs have been reported with infestations with *L. gibbus* only, even with heavy infestations (Paterson, 2006).

PATHOLOGY (GROSS AND MICROSCOPIC FINDINGS)

No pathological findings have been reported with infestations of *L. gibbus* only. The mite can be found on hair specimens.

PATHOGENESIS

This is not applicable.

DIAGNOSIS

Even though the mite causes no clinical signs, it can be easy to find. Fur plucks, brushings, or combing can be used to find the organism (Schoeb, 2007). Optimal areas for looking for the mite include the scapular region and rump. Fur can be placed on dissecting scopes or underneath clean cellophane and examined with a regular microscope. The mite can be identified by its characteristic anatomical features along with the lack of clinical manifestations.

PREVENTION AND THERAPY

As with other ectoparasites, ensuring that rabbit sources are free of the mite is important. The same ivermectin treatment used for *C. parasitovorax* parasitism can be used to effectively treat *L. gibbus* infestations (Schoeb et al., 2007). Some authors recommend against treatment but this parasite is generally considered to be unacceptable within the laboratory animal facility (Paterson, 2006).

Demodex cuniculi

Demodex cuniculi is a follicle mite that is found as a normal resident of rabbit skin. *D. cuniculi* is similar in morphology to *Demodex canis*. Rabbits can be treated with a 0.01% solution of amitraz or a biweekly subcutaneous injections of ivermectin at $500\mu\text{g}/\text{kg}$ body weight until infestation clears (Schoeb et al., 2007).

Trombiculid Mites

The mites of the family *Trombiculidae* (chiggers, red bugs, harvest mites) are found on wild and domestic rabbits which are exposed to vegetation. However, these mites have not been reported in rabbits maintained in modern laboratory facilities (Schoeb et al., 2007). Several species have been found on rabbits including *Trombicula autumnalis*, *T. cavicola*, *T. irritans*, and *T. microti* (Pates and Gerrity, 1997). These mites are not host-specific.

Adult mites are free-living within the soil. Only the larvae are parasitic. Larvae can survive up to 30 days without a host. Once a host is found, the larvae attach to the skin surface and release a highly irritating and pruritic salivary secretion. This secretion results in the destruction of the epithelium and creation of a tubular structure, stylostome, from which the larvae feed on tissue fluid of the host. Once engorged, the larvae fall off the host and enter a period of quiescence in the soil, then molt into non-parasitic nymphs. Unfed larvae are a deep red in color and approximately $210\mu\text{m}$ long. Fully fed larvae are pale yellow in color and approximately $400\mu\text{m}$ long (Pates and Gerrity, 1997).

Larvae are most commonly found on feet, ears, medial canthi, and perineum (Keay, 1937). The saliva released during the feeding process causes intense pruritus (Alexander, 1984) and the development of macules and pustules. Humans are parasitized in a similar fashion as rabbits.

Pentastomids

Linguatula serrata

Lagomorphs are one of the many natural intermediate hosts for the pentastomid, *Linguatula serrata* (James and Harwood, 1969; Soulsby, 1968). The rabbit becomes infected upon consumption of eggs shed by respiratory excretions of the definitive hosts which include dogs and occasionally humans (Schoeb et al., 2007). Upon hatching, larvae will migrate from the intestine to mesenteric lymph nodes, undergo six to nine molts, and become infective nymphs. Nymphs are curved, flattened ventrally, white, 4–6 mm long and contained within small cysts. Infected rabbits have no evidence of clinical disease. Nymphs are often an incidental finding at necropsy.

Ticks

Wild rabbits serve as the host for a multitude of hard (ixodid) and soft (argasidae) tick species. These ticks serve as vectors for several zoonotic diseases including tularemia, lyme disease, and rickettsial diseases. Ticks that feed on wild rabbits rarely bite humans (Schoeb et al., 2007). Ticks rarely infest domestic rabbits but laboratory rabbits may serve as animal models to study tickborne diseases (Hofing and Kraus, 1994).

FAMILY ARGASIDAE (SOFT TICKS)

Otobius lagophilus *Otobius lagophilus* is considered the most common soft tick to affect rabbits (Pates and Gerrity, 1994). The ticks are parasitic in the larval and lymph stages and are found on the face of the affected rabbit. Adult ticks are often found in the nesting areas. Adult female ticks will lay eggs that hatch in 3–8 weeks. *Otobius megnini*, *Ornithodoros parkeri*, and *Ornithodoros turicata* are additional argasid ticks that can affect rabbits (Schoeb et al., 2007).

FAMILY IXODIDAE (HARD TICKS)

Haemaphysalis leporis-palustris *Haemaphysalis leporis-palustris* is a common ixodidae tick that is found on both domestic and wild lagomorphs in North America (Pates and Gerrity, 1994). This tick requires three hosts to complete its life cycle and the rabbit can serve as a host for all three stages. Each stage requires departure from the host to the ground for feeding, thus prohibiting domestic rabbits maintained under proper husbandry conditions from fulfilling the role of a three-host animal. Larvae and nymphs usually attach to the head, especially the ears, and feed from 4–11 days (Soulsby, 1982). A heavy tick burden can lead to anemia and even death in wild rabbits. Diagnosis is made by accurate identification of the tick and treatment requires manual removal of the parasites. Maintaining adequate biosecurity minimizes exposure of domestic rabbits to both infected wild animals and infested vegetation.

Insects

Lice

Haemodipsus ventricosus (Figure 15.12) is the louse of *O. cuniculus* and is reported rarely in pet rabbits (Paterson, 2006). Other species of *Haemodipsus* infest other rabbit species (Durden and Rausch, 2007; Schoeb et al., 2007). The organism and its eggs may be seen with the naked eye. Heavy parasite loads can be associated with anemia, weight loss, alopecia, pruritis, and papules (Hess, 2004). Ivermectin, by subcutaneous injection at 4mg/kg, can be used to treat lice (Hess, 2004; Paterson, 2006). Topical imidacloprid has also been suggested as a treatment (Paterson, 2006).



FIGURE 15.12 *Haemodipsus ventricosus*. (from *Farmers Bulletin* 1568, revised 1931. From Railliet 1895. Used with permission of the University of North Texas Libraries, Digital Collection)

Laboratory rabbits are used in human louse research where the transmission of *Rickettsia* and immunization against human lice as a protective mechanism against infection are studied (Ben-Yakir et al., 1994; Houhamdi and Raoult, 2006; Mumcuoglu et al., 1997).

Fleas

Multiple flea species infect rabbits throughout the world. *Spilopsyllus cuniculi*, known as the rabbit flea (Figure 15.13), is the most frequently mentioned and studied although pet infestations with the dog and cat fleas (*Ctenocephalides canis* and *felis*, respectively) are becoming more commonly noted (Hamilton et al., 2005; Hess, 2004). Other species include *Cediopsylla simplex* (common eastern rabbit flea), *Odontopsylla multispinosus* (giant eastern rabbit flea), *Hystrichopsylla talpae*, *Caenopsylla laptevi*, *Xenopsylla cunicularis*, *Echidnophaga iberica* and *gallinacea*, and *Pulex irritans* (Bowman, 2003; Cooke, 1999; Osacar et al., 2001; Osacar-Jimenez et al., 2001; Schoeb, 2007; Visser et al., 2001). Seasonal variations exist in the population and parasitic potential for these flea species.



FIGURE 15.13 *Spilopsyllus cuniculi*. Cranial portion. (from *Farmers Bulletin* 1568, revised 1931. From Railliet 1895. Used with permission of the University of North Texas Libraries, Digital Collection)

A large concern with rabbit flea infestations is the transmission of pathogenic agents which use the fleas as vectors. Myxoma virus infection, endemic in many populations of *Sylvilagus* and devastating to *O. cuniculus*, is transmitted by fleas along with plague, Rocky Mountain spotted fever, and *Trypanosoma* species (Hamilton et al., 2005; Lowell et al., 2005; Reglero et al., 2007; Merchant et al., 2003a, 2003b, Schoeb et al., 2007; Stanford et al., 2007; von Reyn et al., 1976). Heavy flea infestations have been associated with outbreaks of rabbit hemorrhagic disease (calicivirus) (Lugton, 1999).

Fleas can be diagnosed by direct visualization of them and/or their feces in the fur. Pruritis, alopecia, excoriations, and other signs of trauma can be observed in affected animals. The face and ears are the main areas for clinical signs (Hess, 2004). Imidocloprid (0.4ml topical solution for rabbits weighing less than 4kg and 0.8ml in rabbits weighing greater than 4kg), selamectin, and lufenuron have all been reported to be effective against fleas in rabbits (Bowman, 2003; Hess, 2004). Rabbit environments should also be treated (Hess, 2004).

Flies (Dipterans)

FLIES

None of the parasites in the Order *Diptera* are considered important parasites of laboratory rabbits, however they may be considered relevant in outdoor-raised rabbits (Pates and Gerrity, 1994). Frequently occurring in wild lagamorphs in the western hemisphere are Botflies



FIGURE 15.14 Rabbit bot, *Cuterebra*. (from *Farmers Bulletin* 1568, revised 1931. From Osborn, 1896. Used with permission of the University of North Texas Libraries, Digital Collection)

or Warble flies of the genus *Cuterebra*. Commonly occurring species of *Cuterebra* in wild rabbits include *Cuterebra buccata*, *C. cuniculi*, *C. leporivora*, *C. abdominalis*, *C. jellisoni*, *C. ruficrus*, and *C. lepusculi* (Schoeb et al., 2007). Female flies, which can be up to 20mm long (Soulsby, 1992), deposit eggs near the nesting areas of wild rabbits. Upon hatching, the larvae penetrate the skin of the host directly or enter the host directly via natural orifices or wounds within the skin. The method of host entry is species-dependent. The larvae (Figure 15.14), which can be up to 25mm long (Schoeb et al., 2007), complete a quiescent phase and begin to migrate towards the subcutis. The resulting lesions consist of 2–3cm subcutaneous masses with a painful fistula. Occasionally there is aberrant migration of the larvae such as to the eye (Pates and Gerrity, 1994). Once the larva is ready to pupate it will drop to the group leaving behind a substantial hole in the host skin that should resolve without complication. Infection with multiple *Cuterebra* larvae can be debilitating to the host rabbit which may result in death. Controlling the fly population near any outdoor animal housing can serve to prevent *Cuterebra* infestation. Larvae can be removed from the animal using topical administration of iodine followed by manual extraction of the larvae using forceps (author's experience).

HELMINTHS

Nematodes

Obeliscoides cuniculi

BACKGROUND

The species *Obeliscoides cuniculi* is considered to be two subspecies based on whether it occurs in hares; *O. cuniculi cuniculi* or in cottontail rabbits; *O. cuniculi multistriatus* (Measures and Anderson, 1983). Experimental infection can occur with either species in the domestic rabbit. *O. cuniculi* has a worldwide distribution in wild and domestic rabbits (Schoeb et al., 2007). In experimental settings, guinea pigs are susceptible to infection (Alicata, 1932). There is only one report of an infection in a laboratory rabbit (Jensen et al., 1980) so this nematode is not considered of major importance in laboratory animal settings.

ETIOLOGY/ETIOLOGIC AGENT

Obeliscoides cuniculi is a member of the family Trichostrongylidae. Adult worms are pink and lack a buccal capsule. Females are 546 μ m wide and 15–18 mm long. Males are 230 μ m wide and 10–15 mm long. Eggs measure 75–91 by 42–53 μ m and are thin-shelled and oval (Alcata, 1932).

EPIZOOTIOLOGY

Rabbits become infected by consuming third-stage larvae. Ingested larvae penetrate the gastric mucosa within one day and by 3 days have developed into fourth-stage larvae. A final molt then occurs resulting in adult larvae that emerge on the surface of the gastric mucosa with females capable of dispensing eggs 16–20 days post infection (Alcata, 1932). The prepatent period is 16–20 days. Larvae are capable of maintaining a state of arrested development within the gastric mucosa and may continue development upon loss of adult worms (Schoeb et al., 2007).

CLINICAL MANIFESTATIONS

Clinical disease is generally not associated with natural infections, however heavy parasitemia can result in weight loss, anemia, and diarrhea (Schwartz and Shook, 1928). Similar clinical signs were noted in experimentally infected animals using a large volume of larvae. Animals returned to normal within 1–2 weeks of infection (Pakes and Gerrity, 1994).

PATHOLOGY

Gross pathologic change occurs in the stomach, resulting in a mucosal thickening with a finely granular “cobblestone” appearance with mucous and occasional petechial hemorrhages. The gastric mucosa appears thickened histologically due to glandular hyperplasia,

presence of larval parasites, and an inflammatory infiltrate comprised primarily of lymphocytes and eosinophils (Pates and Gerrity, 1994).

PATHOGENESIS

Infiltration of larvae and adult worms within the gastric mucosa result in glandular hyperplasia and inflammation which may impede the ability of gastric function.

DIAGNOSIS

Observation of *O. cuniculi* eggs within fecal samples can provide a clinical diagnosis. Gross identification of the adult nematode from gastric mucous scrapings can be made at necropsy.

PREVENTION AND THERAPY

Information pertaining to efficacy of treating *O. cuniculi* is limited. A variety of anthelmintic regimens have been used with some success including thiabendazole at 110 mg/kg four times a day on day one followed by eight additional doses at 70 mg/kg (Watkins et al., 1984) and fenbendazole at 50 ppm offered in feed for 5 days (Worley, 1963). Prevention is aimed at minimizing exposure to fecal matter and larvae through effective husbandry.

Trichurids

Trichuris leporis and *Sylvilagus*

BACKGROUND These two whipworms are found in hares (*Lepus* spp.) and cotton tails (*Sylvilagus*), as well as wild rabbits in Europe and the United States (Clemons et al., 2000; Hofing and Kraus, 2004; Schoeb, 2007).

ETIOLOGY/ETIOLOGIC AGENT Adult worms (Figure 15.15) and ova are the same size in both *T. leporis* and *T. sylvilagus*. Adult males measure 19–21 mm long and adult females measure 17.4–20.9 mm long while ova measure 60–65 μ m $\times$ 29 μ m with bipolar plugs (Schoeb et al., 2007).

EPIZOOTIOLOGY Adult whipworms are found in the cecum and large intestines. The mucosa is pierced by the anterior end (Schoeb et al., 2007). Eggs are passed by the animal's feces and become infective approximately 3 weeks later (Schoeb et al., 2007). Transmission is fecal-oral and larvae enter through the small intestinal mucosa eventually making their way to the cecum and large intestines (Schoeb et al., 2007). Several surveys of wild rabbits in the United States have demonstrated prevalence rates of 4–29% for *T. leporis* and *sylvilagus* (Andres and Davidson, 1980; Clemons et al., 2000; Strohlein and Christensen, 1983; Wiggins et al., 1980).

CLINICAL MANIFESTATIONS Weight loss has been associated with high parasite loads of *Trichuris* in snowshoe hares (Keith et al., 1986). Higher worm loads were

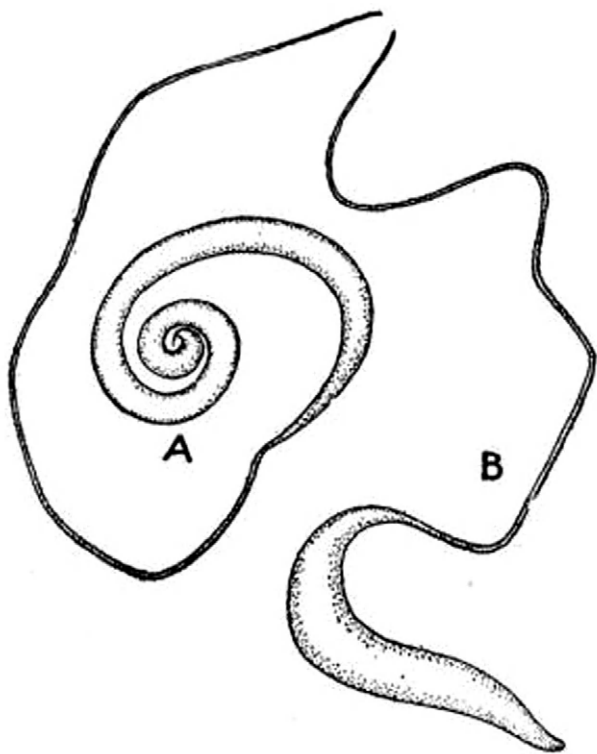


FIGURE 15.15 *Trichuris leporis*. (from Farmer Bulletin 1568, revised 1931. Enlarged. A is female and B is male. Used with permission of the University of North Texas Libraries, Digital Collection).

also reported in juveniles compared to adult snowshoe hares in the same epidemiological study (Keith et al., 1986).

PATHOLOGY There are no reports of lesions associated with these two trichurids (Andrews and Davidson, 1980).

PATHOGENESIS This is not applicable.

DIAGNOSIS Whipworm eggs can be identified in fecal preparations and adults can be found in the intestines at necropsy.

PREVENTION AND THERAPY Treatment regimens for these parasites have not been fully described. Ivermectin was reported as ineffective against *T. leporis* in snowshoe hares in Canada (Sovell and Holmes, 1996). As with other fecal-oral pathogens, good hygiene and eliminating contact with wild lagomorphs are important to controlling these parasites.

Filarids

Dirofilaria scapiceps and *uniformis*

BACKGROUND All members of the Superfamily Filarioidea are transmitted by blood-sucking insects.

Infection can be common among outdoor-housed rabbits (Schoeb et al., 2007). Rabbits can also be an aberrant host for the dog heartworm, *Dirofilaria immitis* (Hofing and Kraus, 1994).

ETIOLOGY/ETIOLOGICAL AGENT Members of this superfamily tend to be rather long and thin white-to-cream-colored worms without marked cuticular ornamentation or lips and have almost no buccal capsule (Bowman, 2003).

EPIZOOTIOLOGY Several species of mosquitoes serve as the intermediate host for these parasites. Mosquitoes become infected with microfilariae as they ingest a blood meal from an infected host. Microfilariae develop into infective third-stage larvae in the mosquitoes and are transmitted to a new host as the mosquito feeds. Larvae migrate to the subcutis, usually in the trunk area, where they mature into fifth-stage larvae. The fifth-stage larvae migrate to their tissue of choice and develop into the adult stage and begin to produce microfilaria. The prepatent period is 137–234 days (Hofing and Kraus, 1994; Schoeb et al., 2007). Adult *Dirofilaria scapiceps* live in the tendon sheaths of the hock joint and less frequently the fascia around the stifle joint (Hofing and Kraus, 1994). Adult *Dirofilaria uniformis* are found in the connective tissue of the trunk.

CLINICAL MANIFESTATIONS Infection is usually subclinical, though it may result in chronic tenosynovitis (Schoeb et al., 2007).

PATHOLOGY Adult forms may be found at necropsy. *D. scapiceps*-infected animals may exhibit chronic proliferative tenosynovitis with fibrinous exudate, hyperplasia, and hypertrophy of the synovial membrane and infiltration of lymphocytes and plasma cells (Hofing and Kraus, 1994).

PATHOGENESIS Infestations with these parasites are typically subclinical. Inflammatory responses to *D. scapiceps* adults in the tendon sheaths have the ability to cause localized disease.

DIAGNOSIS Adults can be identified at necropsy. Circulating microfilaria can be found in the blood.

PREVENTION AND THERAPY Prevention is via indoor housing and/or eliminating exposure to mosquitoes. Treatment has not been reported.

Brugia lepori

BACKGROUND Information on *Brugia lepori* is limited. The original published report is from cottontail rabbits (*Sylvilagus aquaticus* and *floridanus*) in Louisiana

(Eberhard, 1984). Infection of cottontail rabbits in the northeastern United States has also been reported (Eberhard et al., 1991).

ETIOLOGY The etiology is a filarial nematode, *Brugia lepori*.

EPIZOOTIOLOGY The details of the life cycle of *B. lepori* have not been reported. It is thought, however, that like for other members of this superfamily, mosquitoes are an intermediate host (Hofing and Kraus, 1994). Adults are found in abdominal lymphatics and subcutaneous tissue (Hofing and Kraus, 1994).

CLINICAL MANIFESTATIONS No clinical manifestations have been reported.

PATHOLOGY Adult organisms can be found at necropsy. Microfilaria are found in circulating blood.

PATHOGENESIS None reported.

DIAGNOSIS Adult organisms can be found at necropsy. Microfilaria can be found in circulating blood.

PREVENTION AND THERAPY If mosquitoes do serve as an intermediate host, prevention of exposure to infected mosquitoes should offer reasonable prevention of infection. No therapy is reported.

Metastrongylids

Protostrongylus boughtoni

BACKGROUND *Protostrongylus boughtoni* (Goble and Dougherty, 1943) is a metastrongylid parasite of the respiratory tract that occurs primarily in wild rabbits and hares of the northern regions of North America (Hofing and Kraus, 1997).

ETIOLOGY/ETIOLOGIC AGENT Both male and female worms possess six small lips on the anterior end. Male worms are 13–26 mm by 160–250 µm with a copulatory bursa and spicules that measure 260–320 µm. Female worms are larger than males measuring 21–36 mm in length by 200–300 µm in diameter with the vulva located at the abrupt narrowing of the body, 200 µm from the distal end.

EPIZOOTIOLOGY Wild hares and rabbits become infected by consuming an intermediate gastropod host, such as the small land snail *Vallonia pulchella*, while feeding (Hofing and Kraus, 1997). Adult worms inhabit the bronchi where eggs hatch and develop into first-stage larvae. These larvae are coughed up, swallowed, and leave the body via feces.

CLINICAL MANIFESTATIONS Clinical disease has not been adequately described.

PATHOLOGY Pulmonary lesions appear grossly as small, firm, discrete, yellow to gray nodules. Histopathology can reveal peribronchitis and bronchitis which is often more severe in hares than in cottontail rabbits (Goble and Dougherty, 1943).

PATHOGENESIS Infiltration of adult worms within the bronchi can cause inflammation and the formation of parasitic nodules.

DIAGNOSIS *P. boughtoni* larvae can be identified in feces using Baermann floatation methods.

PREVENTION AND THERAPY There have been no reported cases of infection in domestic rabbits. Prevention relies on proper husbandry methods. Ivermectin was reported not to be as effective against nematodes in snowshoe hares compared to other domestic and laboratory animals (Sovell and Holmes, 1996).

Oxyurids

Passalurus ambiguus

BACKGROUND Oxyurids are commonly known as pinworms. *P. ambiguus* is also known as *Oxyuris ambigua* and is the most common pinworm in rabbits in the United States and is distributed in rabbits worldwide (Hofing and Kraus, 1994; Schoeb et al., 2007). Pinworms are uncommonly encountered in SPF rabbits today (Schoeb et al., 2007).

ETIOLOGY/ETIOLOGIC AGENT *P. ambiguus* ova have the typical pinworm appearance being elongated, measuring 93–105 µm long by 43 µm wide (Schoeb et al., 2007). They are flattened on one side (Schoeb et al., 2007). Adults (Figure 15.16) are generally 0.5–1 cm in length (Richardson, 2000; Schoeb et al., 2007).

EPIZOOTIOLOGY The pinworm life cycle is direct and transmission is fecal–oral. Adult worms reside in the cecum and large intestine. Migrating worms may be stuck around the anal area (Richardson, 2000). A viewpoint of some authors is that pinworms should now be thought as normal inhabitants of the rabbit intestines as they are typically non-pathogenic (Richardson, 2000).

CLINICAL MANIFESTATIONS Reports in the veterinary literature note that pinworms are generally non-pathogenic. Granulomatous appendicitis and lymphadenitis and poor reproduction (Düwel and Brech, 1981) have been described in rabbits infected with *P. ambiguus* (Schoeb et al., 2007). Heavy worm

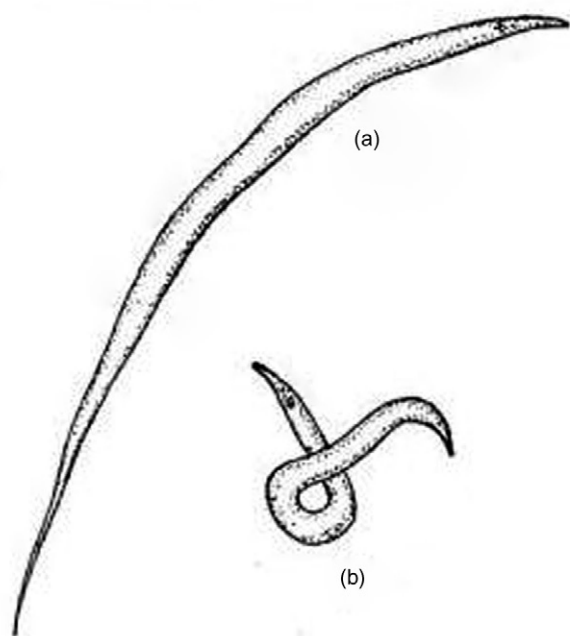


FIGURE 15.16 *Passalurus ambiguus*. (from *Farmers Bulletin* 1568, revised 1931. Enlarged 8 times. A is female and B is male. Used with permission of the University of North Texas Libraries, Digital Collection)

burdens have been associated with perianal pruritis with self-inflicted trauma, and rectal prolapse (Hess, 2004).

PATHOLOGY Limited pathological changes, as previously described, have been noted in animals infested with the pinworms. *P. ambiguus* juveniles can be found in microscopic intestinal sections with normal staining (Schoeb et al., 2007).

PATHOGENESIS Due to the rarity of clinical manifestations, the pathogenesis of the organism and associated disease has not been well described.

DIAGNOSIS Ova, and sometimes adult worms, can be found in fecal samples. At necropsy, adult worms may be seen in the cecum and large intestines (Schoeb et al., 2007). A perianal cellophane tape test could be used to find adults stuck to the anal region (Stein and Walshaw, 1996). Molecular diagnostic approaches (PCR) have been developed (Feldman and Bowman, 2007). Afternoon and evening hours appear to be the best times for fecal collection for the identification of *P. ambiguus* (Rinaldi et al., 2007).

PREVENTION AND THERAPY Main treatments for eradicating pinworm infestations include fenbendazole and ivermectin. Fenbendazole is dosed at 10–20 mg/kg and repeated in 2 weeks (Richardson, 2000). For entire

colonies, fenbendazole at 50 ppm for 5 days in the feed has been reported as efficacious (Düwel and Brech, 1981). The ivermectin dose is the same as for other parasites at 0.4 mg/kg (Richardson, 2000). This ivermectin treatment regimen was described ineffective in ridding snowshoe hares of *P. ambiguus*, however, a recent report of a combined therapy of albendazole (20 mg/kg orally once a day for 14 days) and ivermectin was 100% effective at eradicating *P. ambiguus* in a small colony of New Zealand Red rabbits meant as breeding stock for a laboratory animal vendor (Pritt et al., 2009). Piperazine is reported as a treatment for both young and adult rabbits (Hess, 2004; Schoeb et al., 2007). The anal region should be cleaned at the time of treatment initiation (Hess, 2004).

Good hygiene and ensuring that incoming animals are free of the parasite are important control measures. Due to the shedding of adults and ova that are immediately infective, decontamination of the environment is encouraged when treating for this agent (Hess, 2004).

Dermatoxys veligera

Very little is reported on *Dermatoxys veligera*. It is known to occur in wild rabbits in the United States and infection of domestic rabbits has been reported. Adult parasites live free in the lumen of the cecum and do not attach to the mucosa. Pathology is not reported in conjunction with adult parasites. Fourth-stage larvae attach to the cecal mucosa by hooks and small ulcers may form at the attachment sites. Female parasites are 16–17 mm in length and 600 µm in diameter. Males are 8–11 mm in length and 435 µm in diameter. Eggs are 50 µm by 110 µm with one side slightly flattened (Hofing and Kraus, 1994; Schoeb et al., 2007).

Ascarids

VISCERAL LARVA MIGRANS/CEREBROSPINAL NEMATODIASIS

BACKGROUND Rabbits are not a definitive host but are an aberrant host for ascarid species infecting carnivores. Rabbits have been used to study host immune responses to *Toxocara canis* and the tissue migrating larval stages of the bovine ascarid *T. vitulorum* (Hofing and Kraus, 1994). *T. canis* is an important zoonotic disease of humans, however it is not transmitted by rabbits.

ETIOLOGY *Baylisascaris procyonis*, with the raccoon as its definitive host, is the main causative agent of visceral larval migrans in rabbits. Other sources report *Baylisascaris columnaris* (definitive host is the skunk) and *Toxocara canis* (definitive host is the dog) (Hofing and Kraus, 1994).

EPIZOOTIOLOGY Adult ascarids live in the small intestine of the definitive host and produce eggs which

are shed in the feces. Eggs become infective about 30 days after being passed. Eggs are very resistant to environmental extremes and can remain infective in soil for many years (Bowman, 2009). Rabbits become infected by ingesting infectious eggs. The eggs hatch in the intestine and the larvae migrate throughout the organs and tissues. Because the rabbit is an aberrant host, the migration is not completed and the larvae do not mature (Hofing and Kraus, 1994; Schoeb et al., 2007).

CLINICAL MANIFESTATIONS Clinical signs vary depending upon the location of the parasite migration. The most pronounced clinical signs occur with migration in the central nervous system and include torticollis, ataxia, ear droop, and circling (Hofing and Kraus, 1994).

PATHOLOGY The migrating stage may be seen grossly and, due to its size, one larva can do a significant amount of damage. Histologically, granulomas may be seen along the path the larva has migrated, particularly in the viscera (Hofing and Kraus, 1994; Schoeb et al., 2007). Central nervous system lesions may include necrosis, hemorrhage, and a mixed inflammatory response of lymphocytes and polymorphonuclear cells (Schoeb et al., 2007).

PATHOGENESIS The pathogenesis of the disease is primarily due to damage caused by migrating larva but also to inflammatory response of host.

DIAGNOSIS Visceral larval migrans is typically diagnosed at necropsy via identification of ascarid larvae in tissues.

PREVENTION AND THERAPY Prevention of exposure of definitive host (raccoon, skunk, dog) to caging, bedding, and feed is essential. Treatment with mebendazole (100 mg/kg) for 3 days is reported to be effective against migrating larvae (Cuellar et al., 1990).

Cestodes

Anoplocephalids

BACKGROUND

Anoplocephalids are characterized by lacking a rostellum or hooks (Hofing and Kraus, 1994). Tapeworm infestation in rabbits is considered rare (Richardson, 2000).

ETIOLOGY/ETIOLOGIC AGENT

Several species within the anoplocephalid group have been described as inhabiting rabbits. *Cittotaenia variabilis*, *Mosgovoyia pectinanta americana*, *Mosgovoyia perplexa*, *Monoecocestus americana*, and *Ctenotaenia*

ctenoides have been reported in domestic and wild rabbits in North America (Hofing and Kraus, 1994). Other texts outline the scolex sizes, adult worm length, and approximate number of proglottid for the above species (Hofing and Kraus, 1994; Schoeb, 2007).

EPIZOOTIOLOGY

These cestodes are all located in the small intestines. Oribatid mites are the usual intermediate hosts and rabbits are the definitive hosts, although the life cycles for all anoplocephalid species of rabbits have not been completely documented (Hofing and Kraus, 1994; Schoeb et al., 2007). Transmission is fecal-oral where the rabbit ingests a mite with cysticercoids (Hofing and Kraus, 1994).

CLINICAL MANIFESTATIONS

Enteritis along with emaciation, intestinal perforation, and obstruction has been associated with anoplocephalid infestation (Hofing and Kraus, 1994; Patton et al., 2008). Usually the infestation does not produce clinical signs of disease (Schoeb, 2007).

PATHOLOGY

Clinical signs are associated with higher worm loads. Gross pathological findings would be associated with physical factors involving the number of anoplocephalids present.

PATHOGENESIS

The effects of anoplocephalids are based on the physical presence of excess numbers of organisms.

DIAGNOSIS

Anoplocephalids can be diagnosed in rabbits based on egg identification in the feces and or the gross presence of proglottids.

PREVENTION AND THERAPY

Good pest control combined with keeping infected animals out of research colonies prevent anoplocephalid infestations. Due to the need for an intermediate host, it is unlikely that anoplocephalid infection could continue in rabbits housed in typical laboratory animal facilities (Hofing and Kraus, 1994).

Treatment can be achieved with praziquantel at 5 mg/kg which is repeated in 10 days (Hofing and Kraus, 1994; Richardson, 2000).

Railletina

Several species of *Railletina* occur in wild rabbits, however natural infection of domestic rabbits is not reported (Schoeb et al., 2007). Very little is reported on *Railletina* in general. The species found in wild rabbits

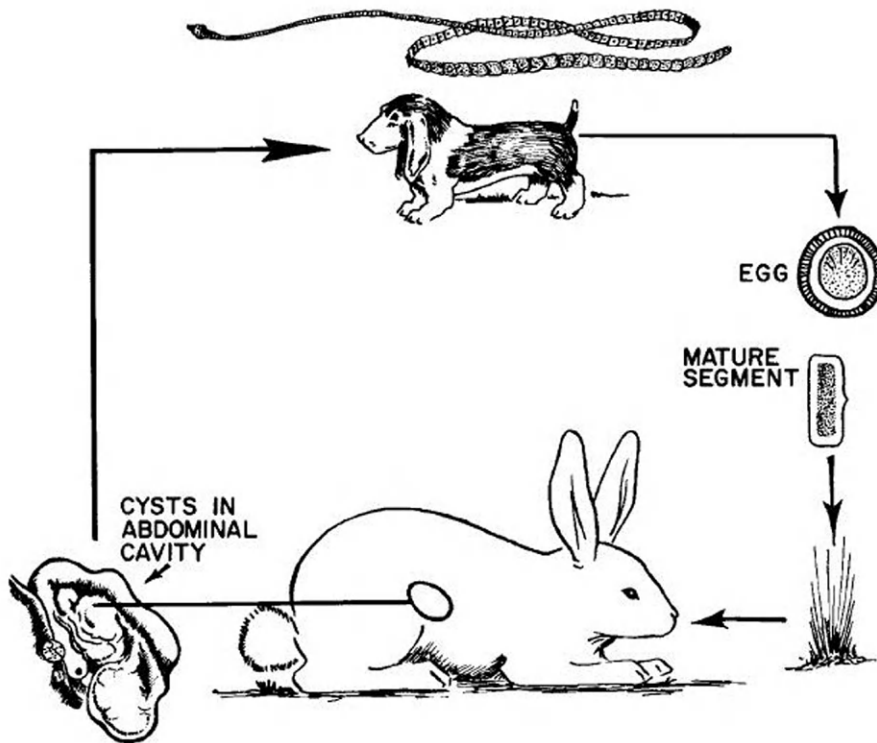


FIGURE 15.17 *T. pisiformis* (adult) and life cycle. (from Patton, N.M., K.W. Hagen, J.R. Gorham, and R.E. Flatt. 2008. *Domestic Rabbits: Diseases and Parasites*. PNW 310. Corvallis, OR: Oregon State University Extension Service. © 1986, 1998 Oregon State University. Used with permission)

include *Raillietina salmoni* (also known as *R. stilesiella*/*R. stilesiella*), *R. selfi*, *R. loeweni*, and *R. retractalis* and are thought to be similar to other members of their class, Cestoda. Members of this class lack an alimentary tract with food absorption and gaseous exchange occurring through the cuticle. The rabbit is the definitive host for members of the genus *Raillietina* (Hofing and Kraus, 1994). *Raillietina loeweni* are known to use ants as intermediate hosts and are transmitted to rabbits via ingestion of cysticeroid-infected ants (Bartel and Hansen, 1967). Most tapeworm infections are subclinical, however large numbers of organisms can result in enteritis, intestinal obstruction, and perforation of the gastrointestinal tract. Adult cestodes are found in the small intestine.

Taenia pisiformis

BACKGROUND

Taenia pisiformis is the most common taeniid tapeworm of dogs in the United States and is also found in foxes and less frequently in other carnivores. Rabbits serve as an intermediate host. *T. pisiformis* is not known to infect humans (Schoeb et al., 2007). Until the middle of the nineteenth century the larval stage and the adult stage were thought to be separate species (Bowman, 2009).

EPIZOOTIOLOGY

Definitive hosts (typically dogs) pass the egg-laden proglottid phase in their feces (Figure 15.17). Rabbits

are infected with the eggs via ingestion of materials contaminated with fecal material from infected animals. Once ingested, the egg hatches and the hexacanth embryo enters the wall of the intestine and migrates to its organ of choice via the portal veins (Bowman, 2009; Schoeb et al., 2007). *Taenia pisiformis* typically prefers the liver and other viscera. The hexacanth embryo grows, cavitates, and differentiates to form the second larval stage which consists of a fluid-filled bladder with a scolex called a cysticercus. This second larval stage is also often called bladderworm and forms 2–4 weeks following ingestion (Bowman, 2009; Schoeb et al., 2007). The cysticercus stage encysts on the liver capsule (Figure 15.18) or other abdominal serosal surfaces and is ingested by the definitive host when it consumes the intermediate host (Schoeb et al., 2007).

CLINICAL MANIFESTATIONS

Infestation is usually asymptomatic. Severe infections, more typical of experimental inoculations, can cause severe hepatitis with chronic wasting or death (Soulsby, 1982).

PATHOLOGY

Liver migration can result in focal granulomatous inflammation and fibrosis. Cysticercus forms can be seen on liver capsule and serosal surfaces of other viscera.

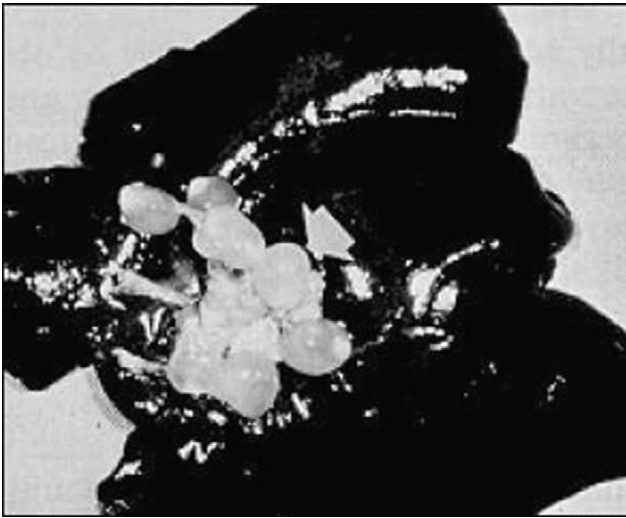


FIGURE 15.18 Liver with cysticerci (arrow). (from Patton, N.M., K.W. Hagen, J.R. Gorham, and R.E. Flatt. 2008. *Domestic Rabbits: Diseases and Parasites*. PNW 310. Corvallis, OR: Oregon State University Extension Service. © 1986, 1998 Oregon State University. Used with permission)

PATHOGENESIS

Typically asymptomatic; severe infections cause disease due to liver damage from migration and resulting inflammation from host response.

DIAGNOSIS

Typically diagnosed at necropsy as an incidental finding. Peri-mortem diagnosis is possible with imaging techniques such as radiography and ultrasound (Schoeb et al., 2007).

PREVENTION AND THERAPY

Prevention is via proper husbandry to prevent contamination of feed, caging, and environment with feces from infected animals. This includes proper washing of fresh foods. Insect control is important also, as some sources report mechanical transmission by blowflies (Lawson and Gemmell, 1990). Treatment with mebendazole is reported to kill both the mature and immature cysticerci (Heath et al., 1975). Other sources recommend praziquantel or the combination of febantel/pyrantel embonate/praziquantel as effective against cestode infections (Mencke, 2007).

Taenia serialis

BACKGROUND

Taenia serialis is less widely distributed and less common than *T. pisiformis* (Schoeb et al., 2007). Rabbits serve as an intermediate host. This agent causes rare cases of human coenurosis but it is not transmitted from rabbits.

EPIZOOTIOLOGY

Definitive hosts (typically dogs) pass the egg-laden proglottid phase in their feces. Rabbits are infected with the eggs via ingestion of materials contaminated with fecal material from infected animals. Once ingested, the egg hatches and the hexacanth embryo enters the wall of the intestine and migrates to its organ of choice via the portal veins (Bowman, 2009; Schoeb et al., 2007). *T. serialis* typically prefers the subcutaneous tissues or viscera. The hexacanth embryo grows, cavitates, and differentiates to form the second larval stage which consists of a fluid-filled bladder with multiple scolices called a coenurus. Each scolex in the coenurus is capable of developing into a mature tapeworm (Bowman, 2009). This second larval stage is also often called bladderworm and forms 2–4 weeks following ingestion (Bowman, 2009; Schoeb et al., 2007). The coenurus stage encysts in the subcutaneous tissue primarily but can also occur in the musculature, abdomen, or brain (Hofing and Kraus, 1994). The coenurus stage is ingested by the definitive host when it consumes the intermediate host (Schoeb et al., 2007).

CLINICAL MANIFESTATIONS

Subcutaneous coenuri usually are asymptomatic. Coenuri in the musculature may affect mobility (Schoeb et al., 2007). Coenuri in atypical locations, such as the brain, may cause neurologic disturbances (Bowman, 2009).

PATHOLOGY

Liver migration of the worm can result in focal granulomatous inflammation and fibrosis. Coenurus forms can be seen in the subcutaneous tissues.

PATHOGENESIS

While infection is typically asymptomatic occasionally severe cases are described as noted above.

DIAGNOSIS

Infection is diagnosed at necropsy usually as an incidental finding. Peri-mortem diagnosis is possible with imaging techniques such as radiography and ultrasound (Schoeb et al., 2007).

PREVENTION AND THERAPY

Prevention is via proper husbandry to prevent contamination of feed, caging, and environment with feces from infected animals. This includes proper washing of fresh foods. Insect control is important also as some sources report mechanical transmission by blowflies (Lawson and Gemmell, 1990). Some sources recommend praziquantel or the combination of febantel/pyrantel embonate/praziquantel as effective against cestode infections (Mencke, 2007).

Trematodes

Hasstilesia tricolor

BACKGROUND

Hasstilesia tricolor is an intestinal fluke which infects wild rabbits in the United States (Hofing and Kraus, 1994).

ETIOLOGY/ETIOLOGIC AGENT

This fluke is small, measuring 350–640µm long and 480–1200µm wide with ventral and oral suckers and a spinous cuticle (Schoeb et al., 2007). Its name stems from three color shades seen on living organisms (Schoeb et al., 2007). *H. tricolor* ova look like typical operculated trematod ova and dimensions of 20–22µm in length by 13–15µm in width (Hofing and Kraus, 1994; Schoeb et al., 2007).

EPIZOOTIOLOGY

Wild rabbits are the definitive host while land snails are the intermediate hosts (Schoeb et al., 2007). Snail or metacercariae ingestion by rabbits results in infestation (Hofing and Kraus, 1994; Schoeb et al., 2007). Prevalence rates in wild rabbit populations range from 1 to 41% (Jacobson et al., 1974).

CLINICAL MANIFESTATIONS

Infestation with *H. tricolor* can result in diarrhea, enteritis, gross hemorrhage in the feces, emaciation, and death (Jacobson et al., 1974). A lack of other descriptions of clinical findings leads many to believe that infections are largely subclinical (Schoeb et al., 2007).

PATHOLOGY AND PATHOGENESIS

When clinical signs are present, infecting flukes destroy intestinal villi and also exhibit fluke-to-fluke attachment through ventral suckers (Jacobson et al., 1974). Physical pressure may be exerted on the intestinal mucosa with heavy infestations (Jacobson et al., 1974).

DIAGNOSIS

Eggs can be observed in fecal samples while adults may be seen in the intestines.

PREVENTION AND THERAPY

Treatment regimens have not been documented. To prevent infection, rabbits should not have access to snails or metacercariae (Schoeb et al., 2007).

Fasciola hepatica

BACKGROUND

Fasciola hepatica is a parasite of herbivorous mammals and humans. Distribution is worldwide and is dependent upon lymnaeid snails as an intermediate

host. These snails require neutral soil which remains moist throughout the year and tend to do better in areas with moderate winters which allow the eggs and juvenile stages to survive (Bowman, 2009). Due to the habitat requirements of the intermediate hosts, distribution may be patchy. The rabbit has been used as a research model for studying *Fasciola hepatica* infection and the host response to infection (Hofing and Kraus, 1994).

ETIOLOGY/ETIOLOGIC AGENT

Fasciola hepatica is a member of the Order Digenea. Members of this order undergo indirect development with sexual and asexual stages parasitizing alternate hosts. (Bowman, 2009).

EPIZOOTIOLOGY

Infection is via ingestion of the metacercaria cyst stage found on the surface of plant material. Following ingestion the cyst is digested in the small intestine to release a young fluke called a marita. The marita penetrates through the wall of the intestine and travels through the peritoneal space to penetrate the liver. The marita travels throughout the liver (typically for several weeks) before it enters the bile ducts and matures into an adult fluke. Egg production begins about a month and a half after infection. Eggs (fertilized ovum and a cluster of vitelline cells enclosed in an operculated capsule) are carried with the bile into the intestinal lumen and then exit the body in the feces. If eggs fall into water it will develop a ciliated larva inside it called a miracidium. The miracidium has a cone-shaped papilla at its anterior end for boring into the intermediate host, the snail, and is covered in cilia for mobility. The miracidium also has a pair of eye spots, a brain, a rudimentary excretory system, and a cluster of germinal cells (Bowman, 2009). After 2–4 weeks at summer temperatures the miracidium is ready to hatch and leaves the egg capsule to swim in search of a lymnaeid snail, its intermediate host. The miracidium needs to find a host within 24 hours of hatching, otherwise it will exhaust its energy supply and die (Bowman, 2009). Once a miracidium finds a suitable host, it bores into the snail body and migrates to the gonad or digestive gland (often called the liver) and forms a sporocyst (Bowman, 2009). The germinal cells grow and divide into germinal balls which develop into redia. The redia grow until they rupture the sporocyst wall and enter the tissues of the snail where they use their mouth and digestive organs to eat their way through the tissues. The redia contain germinal balls also which grow and divide into a third type of larva, the cercaria (Bowman, 2009). The cercaria, a tadpole-like larva with a disc-shaped body and a long tail, develops in 1–2 months at summer temperatures and leaves the redia through a birth pore. The cercaria travels through the tissue of the snail into the surrounding water. The cercaria contains some

adult organs along with secretory cells near the pharynx that are used to secrete a cyst wall around the final larval stage (Bowman, 2009). After leaving the snail, the cercaria swim through the water until reaching plant material. The cercaria travels up the plant material to a level above the surface of the water where it encysts, loses its tail, and becomes a metacercaria and waits to be ingested.

CLINICAL MANIFESTATIONS

Some sources report asymptomatic infection of rabbits with *Fasciola hepatica* (Schoeb et al., 2007). Other sources describe acute and chronic forms most typically seen in large ruminant animals but with the potential to occur in any host (Bowman, 2009). The acute clinical syndrome is attributed to the invasion of the liver by the metacercariae and their subsequent migration through the liver. Damage caused by the migration results in inflammation with clinical signs of abdominal pain and reluctance to move. Other animals with fluke migration and concurrent bacterial infection with clostridial organisms often present as acute death with no outward clinical signs (Bowman, 2009). The chronic clinical syndrome is attributed to the presence of adult flukes in the bile ducts. Clinical signs include progressive weight loss, weakness, anemia, and hypoproteinemia with development of edema (Bowman, 2009).

PATHOLOGY

Gross necropsy findings in the acute clinical syndrome may include blood-stained exudate in the abdominal cavity and an enlarged, friable liver covered with fibrin tags (Bowman, 2009). Microscopic findings include fibrous tracts in the liver due to parasite migration and granulomatous reactions surrounding eggs in the liver parenchyma (Hofing and Kraus, 1994). Gross necropsy findings in the chronic clinical syndrome may include distended, thickened bile ducts with adult organisms present (Bowman, 2009). Microscopic findings include ulcers in the gallbladder, chronic cholangitis, and hyperplasia of the bile ducts (Hofing and Kraus, 1994). Some sources attribute the bile duct hyperplasia to the excretion by *F. hepatica* of large amounts of the amino acid proline (Isseroff et al., 1977).

PATHOGENESIS

Clinical signs are due to the presence of the organism and body response due to fluke migration, foreign body presence, and functional obstruction of the biliary tract.

DIAGNOSIS

Ante-mortem diagnosis is typically by demonstration of eggs in the feces. Post-mortem diagnosis is by observation of flukes in the liver and/or bile ducts.

PREVENTION AND THERAPY

Prevention of infection can be achieved by preventing ingestion of infective metacercariae via careful selection of food sources for laboratory animals, including vegetation used for enrichment. Albendazole and clorsulon can be administered for removal of liver flukes (Bowman, 2009).

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Rabbit Neoplasia

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INTRODUCTION

Preliminary Considerations

Several earlier reviews have summarized the literature dealing with spontaneous neoplasia in the domestic rabbit, *Oryctolagus cuniculus* (Blount, 1957; Cohrs et al., 1958; Courteau, 1935; Dobberstein and Tamaschke, 1958; Dumas, 1953; Fardeau, 1931; Lesbouyries, 1963; Lombard, 1962b; Polson, 1927; Seifried, 1937; Stedham, 1976). This chapter presents new data that have accumulated on the subject from case reports, descriptions of biological aspects of a variety of naturally occurring tumors, and reports of experimentally induced tumor models. This chapter gathers the data and presents an overview of the neoplastic process in the various rabbit species. It should be pointed out that where it appears that information is lacking on particular organs or systems (e.g., in Table 16.3) that is only because it was not encountered in our review.

Sufficient information is available to enable the description of an oncological profile as a function of age and sex for *Oryctolagus*. Although much of the early data originated from rabbit colonies associated with major research institutions, the growing popularity of domestic rabbits as pets allows an increased ability to accumulate data on the spontaneous incidence of tumors in rabbits that are allowed to live a natural life span. Whereas, naturally occurring neoplasms in the rabbit were previously thought to be sporadic, there is evidence for a regular pattern in the incidence of the various tumor types known to be naturally associated with *Oryctolagus*. It should be noted that many pet animals are neutered and this condition may skew the information on the incidence of urogenitary tumors, especially those tumors that are known to be hormone-responsive, such as uterine and mammary adenocarcinomas. More information is available with regard to the neoplastic process in *Oryctolagus* than is the case with species of *Lepus* (hares) or *Sylvilagus* (cottontail rabbits), but this perhaps is fortunate since *Oryctolagus* is the major research, domesticated, food, and companion animal species of the lagomorphs.

Influence of Genetic Predisposition

The influence of genetic background on cancer incidence has been well established in many species. This is clearly evidenced when linebreeding and inbreeding are practiced for the purpose of promoting the occurrence of tumors. This has been the pattern in domestic poultry (Hutt, 1958) and in the mouse, not only with reference to spontaneous tumors but also with neoplasia induced by oncogenic viruses (Gross, 1970), ionizing radiation (Upton and Futh, 1958), and more recently,

in genetically engineered mice. Several lines of evidence lead to the conclusion that this is also the case in domestic rabbits.

In the large rabbit colony maintained by Greene between 1933 and 1955 for the purpose of studying the influence of genetics on a number of disease processes, particularly cancer, records were kept on many stocks (14 "pure" breeds), stock crosses, and familial lines. Conclusive evidence was drawn on the inheritance of susceptibility to adenocarcinoma of the uterus (Greene, 1959); the familial distribution of mammary carcinoma of two distinct (papillary versus adenoid) types (Greene, 1939a, 1939b, 1939c); and on the inheritance of embryonal nephroma (Greene, 1943) in *Oryctolagus*. Similarly, in the Phipps colony maintained for some 30 years by Lurie in which inbred lines were established for studying the inheritance of resistance to tuberculosis (Lurie, 1964) the occurrence of adenocarcinoma of the uterus, as a function of age and familial relationship, was studied as well, with observations similar to those reported by Greene (Adams, 1962; Ingalls, 1964).

The Brown-Pearce carcinoma is also heritably influenced both in terms of transplantability and with regard to tumor antigens that are recognized by host rabbits. As examples, Casey (1939) found that Blue Cross and Lilac Cross breeds of *Oryctolagus* possess natural resistance to growth of Brown-Pearce carcinoma, whereas Chocolate Dutch rabbits were more susceptible and remain the favored breed for transplantation of this tumor. Kidd et al. demonstrated that the carcinoma contained both normal tissue antigens and tumor-specific antigens (Friedewald and Kidd, 1945; Kalfayan and Kidd, 1953; Kidd, 1946a) and showed further that certain host breeds recognized only the tumor antigen(s) as foreign (e.g., Blue Cross and Chocolate Dutch) whereas others recognized, in terms of antibodies produced in response, both types or neither.

From the rabbit colony maintained for the study of genetics at the Jackson Laboratory, a series of reports has indicated that susceptibility to certain malignancies of the lymphoreticular system are inherited (within strains) according to a pattern consistent with that of an autosomal recessive gene (or, alternatively that of a vertically transmitted C-type RNA virus). This pattern was shown to be true for lymphoma in the WH strains (Fox et al., 1970) and for a disease with features of autoimmune hemolytic anemia and thymoma in the X strain (Fox et al., 1971b) similar to that described in the New Zealand Black mouse.

Incidence of Neoplastic Disease

It is difficult to determine the actual incidence of spontaneous neoplasms in the domestic rabbit, *Oryctolagus cuniculus*, as well as wild rabbits of the

Genus *Sylvilagus* and *Lepus*. The normal lifespan of the domestic rabbit *Oryctolagus* is 7–8 years, but they are used at younger ages in most research studies and breeding colonies and are seldom maintained through their natural lifespan. Rabbits are similar to most species in that neoplasia is infrequent in juveniles and young adults. Fragmented data reported from various research laboratories from 1950–1970 reported an overall tumor incidence of 0.5–2.7% in rabbits that were primarily 2 years of age or younger (Weisbroth, 1994). Some authors have suggested that most of the rabbits in these necropsy series were too young to be in an cancer-prone age group, which tended to give an artificially low and misleading picture of the true incidence of neoplasms in this species (Dobberstein and Tamaschke, 1958; Hoffman, 1954; Loliger, 1968). Greene (1959) reported that 16.7% of 849 female rabbits (dying of various causes) were found to have uterine adenocarcinoma. Importantly, the rabbits in the colony were permitted to live until death from natural causes, and the average age was substantially greater (>4 years) than those in the surveys mentioned previously. The importance of the age factor in these data is underscored by the observation that the tumor incidence was 4.2% in female rabbits 2–3 years of age and 79.1% in rabbits aged 5–6 years. Others, notably Ingalls (Ingalls, 1964), have observed the age-associated increase in frequency of uterine adenocarcinoma.

The factors of age and sex are further analyzed in Table 16.1 using data from the tumor series collected in Weisbroth's laboratory over a 5-year period, drawn from a necropsy series of approximately 600 rabbit accessions from a colony of laboratory rabbits. Table 16.2 summarizes the frequency of tumor types, ranked in terms of decreasing frequency, as a function of age and sex. A dramatic increase (seven times) in the incidence of neoplastic disease occurs after the second year of life. The incidence was approximately the same (about 8%) for both males and females over 2 years of age, but the incidence was determined by different types of tumors in the two sexes (see Table 16.2). In the younger age groups, however, the tumor incidence was approximately six times greater in females than males (1.00% compared to 0.16%) and was almost entirely due to adenocarcinoma of the uterus. In fact, if the latter entity were eliminated from the series for females less than 2 years of age, the tumor incidence would be similar to that for young males.

Much of the data presented in the earlier edition of this text originated from colonies of laboratory rabbits kept for use in biomedical research. The tumors with the greatest incidences noted in these animals were uterine adenocarcinoma, lymphoma, embryonal nephroma, and bile duct adenoma. Table 16.3 summarizes reports of 855 naturally occurring tumors which

TABLE 16.1 Incidence of Neoplasia in *Oryctolagus cuniculus*: Influence of Age and Sex^a

Sex/Age	N ^b	Neoplasms	% of Total With Neoplasms	% of Age-Sex Category With Neoplasm	
				>2 Years	<2 Years
M <2 years	217	1	0.16	0.46	
M >2 years	54	5	0.83	9.26	
F <2 years	275	6	1.00	2.18	
F >2 years	53	4	0.66	7.54	
Total F	328	10	1.66		
Total (M + F)	599	16	2.65	8.4	1.4

^aN, number of accession; M, male; F, female.

^bAfter Weisbroth (1972).

TABLE 16.2 Incidence of Tumor Types in *Oryctolagus cuniculus*^a

Tumor Type	N ^b	M		F	
		<2 Years	>2 Years	<2 Years	>2 Years
Uterine adenocarcinoma	5			4	1
Lymphoma	4		1	2	1
Embryonal nephroma	2	1		1	
Bile duct adenoma	2		1		1
Osteosarcoma	1		1		
Osteochondroma	1				1
Leiomyoma	1		1		
Basal cell adenoma	1				1
Rectoanal papilloma	1		1		
Total	18	1	5	7	5

^aAfter Weisbroth (1972).

^bM, Male; F, Female.

includes these earlier reports as well as a large number of more recently reported cases of neoplasms in both laboratory and pet rabbits. Adenocarcinoma of the uterus continues to be the most frequent and important spontaneous neoplasm of *Oryctolagus*, both in the laboratory and in pet animals. It has been the subject of intensive investigation and is well understood biologically. The three next most common tumors occurring in laboratory rabbits in those earlier reports were lymphoma, embryonal nephroma, and bile duct adenoma. This incidence has now changed due to the increased number of reports submitted from pet

TABLE 16.3 Naturally Occurring Tumors of *Oryctolagus cuniculus*

Organ System	Organ	Tumor Type and Site	No. of Cases	Reference
Reproductive system				
Female				
	Uterus			
		Uterine adenocarcinoma		
			1	(Lack, 1900)
			1	(Shattock, 1900)
			1	(Wagner, 1905)
			1	(Selinow, 1907)
			4	(Boycott, 1911)
			1	(Marie, 1911)
			1	(Leitch, 1911)
			1	(Katase, 1912)
			13	(Stilling and Beitzke, 1913)
			2	(Paine and Peyron, 1918)
			1	(Dible, 1921)
			5	(Polson, 1927)
			1	(Rusk, 1927)
			1	(Koyama, 1927)
			1	(Usawa, 1930)
			1	(Watrin, 1930)
			1	(Cutler, 1934)
			1	(Twort, 1937)
			5	(Orr, 1938)
			2	(Witherspoon, 1938)
			2	(Medlar and Sasano, 1937)
			142	(Greene, 1938; Greene, 1939a, 1942a; Greene and Newton, 1948; Greene and Strauss, 1949; Greene, 1959; Greene, 1965)
			15	(Burrows, 1940)
			1	(Biese, 1945)
			1	(Dobberstein and Tamaschke, 1958)
			3	(Lombard, 1959)
			2	(Merrian, 1960)
			86	(Ingalls, 1964)
			2	(Cotchin, 1964)
			8	(Baba and von Haam, 1967a, 1967b)
			2	(Loliger, 1968)
			1	(Flatt, 1969)
			5	(Weisbroth, 1974)
			14	(Baba and von Haam, 1972),

(Continued)

TABLE 16.3 (Continued)

Organ System	Organ	Tumor Type and Site	No. of Cases	Reference
Female		Uterine adenocarcinoma in situ	1	(Kintner, 1980)
			1	(Lansdown, 1980)
			1	(DeSanto, 1997)
			1	(Sommerville, 1998)
			2	(Baba and von Haam, 1972)
			11	(Saito et al., 2002)
			1	(Dirven et al., 2009)
			1	(Kurotaki et al., 2007)
			42	(Asakawa et al., 2008)
		Deciduosa sarcoma	1	(Cooper et al., 2006)
		Mixed Mullerian duct tumor	1	(Goto et al., 2006)
		Choriocarcinoma	1	(Kaufmann-Bart and Fischer, 2008)
		Leiomyoma	4	(Stilling and Beitzke, 1913)
		Leiomyosarcoma		
			1	(Polson, 1927)
			12	(Greene, 1959)
			2	(Ingalls, 1964)
			1	(Baba and von Haam, 1972)
			1	(Irving, 1967)
			1	(Jardine et al., 2004)
			4	(von Bomhard et al., 2007)
			1	(Kurotaki et al., 2007)
	Vaginal squamous-columnar junction			
		Squamous cell carcinoma	3	(Greene, 1947)
	Ovary			
		Hemangioma	1	(Greene and Strauss, 1949)
	Mammary glands			
		Papilloma	1	(Greene, 1939b)
			2	(Burrows, 1940)
			17	(Greene and Strauss, 1949)
		Adenocarcinoma	1	(Bashford, 1911b)
			1	(Marie, 1911)
			1	(Polson, 1927)
			1	(Ball and Douville, 1929)
			1	(Heiman, 1937)

(Continued)

TABLE 16.3 (Continued)

Organ System	Organ	Tumor Type and Site	No. of Cases	Reference
Male	Testicles	Fibroadenoma	19	(Greene, 1939a)
			10	(Pearce and Greene, 1938)
			1	(Burrows, 1940)
			1	(Lombard, 1960)
			1	(Lombard, 1962b)
			1	(Sikoski et al., 2008)
			1	(Blevins et al., 2009)
			1	(Weisbroth, 2010)
	Interstitial cell carcinoma	Seminoma	1	(Paine and Peyron, 1918)
			1	(Brown and Stafford, 1989)
			1	(Anderson et al., 1990)
			1	(Roccabianca et al., 1999)
			1	(Veeramachaneni and Vandewoude, 1999)
			1	(Devaux, 1951-1952)
			1	(DeFaria, 1961)
			2	(Flatt and Weisbroth, 1974)
GI tract	Stomach	Adenocarcinoma	1	(Zwicker and Killinger, 1985)
			1	(Veeramachaneni and Vandewoude, 1999)
			1	(Marino et al., 2003)
			1	(Maratea et al., 2007)
			1	(Hoffman, 1954)
	Intestine	Leiomyosarcoma	1	(Irizarry-Rovira et al., 2008)
	Sacculus rotundus	Papilloma		
	Rectal squamous columnar junction	Papilloma		

(Continued)

TABLE 16.3 (Continued)

Organ System	Organ	Tumor Type and Site	No. of Cases	Reference
Respiratory	Liver	Bile duct system	1	(Geistfeld, 1992)
			1	(Schweizer, 1888)
	Lung	Carcinoma	1	(Niessen, 1913, 1927)
			1	(Greene and Strauss, 1949)
			1	(Kaufmann, 1973)
		Hamartoma	2	(Weisbroth, 1974)
			1	(Starost, 2007)
			1	(Schmorl, 1903)
		Epitheliome primitive	1	(Petit, 1909, 1910)
		Cancer primitive	1	(Barile, 1920)
Central nervous system	Medulla oblongata	Teratoma	1	(Shima, 1908)
			1	(Salaskin, 1929)
	Cervical sympathetic trunk	Neurinoma	1	(Salaskin, 1929)
Urinary tract	Kidney	Embryonal nephroma	1	(Lubarsch, 1905)
			1	(Nurnberger, 1912)
			2	(Bell, 1916)
			1	(Scott, 1917)
			1	(Collela, 1925)
			1	(Oberling, 1927)
			1	(Polson, 1927)
			2	(Eisler, 1938)
			1	(Miyadi, 1940)
			4	(Greene, 1943)
			1	(Flir, 1952)
			2	(Loliger, 1968)
			2	(Weisbroth, 1974)
			1	(Wardrop et al., 1982)
			1	(Lipman et al., 1985)
		Renal carcinoma	1	(Kaufmann and Quist, 1970)

(Continued)

TABLE 16.3 (Continued)

Organ System	Organ	Tumor Type and Site	No. of Cases	Reference
Skin	Urinary bladder			
		Leiomyoma	1	(Weisbroth, 1974)
	Squamous epithelium			
		Papilloma (non-viral)	1	(Dobberstein and Tamaschke, 1958)
		Squamous cell carcinoma	1	(Greene and Brown, 1943)
			1	Pearce (Cited by Greene, 1943)
			3	(Greene and Strauss, 1949)
			1	(Carini, 1941)
			5	(von Bomhard et al., 2007)
		Collagenous hamartoma	26	(von Bomhard et al., 2007)
		Shope fibroma; limbs, head, pinna	19	(von Bomhard et al., 2007)
		Malignant melanoma	11	(von Bomhard et al., 2007)
		Squamous papilloma	4	(von Bomhard et al., 2007)
		Malignant peripheral nerve sheath tumor	8	(von Bomhard et al., 2007)
		Fibrosarcoma	7	(von Bomhard et al., 2007)
			1	(Renfrew et al., 2001)
	Papillomatosis			
		Cutaneous papilloma (verruca)	1	(Schick and Schick, 1994)
	Adnexa			
		Basal cell carcinoma	1	(Groth, 1955)
			1	(Weisbroth, 1974)
		Sebaceous gland carcinoma	1	(Port and Sidor, 1978)
		Trichoepithelioma	1	(Altman et al., 1978)
			4	(von Bomhard et al., 2007)
		Trichoblastoma	59	(von Bomhard et al., 2007)
		Apocrine carcinoma	3	(von Bomhard et al., 2007)
		Apocrine adenocarcinoma (sweat gland origin)	1	(Miwa et al., 2006)
Bone and cartilage				
	Skull			
	Mandible and maxilla			
		Round cell sarcoma	2	(Katase, 1912)
			1	(Schultze, 1913; Schultze, 1914)
		Adamantinoma	1	(Orr, 1939)
		Mucoepidermoid carcinoma	1	(Gillett and Gunther, 1990)

(Continued)

TABLE 16.3 (Continued)

Organ System	Organ	Tumor Type and Site	No. of Cases	Reference
	Appendicular skeleton	Osteosarcoma	1	(Weisbroth and Hurvitz, 1969)
			1	(Walberg, 1981)
			1	(Hoover et al., 1986)
		Osteochondroma	1	(Weisbroth, 1974)
		Osteosarcoma	1	(Salm and Field, 1965)
			1	(Mazzullo et al., 2004)
			1	(Kondo et al., 2007)
	Soft connective tissues	Sarcoma	1	(Bashford, 1911b)
		Spindle cell sarcoma	1	(Kato, 1925)
		Mesothelioma	1	(Lichtensteiger and Leathers, 1987)
		Osteosarcoma	1	(Renfrew et al., 2001)
Lymphoid and hematopoietic system	Various sites	Generalized lymphoma	1	(Aberastury, 1930)
			1	(Schultze, 1913; Schultze, 1914)
			1	(Zschocke, 1914)
			1	(Feldman, 1926, 1927, 1932)
			1	(Greene and Strauss, 1949)
			1	(Llambes and Garcia Mendez, 1954)
			1	(Zbindin and Studer, 1957)
			12	(Loliger, 1966, 1968)
			1	(Van Kampen, 1968)
			29	(Fox et al., 1970)
			1	(Hayden, 1970)
			1	(Kraack, 1971)
			4	(Weisbroth, 1974)
			1	(Flatt, 1972)
			1	(Ubertini, 1972)
			1	(Gupta, 1976)
			1	(Cloyd and Johnson, 1978)
			1	(Finnie et al., 1980)
			1	(Toth et al., 1990)
			1	(Kolappaswamy et al., 2006)
			1	(Ishikawa et al., 2007)
			1	(Reed et al., 2009)

(Continued)

TABLE 16.3 (Continued)

Organ System	Organ	Tumor Type and Site	No. of Cases	Reference
			1	(Shibuya et al., 1999)
			1	(De Carvalho et al., 2007)
			1	(Gomez et al., 2002)
			1	(Volopich et al., 2005)
			1	(Toth et al., 1990)
			3	(White et al., 2000)
			7	(Elvestad and Henriques, 1985)
		Hodgkins like lymphoma	1	(Medlar and Sasano, 1937)
		Lymphoid leukemia	6	(Elvestad and Henriques, 1985)
		Myeloid leukemia	1	(Meier et al., 1972)
			1	(Elvestad and Henriques, 1985)
		Erythroid leukemia	1	(Elvestad and Henriques, 1985)
		Histiocytoma	1	(Yamamoto and Fujishiro, 1989)
		Granulocytic sarcoma, eosinophilic	1	(Perkins et al., 1996)
	Brain			
		Myeloma, plasma cell	1	(Pascal, 1961)
	Spleen			
		Epithelioma	1	(Ball, 1926)
	Vascular			
		Hemangioepithelioma	1	(Pletcher and Murphy, 1984)
		Hemangiosarcoma	1	(Guzman et al., 2000)
	Thymus			
		Thymoma, thymosarcoma	1	(Drieux, 1938)
			1	(Blanchard, 1939)
			1	(Orr, 1939)
			4	(Greene and Strauss, 1949)
			3	(Fox et al., 1971b)
			1	(Clippinger et al., 1998),
			1	(Florizoone, 2005)
			1	(Vernau et al., 1995)
		Thymic carcinoma	1	(Wagner et al., 2005)
Endocrine system				
	Adrenal			
		Adenoma	1	(Boycott and Pembrey, 1912)
				(Ryzhavskii and Tiuriukhanova, 1986)
		Carcinoma	1	(Hueper and Ichniowski, 1944)

(Continued)

TABLE 16.3 (Continued)

Organ System	Organ	Tumor Type and Site	No. of Cases	Reference
Miscellaneous	Pituitary	Eosinophilic adenoma	1	(Polson, 1927)
		Adenoma	9	(Lipman et al., 1994)
	Thyroid	Carcinoma	1	(Dinges and Kovac, 1972)
		Malignant melanoma	1	(Sustmann, 1922)
	Unknown site		1	(Brown and Pearce, 1926)
			1	(Holz and Heutgens, 1955)
				(Hotchkiss et al., 1994)
			1	(Stanley et al., 2003)
		Diaphragmatic endothelioma	1	(Polson, 1927)
		Metastatic "abdominal sarcoma"	1	(Wallner, 1921)
			1	(Baumgarten, 1906)
		Retroperitoneal fibroleiomyoma	1	(Raso, 1936)
	Teratomas	Myxosarcoma	2	(Polson, 1927)
		Spindle cell sarcoma	1	(Polson, 1927)
		Encephaloid carcinoma	1	(Polson, 1927)
	Teratomas	Gonadal	1	(Meier, 1970)
		Intracranial	1	(Bishop, 1978)
			1	(Margulies, 1901)
		Retroperitoneal	1	(Mutinelli et al., 2009)

rabbits seen in veterinary practices. Lymphoma/lymphoid leukemia continues to be the second most commonly reported tumor in rabbits, with the third and fourth most common tumors now being mammary gland tumors (papilloma and adenocarcinoma) and skin tumors (trichoblastomas and collagenous hamartomas). Uterine leiomyosarcoma, embryonal nephroma, and pituitary adenoma were the next most commonly reported tumors, in rank of decreasing frequency. The changes in tumor incidence from the previous text may be attributed to the differences between laboratory and pet rabbits in average age of tumor occurrence, exposure to pathogens (in the case of Shope papilloma virus-related skin tumors), and the relatively larger number of necropsies performed on laboratory rabbits without antemortem clinical signs, which may result in a higher number of tumors reported as incidental findings.

Tumor Models

In addition to reviewing the naturally occurring neoplasms, this chapter summarizes a number of transplantable and experimentally induced tumor models that have been developed in the laboratory rabbit since the previous edition. Surveying the literature in the last 10 years, VX2 carcinoma has become an extremely popular transplantable tumor model (more than 350 publications in the last 10 years), used for studies on interventional radiology, targeted therapy, as well as molecular biology of cancer. However, Brown-Pearce carcinoma and Greene melanoma, which were once popular experimental tumor models, were sparsely used in the last 10 years (<10 Pubmed citations). On the other hand, use of transplantable human melanoma cell lines in the rabbit eye for studies on primary

and metastatic tumors seems to be on the rise (da Silva Correa et al., 2009; Di Cesare et al., 2009; Maloney et al., 2009; Marshall et al., 2007). The animals used were either immunocompetent (studies with tumors in the eye only) or animals with an induced specific state of immunosuppression (e.g., for metastases studies). This could be due to the fact that use of human cell lines has more translational potential.

Another active area of research has been the development of animal models of viral oncogenesis. Naturally occurring pox, papova, and papilloma virus infections in rabbits have been used to answer basic biological questions about viral oncogenesis, and the rabbit has been used as a model for the study of the human papilloma virus (HPV) (Brandsma, 2005; Breitburd et al., 1997; Govan et al., 2008; Wilgenburg et al., 2005). Experimental inoculation of various breeds of domestic rabbits with primate herpesviruses induced generalized lymphoproliferative diseases and lymphoma. These models are used to investigate the pathogenesis of Epstein-Barr virus (EBV) in man as well as for the development of antiviral drugs and vaccines against EBV (Hayashi and Akagi, 2000; Medveczky et al., 1989; Timanovskaia et al., 1988; Wutzler et al., 1995; Yakovleva et al., 1987).

Induction of skin tumors in rabbit through chemical carcinogenesis has allowed investigation into the role of the *ras* family of genes in the multistep process of cancer development. These models allow easy monitoring of tumor development and accessibility to the tumor site (Corominas et al., 1991; Leon et al., 1988).

Finally, transgenic rabbits have been created to study the interaction of the genome with oncogenes and viral DNA inserts (Knight et al., 1988; Sethupathi et al., 1994).

NEOPLASMS OF *ORYCTOLAGUS CUNICULUS*

Table 16.3 summarizes the reported naturally occurring neoplasms in *Oryctolagus*. This section includes descriptions and illustration of the gross and microscopic morphology of the tumors, an assessment of biological behavior, and an estimate of the value they might have as model systems for cancer research.

Tumors of the Female Reproductive Tract

Tumors of the Uterus

UTERINE ADENOCARCINOMA

Adenocarcinoma of the uterus (ACU) continues to be recognized as the most commonly encountered neoplasm of the *Oryctolagus* rabbit. It has received the greatest attention in the literature and has been

the subject of several well-organized and intensively investigated longitudinal studies of individual rabbits so designed as to follow the progression of individual lesion development (Adams, 1962; Baba and von Haam, 1967b; Greene, 1959; Ingalls, 1964). The tumor has been the subject of review articles over the years that deal with both laboratory (Elsinghorst et al., 1984; Greene, 1959, 1965; Weisbroth, 1974, 1994) and pet rabbits (Asakawa et al., 2008; Cutler, 1998; Heatley and Smith, 2004; Saito et al., 2002).

Most investigators have emphasized that metastatic ACU is the final stage of an evolutionary process in tumor development and that the identifying biological and morphological attributes develop slowly over a period of time. Two main lines of evidence lead to this conclusion. The first is that female breeder rabbits with ACU invariably have a preceding case history that includes reproductive disturbance prior to detection of the tumor (Greene, 1959, 1965; Greene and Saxton, 1938). Fertility is diminished, litter size is reduced, there are more stillborn births and desertion by the mother is more common. Dystocia, litter retention in utero, abdominal pregnancy, and fetal resorption are also more likely. The period of altered reproductive performance precedes clinical detection (palpation) by some 6–10 months. Early tumors may be palpated as persistent uterine nodules about the size of a 10-day fetus. They are usually multiple and present in both uterine fundi. In 25% of Greene's cases, cystic changes were also detectable in the mammary glands (Greene, 1959; Greene and Newton, 1948; Greene and Saxton, 1938). The growth rate varies, but the tumor usually progresses from approximately 1 cm initially to 5–6 cm or larger in 6 months. In some cases, the tumors do not enlarge prior to metastasis. In Greene's work, the duration in time between clinical detection and death from metastasis averaged about 12–24 months (Greene and Newton, 1948). Of the rabbit females studied by Ingalls et al. (1964), although 11 of the 12 developed ACU, metastasis did not occur with eight of the 11 during the 3-year period between detection and death (from other causes). This evidence, taken with the recognized age-related factors in tumor incidence, suggests that ACU develops slowly in most cases. Incidence of the tumor does not seem to bear any relationship to degree of parity. Adams (1962) reported no differences in the incidence of the tumor in breeder rabbits as opposed to aged virgin females. Weisbroth's cases all occurred in nulliparous female rabbits (1994).

The other line of evidence is more direct and has been acquired by the technique of periodic laparotomy and uterine biopsy (pioneered by Greene) over the lifespan of individual rabbits (Adams, 1962; Baba and von Haam, 1967a; Greene, 1959; Ingalls, 1964). This work has not only supported the clinical observations

outlined above, but has permitted sequential developmental study of ACU. The normal endometrium of the rabbit should be reviewed for comparison (Baba, 1970; Baba and von Haam, 1967b). Against this normal background, a number of endometrial abnormalities have been characterized, including endometriosis, papillary, cystic and adenomatous hyperplasia, endometritis, pyometra, and senile atrophy (Baba and von Haam, 1967b; Meissner, 1957; Merrian, 1960). Each of these lesions, of course, is characterized by a typical histological appearance. There is general agreement that ACU develops from the glandular epithelium and preserves an identifiable adenomatous appearance of irregular glandular elements embedded in a richly vascular myxoid stroma. The tumors are usually initially situated in the mucosal folds adjacent to the mesometrial insertion and they arise most frequently as multicentric foci irregularly spaced in the fundus of both uteri.

One key question is whether any of the aforementioned endometrial abnormalities are precancerous lesions. There is no uniformity of opinion on this. In studies by Greene (Greene, 1939a, 1942a, 1959, 1965; Greene and Newton, 1948; Greene and Saxton, 1938), based on 140 cases of ACU in which the onset of cancer could be monitored by changes detected by sequential biopsy, every case was preceded by cystic endometrial hyperplasia. In another technically similar study (Baba and von Haam, 1967b), antecedent endometrial lesions (dysplasia and carcinoma in situ) were also described, but the findings could not support cystic hyperplasia as a precancerous lesion in spontaneous ACU. In the case of the Phipps rabbit colony (similarly studied by sequential biopsy) preneoplastic changes were described as including hyperplasia and cystic degeneration, which progressed to focal anaplasia and then ACU (Adams, 1962; Ingalls, 1964).

Greene concluded that mammary carcinoma and ACU were both reflective of pre-existing endocrinologic disturbances in the rabbit (Greene, 1965). The evidence included an incidence of ACU of 48% and 34% in genetic transmitters of pituitary dwarfism and cretinism, respectively, compared to an incidence of 14% in the remainder of his sample group. ACU was not found in the Belgian or Rex breeds, but occurred in other breeds in the following (decreasing) order: Tan, French Silver, Havana, Dutch, Marten, English, Chinchilla, Beveran, Sable, Himalayan, and Polish. Evidence of endocrine disturbance included small adenoma-like thyroids, pronounced accumulations of lipoid substance in the zona fasciculate adrenal, hyperplasia of the pars intermedia of the pituitary in ACU-bearing rabbits, and the statistically significant association of mammary carcinoma in greatest frequency with ACU (when multiple primary tumors were present). Finally, we may cite the observation that breast changes other

than neoplasia (e.g., epithelial hyperplasia, adenosis, and cyst formation) almost invariably accompany ACU (Greene and Strauss, 1949).

These observations have prompted experimentation relating to the carcinogenicity of estrogen in the rabbit, and the issue has been reviewed along with other carcinogens in the rabbit uterus (Baba and von Haam, 1967b). There is little experimental evidence to support the role of estrogen as a factor in spontaneous ACU in the rabbit. Moreover, the model of estrogen-induced glandular hyperplasia that develops in the uterus is known to not involve a precancerous lesion (Baba et al., 1970; Baba and von Haam, 1967b) and to show histochemical changes not characteristic of adenocarcinoma (Baba and von Haam, 1967). The evidence is conflicting, however, because others have reported induction of uterine carcinoma in the rabbit with estrogenic hormones (Meissner, 1957). The data are extremely difficult to interpret because of the naturally occurring high incidence of ACU in untreated rabbits. Estrogen enhances the carcinogenic effect of certain chemicals (Baba and von Haam, 1967b) but in those cases (as in carcinogen alone) the sequence of squamous metaplasia leading to squamous carcinoma is morphologically dissimilar to naturally occurring ACU. Moreover, inasmuch as ACU appears correlated with senescence of the endometrium (Baba and von Haam, 1972) treatment with estrogen appeared to actually reduce the incidence of ACU. More recently, it has been shown that while uterine tissue of most cases of cystic endometrial hyperplasia were ER- α (estrogen receptor- α) and progesterone receptor (PR)-positive, suggestive of stimulation by estrogen, ER- α , and PR positivity correlated with tubular/solid ACUs, but not with papillary ACU (Asakawa et al., 2008). The latter group could find no direct correlation between cystic endometrial hyperplasia and progression to ACU in their series of 88 rabbits studied by biopsy or necropsy. While decidosarcoma is a rarely occurring spontaneous uterine tumor in the rabbit (Cooper et al., 2006), Zook and his coworkers have developed an interesting and reproducible model for induction of decidosarcoma in the rabbit by continuous instillation of contraceptive steroids (Janne et al., 2001; Zook et al., 1987, 2001). Treatment with exogenous estrogen in utero first induces decidualization of uterine epithelium, which then progresses to malignant decidosarcoma. The process was enhanced by cotreatment with progesterone and was reversible by withdrawal of the hormones. The spontaneous tumor tissues of Cooper et al. (2006) were positive for estrogen and progesterone receptors.

The course of ACU is relatively slow, but inexorable. Histologically, the tumor progresses by increasing degrees of dedifferentiation and anaplasia with increase of the vascular, myxoid stroma often at the expense of



FIGURES 16.1 Uterine adenocarcinoma. Note multiple neoplastic foci in uterine horns.

glandular elements. Carcinomatous endometrium loses such cellular elements as cilia and secretory vesicles, and histochemically demonstrates absence of alkaline and acid phosphatases that normally occur in both normal and hyperplastic uterus. Areas of necrosis are common in mature tumors and correlate with capacity to metastasize (Greene and Newton, 1948). The tumor is illustrated in Figures 16.1–16.3. Excellent photographs may be studied in the papers by Greene (Greene and Newton, 1948; Greene and Strauss, 1949). Further histologic classification of ACU into carcinomas with either papillary or tubular/solid cytoarchitecture are illustrated in the paper by Asakawa et al. (2008).

Notwithstanding heredity as a factor, the incidence of ACU has been noted to exceed 50% in certain colonies of Dutch and New Zealand White random bred rabbits kept past the age of 3–4 years. This is a useful feature for investigators seeking model systems for ACU because it means that naturally occurring material to work with experimentally can be obtained with certainty and almost at a predictable rate (Baba and von Haam, 1972). Tumor cell lines for serial rabbit passage and for autologous transplantation have been established (Bieseke, 1945; Greene, 1939a, 1940, 1942b; Ingalls, 1972; Lombard, 1962b). The ability to metastasize appears to be a requisite stage of development before successful passage to homologous or heterologous hosts (Greene, 1942b, 1959). Greene pioneered the technique of using the anterior chamber of the eye for tumor growth (e.g., the Brown-Pearce carcinoma, Greene melanoma, etc.) with the use of the ACU tumor (Greene, 1958; Greene and Saxton, 1938).

At least two other rarely occurring tumors have been described as naturally occurring in the decidualized endometrium of the rabbit. The first of these is the mixed Müllerian tumor (syn.: carcinosarcoma or mixed mesodermal tumor) encountered by Goto et al. (2006)



FIGURES 16.2 Uterine adenocarcinoma. Note metastatic foci in the lung.

as a well-demarcated mass in one of the uterine horns. The contralateral horn was normal. It is distinguished from deciduosarcoma by also having well-differentiated carcinomatous elements of glandular epithelium. The second is the choriocarcinoma described and illustrated by Kaufmann-Bart and Fischer (2008) in a presumptively virgin 3-year-old pet rabbit (Kaufmann-Bart and Fischer, 2008). Choriocarcinomas are highly malignant neoplasms arising from trophoblastic cells of the uterus, ovary, or testis, but may also arise from sequestered loci of totipotent cells of the mediastinum or abdomen. This tumor occurred in the uterus and was

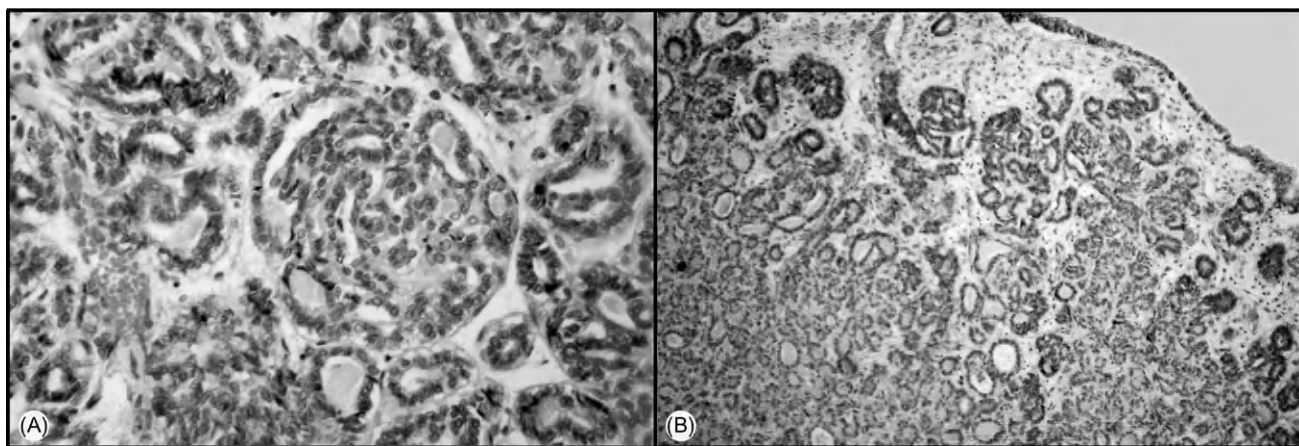


FIGURE 16.3 Uterine adenocarcinoma. Photomicrographs illustrating a premetastatic tumor, with well-differentiated acinar components (A); and a poorly differentiated tumor with loss of tubular differentiation and increased stromal elements associated with increased metastatic potential (B).

metastatic to the lungs and mediastinum, but its origin was not definitively determined. Choriocarcinomas have been induced experimentally in pregnant rabbits (Kushima et al., 1967).

LEIOMYOMA AND LEIOMYOSARCOMA

Both leiomyomas and leiomyosarcomas have been reported from rabbits as sporadic and usually incidental findings at necropsy. Although the benign form is most common, there have been reports of metastasizing leiomyosarcomas (Polson, 1927). These smooth muscle tumors are most frequently associated with the digestive and female reproductive tracts (see Table 16.3). The histology is typical for the same tumor in other species, i.e., whorls and bundles of fusiform cells arranged in a crisscrossing pattern. Malignant forms usually appear less regular, with more abundant nuclei and more mitotic figures. They are generally reported from older rabbits. The collision in the same uterine horn between a malignant ACU that invaded a benign leiomyoma was reported as occurring in a 7-year-old pet rabbit (Kurotaki et al., 2007).

SQUAMOUS CELL CARCINOMA

Squamous cell carcinoma of the vaginal squamocolumnar junction is a comparatively rare tumor. It has been described only by Greene (Greene, 1947) who reported three cases from a necropsy series of 1100 females over the age of 2 years. It is an important tumor, however, because in the rabbit the columnar epithelium of the uterus is continued out over the cervix into the vagina. The transition to squamous epithelium occurs at the level of the urethral meatus. This location would, in the rabbit, appear to be the homolog of the external cervical os in the human female, as that is where the epithelial transition occurs in humans. The difference between

the two species in the location of the transitional zone emphasizes the importance of the junction as a predisposing factor in carcinogenesis. Moreover, perhaps for this reason, cervical cancer has neither been observed nor reported in the *Oryctolagus* rabbit. Greene's rabbits were not related genetically, and all were over 4 years of age. Interestingly, all three had fundic ACU and two also had mammary neoplasia. In all cases the fundic tumor was in a non-metastatic stage of development, but the vaginal squamous carcinomas had metastasized via lymph nodes and/or peritoneal implants to other organs. Two of the tumors were used for transplantation studies and at least 14 anterior chamber-passaged generations were obtained from them.

Tumors of the Mammary Gland

MAMMARY ADENOCARCINOMA

Mammary adenocarcinoma (MAC) has been reported with some frequency in laboratory rabbits (see Table 16.3). The tumor appears to most commonly arise in multiparous females in the third and fourth years of life but occurs often in nulliparous does as well. The clinical appearance of swollen, lumpy, nodular mammae, perhaps with palpable grapeshot structures, often with discoloration of the glands and/or teats and with discharges that may be obvious or expressed requires differential diagnosis because of a number of conditions with similar presentation (Blevins et al., 2009; Harcourt-Brown, 1998). These conditions include pseudopregnancy, prolactin-driven cystic hyperplasia, bacterial mastitis, cyclosporin A-induced hyperplasia (Petraitiene et al., 2001), testicular interstitial cell tumor-induced gynecomastia (Maratea et al., 2007) and, of course, MAC. Greene (1939b, 1939c, 1939d, 1940) described 23 cases of MAC that occurred exclusively in two families of the Belgian and English breeds

from his colony. Of them, 19 were associated with antecedent abnormalities of mammary tissue, including cystic hyperplasia, while four appeared to arise *de novo*, without preceding lesions. Greene further studied the progressive changes leading to invasive MAC over the lifespan of individual rabbits (Greene, 1939c, 1939d, 1940). In one family of the Belgian breed, 19 rabbits were observed to have mammary tissues that progressed from cystic hyperplasia, benign neoplasia (adenosis), to invasive MAC as succeeding events. Considerable individual variation was observed in the time required to pass through the successive stages, varying from 10 to 29 months. Cystic mastitis occurred only in multiparous does and was most common in females 2–3 years of age. Numerous animals of unrelated lines were foster-reared by tumor-bearing rabbits and observed for long periods of time without subsequent development of mammary tumors. This observation, together with the low incidence of tumors in daughters of tumor-bearing dams was interpreted by Greene as evidence against passage in the milk of a virus (analogous to the Bittner virus of mice) influencing MAC development.

Cystic disease in Greene's series was preceded by a variable period of intense mammary congestion that waxed and waned, seemingly according to events in the estrous cycle. Cysts were observed to arise simultaneously from dilated acini and ducts in many areas (which were palpable *in situ* as shot-like structures). Microscopically the cyst epithelium was flattened and as the disease progressed, cystic enlargement occurred by coalescence of acini. Later, fibrous connective tissue accumulated around dilated acini and enlarged cysts. After the variable period of cystic disease, early preneoplastic changes within cysts included sessile growths of either cystic epithelium or uniradicular papillomas. The purely epithelial growths were not seen in later stages, however the papillomata were found in all breasts and all stages of development were found in different sections of the same mammae. The papillomatous structures rapidly became multiradicular and formed epithelium-lined spaces, resembling acini. The walls of the structures gave rise to papillary forms comparable to those seen in humans and referred to as intracystic adenomata (Greene, 1939c). Eventually, malignant changes including disorder in the papillary epithelium, irregularity in the shape and size of neoplastic cells, nuclear hyperchromaticity and more common mitotic figures occurred in widely separated areas of breast tissue. These characteristics of papillomata, in some cases, persisted for more than 6 months before the invasive stages began. In at least four rabbits, in contrast to the picture of progression initiating with cystic hyperplasia, mammary adenomas appeared to begin as atypical proliferations of previously normal acini.

Invasive MAC was the inexorable outcome of the papillomatous stage. Metastatic foci would form neoplastic structures initially identical to those of the primary tumor. In some instances, further dedifferentiation occurred and the cells proliferated in solid, medullary sheets. Characteristically, in different areas, all stages from cystic disease to metastatic carcinoma could be observed. This has been a consistent observation of others describing MAC, as well (Blevins et al., 2009; Burrows, 1940; Heiman, 1937; Pearce and Greene, 1938; Polson, 1927; Sikoski et al., 2008). Invasion to muscle and connective tissue surrounding the mammary gland occurred commonly and was not accompanied by inflammatory response. Metastasis occurred in some cases to the lung and regional lymph nodes, as well as to other organs in different animals.

Although the dominating lesion in affected rabbits was the MAC, pathological changes in other organs reflected profound physiological disturbances that were also observed clinically. The adrenals, pituitary, and uterus appeared to be consistently involved in rabbits with MAC preceded by cystic hyperplasia. The adrenals had numerous foci of large, pale-staining vacuolated cells with pyknotic nuclei. The adenohipophysis was enlarged 3–5-fold owing to chromophobe cell hyperplasia and the uterine epithelium was hyperplastic and cystic. Greene believed that these changes were consistent with a syndrome of hyperestrinism (Greene, 1939d, 1965). He pointed out the frequent involvement of mammary tissues (50%) in the case of ACU, and the unusual frequency of concomitant tumors of these two organs in the rabbit. Of 23 cases of MAC, six also had ACU. Furthermore, he argued, the similar endocrine gland changes (particularly in the adrenal and pituitary) supported a common etiologic basis.

These findings, and the conclusions, as well, were shared by Burrows (1940) who followed 25 female rabbits up through about 3 years of age. Of them, 15 developed ACU, and three developed MAC as well. The females were frequently observed to be pseudo-pregnant owing to the continuous presence of males in the room. The MACs were of the type described by Greene (and others cited above) as being preceded by cystic mastopathy. In different areas of the same breast, all stages described by Greene, including intracystic papillomata and adenocarcinoma were observed. More importantly, however, observation by Greene of (a) cystic mastopathy, (b) cystic hyperplasia of the endometrium, (c) ovarian changes in which the bulk of the organ was changed to luteal tissue, (d) lipid degeneration of the adrenal (similar to that seen in mice by treatment with estrogen), and (e) the unusually frequent association of uterine and mammary tumors as multiple primary neoplasia were all confirmed by Burrows (1940). The fact remains, however, that neither uterine

nor mammary adenocarcinomas of the type that occur spontaneously have been experimentally induced by hyperestrinism, and the role of estrogen, if any, in ACU and MAC in the rabbit remains moot. A more likely endocrine basis has been demonstrated for the role of prolactin. More recently, it has become clear that cystic hyperplasia (mastopathy) of the mammary gland and uterus in rabbits is driven by prolactinemia and may be triggered by progesterone derived from retained corpora lutea (pseudopregnancy), or from hyperplasia of the anterior pituitary or pituitary adenomas with active, prolactin-secreting cells demonstrated by special stains (Blevins et al., 2009; Lipman et al., 1994; Sikoski et al., 2008) and by elevated serum prolactin levels. In addition, Sikoski et al. (2008) were able to diagnostically image pituitary enlargement by tomography in a rabbit with MAC. The hyperprolactinemia observed by Petraiteine et al. (2001) as induced by treatment with cyclosporin A (and methylprednisolone) especially underscores the role of prolactin because of the chemical induction of cystic mastopathy during treatment, and its regression following withdrawal of the drug(s) (Petraitiene et al., 2001). In like manner, the proliferating cells of the gynecomastia (mammary hyperplasia) accompanying the interstitial cell tumor (of the testis) in the male rabbit reported by Maratea et al. (2007) exhibited intense staining for progesterone (PR) receptor.

MAMMARY FIBROADENOMA

Mammary fibroadenoma, although quite common in the laboratory rat (Percy and Barthold, 2007) appears not to have been previously reported as occurring in *Oryctolagus* rabbits. A single case is reported here (Weisbroth, 2010). As in the rat, the tumor was benign, sharply encapsulated from adjacent tissue, well tolerated by the host and, because left untreated, grew to an enormous size (see Figure 16.4). On gross section, the tumor was a pale yellowish color and distinctly lobulated. Microscopically, the architecture of the tumor illustrated above closely resembled that of the counterpart rat lesion, i.e., acinar epithelial elements in a collagenous, fibrous tissue matrix.

Tumors of the Male Reproductive Tract

Tumors of the Testes

Tumors of the testicle are infrequent in the rabbit, most probably owing to the limited population of males over the age of 2–3 years, since most reports have occurred in rabbits even older. Reports of tumors of the male reproductive tract in the rabbit, at present, are limited to testicular neoplasms. Differential diagnosis of the clinical presentation in the rabbit of unilateral (or less commonly) bilateral enlargement of the



FIGURE 16.4 Mammary fibroadenoma in a Dutch Belted rabbit illustrating the very large size that can be attained by this benign tumor.

testicle includes bacterial orchitis, of course, but must also include an increasing array of tumor types, some of which have had interesting physiologic concomitants. It should be noted that enlargement of the testicle does not always accompany neoplasms of this gland (Veeramachaneni and Vandewoude, 1999). Teratoma has also been observed as arising in rabbit testicle, but will be separately described in that section.

INTERSTITIAL CELL TUMOR

Interstitial cell (syn.: Leydig cell) tumors are, arguably, the most common type of testicular neoplasm in domestic rabbits, certainly they are the most commonly reported. Interstitial cell tumors (ICT) can occur as unilateral or bilateral neoplasms (DeFaria, 1961; Devaux, 1951–1952; Flatt and Weisbroth, 1974; Zwicker and Killinger, 1985) and may accompany other primary tumors (Roccabianca et al., 1999; Veeramachaneni and Vandewoude, 1999). Grossly, the tumors occur as enlargements of the testicle and are encapsulated, lobulated, firm, blotchy, and tan or pale in color. Areas of hemorrhage or coagulation necrosis are not uncommon. The tumor initiates as a proliferation of interstitial cells between the seminiferous tubules, which appear to represent a continuum from hyperplasia in early stages to solid sheets and masses of polygonal cells with eosinophilic cytoplasm, separated into lobules by cords of connective tissue as the ICT develops. Examples of these neoplasms are shown in Figures 16.5 and 16.6. The tumor cells stain for Melan A in the cytochrome (Maratea et al., 2007) and may demonstrate Reinke's crystals and vimentin (Marino et al., 2003). The ICT in the rabbit expands radially, but is not locally invasive nor is it known to metastasize. The tumors are hormonally active and, in at least one case, gynecomastia was described as an accompaniment to the primary ICT



FIGURE 16.5 Interstitial cell testicular tumor. Bilateral tumors in a New Zealand White rabbit. Note enlargement of the testicle and multiple encapsulated tumor nodules (arrows).

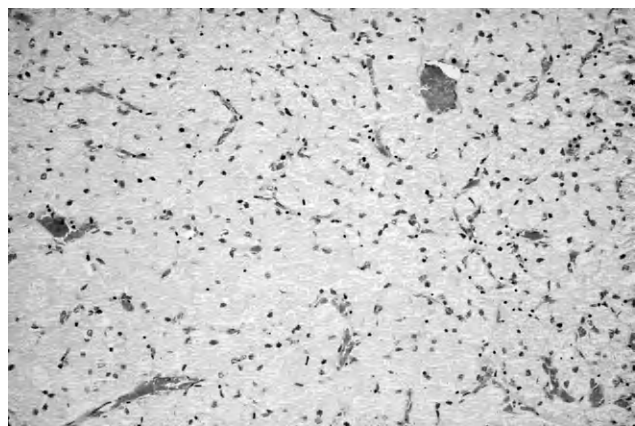


FIGURE 16.6 Interstitial cell tumor. Photomicrograph showing sheets of tumor cells with abundant cytoplasm.

(Maratea et al., 2007). Although neoplastic interstitial cell tumors are known to secrete increased amounts of estrogen, and that estrogen inhibits the cytochrome P450 enzymes necessary for testosterone formation leading to feminization in other species, the relationship of the tumor to gynecomastia in this rabbit was not determined (Maratea et al., 2007). Other reports of ICT in the rabbit have not described status of the breast tissue at necropsy.

SEMINOMA

The seminoma (syn.: germ cell tumor) is a much less frequently reported testicular neoplasm of the rabbit. Although the first case was reported in 1918 (Paine and

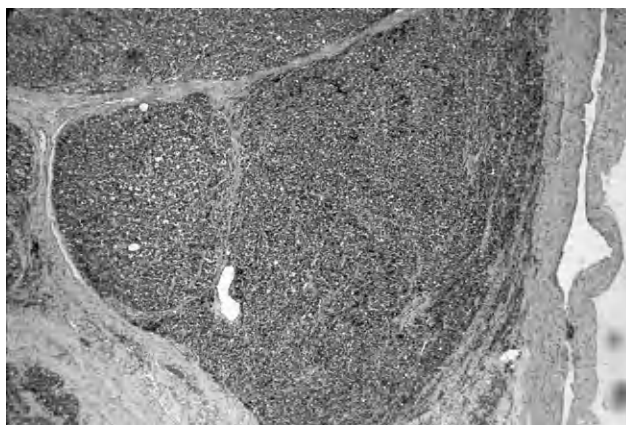


FIGURE 16.7 Seminoma. Low-power photomicrograph showing lobulation and dense cell sheets.

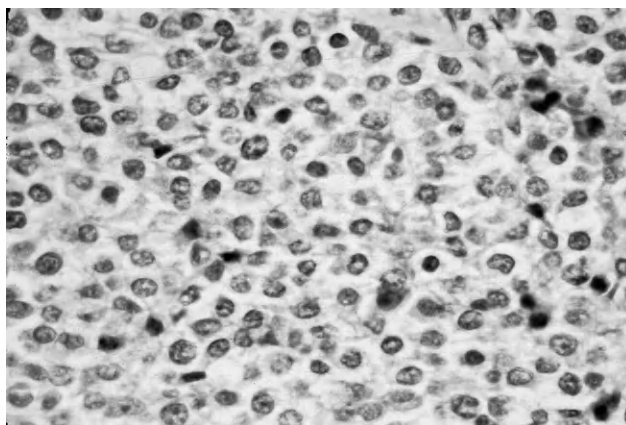


FIGURE 16.8 Seminoma. Higher magnification showing detail of monolithic sheets of polygonal cells with hyperchromatic, basophilic cell nuclei with scanty cytoplasm.

Peyron, 1918), 71 years would elapse before the next report (Brown and Stafford, 1989). These tumors present clinically as usually unilateral enlargements of the testicle in older male rabbits. Grossly, the tumors are firm, nodular, and pale in color, but often are with areas of hemorrhage (Anderson et al., 1990). Microscopically, the tumor is composed of densely cellular packed sheets of large, round or polygonal tumor cells with densely basophilically staining nuclei and scanty basophilic cytoplasm (Figures 16.7 and 16.8). The tumors may invade the capsule of the testis and blood vessels within the organ, but are not known to be malignant. The case described by Veeramachaneni and Vandewoude (1999) additionally had areas of seminiferous tubules with atypically large cells with clear cytoplasm containing PAS-positive material that the authors termed carcinoma in situ. Seminomas, interstitial cell tumors, and carcinoma in situ are well illustrated in the latter article.

GRANULAR CELL TUMOR

Irrizarry-Rovira et al. (2008) have reported the first occurrence of a granular cell tumor (GCT) in the rabbit as a unilateral testicular neoplasm. Clinically, the tumor was seen as an enlargement of the testicle in an 8-year-old male, was encapsulated, tan in color, and there was no evidence of gynecomastia. In other species, including the human, the neoplasm tends to arise in other structures of the female and male reproductive tracts, but this was the first rabbit case observed to occur in the postnatal testicle. The tumor is thought to arise from pluripotential cells of neuroectodermal origin. The authors stress the importance of impression smear exfoliative cytologic demonstration of granular cells in distinguishing between ICT and GCT, which are quite similar in histologic sections. In addition, the tumors may be differentiated by their appearance in transmission electron microscopy. Microscopically, the tumor was seen to consist of sheets of a monotonous population of polygonal cells and dense cellular aggregates in an abundant pink to magenta granular background. Neoplastic cells contained the Melan A and vimentin markers, and there were aggregates of neuron-specific enolase-positive cells.

PAPILLOMATOSIS

Non-viral papillomas in the rabbit have an interest out of proportion to their incidence. The prominence of the Shope papilloma (of *Sylvilagus*) as a research model, on the one hand, and the oral papilloma (of *Oryctolagus*), on the other, has tended to produce in the popular mind an assumption that all papillomas in the rabbit are associated with papova group viruses. Detailed review of virally induced tumors is given in a separate section in this chapter.

Dobberstein and Tamaschke (1958) briefly described papillomatosis of the skin in *Oryctolagus*. They were not able to passage the papilloma in transplantation experiments, and details of the histology were not given. Recently, four cases of non-viral squamous papilloma were reported (von Bomhard et al., 2007). Non-viral origin was concluded based on lack of any features of viral cytopathic effect in the tumor tissues. Tumors occurred on the third eyelid, the pinna, and on the scrotum. These locations seem to match those that also occur with canine papillomatosis. The histologic changes consisted of exophytic papillated projections of mildly pleomorphic squamous epithelium.

Non-viral papillomas of the digestive tract also have been infrequently observed in the rabbit. The single reported case of polyps occurred in the sacculus rotundus (Polson, 1927). In a series of necropsies performed by Weisbroth, one fungating cauliflower-like papilloma was observed at the rectoanal junction of a 5-year-old

male rabbit, and an additional case at the same location was provided by Dr. Barbara Geistfeld (Geistfeld, 1992). Both papillomas were well-differentiated and benign, and inclusion bodies could not be demonstrated.

Tumors of the Gastrointestinal Tract

Reports of primary tumors originating in the GI tract in the rabbit are rare. Shibuya et al. (1999) reported a spontaneous lymphoma with extensive involvement of stomach, liver, and spleen. The majority of the reports of primary GI tumors in laboratory rabbit (*Oryctolagus*) involve the liver.

Tumors of the Liver

Starost (2007) reported a single case of hepatic hamartoma arising from the medial liver lobe of a transgenic New Zealand White rabbit that was diagnosed concurrently with cholelithiasis. Even though the tumor mass showed microscopic similarities to human cases of biliary hemartoma, this tumor was extrahepatic, had no involvement of hepatic parenchyma and was composed of cystic structures.

There is a single report of primary hepatic hemangiosarcoma with pulmonary metastasis in a 5-year-old New Zealand White rabbit (Guzman et al., 2000). Neoplastic nodules of varying size were observed to be distributed on the visceral surface as well as throughout the liver parenchyma. Histologically, blood-filled cavernous spaces of various sizes were separated from the hepatic parenchyma by streams of tightly packed spindle cells that in many cases extended to the central aspects of the cavity to form irregular blood-filled vascular channels. Cells had poorly defined cytoplasmic boundaries with scant eosinophilic cytoplasm and a large, oval, often-cleaved nucleus with finely stippled chromatin and inconspicuous nucleoli. Mitotic figures were rare.

Tumors of the Biliary System

Bile duct adenoma (or cyst adenoma) and its malignant counterpart, the bile duct adenocarcinoma (or cholangiocarcinoma), are relatively common as spontaneous tumors of *Oryctolagus* (see Table 16.3 for reported cases). Bile duct adenomas present as massive solitary growths or as multiple tumors of variable size. The lesions are unencapsulated but sharply circumscribed from normal liver. The tumor is frequently described as a cystadenoma because the gross appearance of multiple intercalating cysts filled with honey-like fluid is matched by the microscopic pattern of variably differentiated ductal forms within a myxoid to fibrotic stroma (see Figures 16.9 and 16.10). The tumor is of some interest because it may be reflective of the hyperirritability associated

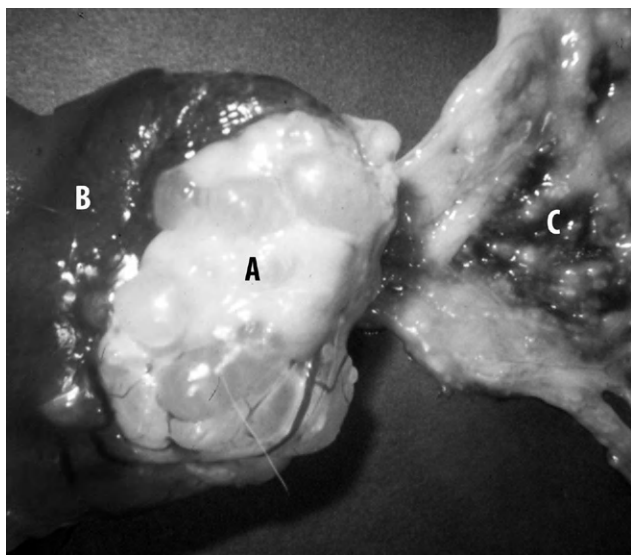


FIGURE 16.9 Bile duct adenocarcinoma. Gross photograph showing adenocarcinoma (A) sharply circumscribed from liver (B). Note metastatic nodules in the mesentery (C).

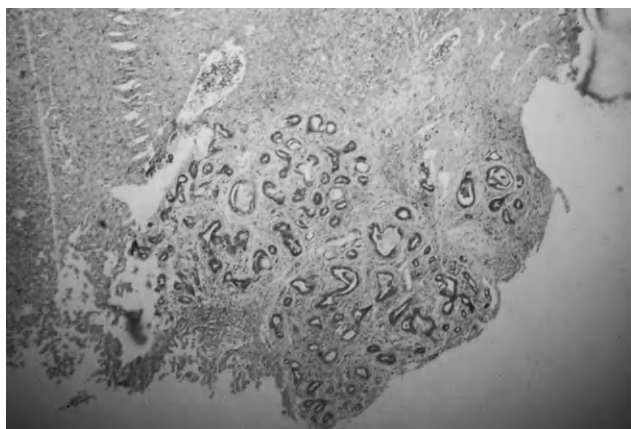


FIGURE 16.10 Bile duct adenocarcinoma. Photomicrograph showing aberrant and incomplete ductal epithelial formations.

with rabbit bile duct epithelium. Since [Schweizer's \(1888\)](#) observation (of coccidia in a liver tumor), it has been known that any of a variety of noxious stimuli may induce the proliferation of bile duct epithelium in the rabbit. For example, bile duct hyperplasia has been associated with *Eimeria steidae* infection. One is tempted to speculate that the genesis of bile duct carcinoma may originate from initially hyperplastic epithelium. This view is consistent with Weisbroth's observation that early developing and small bile duct adenomas are well-differentiated compared to those that are large, presumably more developed, and of longer duration. The histological pattern of the tumor may vary from that of a hamartoma in the most differentiated forms to that of a cystic honeycombed carcinoma with less



FIGURE 16.11 Embryonal nephroma. Gross photograph showing kidney in cut section. Note the sharp demarcation of the tumor nodules from the adjacent renal tissue.

obviously differentiated tubular forms. Metastases in the form of peritoneal, diaphragmatic, and mesenteric implants are common.

All known cases of bile duct tumors have been studied retrospectively from postmortem tissues and have been encountered only sporadically as incidental findings at necropsy. The tumor appears to have little potential as a research model, primarily because of the difficulty of case finding.

Tumors of the Urinary Tract

Tumors of the Kidney

EMBRYONAL NEPHROMA

Embryonal nephromas (syn.: nephroblastomas, Wilms' tumors) are comparatively common tumors in *Oryctolagus* rabbits and have been reported on many occasions (see [Table 16.3](#)). In the review by Dobberstein and Tamsachke (1958), the incidence of this tumor was second only to uterine malignancies. Embryonal nephromas (EMN) are most often encountered as incidental findings at necropsy, indeed, all reported cases in the rabbit have been benign and not obviously a cause of impaired renal function. Cases have been described from both young (18–24 months) and old (60–72 months) rabbits. The EMN tend to enlarge quite slowly, as can be inferred from the observation that tumors from aged rabbits (2cm diameter) are larger than those from younger rabbits (1cm or less). Grossly they are whitish, sharply demarcated nodules of tissue projecting above the cortical surface ([Figure 16.11](#)). The tumors

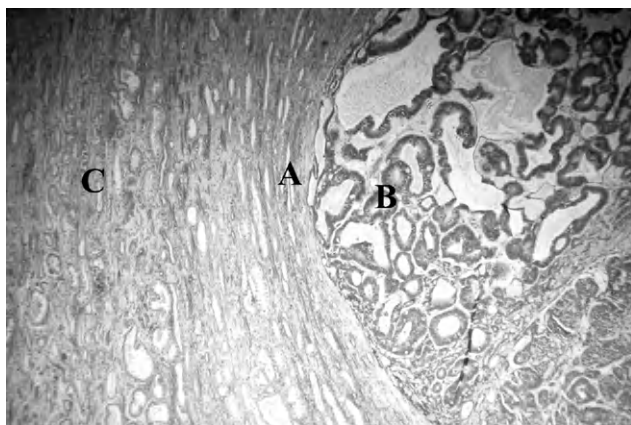


FIGURE 16.12 Embryonal nephroma. Note fibrosis of pseudocapsule (A) by nephroma (B) and compression of adjacent renal tissue (C).

may be single or multiple and appear to occur uni- or bilaterally with equal frequency in either kidney.

Histologically, the EMN of *Oryctolagus* is remarkably similar to that of man (Greene, 1943) and swine (Moulton, 1990). The basic histologic pattern is that of cylindrical cells arranged in tubular formations with spindle-shaped or round stromal elements in indifferent arrangement. The tubular formations are disuniform and many appear cystic. One distinctive feature is the pseudoglomerulus structures seen within tubular formations in the more differentiated areas. Muscle, cartilage, and other tissues common to human Wilm's tumors have not been seen in the rabbit analog. The tumors arise from the cortex and compress the surrounding renal tissue and project above the capsule as they expand radially (Figure 16.12).

Secondary polycythemia has been reported as associated with EMNs in rabbits (Lipman et al., 1985; Wardrop et al., 1982). Interestingly, in the report by Wardrop et al. surgical removal of the neoplasm resulted in correction of the polycythemia. In neither report, however, could a reactive rise in erythropoietin levels be demonstrated, as is commonly the case with human Wilm's tumors. EMN in the human is limited almost entirely to childhood and has an often fatal clinical course. In spite of the remarkably similar microscopic appearance, polycythemia, and youthful incidence, the rabbit EMN, on the other hand is routinely benign and metastases have not been reported. Because the tumor occurs in other species with biologic properties more similar to those of the human EMN (Moulton, 1990), the EMN of the rabbit would seem to offer little of interest as an EMN model. In the four cases encountered by Greene (1943), successful transplants were established in about 20% of rabbit hosts heterografted either in the testicle or anterior chamber of the eye. The very slow rate of growth noted with spontaneous EMNs was characteristic of transplanted tumors as well, even into the fourth transplant generation.

RENAL CARCINOMA

Renal carcinoma (syn.: clear cell carcinoma, hypernephroma) is rare in most animal species and has been reported in the rabbit only once (Kaufmann and Quist, 1970). Grossly, the tumor presented as a large, smooth-surfaced cortical mass on the lateral-posterior aspect of the right kidney. There was a large cyst (10 cm) on one side, but the remainder of the tumor was solid, albeit with necrotic foci. No metastases were observed on necropsy dissection. Microscopically, the low cuboidal to spindle-shaped tumor cells were arranged in irregular tubular formations, or in solid sheets and nests. The cells had eosinophilic cytoplasm, but were extensively vacuolated to form clear cells.

Tumors of the Skin

Based on the literature, there is a significant difference between the type of tumors most commonly seen in pet and laboratory rabbits, which could be attributed to the longer life span of pet rabbits (12 years or more) compared to laboratory rabbits (from a few months to 2 years on average). It is also possible that occurrences of previously reported tumors have been underreported due to lack of novelty. In addition, most of the skin tumors in laboratory rabbits are created by oncogenic viruses or transplanted squamous cell carcinomas. These tumors will be discussed separately later in this chapter.

There are very few reports of primary skin tumors of laboratory rabbits which are non-viral in origin. The majority of the primary cutaneous neoplasm found in laboratory rabbits are squamous cell carcinomas, six cases of which were reported previously (Carini, 1941; Greene and Brown, 1943). Recently, five cases of squamous cell carcinomas were reported in pet rabbits with a mean age of 7.5 years (von Bomhard et al., 2007). There appeared to be no common denominator of significance in the origin of the tumors. All known cases have arisen in different locations, literally from the head (Greene and Brown, 1943) to the tail (Pearce, cited by Greene, 1943). The sites involved haired as well as glabrous skin. The metastatic potential of these tumors was found to be low.

Most reports on skin tumors in the last two decades come from a recent retrospective study in pet rabbits by von Bomhard et al. (von Bomhard et al., 2007). According to the study, the most common cutaneous neoplasm in pet rabbits was trichoblastoma, observed in 58 of 179 reported cases. The incidence was equally distributed in both sexes, with a mean age of 5 years, starting at the age of 1 year. The majority of the cases appeared as solitary neoplasms. In most cases, the epidermis was elevated and frequently ulcerated.

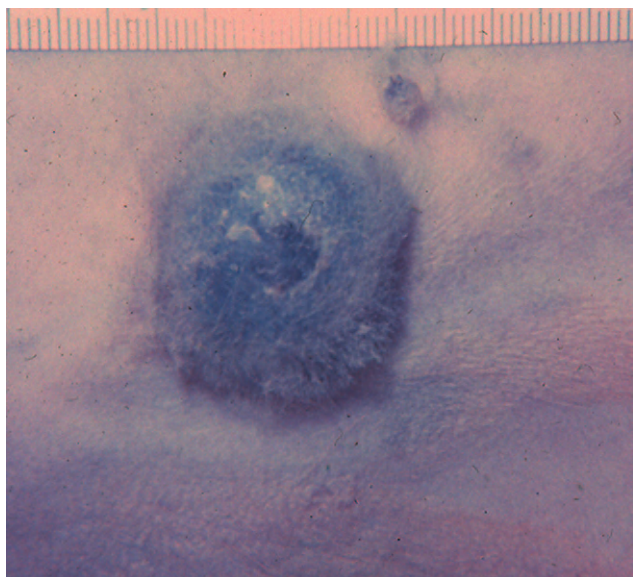


FIGURE 16.13 Basal cell adenoma. Gross photograph showing adenoma projecting above skin of the right dorsal abdomen.

Incidences of cutaneous lymphoma were reported in four pet rabbits recently (Gomez et al., 2002; White et al., 2000). Three of these rabbits were less than 2.5 years old (one New Zealand White, one Netherlands dwarf, and one Dutch dwarf), placing them in the age group of laboratory rabbits. Most of the lesions reported in the young animals (White et al., 2000) occurred in the head and neck region. Histologic studies showed epitheliotropism in two rabbits, suggesting T-cell lymphoma.

Other major cutaneous tumors reported in rabbits include basal cell adenoma (Weisbroth, 1974), trichoepithelioma (Altman et al., 1978; von Bomhard et al., 2007), myxosarcoma, liposarcoma, fibrosarcoma, malignant peripheral nerve sheath tumors, lipoma, and leiomyosarcoma, apocrine carcinomas, and Meibomian adenomas (von Bomhard et al., 2007). Basal cell adenomas (carcinomas) are rare and seen in relatively old rabbits (>4 years). The case reviewed by Weisbroth consisted of two small (2 × 1 cm) pedunculated adenomas of multicentric origin, both on the right abdominal dorsum, projecting above the skin (Figures 16.13 and 16.14) of a doe that also had adenocarcinoma of the uterus (Weisbroth, 1974). Histologically the tumors were similar to those described from other species, especially canine (Moulton, 1990).

Tumors of Bone and Cartilage

Osteosarcoma

Primary bone and cartilage tumors in domestic animals can vary widely, based on the anatomic location,

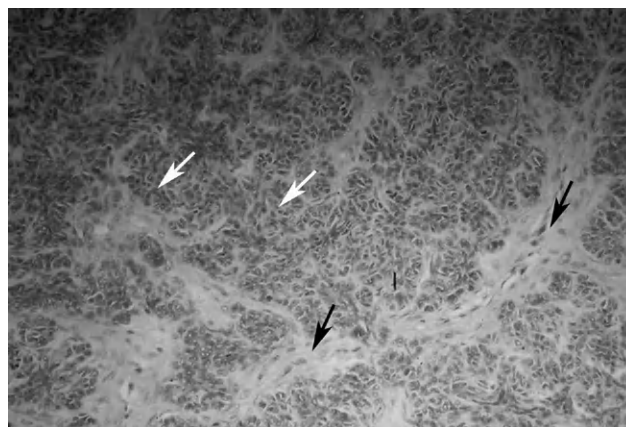


FIGURE 16.14 Basal cell adenoma. Photomicrograph showing clumps of neoplastic basilar cells (white arrows) surrounded by sparsely cellular collagenous stroma (black arrows).

tumor cell characteristics, as well as the tumor matrix as given in Meuten (2002). Even though spontaneous osteosarcomas are rare in rabbits, the reported cases show similar heterogeneity. Osteosarcomas can be experimentally induced in rabbits by various techniques, which will be discussed later in this chapter (Barnes et al., 1950; Coventry et al., 1959; Hulse, 1969; Janes et al., 1954; Sabin et al., 1932; Schurch and Uehlinger, 1931; Tapp, 1966, 1969a, 1969b; Ubertaini, 1972).

The “round cell tumors” of bone reported by Katase (1912) are difficult to evaluate in terms of present-day descriptive terminology for osteosarcomas because of the insufficient description provided by that author. Orr (1936) described a multicystic tumor of the mandible that he classified as an adamantinoma. The cysts were within a loose fibrous tissue matrix, were lined with stratified squamous epithelium, and had numerous desquamated cells in the cyst cavities. Other areas of the tumor consisted of solid alveolar masses of cells of squamous (non-keratinized) type. The description and illustrations provided by Orr were not typical of adamantinomas in other species (Moulton, 1990) and indeed, when critically reviewed by Gillett and Gunther (1990), Orr’s description was held to closely resemble the tumor more properly described as a mucoepidermoid carcinoma.

Even though a number of cases of osteosarcoma in rabbits have been reported (Kondo et al., 2007; Mazzullo et al., 2004; Renfrew et al., 2001) there are still only very few reports of primary neoplasia of bone and cartilage in rabbit. Kondo et al. (2007) described a primary osteosarcoma in rabbit that was anatomically and histologically similar to osteosarcoma in dogs. The tumor lesions extended from the distal scapula to the diaphysis of the humerus. However, it was different from most osteosarcomas in that the proliferative and destructive lesions extended into the glenohumeral

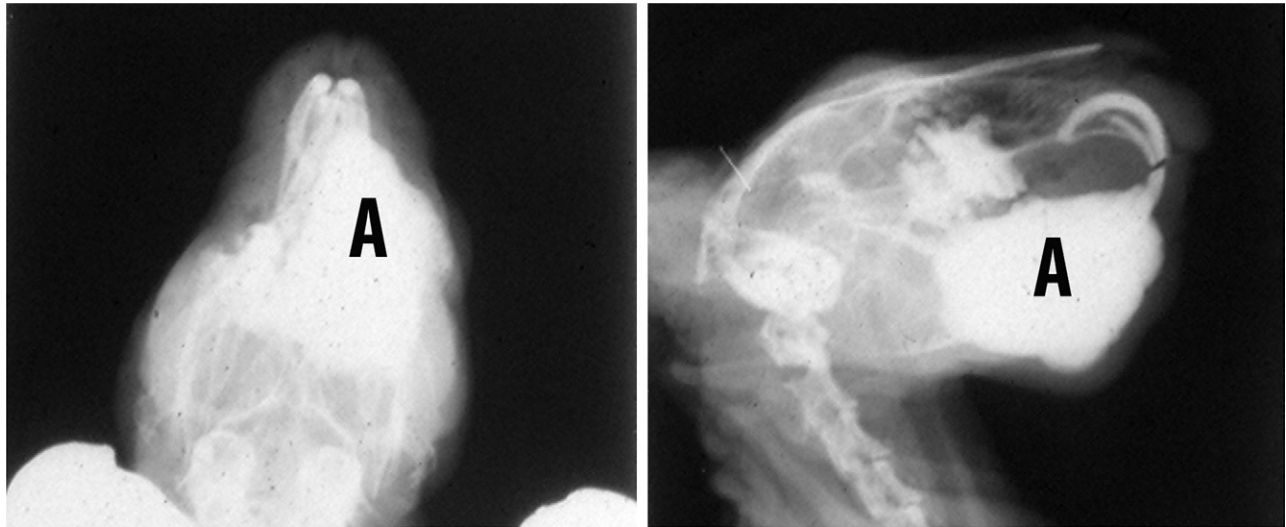


FIGURE 16.15 Osteosarcoma. Radiographs of mandibular osteosarcoma (A) in lateral and ventrodorsal orientation.

joint space. Histologic description of these lesions varied from giant-cell-type osteosarcoma (Kondo et al., 2007) that is similar to canine osteosarcoma of the same category (with similar locations as canine) to primary extraskeletal osteosarcomas (Renfrew et al., 2001) with neoplastic foci occurring in multiple soft tissue sites including the subcutaneous tissue and organs. Examples of osteosarcoma are shown in Figures 16.15–16.17.

Radiographic descriptions of osteosarcoma, which are important from a clinical stand point, are rare in reports on rabbits. Alkaline phosphatase (ALP) was found to be elevated in some reports (Ishikawa et al., 2007; Weisbroth and Hurvitz, 1969). Kondo et al. (2007) suggested that ALP might be useful as a diagnostic and prognostic factor in rabbits with primary osteosarcoma.

Tumors of the Hemolymphatic System

Lymphoid Tumors

LYMPHOMA AND LYMPHOID LEUKEMIA

Lymphoma is the second most common spontaneously occurring tumor in domestic rabbits (*Oryctolagus cuniculus*) and the most common tumor in young and juvenile rabbits. Most early cases were studied retrospectively from postmortem material which impeded progress in the characterization of lymphoma in the rabbit because the early cases occurred sporadically and with unknown histories. There has been an increase in the numbers of pet rabbits of diverse genetic background that are allowed to achieve a natural lifespan and cases have been reported in different breeds and ages of rabbits. Lymphoma has been reported in a variety of domestic breeds including New Zealand White,



FIGURE 16.16 Osteosarcoma. Gross photograph of mandibular osteosarcoma lesions (A) shown in Fig. 16.15.

multicolor English type, Japanese White, Dutch dwarf, and domestic cross-bred rabbits as well as in *Sylvilagus* and *Lepus* spp. in North America and Europe (Finnie et al., 1980; Gomez et al., 2002; Ishikawa et al., 2007; Reed et al., 2009; Shibuya et al., 1999; White et al., 2000). The most common age of occurrence of naturally occurring tumors is young, from 6–18 months of age, although increased cases of lymphoma have been

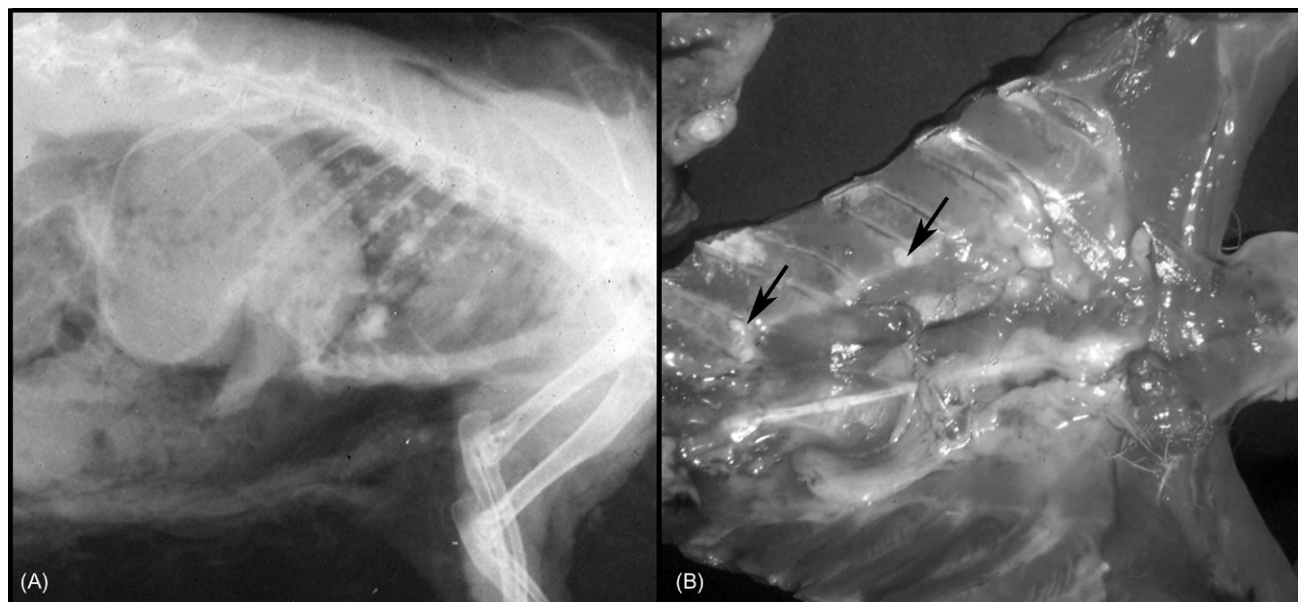


FIGURE 16.17 Osteosarcoma. Radiograph of thorax showing extensive “cotton ball” metastases (A) and gross photo showing pleural metastases (B, arrows).

reported in rabbits as old as 9.5 years of age (Ishikawa et al., 2007; Reed et al., 2009; White et al., 2000). Most early cases of lymphoma were identified in laboratory rabbits which generally do not achieve a natural lifespan since they are consumed for research purposes before reaching 2 years of age. The larger number of pet rabbits now seen in clinical practice may be resulting in an increased volume of case reports of lymphoma in older animals. The disease incidence does not appear to have a sex predilection. Cases of lymphoma characterized in early literature (Fox et al., 1976; Weisbroth, 1976) were uniformly aleukemic but more recent cases reported leukemia associated with multi-organ involvement (Cloyd and Johnson, 1978; Finnie et al., 1980; Toth et al., 1990). In these cases, clinical pathology findings included anemia, leukocytosis due to lymphocytosis, and sporadic hypercalcemia, which might have been attributable to diet. Other laboratory findings may occur with or without the presence of overt leukemia and include depressed hematocrit, low hemoglobin attributable to infiltrative obliteration of the marrow by tumor cells and, late in the course, elevated blood urea nitrogen attributable to infiltrative destruction of the renal cortex (Fox et al., 1976; Kolappaswamy et al., 2006; Toth et al., 1990). Clinical signs depend somewhat on organ involvement and include anorexia, weight loss, depression, alopecia, cutaneous nodules, with and without ulceration, blepharitis, swollen palpebrae, sudden onset of paralysis, or lymphadenopathy (Ishikawa et al., 2007; Kolappaswamy et al., 2006; Reed et al., 2009; Shibuya et al., 1999; Volopich et al., 2005; von Bomhard et al., 2007; White et al., 2000). Necropsy findings commonly



FIGURE 16.18 Lymphoma. Gross lesions illustrating hepatomegaly, splenomegaly and enlarged, pale renal kidneys.

include hepatosplenomegaly, enlarged, pale kidneys, enlarged thoracic and mesenteric lymph nodes, pulmonary nodules, pale bone marrow, and skin nodules. The presence of four gross lesions occurs consistently in cases reported in the literature and can be considered pathognomonic for lymphoma in *Oryctolagus*. This common tetrad of signs is enlarged, light tan kidneys with an irregular lumpy surface and thickened whitish cortex but normal medulla on cut surface, hepatomegaly with diffuse small (0.5mm) pale foci, splenomegaly, and lymphadenopathy (Weisbroth, 1994) (Figure 16.18).

Although practically any organ of the body may be susceptible to lymphomatous infiltrates, the most



FIGURE 16.19 Lymphoma. Infiltration of the cornea, orbital and periorbital structures.

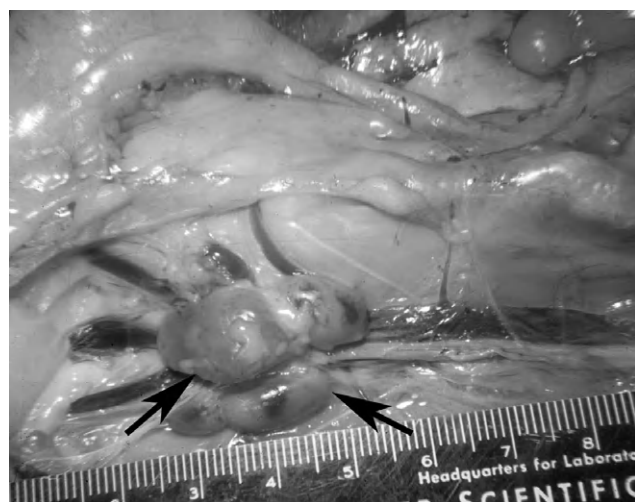


FIGURE 16.20 Lymphoma. Iliac lymphadenopathy. Note enlarged iliac lymph nodes (arrow).

commonly involved organs are the spleen, liver, kidneys, lymphoid tissues of the GI tract, and lungs, and the structures of the eye, particularly the choroid, ciliary body, iris, and anterior chamber (Figures 16.19 and 16.20). Histopathologic findings generally include diffuse infiltration of neoplastic lymphocytes into multiple organs. Neoplastic lymphocytes may infiltrate the skin, occasionally occupying the entire dermis, epidermis, and periadnexal regions. Eyelids and the Harder's gland may be affected (Volopich et al., 2005; White et al., 2000). The lymphoid tissues of the alimentary tract, including the pharyngeal tonsils, stomach, Peyer's

patches, and mesenteric lymph nodes, appear to be routinely involved in the sarcomatous process. Pulmonary lymphoma often appears as exaggerated peribronchial lymphoblastic infiltrates that may obliterate the normal aggregates of lymphoid tissue. The interalveolar septa may be infiltrated as well. Liver infiltration typically starts with periportal aggregates which progressively extend into hepatic sinusoids. Over 50% of hepatic tissues may be obliterated by solid lymphoma. The spleen has been involved in most cases but was essentially normal in the case reviewed by Van Kampen (Van Kampen, 1968). The splenic white pulp becomes uniformly obliterated by neoplastic cells which move by radial extension of the white pulp through the spleen. Hematopoietic activity in the red pulp is an apparent reflection of decreased functional ability of infiltrated bone marrow. Virtually all of the lymph nodes in the body may be involved in the neoplastic process, often bringing to prominence lymph nodes difficult to discern in normal rabbits. The normal architecture of the node usually is entirely obliterated by masses of neoplastic lymphoblasts (Weisbroth, 1994). The architecture of the mesenteric and peripheral lymph nodes is often effaced due to the extensive invasion of neoplastic cells. Lymphoid tissue involvement in the lungs appears as robust peribronchial lymphocytic expansion that exceeds and replaces the normal lymphatic tissue. The pattern of renal lesions is distinctive with the cortex usually extensively involved but the medulla relatively spared. Neoplastic cells invade the renal cortical interstitium and widely separate the normal glomeruli, causing the normal-appearing glomeruli and cortical tubules to stand out in relief as widely separated structures (Figure 16.21). The ovary and adrenals are usually involved in the sarcomatous process, frequently to the point where only scattered remnants of organ-distinctive tissue may persist.

The neoplastic lymphocytes are typically larger than normal lymphocytes, ranging in size from 5 to 35 μm in diameter, with large basophilic nuclei, two or more nucleoli, and scanty cytoplasm (Figure 16.22). Regardless of the organ, mitotic figures are common and indicative of the rapidity with which lymphomas progress in *Oryctolagus*. Lymphomas of B cell, T cell, and mixed cell origin have been described that present with these same gross lesions. T and B cell lymphomas appear to be reported in approximately equal numbers, with only one report of a mixed cell type (Gomez et al., 2002; Ishikawa et al., 2007; Reed et al., 2009; Toth et al., 1990; Volopich et al., 2005; White et al., 2000). B cell lymphoma cells were routinely positive for the cell surface marker CD79 α . and T cell lymphomas were routinely positive for the CD3 cell surface marker. The single case of a mixed cell lymphoma was a naturally occurring case in a 2.5-year-old pet Dutch dwarf rabbit that

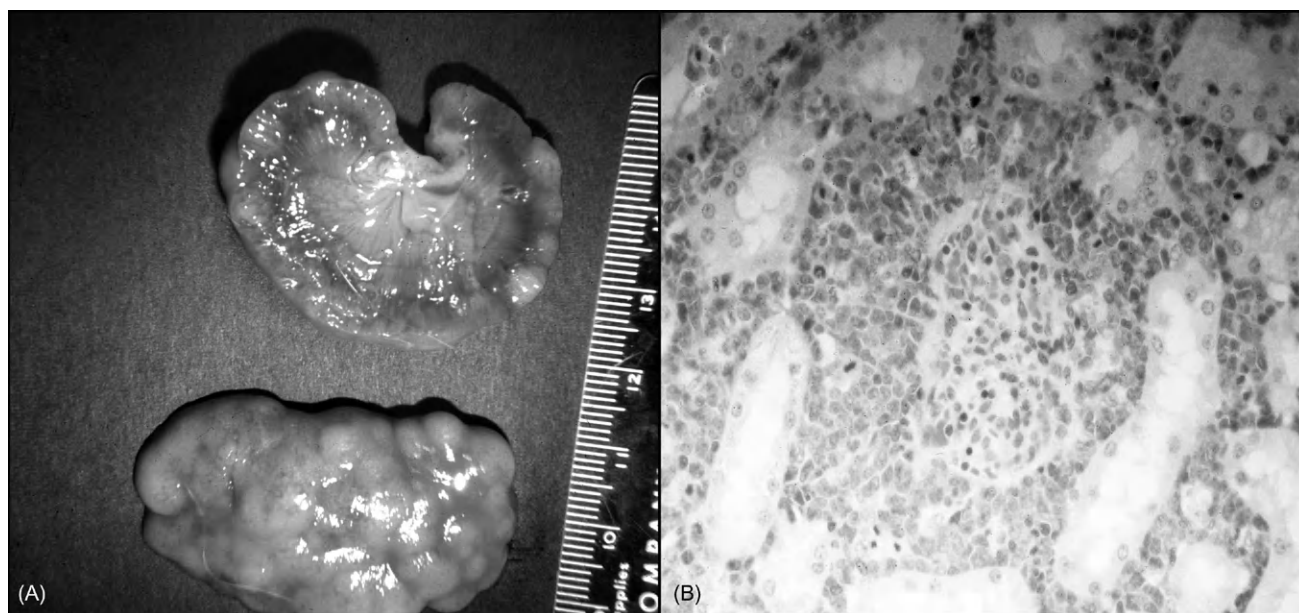


FIGURE 16.21 Lymphoma. Lymphocytic infiltration of renal cortex. Photograph of gross lesions showing irregular, “lumpy” subcapsular contour (A) and photomicrograph of cortex showing interstitial distribution with sparing of glomeruli and tubules (B).

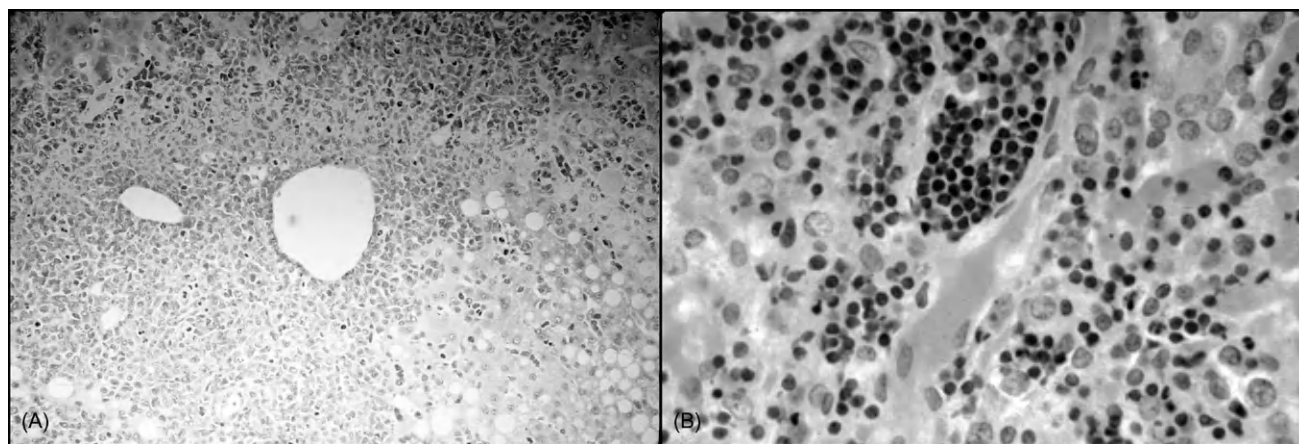


FIGURE 16.22 Lymphoma. Low-power photomicrograph showing diffuse infiltration of the liver (A) and higher magnification showing packing of hepatic canals and lymphatics with neoplastic lymphocytes (B).

was classified as a T-cell-rich, B cell lymphoma (Gomez et al., 2002).

Medlar and Sasano (1937) described a single case of retroperitoneal sarcoma involving the appendix, adrenals, mesentery, pancreas, and left ureter in a solitary large neoplastic mass. Microscopically, the tumor mass itself and focal infiltrates in several organs were not typical of lymphoma in the rabbit. It is possible that the “abdominal sarcomas” of Wallner (1922) and of Baumgarten (1906) were of this type.

The cause of lymphoma in rabbit has not been established. Although most reports of lymphoma describe sporadic occurrences, a genetic susceptibility has been described in colonies of Wirehaired and laboratory

rabbits (Fox et al., 1970). Fox et al. reported well over 100 lymphomas in the series at the Jackson Laboratory (Fox and Meier, 1976; Fox et al., 1970, 1971a, 1971b; Meier and Fox, 1973). Loliger (1966, 1968) reported ten cases from a necropsy series of 602 from a single rabbitry, too great an incidence for a random distribution. The data revealed a pattern of incidence that led to the conclusion that an autosomal gene (designated *ls*) confers, in homozygosity, susceptibility to vertically transmitted lymphoma.

The occurrence of hemolytic anemia in the same strains was also reported by Fox and group (1971b). This condition segregated similarly to the autosomal recessive pattern of *ls*, and it was interpreted as

an allelic alternative and given the designation *ha*. Evidence was submitted to show that the gene designated *ls* in strain WH rabbits (associated with lymphoma) and the gene designated *ha* in the strain X rabbits (associated with hemolytic anemia and thymoma) might in fact be the same gene (at the same locus) expressing itself differently in the two substrate strains (Fox et al., 1971b; Meier and Fox, 1973). Supporting evidence included not only common ancestry for the two strains, but also the fact that blood cells from *ls* individuals were Coomb's test positive and 21% of all individuals in both strains were Coomb's test positive. The data presented were compatible with a concept of genetic susceptibility to vertically transmitted oncogenic virus analogous to the pattern now known for the C-type RNA genome. The authors hypothesized that the conditions might result from the interactions of *ls* and *ha* genes with the remainder of the host genotype and with a (posited) vertically transmitted C-type RNA virus, with the presence of the viral genome in the WH strain being expressed as lymphoma, whereas in the X strain it is manifested as an immunopathy similar to that described in the New Zealand Black mouse and culminating in thymoma when sublethal and chronic. This was an extremely important finding because the variables of age, sex, and genetic background can be purposefully manipulated in order to gain insight into the mechanism of the disease.

Support for interaction with a retrovirus was provided by the observation of Gupta that rabbit lymphoma tissues had virus-like particles on electron microscopic examination (Gupta, 1976). Shortly afterward, marker enzyme for the presence of type C retrovirus (RNA-directed DNA polymerase, or RDDP) was demonstrated in rabbit lymphoma tissues (Bedigian et al., 1976) and, finally, type C retrovirus was isolated in culture from rabbit lymphoma cells (Bedigian et al., 1978). A causative type C retrovirus has not been identified and no further significant work has been done since then to further study the role of C-type retroviruses in rabbit lymphoma or to identify the genes involved in rabbit leukemia.

Lymphoma has been encountered as an unanticipated complication of drug treatment. In the search for nitroarene trypanocides in the treatment of Chagas disease, Teixeira 1990 produced malignant, mixed cell, non-Hodgkins type lymphomas in 25–42% of rabbits treated with nitroimidazole compounds (Teixeira et al., 1990). A developing area of study is the role of retroviruses and Herpesviruses in rabbit lymphoma.

PLASMA CELL MYELOMA

A single case of plasma cell myeloma has been described from the rabbit (Pascal, 1961). It occurred as a large solitary mass lying on the left ventral side of the

brain, extending from the medullopontine groove to the pyramidal decussation and projecting laterally to the edge of the cerebellum. It is not known if the tumor had the functional characteristics (gammopathy) of plasma cell myelomas in other species because the diagnosis was established retrospectively on the solely histopathological criteria. The microscopic description of this tumor was not typical for plasma cell myelomas of other species (Moulton, 1990).

Tumors of the Spleen

No primary tumors of splenic tissue have been reported in rabbits. The small (5mm) tumor within the spleen described by Ball (1926) was composed of normal-appearing intestinal epithelium, including goblet cells. This finding, and a lack of any other lesions, suggests the tumor was a fetal nest or hamartoma of intestinal tissue lodging in the spleen.

Tumors of the Thymus

Tumors of the thymus have been reported infrequently in the rabbit (Blanchard, 1939; Clippinger et al., 1998; Dirven et al., 2009; Drieux, 1938; Florizoone, 2005; Orr, 1939; Greene and Strauss, 1949; Vernau et al., 1995). Thymic tumors typically occur in mature rabbits between 1 and 9 years of age. Complicating the diagnosis of thymic sarcoma in rabbits is the observation that hyperplasia of the thymus in adult rabbit is not uncommon; and enlargement of the organ up to 3–4 times normal size without acquisition of neoplastic characteristics can lead to a gross diagnosis of tumor that may not be supported by careful microscopic observation. Three cases of thymic hyperplasia were observed in Weisbroth's necropsy series of approximately 600 rabbits (Weisbroth, 1974).

THYMIC CARCINOMA

A thymic reticulum cell tumor described by Orr (1939) was metastatic to thoracic organs and abdominal lymph nodes. Another case of a thymic carcinoma was reported in 2005 that occurred in a 5-year-old castrated male chinchilla rabbit. The rabbit presented for bilateral exophthalmos and a mediastinal mass was identified on thoracic radiographs. Necropsy findings revealed a large cystic mass in the cranial thorax with nodules present in both kidneys. The masses were diagnosed as metastasizing thymic carcinoma. It should be noted that the diagnosis of malignancy was based on evidence of epithelial metastases to the kidneys rather than the histologic appearance of the tumor, which was difficult to distinguish from a thymoma (Wagner et al., 2005).

THYMOMA

Thymomas in rabbits and other species have been associated with the occurrence of a paraneoplastic

syndrome with immune dysregulation. Three cases of thymoma were reported in association with systemic immunopathy (Fox et al., 1971b; Meier and Fox, 1973). The disease occurred in the X strain and was characterized in homozygotes (for a newly designated gene, *ha*) by hemolytic anemia. Disturbances in the peripheral blood included regenerative anemia characterized by polychromatophilia, anisocytosis, decreased numbers of erythrocytes, and numerous nucleated erythrocytes and lymphocytes. The erythrocytes of *haha* rabbits were Coomb's test positive. Lesions and signs included petechiation of most tissues, darkened feces, icterus, hemoglobinemia, pale liver, hemorrhagic spleen and kidney, hemoglobinuria, and edema of most tissues. The age at death in homozygous ranged from 1.5–12 months, with an average of 4–5 months. Thymoma occurred only in protracted cases. Although key kidney lesions were lacking in *haha* rabbits, the authors believed the condition to be an excellent model for systemic lupus erythematosus and to have most of the pathological features of the canine and murine models. More recent sporadic cases of thymoma have been reported in pet rabbits with diverse genetic backgrounds (Clippinger et al., 1998; Florizoone, 2005; Vernau et al., 1995). The cases reported by Clippinger and Vernau were diagnosed by identification of a mediastinal mass on thoracic radiographs and were not associated with immunopathy. Florizoone (2005) reported a case in a 5-year-old pet rabbit that presented for exfoliative dermatitis; thymoma was diagnosed at necropsy. Exfoliative dermatitis has been reported in conjunction with thymomas in cats, and may be part of the paraneoplastic syndrome that accompanies this disease.

Myeloid Tumors

Granulocytic sarcomas are rare neoplasms of domestic animals and are the result of solid extramedullary growth of myelogenous stem cells. These tumors occur most in dogs and are typically located in the lungs, gastrointestinal tract, and skin. Most cases are non-leukemic initially, but may progress to acute myelogenous leukemia. A single case of an eosinophil granulocytic sarcoma was reported in an 18-month-old, female New Zealand White laboratory rabbit that was used for polyclonal antibody production. The animal developed a rapidly growing perianal mass and was euthanized. The perianal mass was composed of neoplastic myeloid cells that contained large eosinophilic granules in varying stages of maturation. Special stains and electron microscopy confirmed the presence of eosinophil granules. Bone marrow aspirates featured a prominent population of eosinophils. Although no hematological analysis was performed to document the presence of myelogenous leukemia, numerous neoplastic cells were present in the bone marrow, cardiac chambers, and lung

which suggested that rabbit was leukemic (Perkins et al., 1996).

Vascular Tumors

Tumors of vascular origin are very rare in domestic or wild rabbits. One case of malignant peritoneal endothelioma was reported in 1927 and Green described a single case of benign ovarian hemangioma in a rabbit in 1949 (Greene and Strauss, 1949; Polson, 1927). Pletcher and Murphy (1984) reported on two cases of naturally occurring metastatic hemangioendotheliomas in rabbits submitted to the Armed Forces Institute of Pathology. One case occurred in a wild rabbit (presumed to be *Sylvilagus* sp.) of unknown age and sex that was found dead. Multiple tumorous masses were present in subcutis, adjacent skeletal muscle, and visceral organs. The other animal was an adult female New Zealand White rabbit from a research laboratory. The animal did not exhibit antemortem clinical signs and multiple nodules were seen grossly in the liver and lung during a standard necropsy. Guzman et al. (2000) reported diagnosis of a primary hepatic hemangiosarcoma that occurred in a 5-year-old New Zealand White male laboratory rabbit. The animal was found dead in the cage without antemortem clinical signs. Postmortem exam revealed good body condition, a fluctuant abdomen, and pale mucous membranes. At necropsy the abdomen contained partially clotted blood and the liver (primary site) and lung contained multiple dark red nodules. The liver and lung contained multiple blood-filled cavernous spaces that were formed by disorganized, tightly packed spindle cells. Immunohistochemistry of the neoplastic cells for von Wildebrand's (factor VIII-related antigen) was inconclusive due to intense background staining but the neoplastic cells were positive for CD31 (platelet endothelial cell adhesion molecule) which is a useful marker for endothelial cells in human and canine tissue.

Tumors of Endocrine Glands

Tumors of the Pituitary Gland

PITUITARY GLAND ADENOMA

Tumors of the endocrine glands have been rarely reported in *Oryctolagus*. Several cases of teratoma arising in the pituitary are described ahead in the section on teratomas. Polson (1927) reported an adenoma of the anterior pituitary. The tumor was small (2mm), clearly demarcated from the normal tissue, and not associated with clinical signs. Histologically it was classified as an eosinophil adenoma.

Lipman (1994) described the first report of prolactin-secreting acidophil adenomas in a group of rabbits that were also associated with mammary dysplasia. Female New Zealand White rabbits from a specific

pathogen-free colony were used for antibody production. The animal's ages ranged from 2–4.75 years, with an average age of 3.2 years. Clinical signs included enlarged mammary glands, that were cystic on palpation. Some of the rabbits had discolored teats that expressed red or yellow fluid. All of the rabbits had significant serum prolactin elevations, ranging from 22.4 ng/ml–2.21 µg/ml, which was 10–1000-fold higher than normal (1.7–2.1 ng/ml). All rabbits had enlarged pituitaries at necropsy and acidophil adenomas were subsequently diagnosed in all rabbits. The tumor tissue stained positive for prolactin. Anterior pituitary tumors are relatively frequent occurrences in man, dogs, rats, and have also been reported in cats, sheep, mice, and non-human primates, but are rarely reported in rabbits. Prolactin-secreting anterior pituitary adenomas (prolactinomas) are the most common pituitary tumors in humans, where their occurrence is associated with irregular menses and galactorrhea. Rats frequently have prolactin-secreting tumors, but mammary changes are not usual in other species with prolactinomas. Mammary changes may occur more frequently in rabbits with prolactin-secreting tumors because rabbit mammary tissue is more sensitive to the lactogenic properties of prolactin and rabbits are more dependent on prolactin for mammary functions than other species. (See also the section on mammary tumors.)

Tumors of the Adrenal Gland

ADRENAL CORTICAL CARCINOMA

Carcinoma of the adrenal cortex has been reported twice in the literature. The case described by [Boycott and Pembrey \(1912\)](#) occurred in a feral *Oryctolagus* rabbit, was bilateral, and appeared to involve the anterior pole of each kidney and the corresponding adrenal cortex. The normal tissue within each adrenal was separated from the tumor by a well-developed capsule. The cell type of the tumor (described as “round cell”) was not characteristic of adrenal cortical tumors described later from the rabbit or from other species ([Moulton, 1990](#)). Asymptomatic carcinoma of the adrenal cortex (also occurring bilaterally) was described as an incidental finding in a male rabbit of unknown age by Hueper and Ichniowski ([Hueper and Ichniowski, 1944](#)). The tumors were located primarily in the adrenal glands, which had been largely replaced by tumor tissue consisting of sheets of very large foamy cells with small, oval nuclei. The tumors had metastasized radially to the subserosa of the duodenum and periadrenal fat and connective tissue. Cholesterol crystal clefts were scattered throughout the tumor tissue. The carcinoma was typical of adrenal cortical tumors reported in other species ([Moulton, 1990](#)). The authors emphasized that the tumor tissue was not separated from the normal

adrenal cortex by a capsule. This observation is perhaps worth emphasizing because non-neoplastic nodular hyperplasia of the adrenal cortex is a very common lesion in *Oryctolagus*, but in all cases the nodules appear to be well demarcated from the remainder of the organ by a capsule.

Melanomas

At least 12 cases of malignant melanomas in rabbits are reported in the literature, including the Brown-Pearce carcinoma, which was later used for creating transplantable tumor models. The case described by Brown and Pearce arose in the eye of a 10-month-old male rabbit that had been infected with *Treponema pallidum* 2 months previously ([Brown and Pearce, 1926](#)). The authors believed the treponemal infection to have been of significance in predetermining the neoplasm. The cases described by [Sustmann \(1922\)](#) and [Holz and Heutgens \(1955\)](#) were more typical of malignant melanomas in other species. More recently, in a retrospective study based on tumor specimens submitted, [von Bomhard et al. \(2007\)](#) reported eight cases of melanoma in pet rabbits, predominately in male (six males, two females). The lesions (11 sites) were distributed in ear pinna (three), eyelid (three), head (one), perivulvar haired skin (one), scrotum (one), thigh (one), and stifle (one). Out of eleven tumors, all except three neoplasms contained abundant melanin. Histologically, all tumors had a high mitotic index, marked anisocytosis, and anisokaryosis. Lymphatic invasion was present in four tumors. Malignant melanoma on the upper eyelid of an 8-year-old Himalayan dwarf rabbit was reported by [Stanley et al. \(2003\)](#). The tumor regressed after the use of radiation through brachytherapy.

Interestingly, there was only one report of melanomas in laboratory rabbits for the 55 years prior to this review ([Hotchkiss et al., 1994](#)). One reason could be that melanoma seems to occur in older rabbits and most of the laboratory rabbits are used at a younger age. Except the case described by [Brown and Pearce \(1926\)](#) and Holz and Heutgens (1955), all reported cases of melanoma have been in rabbits 2 years of age or older. The mean age of rabbits with melanoma, based on all 12 melanoma cases reported so far, is around 4 years. It is possible that, as in the case of other rabbit tumors, underreporting also played a role in the scarcity of melanoma reports in laboratory rabbits. Increased exposure of pet rabbits to sunlight compared to laboratory rabbits which are usually kept indoors could be another compounding factor. It is worth pointing out here that literature references to the Greene melanoma in rabbits are actually to a tumor of hamster origin adapted for propagation in rabbits (see the section on Experimental Tumor Models).

Miscellaneous Neoplasia

Teratomas

Teratomas are seen infrequently in animal species, including the rabbit. They arise as generally benign, slow-growing tumors containing often mature derivative tissues, e.g., bone, muscle, hair, teeth, neuronal aggregates simulating ganglia, glandular formations, etc. arising from totipotential cells of at least two of the three embryonic layers (ectoderm, mesoderm, and endoderm). Teratomas develop from germ cells in the gonad (and that is the most common location of teratomas), or from aberrant ectopic nests of germ cells in the abdominal midline or central nervous system. Mutinelli et al. (2008) reviewed the presumptive differences in histogenic origin of gonadal versus extragonadal teratomas. The clinical signs associated with the cases were related to their location, but they were often asymptomatic and detected as incidental findings at necropsy. A pituitary gland teratoma, described by Margulies (1901), was not associated with clinical signs. The tumor was approximately 1 cm in diameter, located at the pituitary infundibulum, and had infiltrated the brain. Histologically the tumor was cystic and composed of mucus-secreting columnar epithelium, hairs, smooth muscle cells, connective tissue, and glands resembling gastric epithelium and cartilage. The teratoma described by Bishop (1978) was also intracranial (but was the cause of CNS signs), as were a number of the older, particularly German references he cited. The teratomas reported by Meier et al. (1970) and by Mutinelli et al. (2008) were gonadal, and in a retroperitoneal location, respectively.

Experimental Tumor Models

Transplantable Tumors

VX2 CARCINOMA

The rabbit VX2 tumor originated from a papilloma induced by Shope's papilloma virus. The Shope papilloma virus was originally limited to cottontail *Sylvilagus* rabbits indigenous to the American Midwest and Mississippi basin region. The disease occurs occasionally in laboratory rabbits of genus *Oryctolagus*. The natural disease causes cutaneous, pigmented warts on the head and body, which regress spontaneously in approximately 33% of animals. The papillomas progress to squamous cell carcinomas in approximately 25% of affected animals. The original tumor occurred in a Dutch Belted male rabbit in the 1940s that carried a Shope papilloma for 9–11 months (Kidd and Rous, 1940). The tumor progressed from an aural papilloma to a squamous cell carcinoma. The axillary lymph node of the rabbit contained metastatic carcinoma that

was minced to provide transplantation fragments. The tumor was then serially passaged through donor rabbits. The success of transplantation averaged only about 5%, even in genetically related Dutch Belted rabbits, until after the fifth transplant generation. The tumor, described originally as a squamous cell carcinoma, became increasingly anaplastic in the course of serial transfer, but, at least through the twenty-second transplant generation (Kidd and Rous, 1940), it unfailingly induced neutralizing antibodies against Shope papilloma virus (SPV) in homografted rabbits. Serological testing in the rabbits was suspended during World War II, and, when resumed after the war (subsequent to the forty-sixth transplant generation) it was found that antibody to SPV was no longer present in the sera of rabbits bearing the carcinoma (Rous et al., 1952). Although phenolic nucleic acid extracts of papillomas and other papilloma-derived carcinomas (e.g., VX7) retain the ability to induce papillomas in susceptible hosts, such extracts of VX2 carcinomas do not (Chambers and Ito, 1964; Hodes et al., 1963; Ito, 1960, 1970; Ito and Evans, 1965a, 1965b). The tumor take rate has increased in accordance with the increased anaplastic appearance.

In its present form, the tumor is reliably transplantable in laboratory rabbits of genus *Oryctolagus*, with a take rate of over 90% without immunosuppression (Tinkey and Uthamanthil, 2009). Following implantation, the tumor aggressively invades the host organ parenchyma, surrounding muscle, and connective tissue. The tumor does not become encapsulated, but does cause inflammation in the surrounding tissue, with variable desmoplastic reaction. The tumor frequently develops central areas of necrosis but there is little associated hemorrhage. The tumor grows rapidly, progressing from the volume of inoculum (0.5 cc) to a solid tumor nodule measuring ~1.5 cm × 1.5 cm by 14 days post-implantation (Chen et al., 2004; Tinkey and Uthamanthil, 2009). The tumor's invasiveness has been linked to the expression of high levels of tumor-associated hyaluronan (Knudson et al., 1989). The tumor reliably metastasizes to regional lymph nodes and lungs within 21 days post-implantation, but only occasionally to other organs despite the tumor's aggressive nature (Figure 16.23). The apparent barrier to unrestricted metastasis has been the subject of several investigations (Engzell et al., 1968; Fisher and Fisher, 1966; Wood et al., 1967). Greene (1953) found the host range of transplantability to include the brain of guinea pig, mouse, rat, and hamster, anterior chamber of guinea pig eye, and subcutaneous tissues and testis of hamster and mouse.

On histopathologic examination, the cells are organized into uniform sheets separated by thin cords of stroma and blood vessels with a moderate population of inflammatory cells. Nuclei are hyperchromatic and uniform in size and shape, and giant cells are

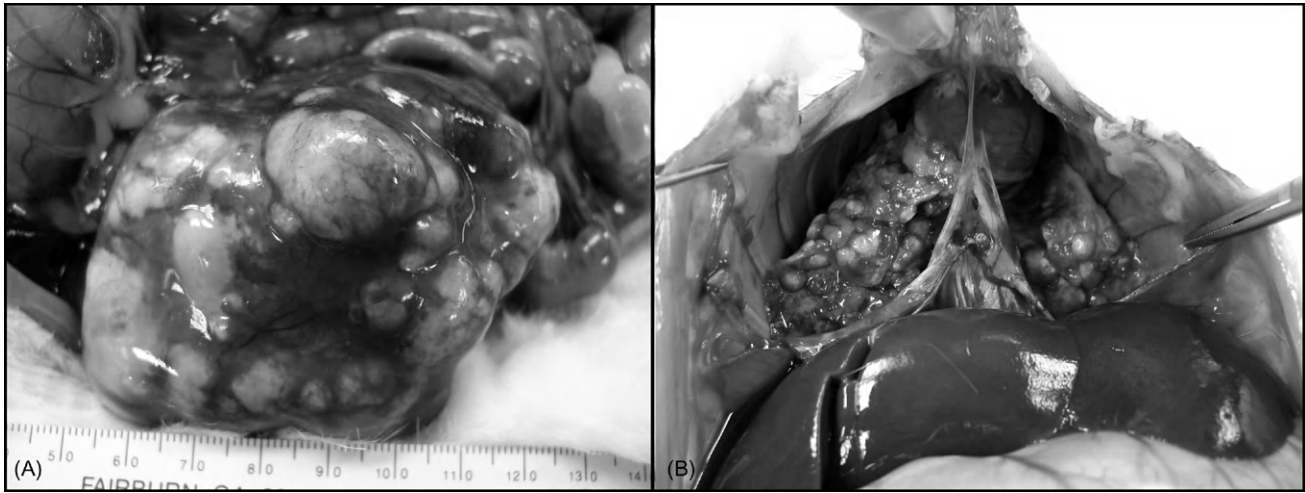


FIGURE 16.23 VX2 carcinoma. Primary inoculation site in the kidney (A) with extensive metastases to the lung (B) about 4 weeks after inoculation.

infrequent. The tumor is known to secrete a hormone-like substance that depresses the renal tubular reabsorption of phosphorus and causes hypophosphatemia, hypercalcemia, and dystrophic calcification of the tumor and soft tissues (Gertner et al., 1964; Shtacher, 1969; Vogel et al., 1967; Wilson et al., 1964, 1965). Typical features of the tumor are illustrated in Figures 16.24 and 16.25, and the excellent photomicrographs in the monograph by Stewart et al. (1959) can be used as a reference. Other carcinomas (e.g., VX7) have been developed in like manner from Shope papillomas by the same and other laboratories. Some have used newborn rabbits to develop tumor strains of greater malignancy (Fischer and Syverton, 1951; Smith et al., 1952).

The rabbit VX2 carcinoma has remained a widely used model of transplantable malignancy and has been used extensively in rabbits for a variety of research purposes (Kreider and Bartlett, 1985; Strauli and Haemmerli, 1984). Utilization has shifted from that of intrinsic exploration of the papilloma-to-carcinoma transition to employment as a reproducible, easily carried model system in random-bred hosts for exploration of treatment modalities. The tumor has been implanted in diverse anatomic locations, including liver, kidneys, intestines, brain, tongue and larynx, and extremities (subcutaneous and intramuscular locations) (Boehm et al., 2002; Carson et al., 1982; Davidson and Wallace, 1986; Davidson et al., 1986; Kim et al., 2004; Nakase et al., 1977). One common research use is to create a solid tumor for imaging studies. Imaging modalities used include radiography, CT, US, MR, and PET imaging. The tumor has been used to study various approaches to therapies, including chemotherapy, interventional radiology, and immunotherapy (Rosales et al., 2000). This model has also been used to investigate

tumor angiogenesis and paraneoplastic syndromes, such as tumor-associated hypercalcemia, granulocytosis, and cachexia (Doppelt et al., 1982; Hough et al., 1983; Ishiko et al., 1999; Tamargo et al., 1990). At The University of Texas M.D. Anderson Cancer Center, this tumor model has been implanted into skeletal muscle in the rear leg, liver, and kidney, and used extensively to study the efficacy of treatment using embolic compounds delivered via intravascular catheters, development of devices for non-surgical ablative techniques, such as radiofrequency and MR-guided focused ultrasound, and as a model to allow correlation of images with histopathology. Although an exhaustive cataloging of references to the VX2 carcinoma is beyond the scope of this chapter, a review of recent uses of this important tumor model is presented in Table 16.4.

The tumor cell line has never been reliably established in cell culture and is generally maintained by serial passage in rabbits. There have been two reports of establishment of cell lines from the VX2 tumor, but no additional references have been published and the cell lines are no longer available from either source (Easty and Easty, 1982; Georges et al., 1985). The most reliable implantation site is intramuscular and it is typically implanted in one or both caudal thigh muscles of the donor animal. The tumor is generally harvested 14 days postimplantation, when the tumor nodule has reached ~2.0 cm × 2.0 cm. Delaying harvest past 14 days results in morbidity due to pulmonary metastasis and poor quality of tumor due to increased amounts of necrosis. The tumor can be minced, placed in media, and frozen at -80°C to preserve inocula for propagation of the tumor. Implantation of frozen tumor inocula results in reliable but slightly lower tumor take rates and slightly slower growth rates (Tinkey and Uthamanthil, 2009).

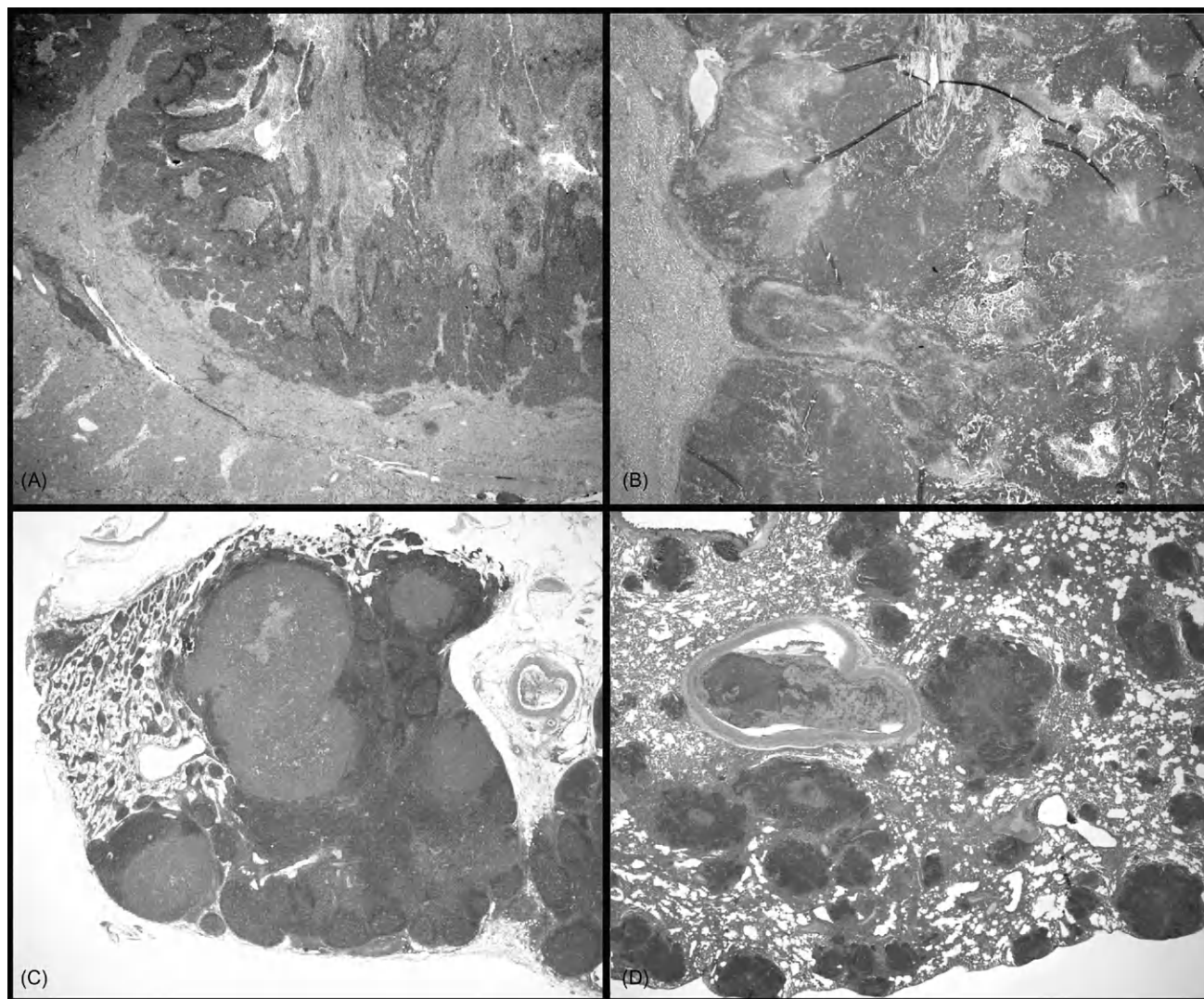


FIGURE 16.24 VX2 carcinoma. Typical histologic appearance of primary inoculation sites in muscle (A) and liver (B) and metastatic lesions in lymph nodes (C) and lung (D).

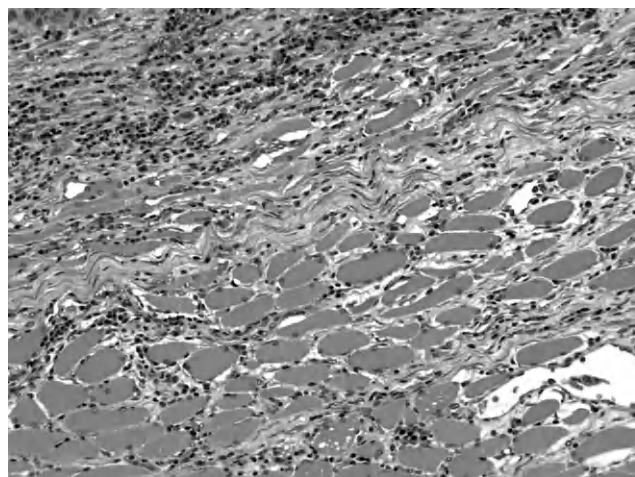


FIGURE 16.25 VX2 carcinoma. Photomicrograph of primary inoculation site. Adjacent muscle tissue shows extensive infiltration by the tumor between muscle bundles.

BROWN-PEARCE CARCINOMA

The Brown-Pearce carcinoma, one of the most useful of the transplantable rabbit tumors, has great historic importance and was used extensively to study tumor immunogenicity. The Brown-Pearce carcinoma is an anaplastic carcinoma that was originally derived from a syphilitic chancre of an experimental rabbit used to study *Treponema pallidum*. Despite the exhaustive description provided by Brown and Pearce (Brown and Pearce, 1923a, 1923b, 1923c, 1923d, 1923e, 1923f, 1923g, 1924) an understanding of the antecedent tissue from which the carcinoma originated is destined to remain somewhat ambiguous. For that reason it is worth describing the circumstances of the rabbit in which the tumor arose. Wade Brown and Louise Pearce of the Rockefeller Institute were intensively engaged in the study of syphilis. In June, 1916, a rabbit, injected

TABLE 16.4 Research Uses of the VX2 Carcinoma

Type of Use	Reference
As a model for tumor therapy	
Localized chemotherapy, vascular embolization, or combination therapies	(Hsieh, 1990; Konno, 1990; Koyama et al., 1990; Tromberg et al., 1990; Hasegawa et al., 1991; Berger et al., 1993; Curley et al., 1993; Kamei et al., 1993; Sakane et al., 1993; Koishi et al., 1994; Koyama et al., 1994; Komatsu, 1995; Mugitani et al., 1995; Yanai et al., 1995; Hoshi et al., 1997; Nagamitsu et al., 1998; Alexiou et al., 2000; Nakamura et al., 2000; Tabaru et al., 2001; Liu et al., 2002; Zhang et al., 2002; Yoon et al., 2003; Pohlen et al., 2004a; Pohlen et al., 2004b; Zhang et al., 2004; Budzinska et al., 2005; Ling et al., 2005; Chen et al., 2008)
Radiofrequency ablation	(Goldberg et al., 1996; Merkle et al., 1999; Polascik et al., 1999; Boehm et al., 2001; Miao et al., 2001; Boehm et al., 2002; Mia et al., 2002; Munver et al., 2002; Lee et al., 2003; Nakada et al., 2004; Eichel et al., 2005; Frieser et al., 2007; Luo et al., 2008; Mostafa et al., 2008)
Hyperthermia or cryotherapy	(Isbert et al., 2001; Nakada et al., 2004)
Focused ultrasound	(Kigure et al., 1995; Prat et al., 1995; Adams et al., 1996; Hazle et al., 2002; Luo et al., 2008)
Approaches to immunotherapy	(Carroll et al., 1995; Gnant et al., 1999; Sano et al., 2000; Winter et al., 2008)
As a model for tumors of particular anatomic structures	(Tabuchi et al., 1991; Sueyoshi 1992; Tada et al., 1992; Wunderlich et al., 1992; Kim et al., 1993; Jiang et al., 1996; Wilkinson et al., 2001; Lin et al., 2002; Dunne et al., 2003; Chen et al., 2004; Kim et al., 2004; Takahashi et al., 2004; Quan et al., 2006; Lv et al., 2007; Duan et al., 2008; Virmani et al., 2008; Lee et al., 2009; Zhao et al., 2009)
As a model for imaging studies	(Parker et al., 1990; Phillips et al., 1991; Pillai et al., 1991; Suga et al., 1991; Choi et al., 1993; Shmulewitz et al., 1993; Taupitz et al., 1993; Forsberg et al., 1995; Ra et al., 1995; Robinson et al., 1995; Leander 1996; Oya et al., 1996; Runge et al., 1996; Kono et al., 1997; Runge et al., 1997a; Runge et al., 1997b; Tajiri et al., 1997; Brown et al., 1998; Runge and Williams, 1998; Brown et al., 1999; Moon et al., 2000; Purdie et al., 2001; Sayegh et al., 2001; Herborn et al., 2002; Herborn et al., 2003; Maehara and Maehara, 2003; Malich et al., 2003; Purdie et al., 2003; Winter et al., 2003; Wyman et al., 2003; Kondo et al., 2004; McDannold et al., 2004; Kim et al., 2005; Yuan et al., 2005; Yuan et al., 2005; Choi et al., 2006; Hu et al., 2007; Choi et al., 2008; Jiang et al., 2008; Youn et al., 2008; Yuan et al., 2008; Ohira et al., 2009)
As a model for tumor behavior and physiology	(Wunderlich et al., 1992; Yoshikawa et al., 1993; Gadeholt-Gothlin and Gothlin, 1994; Kubota et al., 1994; Thorstensen et al., 1994; de Baere et al., 1996; Fujimiya et al., 1997; Hatton et al., 1999; Kaneko et al., 1999; Prudhomme et al., 1999; Ishida et al., 2000a, 2000b, 2000c; Kuszyk et al., 2000; Murata et al., 2000; Ishiko et al., 2001; Wilkinson et al., 2001; Chikui et al., 2002; Obata et al., 2003; Seki and Fujimura, 2003; Wissniowski et al., 2003; Zhao et al., 2004; Li et al., 2006; Quan et al., 2006; Saad-Hossne et al., 2007; Weinberg et al., 2007; Qi et al., 2008; Lee et al., 2009)

with testicular tissues from another rabbit infected with *Treponema pallidum*, developed typical syphilis chancres at each of the intra-scrotal sites. In the intervening period between 1916 and 1919, the testicles of the rabbit became fibrotic and atrophic. In April 1919, the rabbit developed generalized cutaneous lesions and, by 1920, an eczematoid dermatitis of the left scrotum. This process became chronic but was marked by exacerbations and remission. In October of 1920, a nodule was noted at the site of the primary chancre in the left scrotum. The nodule, which rapidly increased in size, proved to be a malignant tumor. Treponemes, although demonstrable in a popliteal lymph node as late as 1 month prior to the appearance of the nodule, could not be demonstrated in dark-field preparations from nodule biopsies. The nodule was removed twice by surgical excision but recurred.

The rabbit was euthanized in March 1921. Necropsy findings were those of metastatic cancer and chronic syphilis. Tumor metastases were present in liver, bone, bone marrow, lung, spleen, kidneys, left testicle, inguinal adipose tissue, and the iliac, inguinal, and

retroperitoneal lymph nodes. The eczematoid dermatitis of the scrotum extended cranially to the skin of the abdominal ventrum. Chronic degenerative and inflammatory changes were evident in the epithelium of the tongue, oral cavity, esophagus, respiratory tract, and testes. Elsewhere, the skin was fibrotic with atypical pilofollicular proliferations, and the endocrine glands were abnormal.

Neoplastic tissue from the left inguinal lymph node was minced and injected into the testes of other rabbits. Brown and Pearce described the biological behavior of the transplanted tumor (Brown and Pearce, 1923b, 1923c, 1923d, 1923e, 1923f, 1923g, 1924) and noted the characteristic variable results. Tumor take rates varied from 100% in the testicle, anterior chamber, and brain, to 80% intramuscularly to 20% by the subcutaneous or intravenous route. Spontaneous regression of the tumor was common, even after the onset of metastasis, and varied with the genetic background of the recipient. The course might be fulminating with death 6–7 weeks after inoculation or prolonged to more than 6 months. Most commonly death occurred 8–12 weeks after

transplantation. The tumor was remarkably stable, and after some 15 years of continuous transplantation Casey (Casey, 1939; Casey and Pearson, 1939) wrote that the tumor had undergone no change with regard to metastatic distribution. It was noted that rabbits in which the tumor had regressed were resistant to subsequent transplants.

Neither desiccated tumor nor cell-free extracts were successful in establishing tumors (Pearce, 1927). Greene and colleagues (1949), who pioneered the technique of anterior chamber preparations for tumor transplantation, reported 100% success with the Brown-Pearce carcinoma (Ellerbrook et al., 1952; Saphir, 1941). Growth in the anterior chamber was rapid, often filling the chamber within 7–10 days and producing metastases within 4–6 weeks. Greene (1949) was unable to transplant the tumor to guinea pigs, and, although initial growth occurred in testicle, eye, and subcutaneous sites in rats, mice, and hamsters, regression was the invariable rule in these hosts. Growth of the tumor is supported on embryonating chicken chorionic allantoic membrane (Schechtman, 1950). Rabbits transplanted with the tumor may die as early as 10 days after inoculation or may survive for over 100 days (Stewart et al., 1959).

The tumor itself is soft and friable, has areas with hemorrhage, and may be necrotic, particularly toward the center. Microscopically the tumor is an anaplastic carcinoma with a monotonous medullary pattern of cords of carcinoma cells supported in a scanty stroma of connective tissue with a few reticular fibers and blood vessels. The tumor cells are typically round or oval with hyperchromatic centrally located nuclei in a slightly basophilic but pale cytoplasm. The prominent nuclear membrane encloses two or four acidophilic nucleoli, dense chromatin bars, and a lace-like meshwork. Mitotic figures are common. Necrosis is more common centrally than at the periphery. The tumor provokes little inflammation unless regression supervenes. The tumor is illustrated in Figures 16.26 and 16.27. Excellent illustrations of the tumor are to be found in the original reports by Brown and Pearce, the reviews by Snell (1953) and Stewart et al. (1959), and the investigation by Appel et al. (1941).

The Brown-Pearce carcinoma elicits tumor-specific antibody response in some rabbits (with donor-specific responses in some rabbits depending on genetic background), thus the carcinoma has been used extensively as a model of tumor immunity (Snell, 1953). Casey observed in 1932 that rabbits injected with killed Brown-Pearce carcinoma tissues were rendered more susceptible to malignant consequences when later inoculated with the tumor (Casey, 1932, 1933). He believed there were “enhancing materials” or an “XYZ factor” in the preserved tumors which was responsible for the specificity of the effect (Casey, 1941). The effects could

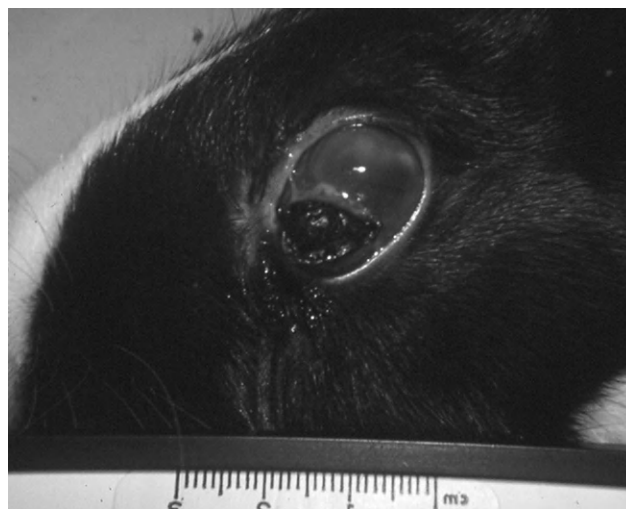


FIGURE 16.26 Brown-Pearce carcinoma. Carcinoma explant in anterior chamber of the eye, approximately 30 days after inoculation. Note corneal perforation by the tumor.

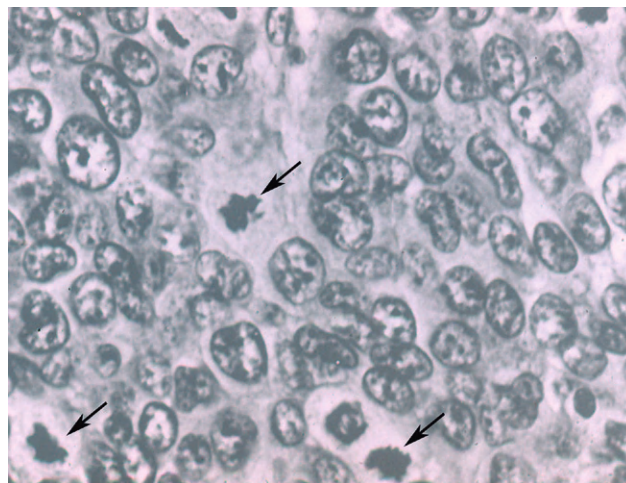


FIGURE 16.27 Brown-Pearce carcinoma. Photomicrograph of tumor. Note abundant mitotic figures (arrows).

last as long as 7 months after injection (Casey, 1934a, 1934b) and provided important support for the general theory of immunologic enhancement of tumor growth developed by Nathan Kaliss. Kaliss, in a review of this evidence, emphasized the conditions required for maximal expression of immunologic enhancement and the dependency of the phenomenon on late-appearing immunoglobulin G (IgG) antibody (Kaliss, 1969).

Even before it was realized that the XYZ factor(s) was an immunogen of the tumor, it was generally recognized that there were other immunologic consequences of transplantation. Saline extracts of the tumor contain antigens that fixed complement when mixed with sera from rabbits bearing the tumor (Appel and Strauss, 1942; Cheever, 1940; Ellerbrook et al., 1952; Friedewald

and Kidd, 1945; Jacobs and Houghton, 1941; Kidd, 1938). Rabbits implanted with the tumor developed antibodies that were either tumor-specific or donor-specific, or both, depending on the genetic background of the recipient. Blue Cross and Chocolate Dutch rabbits formed antibodies only against the tumor-specific antigens, and other breeds, if they produced antibody at all, produced both types (MacKenzie, 1945). It has been generally concluded that the Brown-Pearce carcinoma contains antigens different from those of normal tissues. The antigen is probably a ribonucleoprotein (MacKenzie, 1945) and located on tumor cell microsomes (Kidd, 1946b). Certain methods of antigen extraction (e.g., methanol precipitation) preferentially favor release of tumor-specific antigens (Zilber, 1958). Tumor-specific antibodies inhibit culture of the tumor in vitro (Kidd, 1944, 1946a). Despite this extensive early work performed with this model, research use has decreased substantially and from 1990 to 2009 there have only been less than five publications describing the use of the Brown-Pearce carcinoma.

GREENE MELANOMA

In an initial report, Greene (1958) described a spontaneous melanoma from the pigmented flank organ of a 2.5-year-old Syrian golden hamster (*Mesocricetus auratus*). The tumor was transplantable to homologous hosts and heterologously to DBA mice and guinea pigs, but not to rabbits. In certain recipients melanomas developed with amelanotic foci, whereas other developed foci that appeared sarcomatous. This feature led Greene to consider the tumor to be a carcinosarcoma in which the sarcomatous elements were thought to arise from neoplastic transformation of the stroma of the primary melanocarcinoma.

Further study of the tumor revealed that metastatic foci from primary subcutaneous melanomas (or from intravenous injection of tumor suspensions) arose in the lung and were invariably melanotic (Greene and Harvey, 1966). On the other hand, tumor suspensions injected into the left heart, or aorta, produced tumor foci that were partially or completely amelanotic. Subsequent transplants of an amelanotic tumor line were transplantable as xenografts to additional hosts, including the rabbit.

The tumor has been a useful model in the rabbit for studying the physiology and treatment of ophthalmic tumors, including human ophthalmic melanomas. Literature references to animal "uveal melanoma" or "choroidal melanoma" refer, in fact, to the Greene melanoma (Panagopoulos et al., 1989; Swift et al., 1990). More recently, a refined model for ocular tumors using Greene melanoma was described using immunosuppressive agent, cyclosporine (de Waard-Siebinga et al., 1994).

The only experimental melanoma successfully implanted in the rabbit eye without immunosuppression

is the Greene melanoma (Hu et al., 1994), which makes it a good model for immunotherapy studies. The limitations of this model in ophthalmic tumor research include its lack of melanin pigments as well as the clinical course, which is different from human uveal melanomas (Romer et al., 1992). To fill this gap, highly melanotic tumor cells of mouse (B16F10 cells) (Hu et al., 1994; Pineda et al., 1998) and human (Lopez-Velasco et al., 2005; Maloney et al., 2009; Marshall et al., 2007) origin are used more often.

OTHER TRANSPLANTABLE TUMORS

A transplantable squamous cell carcinoma cell line, named "RSCC-1" was recently reported by Wulong et al. (2008). The cell line was established from tongue tumors of epithelial origin, which had been induced in New Zealand White rabbits by topical application of dimethyl benzanthracene (DMBA) and repeated mechanical stimulation of the site. The resulting tumors were harvested and serially passaged into additional NZW rabbits. The tumor take rate reached 100% in rabbits after 70 passages, and was also 100% successful when implanted in immunodeficient mice. The cells transplanted to the lateral margin of tongue of ten rabbits grew to solid nodules adhered tightly to lingual mucosa within 1 week. Later stages demonstrated cervical lymph node metastasis in 50% of animals as well as lung metastasis in 20% of animals. No spontaneous regression was noted in any animals bearing this tumor.

Oncogenic Studies

Virally Induced Tumors

NATURALLY OCCURRING

Certain Pox and Papova group of viruses, especially cottontail rabbit papillomavirus (CRPV) (Breitburd et al., 1997; Govan et al., 2008), rabbit oral papilloma virus (ROPV) (Wilgenburg et al., 2005) and Shope fibroma have played an important role in furthering the understanding of viral oncogenesis as a basic biological process. The rabbit has been used as a model for study of human papilloma viruses (HPV). Recombinant mutants of cottontail rabbit papilloma virus (CRPV) harboring the E6 or E7 genes from IIPV induced papillomas in rabbit hosts. By this means, Brandsma (2005) demonstrated that the E7 gene of HPV is required for induction of papillomas. It has been observed that among commonly known oncogenic papilloma model systems including canine and bovine papillomas, only ROPV had a tissue tropism and a life cycle organization that resembled those of the human mucosal papilloma types (Peh et al., 2002). ROPV and CRPV and the lesions they induce have been among the most intensively investigated in the entire field of viral oncogenesis. Early concepts of viral oncogenesis in mammalian species

were formed on the basis of experimentation with these tumors and they still remain as important models of viral tumorigenesis, and the concept of coevolution between host and virus was generated largely from investigations employing these viruses. The natural history, host range, epizootiology, virology, pathology, and immunology of the viruses are reviewed in Chapter 9. Additional reviews with important bibliographical resources include the monographs by Fenner (1965), Gross (1970), and Moulton (1990) and the texts edited by Dalton and Haguénau (1962) and Stewart et al. (1959). All of the viruses (with the exceptions of hare fibroma and oral papilloma viruses), namely, myxomatosis, Shope fibroma viruses, Shope papilloma virus, the herpesvirus lymphoma of Hinze, and the Dos Santos hepatoma, have been discovered in and limited in natural distribution to the American continents.

Malignant rabbit fibroma virus (MV) is a poxvirus related to the Shope fibroma virus and the Moses strain of rabbit myxoma virus. Rabbits receiving intradermal inoculation with MV developed a primary tumor at the site of inoculation, with subsequent rapid development of secondary tumors in the extremities including the ears, nose, and feet. The tumors were malignant and resembled myosarcomas. The rabbit developed a profound virus-associated immunosuppression and succumbed to secondary opportunistic infections by commensal Gram-negative organisms within 12–14 days after inoculation. The immunosuppression affected both B and T lymphocytes. This has been touted as a model for AIDS in man (Strayer, 1985).

EXPERIMENTALLY INDUCED MODELS

Models of rabbit lymphoma have been induced by inoculation with oncogenic viruses from various species of animals. A rabbit model for adult human T-cell leukemia was developed in 1990 by Isono. The model was produced by inoculation of B/J X Chbb:HM rabbit F1 hybrids with a transformed T-cell line maintained in culture. The cell line was established from peripheral blood lymphocytes of an F1 hybrid infected with human T-cell leukemia virus 1 (HTLV-1). This HTLV-1 transformed cell line, 647a, is leukemogenic in newborn F1 rabbits, with a median lethal dose of 10^7 cells. The animal model closely resembles the human disease in the development of leukocytosis associated with leukemic infiltration of the major organs. The cell line was used to reproducibly supply leukemia rabbits for exploration of treatment modalities, for example, zidovudine (AZT) (Isono et al., 1990). In the early 1990s Hayashi and Simpson reported the induction of lymphomas in rabbits after the inoculation of HTLV-1 producing leukocyte cell lines. A confounding factor was that DNA analysis of the resulting rabbit lymphomas did not show any integration of the HTLV genome. Although it has been shown that the

HTLV-1 virus consistently infects rabbits, the majority of animals are asymptomatic for clinical disease and do not develop neoplasia. However, these models are useful for the study of viral transmission and vaccination (Akagi et al., 1985; Lairmore et al., 2005). There have been some reports of production of an “ATL-like” disease in rabbits following intraperitoneal or intravenous inoculation of HTLV-1 transformed cells, but the studies did not conclusively demonstrate whether the leukemia cells were from the inoculums or generated de novo (Ogawa et al., 1989; Seto et al., 1988; Zhao et al., 1993). There have been sporadic reports of neoplasia occurring in HTLV-1-infected rabbits. Zhao et al. (2002) reported the occurrence of a thymoma 5.5 years after inoculation with an HTLV type 1 molecular clone. In this case, an HTLV-1 provirus was demonstrated in thymocytes and thymic epithelium. A second rabbit in the study developed an endometrial carcinoma 2.5 years after inoculation.

Further study by Hayashi and Akagi (2000) demonstrated that the HTLV-1 producing cell line was also producing an EBV-like gamma herpesvirus which was later shown to be the causative agent of the lymphomas. A significant amount of work has now been done to establish rabbits as a model of Epstein-Barr-virus-associated diseases in man. Epstein-Barr virus (EBV) is one of several human herpesviruses and is a member of gamma herpesvirus family (lymphocryptovirus). EBV is closely linked with the development of human neoplasms including Burkitt's lymphoma, nasopharyngeal carcinoma, oral hairy leukoplakia in AIDS patients, and a number of other cancers, but no firm causal relationship has been established to date. Work by several laboratories showed the macaque herpesviruses caused lymphoma or lymphoproliferative diseases in domestic rabbits. In a study by Yakovleva et al. (1987), six of six young gray rabbits developed generalized lymphoma following inoculation with *Macaca arctoides* peripheral lymphocyte cell cultures producing B-lymphotropic herpesvirus and C-type retroviruses. Yakovleva concluded that the lymphoma development was related to co-inoculation of the EBV-like herpesvirus (HVMA) and the C-type retrovirus similar to HTLV1. Subsequent studies by Timanovskaia et al. (1988) and Wutzler et al. (1995) were conducted in which rabbits of various breeds were inoculated with Herpesvirus of *Macaca arctoides* (HVMA) from either HVMA-producing cell lines or cell-free virus. Around the same time, a study was performed by Medveczky et al. (1989) in which New Zealand White rabbits were inoculated with Herpesvirus saimiri. In these three studies, inoculation of various breeds of rabbits with these different primate herpesviruses induced generalized lymphoma with multiple organ infiltration. Wutzler (1995) showed definitely that the development of malignant lymphoma in New Zealand White, black, and gray rabbits was caused

by the HVMA virus by demonstrating that the disease occurred in animals that were directly inoculated with the virus rather than the virus-producing cell line. In that study, 17 of 32 rabbits infected with HVMA derived from the simian cell line MAL-1 developed lymphoproliferative disease; 13 developed malignant lymphoma and four developed lymphoid hyperplasia. Wutzler demonstrated the expression of EBER-like transcripts and HVMA-DNA in the lymphoma cells. This model was reported to be a suitable animal model for studying antiviral drugs and vaccines against EBV.

Hayashi and Akagi (2000) reported a rabbit model of EBV-associated lymphomagenesis in the rabbit created by inoculation of EBV-like viruses from macaques. Malignant T cell lymphomas developed in 80–90% of the rabbits 2–5 months following either intravenous infection or peroral spray of EBVs from cynomolgus monkeys (Si-IIA-EBV or Cyno-EBV). This rabbit model of Herpesvirus-induced lymphoma and lymphoproliferative disease has been used to study the oncogenesis of herpesviruses and the efficacy of drug therapy. These models are good models for fatal lymphoproliferative disease seen in fatal infectious mononucleosis/X-linked lymphoproliferative disease and EBV-pos T cell lymphoma. This rabbit model offers a good substitute for monkeys for studying the biology and pathogenesis of EBV, and can be used to study the mechanism of the oropharyngeal route of infection.

A rabbit model of T-cell lymphoma was produced by inoculating New Zealand White rabbits intravenously with primate T cells that were infected with a naturally occurring herpesvirus (HV-MNE) or by direct IV inoculation of cell-free virus. HV-MNE is novel Epstein-Barr-like virus that was isolated from a pig tailed macaque (*Macaca nemestrina*) that was diagnosed with a T cell mycosis fungoides-T cell lymphoma. Eight of 14 rabbits developed T cell lymphoma with lymphocytic infiltrates in spleen, liver, lungs, kidney, heart, lymph nodes, thymus, skin, and subcutaneous tissues. Viral sequences from lymphomatous tissue were detected by PCR; therefore this is a valuable model for studying the genetic determinants of T-cell transformation by this EBV-like primate virus (Ferrari et al., 2001).

Hemophagocytic syndrome (HPS) is a syndrome seen in human Epstein-Barr-virus-associated disease in man. HPS is a systemic lymphohistiocytic proliferative disorder characterized by systemic activation of macrophages to undergo phagocytosis and is characterized by fever, liver dysfunction, coagulopathies, and pancytopenia. Hayashi et al. (2001) described a new model of this disease in rabbits. Rabbits were inoculated with Herpesvirus papio either by intravenous inoculation of virus-producing cells or by peroral spray of cell-free HPV. Rabbits inoculated by either of these routes developed lymphoproliferative disease with hemophagocytic

syndrome. For a further description of these models, the reader should read the two-part review written by Hayashi in 2005 which reviewed the existing models of EBV-associated diseases and their applications (Hayashi, 2005a, 2005b).

The rabbit has also been used as a model for study of human papilloma viruses (HPV). Recombinant mutants of cottontail rabbit papilloma virus (CRPV) harboring the E6 or E7 genes from IIPV induced papillomas in rabbit hosts. By this means, Brandsma (2005) demonstrated that the E7 gene of HPV is required for induction of papillomas.

Malignant rabbit fibroma virus (MV) is a poxvirus related to the Shope fibroma virus and the Moses strain of rabbit myxoma virus. A rabbit receiving intradermal inoculation with MV developed a primary tumor at the site of inoculation, with subsequent rapid development of secondary tumors in the extremities including the ears, nose, and feet. The tumors were malignant and resembled myosarcomas. The rabbit developed a profound virus-associated immunosuppression and succumbed to secondary opportunistic infections by commensal Gram-negative organisms within 12–14 days after inoculation. The immunosuppression affected both B and T lymphocytes. This has been touted as a model for AIDS in man (Strayer et al., 1985).

Chemically Induced

Chemical induction of tumor is a commonly used technique to study various aspects of oncogenesis in rodents. Chemical carcinogenesis is known to activate various oncogenes that are important in the initiation, promotion, and progression of cancer. The laboratory rabbit has received limited attention as a model for exploration of oncogene activation in neoplasia. The chemical compounds that have been used for induction of tumors in rabbits include dimethylbenzanthracene (DMBA) (Corominas et al., 1991; Ghadially, 1958; Wulong et al., 2008), dimethyl nitroso-amine (DNA) (Reznik and Padberg, 1991), and beryllium salts (Barnes et al. 1950; Janes et al., 1954; Tapp, 1969b).

Members of the ras gene family (H-ras, K-ras, and N-ras) are one of the most commonly active oncogenes in human tumors, and are activated in about 26% of human tumors (Corominas et al., 1991). Cellular oncogenes are highly conserved in vertebrate species, and the K-ras-2 locus, which has been characterized in both humans and mice, has been located to the centromere area of the rabbit chromosome 16 by use of the labeled human cDNA probe (Martin-DeLeon and Picciano, 1988). In like manner, Xu and Hardison (1989), have localized the H-ras-1 proto-oncogene onto rabbit chromosome 1 and demonstrated synteny of the Harvey-ras-1 (H-ras-1) gene with those for parathyroid hormone and beta-like globins in a number of mammalian orders,

underscoring the strong conservation of the gene groups in the evolution of chromosomes. There is a marked correlation between the type of ras gene activated and the tissue type affected, with the H-ras gene activated in epithelial tumors and either K-ras or N-ras genes activated in mesenchymal tumors.

Dimethylbenzanthracene-induced keratoacanthomas (KA) and squamous cell carcinomas (SCC) of rabbit ear skin have been employed to investigate activated oncogenes (H-ras) in both developing and regressing KA and metastasizing SCC (Corominas et al., 1991; Leon et al., 1988). Application of DMBA on the inner ear skin of the rabbit causes development of keratoacanthomas, which are benign tumors. Keratoacanthomas induced with DMBA on the ear spontaneously regress after some time, thus the rabbit model is considered useful for investigation of the ras genes because it results in an array of both developing and regressing tumors and thus offers a unique model in which to identify and study oncogenes that are not sufficient to maintain tumor growth. The rabbit tumors were regarded as morphologically and histologically identical to human keratoacanthomas (Ghadially, 1958; Prutkin, 1967; Prutkin and Gerstner, 1966). Corominas et al. (1991) showed that these keratoacanthomas has high genetic similarity to human keratoacanthoma. The DMBA-induced keratoacanthomas show a high frequency of H-ras-activated genes carrying a mutation in codon 61. The same is observed in human keratoacanthomas, although in humans mutations they are in both the 12th and the 61st codons of the H-ras gene. Other benefits of this model include the accessibility of the tissue to chemical carcinogenesis, and the ease of monitoring tumor development.

More recently, DMBA was used to induce tumors on the tongue of New Zealand White rabbits (Wulong et al., 2008). Out of 100 rabbits used for the study, two rabbits developed an aggressive, poorly differentiated, squamous cell carcinoma that was transplantable to other rabbits. The authors also report a transplantable tumor cell line developed from this model, which is discussed elsewhere in this chapter.

The chemical carcinogen diethylnitrosamine (DENA) is a potent carcinogen, and has been used to induce tumors in many species of animals, including rodents, swine, and non-human primates. In a report from a single laboratory (Reznik and Padberg, 1991), rabbits were administered oral DENA three times weekly over 14 months. Eleven rabbits (100%) developed liver tumors by the end of the study; hepatocellular carcinomas were the most common tumor found in ten rabbits, with one rabbit developing hepatocellular adenoma. The hepatocellular carcinomas metastasized to the lungs in 70% of the affected animals. Five of the animals also developed additional primary tumors, including adenomas and carcinomas of the nasal cavity, kidney, and uterus. This

study was done to create a model which allowed study of the clinicopathological changes that occurred during the development of hepatocellular carcinoma. The study was not repeated and no cell line was established from these tumors.

Beryllium salts were found to be highly carcinogenic to rabbits, inducing osteosarcomas (Fodor, 1977; Groth, 1980; Hiruma, 1991; Komitovski, 1971; Mazabraud, 1975). The tumors typically originate from medullary area of the bone. Various refinements of the technique was reported including a single injection to the epiphysis (Mazabraud, 1975) and deposition of hydroxypropylcellulose mixed beryllium oxide pellet into the fracture callus (Hiruma, 1991).

Radiation-Induced Models

There is one report of radiation-induced tumorigenesis in rabbits (Buchholz et al., 1999). One hundred and two female New Zealand White rabbits underwent mandibular radiation treatments with a total dose of 64Gy in 20 treatment fractions. Nineteen percent of the surviving animals developed osteosarcoma. The report suggested that TP53 mutations may have played a major role in development of these tumors.

Genetically Engineered Models

Genetically engineered rabbits were created by Sethupathi et al. (1994) by conjugating c-myc to the Ig k-chain enhancer gene Ek. Eight of 19 progeny from the resulting transgenic line developed a variety of tumors, including four rabbits with lymphoma, one with embryonic nephroma, one with hepatoma, one with ovarian carcinoma, and one with basal cell carcinoma. The lymphomas were of B-lymphoid lineage. Becker et al. (1990) created Emu-myc transgenic rabbits which developed Polyclonal B cell leukemias at 17–20 days of age. Rabbits have also been used as recipients of oncogenes fused with DNA segments that enhance or regulate gene transcription in a tissue-specific manner (Knight et al., 1988). This approach was used to produce transgenic rabbits with the c-myc oncogene fused with DNA for the rabbit immunoglobulin heavy-chain enhancer region (E_H). Single-cell zygotes microinjected with the above construct and implanted into pseudopregnant does developed (in three of 21 cases) malignant lymphocytic (B-cell) leukemia as juveniles. Finally, Peng et al. (1993) developed transgenic rabbits carrying multiple copies of CRPV genomic DNA. The tumor development was restricted only to the skin in these rabbits. CRPV DNA was detectable in all tissues of the transgenic rabbits, however, the transcripts of CRPV genes only were found in skin and skin tumors. It was suggested that extensive methylation of CRPV genome, especially in the URR, might be a factor in controlling its tissue-specific expression (Peng, et al., 1995). Table 16.5 summarizes the experimentally induced tumor models.

TABLE 16.5 Induced Rabbit Tumor Models

Type of Tumor	Reference	Biology	Induction Method
Tongue squamous carcinoma cell line	(Wulong et al., 2008)	Transplantable	Initially developed by local DMBA application
Hepatocellular carcinoma	(Reznik and Padberg, 1991)	Primary and metastasizing	Oral DENA
Bone and cartilage sarcomas	(Sisson, 1950; Kelly et al., 1961; Higgins et al., 1964; Mazabraud, 1975; Hiruma, 1991)	Primary tumor development	Beryllium salts
Keratoacanthoma	(Matesanz et al., 1995)	Benign – self regressing	DMBA – skin application
Keratoacanthomas and squamous carcinoma	(Corominas et al., 1991)	Primary	Diethybenzanthracine on pinna
Splenic and ovarian decidosarcomas	(Janne et al., 2001)	Primary	Estrogen/progestrone
Radiation induced			
Osteosarcoma	(Buchholz et al., 1999)	Primary	Radiation (60Gy total, 19% incidence)
Viral			
Malignant lymphoma	(Koirala et al., 1997; Hayashi, et al., 1994)	Primary and metastasizing	IV or oral spray of cells producing Epstein-Barr-related herpes virus or direct virus
Transplantable neoplasia			
VX2 carcinoma	(Kidd and Rous, 1940; Rous et al., 1952; Chen et al., 2004)	Transplantable	
VX7 carcinomas	(Ito and Evans, 1965; George et al., 1992; Hamaguchi et al., 2003)	Transplantable	
Brown-Pearce carcinoma	(Greene, 1949; Shivas and Gillespie, 1969; Harzmann et al., 1979)	Transplantable tumor of rabbit origin – allograft	Used as tumor model for multiple sites – bladder, liver, and other organs
Choroidal melanoma	(Lopez-Velasco et al., 2005; Hu et al., 1994)	Xenografts – human melanoma cells	Rabbit's immunosuppressed (cyclosporin). Metastasis in two tumor cell types
Transplantable rabbit hepatoma	(Tanaka et al., 1950)		May be transplantable, however, no further reference found
Uveal melanoma	(Blanco et al., 2005)	Xenografts – human uveal melanoma cells	
Extrascleral extension of ocular melanoma	(Pineda et al., 1998)	Surgical technique of extension	B16F10 melanoma cell line
Hamster green melanoma	(Hahn and Spitznas, 1981; Liu and Ni, 1983; Shikishima, 2004)	Xenograft	
Transgenic rabbits with tumor susceptibility			
Lymphoma and other tumors	(Sethupathi et al., 1994)	E kappa-myc transgenic rabbits	Other tumors also seen – hepatoma, ovarian carcinoma, etc.
B-cell leukemia (polyclonal)	(Becker et al., 1990)	Emu-myc transgenic rabbits	
Papillomas and carcinomas	(Peng et al., 1993)	EJ-ras transgenic rabbits with papilloma virus DNA	Mostly skin tumors
Hemangiomas of liver	(Kitajima et al., 2005)	Transgenic – increased VEGF in liver	

Other Tumors of Historical Interest

Rabbit Tumor Necrosis Factor

The serum of rabbits (and a range of laboratory rodents) injected with the BCG strain of *Mycobacterium bovis* (or with other forms, including *Corynebacterium* or *Propionibacterium* species) and then challenged with bacterial lipopolysaccharide (endotoxin) develop a transiently circulating oncolytic factor termed tumor necrosis factor or TNF (Ostrove and Gifford, 1979). The factor has been so designated because TNF will cause hemorrhagic necrosis of certain transplanted solid tumors and is cytotoxic for transformed or tumor cell lines in culture, but not for normal cells. TNF is also inhibitory to a range of viruses and parasites (Gifford and Flick, 1988). A macrophage-derived product, TNF may be classed as a low-molecular-weight protein or cytokine (Ruff and Gifford, 1980). Recombinant murine interferon will serve to prime macrophages, in vivo, for TNF production (Lorence et al., 1990). The inflammatory cytokines include the interleukins (e.g., IL-1, IL2), cachectin, and TNF as a group of closely related factors with profound and diverse biological effects on both host and invading foreign forms (Langstein and Norton, 1991).

The amino acid sequence of rabbit TNF is known (estimated molecular weight 39000), and it is immunologically distinct from mouse TNF (Haranaka et al., 1985). Although the mechanism for oncolysis by TNF is non-immunologic, the actual means is still somewhat uncertain but may relate to the wide spacing between neoplastic cells, compared to more closely packed normal cells (Matthews and Neale, 1989).

Dos Santos Hepatoma

Dos Santos reported 35 fatal cases of hepatoma in a necropsy series of 120 rabbits (*Oryctolagus*) from a breeding colony in Brazil (Dos Santos, 1961). The hepatoma was limited to the liver in ten cases, but metastases were observed in 25 cases, most commonly to the lung. The tumors were more common in the females by a ratio of 1:6. Thirty-two tumors were hepatomas, two were mixtures of hepatic cell and bile duct elements, and one appeared to be a bile duct carcinoma. In ten of the tumors, intranuclear inclusion bodies were observed in hepatoma cells. The latter finding led the author to conclude that the tumors were induced by a virus.

Kato Sarcoma

The older literature contains occasional references to a transmissible tumor discovered as a naturally occurring sarcoma by T. Kato (1925). The tumor, which bears his name, was described as a spindle cell sarcoma with pronounced metastatic properties. The tumor was used in experiments to study the nature of metastasis (Nakano,

1929; Tomozowa, 1931), interaction of the sarcoma with certain endocrine glands (Hayashi, 1930; Maeda, 1930; Murohara, 1930), and several biochemical properties (Mori, 1939a, 1939b). It does not appear in the literature after the reports by Mori, and it is doubtful if the sarcoma has been maintained and is still available.

Andrewes Rabbit Sarcoma

In studies on the generalization of fibromatosis in tar-treated rabbits, Andrewes and Ahlstrom (1938) happened on an unusual occurrence. In one rabbit given two intramuscular injections of tar 4.5 months apart and 15 topical treatments of tar during the intervening period, a tumor developed at the intramuscular tar injection site 7 days following the intravenous injection of OA strain Shope fibroma virus. The tumor was described initially as a transplantable fibroma (Andrewes et al., 1937); however, subsequent changes led to its reclassification as a sarcoma (Andrewes and Ahlstrom, 1938), and the tumor has since come to be known as Andrewes rabbit sarcoma I (RSI). The tumor was passed for 12 transplantation generations in rabbits during the "fibroma phase." During this phase the tumor had the histological characteristics of a typical fibroma: elongated, spindle-shaped cells arranged in bundles and whorls with abundant fibrils. As a rule the tumor grew to an average size of only 20 × 10 × 10 mm, with central necrosis beginning by the fourteenth day.

The tumor, which had begun to fail by the twelfth transplant generation, was "restored" by injection of OA fibroma virus into rabbits with regressing fibromas during the thirteenth passage. During the next five transplant generations (the "sarcoma phase"), the tumor became less differentiated and more cellular, fibrils were less frequent, and mitoses more numerous. The transplanted sarcoma underwent growth in only about one-third of inoculated rabbits, but in them metastasis was not infrequent, occurring most commonly to the inguinal and iliac lymph nodes.

All attempts to demonstrate either fibroma virus (in RSI) or antifibroma antibodies in rabbits bearing RSI fibrosarcomas met with failure. Inclusion bodies typical for fibroma virus were never observed in RSI. Interestingly, several years later (Andrewes, 1940), the sarcoma became infected with Herpesvirus cuniculi (virus III of Rivers and Tillett (1923)). Virus III does not influence the course of RSI transplant growth, although it does abort or ameliorate the course of OA fibroma virus infection, apparently by the process of the interference phenomenon. This finding further strengthened the concept that fibroma virus was no longer detectable in RSI fibrosarcomas. After the 1940 report by Andrewes the tumor does not appear in the literature, and it is assumed that it is now extinct.

Kondrateva Osteogenic Sarcoma

In the earlier section on naturally occurring osteogenic sarcoma, it was mentioned that transplantable bone sarcomas have been reported from the Russian literature. The first of these was described by A.F. Kondrateva in the course of several experiments (Kondrateva, 1958; Kondrateva and Melnikov, 1965). The tumor arose 890 days after a paraffin pellet containing 9,10-dimethyl-1,2-benzanthracene had been inserted into a bone. The tumor was found to be transplantable. By 1958 it was in the fortieth transplantation generation and had an overall transplant success rate of at least 75%.

The tumor typically caused death 30–95 days after transplantation by both extensive local invasion and metastasis. The incidence of pulmonary metastasis was 100% in early transplant generations but became less frequent later. Transplantation of the tumor to bony tissues (virtually 100%) was more successful than that to soft tissues. Similarly, the rate of tumor growth was faster in bony tissues although tumors generally obtained a larger size in soft tissues. Tumor growth (and success of transplantation) was higher in muscle than in liver, and transplantation to testicle or subcutaneous sites was not generally successful. The morphology of the tumor (osteolytic osteosarcoma) was similar irrespective of the host tissue to which it was transplanted (Kondrateva, 1958).

The Kondrateva osteosarcoma has been used to some degree in experimental oncology. It has been exploited as a model for the study of tumor vascularization by making use of the technique of intraosseous phlebography (Kleinman and Chernomordikova, 1964). The intra-arterial

injection of a contrast medium (Diodon) has been reported to exert a marked inhibitory effect on the tumor, culminating in regression and cure (Ivanov and Pavlov, 1963). Several similar tumors including the LOI osteoblastic osteosarcoma and the UzIKEM osteolytic osteosarcoma have been reported in Russian journals and used for a variety of purposes (Karminov and Subkhankulova, 1963). The relation of the LOI and UzIKEM tumors to the Kondrateva osteosarcoma is not certain.

NEOPLASMS OF SYLVILAGUS

Although neoplasms induced by oncogenic DNA viruses have been frequently reported as spontaneous findings in *Sylvilagus* rabbits, reports describing other tumors in cottontail rabbits are rare. Tumors of the former type (Shope fibromas and Shope papillomas and malignant derivatives) are described in further detail in Chapter 1. It is generally believed that neoplasms of all types have a very low incidence in wildlife. Numerous factors contribute to such an observation, but some we may cite include the facts that the rigors of life in the wild are such that rabbits rarely survive into old age; that most animals which die naturally are not available for prosections; that ill and tumor-bearing rabbits are rarely encountered by hunters; and also the *Sylvilagus* rabbits have not been successfully domesticated so as to enable the longitudinal lifetime observation of colonized cottontails. Accordingly, the infrequent reports of neoplasms (of non-viral origin) in *Sylvilagus* rabbits have been summarized in Table 16.6. Additional

TABLE 16.6 Naturally Occurring Tumors of *Sylvilagus*

Organ System	Organ	Tumor Type and Site	No. of Cases	Reference
Urinary tract	Kidney	Embryonal nephroma	1	(Lopushinsky and Fay, 1967)
		Hamartoma	1	(Carlton and Dietz, 1977)
		Renal adenocarcinoma	1	(Carlton and Dietz, 1977)
Lymphoreticular system	Lymph tissue	Generalized lymphoma	6	(Lopushinsky and Fay, 1967)
		Hodgkins'-like lymphoma	1	(Bell and Chalgren, 1943)
Connective tissue	Subcutaneous tissue	Lipoma	1	(Lopushinsky and Fay, 1967)
Other	Cardiovascular	Hemangioendothelioma	1	(Pletcher and Murphy, 1984)

TABLE 16.7 Naturally Occurring Tumors of *Lepus*

Organ System	Organ	Tumor Type and Site	No. of Cases	Reference
REPRODUCTIVE SYSTEM				
Female				
	Uterus	Fibroma	12	(Flux, 1965)
			1	(Jardine et al., 2004)
	Vaginal squamous-columnar junction	Squamous cell carcinoma	1	(Cheatum and Bunting, 1951)
	Ovary	Teratoma	1	(Goyon, 1959)
			6	(Flux, 1965)
	Mammary gland	Adenocarcinoma	1	(Flux, 1965)
Urinary tract				
	Kidney	Renal carcinoma	1	(Cheatum and Bunting, 1951)
HEMOLYMPHATIC SYSTEM				
	Lymph tissue	Generalized lymphoma	1	(Piening, 1965)
			7	(Elvestad and Henriques, 1985)
		Lymphoma/leukemia	6	(Elvestad and Henriques, 1985)
		Hodgkins'-like lymphoma	1	(Salomon, 1933)
			1	(Nicod, 1961; Nicod and Burgisser, 1964)
		Myeloid leucosis	5	(Burgisser, 1957)
			1	(Guillon et al., 1963)
		Myeloid leukemia	1	(Elvestad and Henriques, 1985)
		Erythroid leukemia	1	(Elvestad and Henriques, 1985)
	Connective tissue			
	Subcutaneous tissue	Fibroma	1	(Flux, 1965)
GI tract				
	Liver	Hemangioma	4	Haberman (cited by Cheatum)
	Liver and mesentery	Mesenchymal tumor (metastatic – primary site not identified)	1	(Jardine et al., 2004)
Respiratory tract				
	Lung	Leiomyosarcoma (metastatic – primary site not identified)	1	(Jardine et al., 2004)

reports may be found in the compilation by [Stedham \(1976\)](#) as well. The bulk of the reported tumors of *Sylvilagus* are lymphomas and renal tumors, neoplasms associated with adolescence in *Oryctolagus* rabbits.

The published description of the embryonal nephroma by [Lopushinsky and Fay \(1967\)](#) was not typical of the same tumor type in *Oryctolagus* rabbits. Although it had not metastasized, the tumor was

extremely large (55g) and had almost completely replaced all normal renal structures in the affected kidney. The descriptions of lymphomas by Lopushinsky and Fay, however, appeared to be similar to those observed in *Oryctolagus*, involving diffuse infiltrative destruction of the liver, spleen, and stomach. The extent of renal involvement was not reported. The lymphoma reported by Bell and Chalgren (1943) was more of a Hodgkin's type with sarcomatous infiltration of cervical, mesenteric, axillary, and cervical lymph nodes, but with sparing of the liver, spleen, and kidney. Histological descriptions and illustration were not provided.

NEOPLASMS OF *LEPUS*

The comments above with reference to the difficulty of case finding and assessment of the true incidence of neoplasms in *Sylvilagus* apply equally to *Lepus* (hares); most observations are obtained in a casual random way from wild populations. Although an insufficient number of cases have been described and reported to enable a confidence profile of the tumors affecting the genus to be outlined, preliminary evidence (Table 16.7) indicates the tumors' distribution in *Lepus* spp. to be similar to that of *Oryctolagus*. This is supported by the retrospective study submitted by Elvestad and Henriques (1985) which categorized cases of leukemic neoplasia submitted as diagnostic necropsy samples to the State Veterinary Diagnostic Laboratory in Denmark from 1934–1984. The samples were submitted from 15 brown hares presumed to be *Lepus europaeus*. The majority of the animals were diagnosed with lymphoma, with lesser numbers exhibiting an accompanying lymphoid leukemia. One important exception is the finding of myeloid leukemia in six separate accessions of *Lepus* rabbits reported from France and Switzerland (see Table 16.5) (Burgisser, 1957; Guillon et al., 1963). The gross pattern of splenomegaly, hepatomegaly, and generalized lymphadenopathy was typical for all six cases. Although no individual was observed prior to death, the infiltration of most major organs and tissues with neoplastic cells typical of the myeloid series suggested a leukemic state.

Another exception includes the ovarian teratomas found in Europe (Goyon, 1959) and Australia (Flux, 1965) by investigators surveying wild *Lepus* populations. This tumor has not been observed in *Oryctolagus* (or *Sylvilagus*). Flux (1965) has suggested that the high incidence of ovarian cysts and teratomas in wild hares may be attributed to the proximity of the animals to agricultural insecticides (e.g., DDT).

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Mycoses and Non-Infectious Diseases

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INTRODUCTION

This chapter discusses those disorders and diseases of the rabbit that are not readily classified into the other chapters. Much of the initial information for this chapter was obtained from chapters in earlier volumes in the American College of Laboratory Animal Medicine series (Bergdall and Dysko, 1994; Lindsey and Fox, 1994). With regard to the genetic diseases of rabbits discussed throughout this chapter (and also in previous texts), some may have “disappeared” through loss of breeding stock or discontinuation of the inbred strain in which the mutation was seen. The authors endeavored to include only those inherited diseases of significance that might be seen in existing outbred colonies or in inbred strains that were still maintained at one or more global locations. We acknowledge that some disorders noted in previous editions may have been omitted accidentally, and that some mutation/strain combinations may have been included that are no longer in existence.

MYCOTIC DISEASES

Superficial Mycoses

Dermatophytoses (ringworm, favus) are uncommonly encountered in the domestic rabbit. The disorders are comparatively rare under laboratory conditions or in commercial rabbitries with satisfactory hygienic standards. Under these conditions, dermatophytoses are most often encountered as individual infections of a sporadic nature, although epizootics have been reported (Banks and Clarkson, 1967; Cohrs et al., 1958; Hagen, 1969). Most reports indicate that younger or immunosuppressed rabbits are most susceptible.

Ringworm is caused by infection of the epidermis and adnexal structures such as hair follicles and hair shafts with pathogenic dermatophytic fungi. *Trichophyton mentagrophytes* is the dermatophyte most commonly encountered in ringworm infections of both *Oryctolagus* (Banks and Clarkson, 1967; Hagen and Gorham, 1972; Saito et al., 2001; Van Rooij et al., 2006), and wild lagomorphs (Adams et al., 1959; Gallo et al., 2005). Infections with other species of dermatophytes, such as *Microsporum canis*, *M. gypseum*, *M. cookei*, *T. terrestre*, and *T. ajelloi* have also been documented (Gallo et al., 2005). The latter four fungal species are geophilic and not as commonly isolated. In a sampling of *Sylvilagus* sp., seasonal variation in the prevalence of geophilic fungi was identified and assumed to be due to environmental effects on fungal survival. Environmental contamination is a significant risk factor for spread of clinical dermatophytosis within a population, as well as to other species occupying the area. With regard to pet rabbits, lesions have been

detected on both the rabbit and the owner (Van Rooij et al., 2006).

Dermatophytic infections usually arise on or about the head in rabbits. The lesions are pruritic and may secondarily spread to the feet, especially the toenail beds, and to other areas of the body. The lesions usually appear as areas of patchy alopecia, erythema, and crustiness with an underlying inflammatory reaction. Hyperkeratosis, acanthosis, and diffuse infiltration of the dermis with polymorphonuclear leukocytes are observed histologically. Lymphocytes and plasma cells are also present in the dermis and adnexal structures. Abscessation of hair follicles by secondarily invading bacteria is common. Mycotic elements can be identified with Gridley fungus stain, the periodic acid-Schiff (PAS) reaction, or Gomori methanamine silver stain.

Diagnosis of dermatophytosis involves differentiation from other causes of crusty alopecia of the head and ears. Incidental lesions such as genetic hairlessness, trauma, hair clipping or depilatory hair loss, and fur pulling should be ruled out by obtaining a thorough history. Routine dermatophyte tests such as hair/skin scrapings mounted in 10% potassium hydroxide, Wood's light (ultraviolet) illumination, and culture on a suitable dermatophyte media should be performed. Reliance cannot be placed on culture alone to substantiate a diagnosis of dermatophytosis because rabbits have been shown to be asymptomatic carriers of dermatophytic fungi (Balsari et al., 1981; Franklin et al., 1991). Substantiation of the diagnosis must include histological evidence of epithelial invasion by the fungus. The use of polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) for diagnosis has been successfully used for the rapid identification of *T. mentagrophytes* from clinical specimens (Arabatzis et al., 2006). In this report, one of the sexual forms of *T. mentagrophytes*, *Arthroderma vanbreuseghemii*, was identified as the cause of dermatophytosis in several commercial rabbitries. It was proposed that PCR-RFLP might be useful in epidemiological investigations of outbreaks since there is some evidence to support species-specificity associated with different teleomorph forms of *T. mentagrophytes* (Summerbell et al., 2002).

Treatment of dermatophytosis involves isolation of infected animals owing to the zoonotic hazard. Untreated cases should be euthanized and the immediate environment disinfected. Enilconazole is reported to be effective for environmental decontamination when used in a spray or fogger application (Rochette et al., 2003). Experimentally induced rabbit dermatophytosis (*T. mentagrophytes*, *M. gypseum*, and *M. canis*) responded well to orally administered griseofulvin (25 mg/kg in aqueous suspension by gastric intubation) once daily for 14 days (Hagen 1969). Colonies may be mass treated by providing a ration containing 0.375 g of powdered

griseofulvin per pound of feed for 14 days (Hagen, 1969). Other treatments suggested for colony outbreaks included topical azole antifungals such as enilconazole (Rochette et al., 2003), or a 1:10 dilution of a MECA spray (metastabilized chlorous acid/chlorine dioxide), twice weekly for 3–4 weeks using about 8 ounces per rabbit at each application (Franklin et al., 1991). In most cases, euthanasia of infected laboratory rabbits is the most prudent approach due to the potential for spread and the research side effects associated with treatment. The development of vaccines shows promise as another potential treatment option for dermatophytosis (Chermette et al., 2008).

Deep Mycoses

Rabbit species of all genera appear to be remarkably resistant to deep or systemic mycotic infections (Schwartz, 1954). Only two naturally occurring mycoses seem to be significant: aspergillosis of the domestic rabbit and adiaspiromycosis of several wild lagomorph species. Isolated case reports of other deep mycotic infections have been published (Barrington, 1921; Soerensen and Saliba, 1961; Turn and Eveleth, 1954).

Clinical aspergillosis is primarily of historical significance as a disease of *Oryctolagus* rabbits (Blount, 1957; Cohrs et al., 1958; Dumas, 1953; Lesbouyries, 1963; Seifried, 1937), particularly as the standards of husbandry have advanced. *Aspergillus fumigatus* had been the primary species isolated, but *A. niger* and *A. flavus* were also associated with clinical cases. The course of the disease is commonly chronic in nature with gradual onset of cachexia and dyspnea as the chief clinical findings. The disease has also been observed as a subclinical problem in young rabbits (less than 5 weeks of age) obtained from a commercial rabbitry (Matsui et al., 1985).

Lesions of aspergillosis are typically confined to the lungs. Focal caseous necrosis is the primary lesion; cases must be differentiated from tuberculosis and necrobacillosis (Schmorl's disease). Diagnosis is established by histopathological demonstration of the organism and is confirmed by cultural isolation of *Aspergillus*. The disease in rabbits is similar in most pertinent details to avian infections (Chute, 1972).

Adiaspiromycosis (haplomycosis) is caused by fungi of the genus *Emmonsia* (*Haplosporangium*). Two species, *E. parva* and *E. crescens*, are distributed worldwide and recognized as important agents of feral animal infections. Adiaspiromycosis has been described as a natural infection in a domestic rabbit (Dvorak et al., 1966). *Emmonsia* spp. have been encountered principally in the western United States as pathogens of wild rodents, carnivores, and lagomorphs of the genera *Sylvilagus* and *Ochotona*. Naturally occurring infections appear to be benign and generally confined to the lungs (Jellison, 1970).

Cultures of *Emmonsia* on artificial media grow as typical fungi with profuse spore production. *Emmonsia parva* isolates produce spores of 10–40 µm in diameter, whereas *E. crescens* spores are unusually large at 250–500 µm. The large spores (adiaspores) correspond to the form found in infected tissues. Jellison (1970) provides a more thorough discussion of the disease.

Pneumocystosis

Pneumocystis species are well-recognized opportunistic pathogens infecting the lungs of many animals. This genus of fungi has been investigated using molecular genotyping techniques and strong host specificity has been identified among *Pneumocystis* isolates. The isolate which infects rabbits is *Pneumocystis oryctolagi* sp. nov., and it does not appear to cross to other species (Dei-Cas et al., 2006).

Clinical signs occur in weanling rabbits and consist of respiratory distress with complete recovery in 3–4 weeks. *P. oryctolagi* sp. nov. has been isolated from diverse geographical areas and rabbit breeds, indicating that this agent has been well adapted to the rabbit for a relatively long time.

Diagnosis of the agent can be made using nasal lavage with sterile saline followed by PCR, or via histopathology at necropsy. Special stains such as toluidine blue O can be used to identify the organisms lining the alveolar epithelium. One unique feature of this disease in rabbits is the lack of foamy eosinophilic honeycomb material in the alveoli on histopathologic analysis, a common finding of *Pneumocystis* infection in other species. Because rabbits are infected and develop clinical signs without the use of corticosterone for immunosuppression, they represent an important natural animal model for investigations of pneumocystosis (Dei-Cas et al., 2006).

NON-INFECTIOUS DISEASES

Ocular

Buphthalmia

Buphthalmia (synonyms: hydrophthalmia, congenital or infantile glaucoma) is recognized as one of the most common inherited diseases of contemporary domestic rabbits. Although information on frequency of occurrence in various breeds is generally lacking, the condition is regularly seen in some New Zealand White stocks bred for laboratory purposes (Hanna et al., 1962). The term buphthalmia is of Greek origin and literally means "ox eye." Originally used as a descriptive term for enlargement of the eye, buphthalmia has become the accepted term for this particular disease of rabbits.

Buphthalmia has been observed in rabbits of many stocks around the world (Aurichio and Wistrand, 1959; Greaves and Perkins, 1951; Hanna et al., 1962; Pichler, 1910; Rochon-Duvigneaud, 1921; Schloesser, 1886; Smith, 1944; Vogt, 1919). Grossly visible abnormalities of the eye may appear as early as 2 or 3 weeks of age, but most appear much later. Initially, one may see an increased anterior chamber size with either a clear cornea or a slight cloudiness and very delicate bluish tint to the cornea. Subsequent changes include progressive opacity and flattening of the cornea, increased prominence of the ocular globe, and conjunctivitis. Corneal ulceration may develop, and ultimately result in traumatic rupture of the cornea with scarring and vascularization. General health, appetite, and libido are reported to be reduced (Geri, 1954; Nachtsheim, 1973).

The development of buphthalmia in rabbits seems to be related to abnormalities in production and removal of aqueous humor from the anterior chamber, as in congenital glaucoma of humans. Evidence suggests that affected rabbits have an abnormal trabecular meshwork and may develop chronically increased intraocular pressures as early as 3 months of age (Burrows et al., 1995; Gelatt et al., 1998; Ueno et al., 1999). Because of the similarities in development of the disorder, buphthalmic rabbits serve as a useful experimental model of human congenital glaucoma. A major difference, however, is that the scleral coat of the rabbit is less mature (more elastic) at the onset of increasing pressures, thus permitting the globe of the eye to enlarge (i.e., buphthalmia).

Buphthalmia is inherited as an autosomal recessive trait (*bu/bu*). It has been suggested that this mutation and resulting loss of appropriate gene function perturbs differentiation of the trabecular meshwork, resulting in the buphthalmic phenotype (Knepper et al., 1997). Unfortunately, attempts to completely characterize the effects of the *bu* gene have met with variable results. There appears to be incomplete penetrance of this gene, as clinical disease appears in some but not all homozygotes. The eye lesions may be unilateral or bilateral. Age of onset of recognizable disease is extremely variable, as is true of several physiological parameters associated with production and removal of fluid from the anterior chamber of the eye. The variable age of onset may account for some of the incomplete penetrance. In general, however, there is demonstrable decrease in outflow of aqueous humor from the anterior chamber of the eye by 3 months of age and an increase in intraocular pressure by 5 months of age (Fox et al., 1969; Kolker et al., 1963; McMaster, 1960; McMaster and Macri 1967). Even at birth there is reported to be an underdevelopment or absence of outflow channels in the ciliary body and sclera (Hanna et al., 1962). As the disease progresses, there is widening and even complete loss of the drainage angle, thickening of Descemet's membrane, increased

corneal diameter, increased vascularity and opacity of the cornea, atrophy of the ciliary processes, glaucomatous excavation of the optic disk, and possible atrophy of the optic nerve (as a secondary effect). Striking differences in cytological features and turnover rate of corneal epithelial cells have been observed in buphthalmic rabbits as compared to unaffected ones (Babino and Fox, 1967; Fox and Babino, 1965; Sheppard and Shanklin, 1968; Sheppard et al., 1971).

Fox et al. (1969) obtained suggestive evidence that buphthalmic males have reduced spermatogenesis. Administration of ascorbic acid has been reported to have no effect on buphthalmic rabbits although it apparently facilitates outflow of anterior chamber fluid in normal rabbits (Noah and Geeraets, 1971). Lam et al. (1989) suggested that the deposition of fibrin in the trabecular tissue contributes to the obstruction of aqueous humor drainage in hereditary buphthalmic rabbits. Glucose metabolism in buphthalmic animals is probably normal; however, buphthalmic rabbits have been shown to be significantly more resistant to insulin shock than normal controls (Fox et al., 1971b).

As already mentioned, incomplete penetrance is an important factor in the inconsistent clinical presentation of buphthalmia. Another possible reason that fewer than the expected number of buphthalmics are produced may involve losses in utero. Hanna et al. (1962) found that larger litters (≥ 6) yielded fewer than predicted buphthalmic littermates when compared to smaller litters (≤ 5), which had affected offspring at the calculated rate of incidence.

Specific treatment for buphthalmia has not been described for rabbits; however affected animals should not be used for breeding purposes (Suckow et al., 2002).

Aberrant Conjunctival Stricture (*Pseudopterygium*)

Pterygium, a condition that occurs in humans, is a limbal-based, triangular fold of conjunctival membrane that protrudes onto the cornea. Generally, it is a progressive lesion that advances to the pupillary area. True pterygium adheres closely to the cornea resulting in invasion of the corneal stroma and destruction of Bowman's membrane. Its growth is due primarily to the proliferation of connective tissue fibroblasts.

A similar condition in rabbits has been reported repeatedly throughout the literature. It has been referred to by numerous terms including pseudopterygium, epicorneal conjunctival membrane occlusion, conjunctival centripetalization, membranous occlusion of the cornea, and aberrant conjunctival stricture (Allgoewer et al., 2008; Arnbjer, 1979; Dupont et al., 1995; Roze et al., 2001). Consistently the lesion is described as a membrane that originates from the bulbar and limbal conjunctiva. Though similar, it is a different entity to pterygium in humans. It does not have the triangular shape as seen in

humans, but progresses centripetally and symmetrically across the cornea towards the pupil. Most significantly, it does not alter, nor is it adhered to the underlying cornea (Allgoewer et al., 2008; Roze et al., 2001). Clinically, visual impairment occurs as the membrane moves towards the central aperture.

The pathogenesis and etiology of this condition have not yet been elucidated. There is an increased incidence in young, male dwarf and pygmy rabbits. Though originally thought to be a congenital condition, it has presented in both adult and juvenile animals (Allgoewer et al., 2008). Causative factors in humans have included infection, ultraviolet (UV) radiation, and microtrauma. Histologically, the lesion in the rabbit is described as a focal excess and dysplasia of conjunctival collagen. This is consistent with a type of collagen nevus that has been described as a cutaneous lesion in both humans and dogs. These nevi are thought to be secondary to decreased collagenase production and increased fibroblast proliferation, but the mechanism and inciting factor is unknown (Roze et al., 2001). Treatment in the rabbit consists of removing and preventing the obstruction. Arnbrjer (1979) discussed two cases of pseudopterygium in the pygmy rabbit (*Brachylagus idahoensis*). One case was successfully treated by detachment of the conjunctival tissue with a blunt instrument, subconjunctival injection of methylprednisolone acetate, and a 3-week course of a topical steroid/antibiotic ophthalmic solution. The other case received only topical antibiotics after pseudopterygium detachment and the condition recurred within a few weeks.

Roze et al. (2001) described four cases in dwarf rabbits where the abnormal conjunctival fold was excised at the limbus. Follow-up treatment with 0.1% topical dexamethasone was provided for two of the four animals and recurrence was noted 3 and 4 weeks post-operatively. The remaining two rabbits received topical dexamethasone and topical 0.2% cyclosporine ointment. At 3 months post-operatively there was no recurrence. Based on this study, it was concluded that there may be local immunity factors involved in the pathogenesis as suggested by the efficacy of the cyclosporine.

Most recently, Allgoewer et al. (2008) reported a centrifugal surgical technique that provided efficacious long-term resolution. This technique included six radial incisions of the conjunctival fold with fixation via sutures to the eyelids. There were no incidences of recurrence following six cases (ten eyes) where this technique was utilized.

Corneal Dystrophy

True corneal dystrophies differ from other corneal disorders in that they are non-inflammatory, typically bilateral, and involve the central cornea. They are also unassociated with any systemic disease, such

as hyperlipidemia, and primarily involve a single corneal layer with other layers only secondarily involved (Moore et al., 1987). Two reports of variants of corneal dystrophy in domestic rabbits have appeared in the literature.

Port and Dodd (1983) described a unilateral epithelial dystrophy in two New Zealand White rabbits. The lesion appeared as an opaque membranous ring originating at the limbus and extending towards the center of the cornea. Histologically, the membrane was an irregular accumulation of corneal epithelial cells, interspersed with a pathologically thin epithelium. No inflammation was present, and the center of the cornea was normal, as were the other component structures of the eye. The authors refuted the possibility of trauma or chemical injury as a cause of the lesion.

Moore et al. (1987) described primary anterior corneal dystrophy in members of a colony of American Dutch belted rabbits. In these cases, the corneal opacities were irregularly shaped lesions in the superficial layers of one or both corneas. The lesions were found to be caused by thickened and irregular sections of the corneal basement membrane, with occasional disorganization and detachment of the basal epithelium. Genetic involvement was suspected, based on the breeding records of the colony. The authors postulated about the usefulness of the animals as a model of anterior corneal dystrophies in humans.

Band Keratopathy

Band keratopathy, a degenerative disease of the human cornea, is characterized by the deposition of calcium salts in the subepithelial corneal stroma. The first description of the disease in rabbits was in a colony which had received an intraocular injection of albumin to produce experimental uveitis (Economon et al., 1963). In addition to the uveitis, which was produced intentionally, the rabbits also had calcium deposits in several visceral organs. The metastatic calcification was presumed to have been caused by an accidental hypervitaminosis D as the result of an error in feed formulation. Band keratopathy was observed only in rabbits that had both uveitis and hypervitaminosis D. Rabbits with either condition alone did not develop band keratopathy. The development of the corneal disease, therefore, appeared to depend on the concomitant hypercalcemia.

Doughman et al. (1969) described two models for the production of band keratopathy. The first model reproduced conditions similar to those that occurred in the case report by Economon et al. (1963): creation of immunologic uveitis following vitamin D intoxication. Band keratopathy developed 100% of the time under these conditions. The authors found, however, that this model did not feature hypercalcemia (as proposed by Economon et al., 1963) and that the deposition of

calcium in the cornea was best explained by the theory of calciphylaxis. Calciphylaxis was defined as “a condition of induced systemic hypersensitivity (vitamin D intoxication) in which tissues respond to appropriate challenging agent (uveitis) with local calcification.” It was proposed that the uveitis might alkalinize the cornea, altering metabolism to promote calcium deposition. The second model involved the development of band keratopathy in 35% of the eyes injected with polyethylene sulfonate.

Lipid Keratopathy

Lipid keratopathy is characterized by intracellular or extracellular lipid deposition within the cornea, iris, and ciliary body. This disease has been reported in both humans and animals as a sequela of hyperlipidemia and hypercholesterolemia. Rabbits do not have an effective mechanism for processing excess cholesterol and thus have been utilized as an animal model for hypercholesterolemia (Kruth, 1987; Terpstra et al., 1981). Due to the ease and severity with which rabbits can be rendered hypercholesterolemic, this species has been also been targeted as a model for studying the pathogenesis of lipid keratopathy.

Diet-induced corneal lipidosis has been reported in New Zealand White (NZW) rabbits. In one publication (Sebesteny et al., 1985) this ocular lesion was noted in rabbits that were accidentally fed a fishmeal diet; affected rabbits also had elevations in their serum cholesterol and atheromatosis. Once the fishmeal diet was replaced with a standard vegetarian diet, the presence of these lesions in the colony resolved. In other reports (Reddy et al., 1987; Roth et al., 1988; Stock et al., 1985), the lesions were created intentionally utilizing a high-cholesterol diet in conjunction with corneal suture placement. In these studies, rabbits that were fed a high-cholesterol diet, but did not have corneal suture placement, developed the lipid keratopathy more slowly, and the lipid deposition only occurred circumferentially at the corneal limbus. This indicated that for the NZW rabbit model for lipid keratopathy, combination with a corneal neovascularization stimulant (suture) promoted more significant lipid deposition than did diet alone.

The pathogenesis of experimentally induced lipid keratopathy involves an increased intracellular uptake of LDL and cholesterol esters within the stromal keratocytes, pericytes, and endothelial cells. Cell death occurs when the intracellular lipid metabolic mechanisms of these cells are overwhelmed. The lipid and protein debris then enters the corneal stroma. This debris is phagocytosed by macrophages, thus creating the characteristic “foamy” cells that are seen histologically (Roth et al., 1988).

The Watanabe heritable hyperlipidemic (WHHL) rabbit is commonly utilized as a model of human

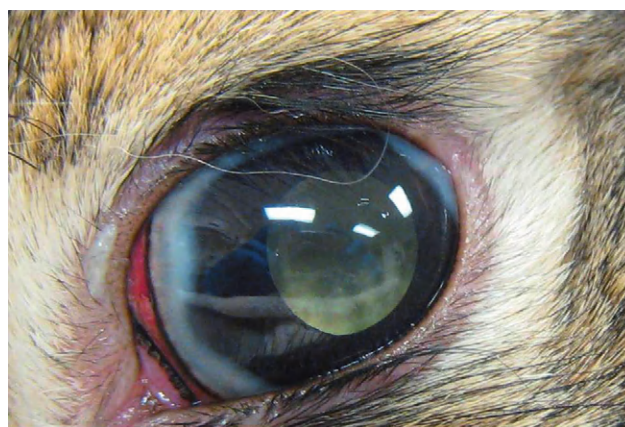


FIGURE 17.1 Eye of a WHHL rabbit with circumferential lipid keratopathy at the margin of the cornea and sclera. This rabbit also has a detached retina. Courtesy of The Ohio State University.

familial hypercholesterolemia. Homozygous WHHL rabbits have a genetic deficiency of low-density lipoprotein (LDL) receptors and as a result develop high serum cholesterol levels and atherosclerotic plaques. Additionally, these animals have been reported to develop a lipid keratopathy without either surgical or dietary intervention (Figures 17.1 and 17.2). Garibaldi and Pecquet Goad (1988) describe a lipid keratopathy in these animals as a pearly corneal opacity comprised histologically of lipid laden, or “foamy,” macrophages. Kouchi et al. (2006) described the corneal opacities as yellowish-white lesions predominantly found along the corneoscleral junction. No ophthalmoscopic lesions of the fundus or retina have been reported; however, histopathologically clusters of “foamy” cells and crystalline clefts were present in the choroid and sclera in association with blood vessels (Figures 17.3 and 17.4). In WHHL rabbits with severe and prolonged hyperlipidemia, retinal involvement, including complete bullous retinal detachment, has been observed (Figures 17.1 and 17.3) (L. Gallagher, personal observation, 2007).

Cataracts

Historically, at least two types of inherited cataracts have been reported in rabbits. Nachtsheim and Gürich (1939) described one type (gene symbol *Cat-1*) that was transmitted as a simple recessive allele. Slight bilateral dullness was present in the posterior wall of the lens at birth and subsequently progressed to complete opacity of the lens by 5–9 weeks of age. The rate of progression was slower if animals were fed dry diets as opposed to foods with high water content. A second type of hereditary cataract (gene symbol *Cat-2*) was described by Ehling (1957); it was considered an incomplete dominant trait with 40–60% penetrance. In contrast to the *Cat-1* version of cataract, the *Cat-2* form was often unilateral in occurrence.

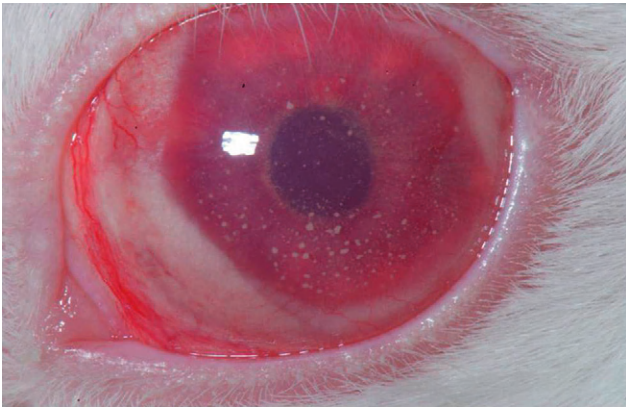


FIGURE 17.2 Eye of a WHHL rabbit with more diffuse lipid keratopathy, as well as anterior uveitis. Courtesy of The Ohio State University.

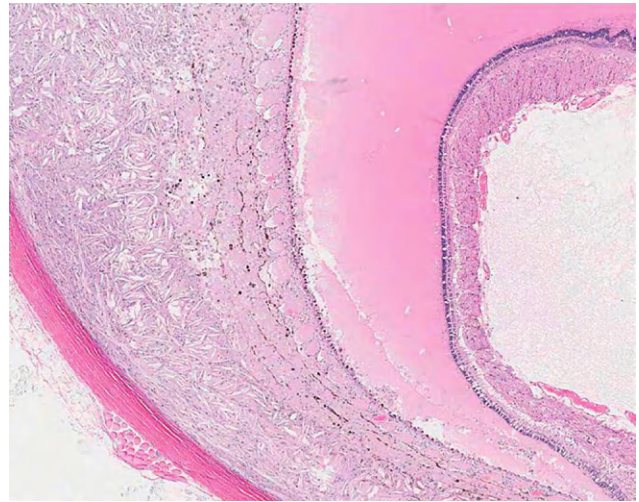


FIGURE 17.4 Histopathologic section of the eye of a WHHL rabbit; note the cholesterol clefts in the subretinal lipid deposition. Courtesy of The Ohio State University.



FIGURE 17.3 Histopathologic section of the eye of a WHHL rabbit; note the lipid deposition at the corneo-scleral junction and the subretinal lipid deposition resulting in retinal detachment. Courtesy of The Ohio State University.

In addition to the reports above, [Munger et al. \(2002\)](#) and [Jeong et al. \(2005\)](#) described spontaneous cataract formation in New Zealand White (NZW) and New Zealand White–New Zealand Red (NZW × NZR) hybrid rabbits that was consistent with an autosomal recessive inheritance. In their study, [Munger et al. \(2002\)](#) demonstrated a higher incidence of cataracts in NZW than in the NZW × NZR rabbits, further supporting this mode of inheritance. Additional studies are needed to fully characterize this condition and its potential similarity to the condition previously described by [Nachtsheim and Gürich \(1939\)](#).

Skeletal Diseases

Hydrocephalus

Hydrocephalus is generally considered a hereditary disease in most species; although it has been

well-documented that hydrocephalus is also caused in rabbits by both vitamin A deficiency and toxicity (see below). [Nachtsheim \(1939\)](#) studied a family of rabbits in which hydrocephalus segregated as an autosomal recessive trait (*hy*), and was frequently associated with dwarfing (*nan*) and brachygnathia. A second, probably different, inherited hydrocephalus was observed by [Rosa \(1946\)](#) to behave as an autosomal trait with incomplete dominance. In those rabbits, hydrocephalus was commonly associated with several anomalies including anophthalmia, microphthalmia, ectopia of the eyeball, coloboma of the iris and choroid, and cataracts. Unfortunately, the studies of [Nachtsheim \(1939\)](#) and [Rosa \(1946\)](#) were based mainly on detection of cases of hydrocephalus by gross examinations. As such, their analyses would have been unable to detect mild cases that survived and appeared clinically normal. Cases of hydrocephalus without a clear pattern of inheritance are known to occur sporadically in many contemporary stocks ([Sawin, 1955](#)).

Whether abnormal vitamin A metabolism was a factor in any of the above examples remains unknown, although [Greene \(1965\)](#) described a form of hydrocephalus in rabbits apparently based on an inherited defect in vitamin A metabolism. However, it has been well established that both vitamin A deficiency ([Harrington and Newberne, 1970](#); [Lamming et al., 1954](#)) and toxicity ([Cheeke et al., 1984](#)) in pregnant does regularly produces hydrocephalic young. Vitamin A is required for maintenance of pregnancy in rabbits, and deficiency results in abortion, fetal resorption, neonatal death, and small, weak litters ([Cheeke, 1987, 1994](#)). Interestingly, the same reproductive effects, including the development of hydrocephalus, are seen with vitamin A toxicity.

Rabbits appear to be especially sensitive to high levels of this vitamin. Cheeke et al. (1984) observed the reported reproductive complications and hydrocephalic young with a diet containing 191 180 IU per kg feed; Scott et al. (1982) reported the toxicity of vitamin A (as retinol and its esters) in "animals" to be greater than 1 million IU per kg feed. Hydrocephalus is rarely seen in laboratory colonies now due to the production of high-quality commercially available feed, but as with all nutritionally based diseases, it could recur due to accidental errors in diet formulation.

Mandibular Prognathism

Mandibular prognathism (synonyms: malocclusion, underbite, walrus teeth, buck teeth) is probably the most common of the known inherited diseases in the rabbit. Few laboratory or commercial breeding stocks are free of the problem. The term mandibular prognathism refers to an abnormally long mandible relative to the length of the maxilla. However, in rabbits the primary defect is actually a short maxilla in relation to a mandible of normal length, so the common name is actually a misnomer. Both conditions are known to occur in humans (Rubbrecht, 1939), dogs, and sheep (Nordby et al., 1945).

Mandibular prognathism in rabbits is somewhat unique because of peculiarities of the dentition in this species. Rabbits have open-rooted adult teeth which continuously grow, and are one of only five species classified as full elodonts (Legendre, 2002). The dental formula for the normal rabbit is I 2/1, C 0/0, PM 3/2, M 3/3 (Craigie, 1960). There is a single pair of large chisel-shaped lower incisors. The upper incisors consist of a similar pair of large primary incisors and a pair of smaller secondary incisors, also called *peg teeth*, positioned directly behind the primary incisors. Normally, the lower incisors occlude behind the primary upper incisors and against secondary incisors, which protrude only slightly beyond the gingiva. The lower incisors wear at approximately right angles to the secondary incisors, and the primary upper incisors wear along the arc followed by the lower incisors. This is due in part to the fact that the enamel layer in the rabbit incisor tooth is not distributed evenly around the tooth. The anterior edge has the heaviest layer of enamel, progressing to little, if any, enamel on the posterior surface (Weisbroth, 1973). Thus, a sharp cutting edge is present at the labial surface of the lower and primary upper incisors. The primary upper incisors curve posteriorly and laterally, whereas the lower incisors grow in an anteromedial direction. Between the incisors and first premolars (where canine teeth would normally be located) is a toothless space known as the diastema. The premolars and molars meet evenly to form a flat occlusal surface (Fox and Crary, 1971; Weisbroth and Ehrman, 1967).

The large incisors of the rabbit grow throughout life at average rates of about 2.0 and 2.4 mm per week for upper and lower incisors, respectively. On an annual basis this is approximately four inches for each upper incisor and five inches for each lower incisor (Shadle, 1936). Optimal occlusion of incisors is critical for counterbalancing the rapid extrusive growth with normal attrition. Absence of good occlusion from any cause quickly leads to overgrowth of incisors and/or cheek teeth in the rabbit. Clinically significant malocclusions may result from a variety of hereditary and environmental causes.

In the rabbit with hereditary mandibular prognathism (*mp/mp*), malocclusion of incisors is usually first observed after the third week of life. Initially there is an edge-to-edge bite of the incisors with resultant blunting of the cutting edges; this eventually progresses to positioning of the lower incisors anterior to the upper ones. In accordance with the usual planes of growth, the primary incisors tend to curl within the mouth, whereas the lower incisors grow anteriorly to protrude from the mouth. The upper incisors may pierce the gingival or buccal mucosa, leading to ulceration and/or abscess formation. Impaired closure of the mouth also interferes with normal attrition of molars and premolars. If the incisors are not trimmed, an affected animal will have difficulty eating and may die of inanition.

Fox and Crary (1971) reported evidence of autosomal recessive inheritance of mandibular prognathism with incomplete (81%) penetrance. Affected animals were also found to have reduced length measurements for the skull and maxillary diastema, without significant deviation from normal length of mandibles. Thus a postulated genetic mechanism involves a gene (*mp*) causing differential growth of dorsal and basal skull bones, with resultant anterior displacement of the mandible. The condition in rabbits has been compared to class III malocclusion, a similar abnormality in humans (Nepola, 1969).

Mandibular prognathism with attendant malocclusion of incisors should be distinguished from the condition involving malocclusion of premolars and molars, which has been reported in a few older rabbits (Ireson, 1968; Pollock, 1951; Zeman and Fielder, 1969). It has been speculated however that the latter condition may also have a genetic basis.

Absence of Second Upper Incisors

The absence of the second upper incisors (peg teeth) has been reported (Crossley, 2003; Nachtsheim, 1938, 1958) in domestic rabbits and occasionally in wild specimens. There are no known ill effects associated with this phenotype. Affected animals develop small rudimentary structures that resemble teeth, but in most instances disappear very shortly thereafter. The absence of the second

upper incisors described by Nachtsheim was considered to be a dominant trait, however other cases indicate that there may be multiple modes of inheritance (Crossley, 2003).

Scoliosis and Torticollis

Scoliosis is a term meaning lateral curvature of the spine. Serious investigations of scoliosis are very few. In the limited rabbit studies to date it is considered familial and perhaps polygenic in inheritance. Anatomically, the findings in affected rabbits have included missing, reduced, or extra half-vertebral units and bifurcations or fusions of ribs. Depending on the breed of rabbit in which the anomaly occurs, vertebrae in either the cervicothoracic, thoracic, or lumbar regions of the spine may be deformed (Sawin and Crary, 1955, 1964).

Torticollis, also called wryneck, is a term that refers to twisting of the neck with associated abnormal positioning or carriage of the head. It generally implies an etiology involving muscular, neurological, or inner ear disease. Although the term has been applied frequently to diseased rabbits in the past, little is known about the underlying etiology. However, torticollis has been associated with *Pasteurella multocida* infection of the middle ear (Fox et al., 1971e), and *Encephalitozoon cuniculi* infections (Valencakova et al., 2008). Thus, there is a distinction between the clinical sign of torticollis, which occurs secondary to disease processes, and the anatomic condition of scoliosis caused by vertebral defects as described above.

Traumatic Vertebral Fracture

Posterior paralysis as a consequence of mechanical damage to the spinal cord following vertebral fracture or dislocation occurs commonly in the domestic rabbit (Roe and Stiff, 1962; Templeton, 1946). This seems to be a result of the attachment of powerful hindlimb muscles to a relatively light and fragile skeleton (Harkness and Wagner, 1989); the skeleton of the rabbit is approximately 7–8% of the body weight, as compared to 12–13% for cats (GeoChemBio, 2011). The injury may be referred to as broken back, paralysis of the hindquarters, spinal fracture, or traumatic vertebral dislocation. Onset is generally acute and coincides with struggling or inadequate hindquarter support when animals are handled. The heavy hindquarters can twist about the lumbosacral junction when improperly supported; this acts as a fulcrum, applying leverage to the vertebral column. Often the incident is not immediately observed (or recognized), and the rabbit is later found paralyzed in the cage. The L7 vertebral body or its caudal articular processes are considered to be the most frequent sites of such fractures, with fractures of these vertebrae more common than dislocation (Flatt et al., 1974). Of ten paralyzed rabbits with survey radiographs taken

at the University of Michigan between 1986 and 1990, only two had fractures of L7, specifically one compression fracture and one transverse fracture of the vertebral body. Three rabbits had compression fractures of L6, one had a transverse fracture of L6, and four rabbits had no detectable skeletal lesions. A transverse fracture of L6, resulting in posterior paralysis, was previously reported by Jones et al. (1982).

Diagnosis may be established on clinical criteria and confirmed by radiography. Clinical criteria include complete or partial rear limb paralysis and loss of sensory innervations. If the cord is completely severed (paraplegia), motor control of the anal sphincter and urinary bladder is lost as well. This condition results in urine retention and bladder distention, followed by clinical signs of uremia if the bladder is not expressed frequently. It is important, therefore, to distinguish overflow urination from normal micturition. Other common findings include staining of the perineum with liquid feces, owing to the loss of anal sphincter function, and decubitus ulcers. Serious cases that affect urination and defecation should be considered for euthanasia if no improvement in condition is observed after several days.

Frequently, however, the signs of spinal cord injury are related to transient edema at the site of trauma if the fractured bone ends do not become displaced. Corticosteroid and diuretic therapy may be initiated for the treatment of spinal edema, if warranted (De Lahunta, 1985). Over the course of 1–2 weeks, resolution of spinal shock may result in return of locomotor function such that the rabbit can independently move about the cage. Euthanasia need not be considered in cases where control over the urinary bladder and anal sphincter remains unimpaired and where partial to complete motor recovery follow an observation period of 2 weeks. Frequently an episode of spinal fracture with substantial recovery may be recognized months or years later as hyperflexion of the sacrum.

Splay Leg

The term “splay leg” is the descriptive name commonly applied to rabbits that lack the ability to adduct one or more legs and come to a standing position. Affected animals assume the typical splay leg appearance when ventrally recumbent and, at best, are able to move horizontally for only short distances by making weak, clumsy leg movements. In more severe cases all legs may be completely non-functional. Affected animals usually appear mentally alert. Although use of the term suggests a specific disease, “splay leg” is actually the leading clinical manifestation of several independent disease entities of rabbits, including the mutants syringamelia (*sy/sy*), hypoplasia pelvis (*hyp/hyp*), femoral luxation (*lu/lu*), and hereditary distal foreleg curvature (*fc/fc*).

Innes and O'Steen (1957), who studied ten cases in rabbits of the New Zealand White and Dutch breeds, advanced the idea that splay leg is a recessively inherited achondroplasia with pathological effects restricted to the hip and shoulder joints. Partial and complete dislocations were regarded as late sequelae. In contrast, Arendar and Milch (1966) studied 30 cases from a strain of inbred lop rabbits and found that the primary pathological defect was anteversion (forward tipping) of the femoral neck. This was sometimes secondarily associated with subtrochanteric torsion of the femoral shaft. As a consequence of both processes, the femoral heads projected from the anterior rather than medial surfaces of the femoral shafts. Rabbits with these femoral malformations consistently had subluxation of the hip joint; complete dislocation was observed only once. Arendar and Milch also considered the disease in their rabbits to be a recessive trait. Pedigree analysis and genetic mating tests by Joosten et al. (1981) showed that, in contrast to previous reports, splay leg is not determined by a single recessive gene with complete expressivity. They state that the underlying genetic mechanism is composed either of one recessive gene with reduced expressivity or of multiple genes with the probable involvement of environmental factors.

A possible environmental component to the clinical condition of splay leg was suggested by Owiny et al. (2001). The authors noted the development of hip dysplasia (coxofemoral luxation) in 2–4-week-old Dutch-belted rabbits born as part of a reproductive toxicology study. The incidence of the problem was significantly influenced by the type of flooring on which the rabbits were raised; non-slippery flooring during the post-natal period was associated with reduced hip dysplasia and splay leg-associated pathology in the young rabbits.

Splay leg remains a descriptive clinical term without clearly established pathological meaning(s). Further systematic investigation of the problem is needed.

Hereditary Distal Foreleg Curvature

Pearce (1960a) described a deformity of the forelegs in rabbits of the Beveren, Belgian, French silver, and Dutch breeds. Beginning at 2–3 weeks of age, the forelegs were observed to bow inwardly while the paws deviated laterally in a seal-like manner. The condition usually reached full expression by 2–3 months of age. There was marked widening of the distal epiphysis in each ulna. Histologically, this epiphysis showed retarded calcification and an abundance of osteoid tissue. The abnormally wide plate of cartilage usually diminished in thickness by about 2.5 months of age and became histologically normal by about 4 months of age. The forelegs remained permanently deformed by the inward bowing of radii and ulnae and the lateral deviation of the paws. Bones of the carpal joints and paws were considered normal.

Pearce (1960b) described the effects of a single autosomal recessive gene, which at least strongly predisposed the rabbits to the foreleg deformity. However, a similar condition is recognized among rabbit breeders when young rabbits are raised on smooth surfaces, such as when a doe persistently removes bedding from the hutch. Some of these affected rabbits have been observed to “return to normal” when transferred to cages with wire floors (Lindsey and Fox, 1994). The relationship of such cases to the mutation described by Pearce is not clear, and the overall contributions of environment versus genetic predisposition have not been discerned.

Dwarfism

Pituitary dwarfism, the earliest reported form of dwarfism in the rabbit, has been noted in both the United States (Greene, 1940; Greene et al., 1934) and Europe (Kröning, 1939; Nachtsheim, 1973; Schnecke, 1941). The American (gene symbol *Dw*) and European (gene symbol *nan*) mutations appear to be similar, and may well be the same mutation occurring in different stocks.

Both were originally reported as autosomal recessive mutations symbolized by *dw* and *nan*, respectively. The homozygous animals are typically unable to nurse and die within a few days of birth, although some have survived for longer. Because the mutation came to be considered an incomplete dominant lethal or semi-lethal, the mutant symbol was changed from *dw* to *Dw* (Latimer and Sawin, 1955; Sawin, 1955). The birth weight and adult body size of heterozygous animals are approximately one-third that of unaffected littermates (Castle and Sawin, 1941; Latimer and Sawin, 1955).

The affected animals are perfectly proportioned miniatures, with the exception of shortened ears and a disproportionately large central nervous system (CNS) (Castle and Sawin, 1941; Latimer and Sawin, 1955). Thus, the animals have bulging foreheads and protuberant eyes, and the skulls of those surviving more than 2 or 3 days have numerous large foramina (Sawin and Crary, 1964). Cause of death is attributable to either the CNS lesions or to hydronephrosis, which also develops in many of these animals.

Another dwarf mutation was observed in 1957 at the Institute of Human Genetics at the University of Münster (Germany). It was described by Degenhardt (1960) as a single autosomal recessive gene and designated *zw*. Hydrocephaly was observed as a common occurrence with the *zw* gene. The animals had features similar to the *Dw* and *nan* mutations and may well be another variant of the same gene.

Osteopetrosis

The autosomal recessive mutation osteopetrosis was discovered in a Dutch stock of rabbits and extensively studied by Pearce and Brown (1948) and Pearce (1948,

1950). This same line, later designated as the OS strain, was maintained at the Jackson Laboratory.

At birth affected animals are normal in appearance except for slightly reduced body size and abnormalities of the teeth. The incisors, particularly the uppers, are retarded in development, abnormally shaped, and usually rudimentary in appearance. One or all incisors may be completely absent. At necropsy changes similar to those in incisors are observed in molars. At birth, radiographs reveal a marked increase in density of the skeleton with practically no internal detail in bones throughout the body. For the first 2–3 weeks of life affected rabbits grow at a rate only slightly less than normal. Thereafter, growth ceases, and animals become cachectic, have diarrheal stools, and usually die at about 4 or 5 weeks of age.

The outstanding anatomic findings are persistence of spongy bone and failure to form normal marrow cavities. Marrow cavities that do form are filled with undifferentiated mesenchymal tissue (Walker and Fox, 1973). The spongy bone is composed of bony trabeculae having an irregular arrangement and variable staining. Although little compact bone is present, the bones are harder and more brittle than normal. Only sparse hematopoietic tissue ever appears in the bones, and despite the persistence of centers of extramedullary hematopoiesis in other tissues, such as liver and spleen, there is progressive macrocytic anemia with thrombocytopenia.

Pearce (1948, 1950) also observed that osteopetrotic rabbits had enlarged parathyroids and suggested that this finding was in some way related to the cause of the disease. Affected animals initially had normal serum calcium levels, which declined with age. Serum phosphorus levels were low initially but slightly exceeded normal values by the fourth week of age. These and other findings in osteopetrotic rabbits bear a close similarity to osteopetrosis seen in humans (synonyms include marble bone disease and Albers-Schönberg disease) and the gray lethal mutation of mice (Bateman, 1954). Familial osteopetrosis also has been reported in calves (Leipold et al., 1970). Although definitive evidence is not available except for the mouse (Marks and Walker, 1969), it seems extremely probable that all these diseases eventually will be explained by a defect(s) in parathormone and/or thyrocalcitonin regulation of bone metabolism.

Popoff et al. (1989) used osteopetrotic mutant rabbits to study the effects of calcitriol on elevated serum values of 1,25-(OH)₂ vitamin D (which are detected in some osteopetrotic children and animal models) and osteoclast-mediated bone resorption. Calcitriol was able to increase the osteoclast populations in both normal and osteopetrotic rabbits, but increased bone resorption and improvement of skeletal sclerosis were not observed.

Integumentary

Moist Dermatitis

Moist dermatitis is a chronic progressive disease of the skin that occurs with sporadic distribution (Casady et al., 1971; Pollock, 1951; Templeton, 1962; Vail and McKenny, 1943). The disease is also known as slobbers, ptyalism, and wet dewlap. It is thought that the dermatosis resulting from chronic wetness of the chin, intermandibular space, and cervical ventrum predisposes these sites to secondary bacterial tissue invasion. The condition can also present on other areas of the body if chronic wetness occurs (Garibaldi et al., 1990). Predisposing factors include drooling as a result of chronic dental diseases (especially malocclusion), drinking from crocks or water pans which cause continual wetting of the skin, presence of cold, damp contact bedding, and general unsanitary environmental conditions. Caging with ledges that retain water or sipper tubes that leak may also contribute to the problem.

A variety of bacterial species can invade chronically wet skin, and as the dermatitis progresses, gross pathological features consist of inflammation, partial alopecia, ulceration, and necrosis of the affected dermis. Microscopic examination demonstrates irregular patches of ulceration, coagulative necrosis, abscessation, and colonization of the dermal layers with bacteria. Cellular elements of both acute and chronic inflammation are found in the tissues surrounding the ulceration. Lymphatic and hematogenous extension may occur, especially to the ventral cervical and pulmonary lymph nodes. It has been suggested that Schmorl's disease (necrobacillosis) represents a special variant of moist dermatitis with more extensive invasion and tissue destruction.

Unless the bacterial invasion has become systemic, the prognosis for individual cases of moist dermatitis is good if treated. Treatment consists of eliminating the cause of chronic wetting, clipping the affected area, and scrubbing with a surgical scrub. A topical broad-spectrum antibiotic should be applied daily for a 10–14-day course. If the infection appears extensive, a course of systemic antibiotics should be administered as well.

Ulcerative Pododermatitis

Traumatic ulcerative dermatitis of the plantar surface of the metatarsal region, and less commonly the volar surface of the metacarpal-phalangeal region, is commonly referred to as sore hocks. The name "sore hocks" is actually a misnomer since the affected area is most commonly the metatarsus, not the hock (tibio-tarsal area). The condition is caused by pressure necrosis of the skin, usually resulting from the bearing of heavy body weight on wire-bottom cages (Ostler, 1961). Immature rabbits and rabbits of smaller breeds are seldom affected (Templeton, 1962; Vail and McKenny, 1943). Wet cage

floors, especially from urine-soaked feces, may predispose to development of pododermatitis (Ostler, 1961; Schwartz and Shook, 1928; Templeton, 1962; Vaida, 1959; Vail and McKenny, 1943), but it can also occur despite excellent sanitation practices. Other predisposing factors include excessive thumping, thin plantar fur pads (Harkness, 1987), and certain forms of wire crimp in the floor.

The lesions consist of circumscribed ulcerated areas in the skin covered by a dry, crusty scab. Secondary bacterial dermatitis adjacent to the ulcers and abscessation under the crusty debris sometimes occurs. *Staphylococcus aureus* is a common cause of such bacterial abscesses. Affected rabbits may appear healthy in every regard except for the lesions described; however, anorexia, weight loss, humping of the back, stilted movements, and death (Vaida, 1959) have been described in association with this condition. Frequent shifting of body weight from limb to limb has also been observed (Templeton, 1962; Vail and McKenny, 1943). Lesions of the front feet are thought to be a result of the shifting of weight from sore hind feet to the front feet.

Use of solid-bottom cages with frequently changed soft bedding or placement of resting boards in wire-bottom cages have been used to treat ulcerative pododermatitis. These approaches minimize trauma to the affected limbs and allow healing to proceed. Resting boards tend to prevent feces from falling through the cage floor, however, and thus can increase the problem of ensuring adequate sanitation. Topical use of zinc and iodine ointments and 0.1% solution of aluminum acetate have been described, and topical antibiotics are occasionally used. If abscesses are present they should be drained and systemic antibiotics administered.

Pulmonary and Cardiovascular

Pulmonary Emphysema

Naturally occurring pulmonary emphysema in rabbits was first reported by Strawbridge (1960a). The disease was initially observed in rabbits which were control animals for a study on the experimental induction of emphysema by the intravenous injection of a particulate dye. More than 50% of rabbits over 2½ years old were found to have spontaneous generalized pulmonary emphysema. The histological lesions were characterized by fenestrations of the alveolar wall which progressed to alveolar wall atrophy or destruction. Young rabbits given the particulate dye had similar lesions, and the author suspected that ischemic atrophy of the alveolar walls was the cause of the emphysema (Strawbridge, 1960b). An electron microscopy study of the alveolar walls in naturally affected rabbits supported the contention of an ischemic pathogenesis, as affected tissue

had a loss of papillary endothelium, partial or complete filling of capillaries with collagen, and recanalization of some damaged capillaries (Boatman and Martin, 1965). Strawbridge (1960a) proposed that spontaneous pulmonary emphysema in the rabbit could serve as a model for the human disease. This has apparently not been the case however, as an extensive literature search identified only chemically induced rabbit models of pulmonary emphysema. Further study of the spontaneous condition in rabbits has not been reported, and so it appears either that the cases were a unique circumstance, or that the practical significance of pulmonary emphysema in rabbits is minimal.

Hypertension

Inherited strain differences in blood pressure have been observed in rabbits (Fox et al., 1969). A population of hypertensive rabbits has been developed by selection (Alexander et al., 1954), with a high percentage of the progeny in subsequent generations also having hypertension (Alexander et al., 1956). Direct arterial pressure measurements in the selected population of hypertensive rabbits showed the mean systolic blood pressure to be 30 mmHg higher than normotensive stock rabbits. The hypertensive rabbits also had larger Traube-Hering waves than did normotensive rabbits (Alexander et al., 1956). A review of the literature (Schlager, 1972) suggests that the heritability of this condition in various laboratory animal species is in the order of 20%.

Aortic Arteriosclerosis

Spontaneous arteriosclerosis of the aorta in rabbits was first described by Israel in 1881, and many additional reports have been subsequently published (Bragdon, 1952; Kesten, 1935; Levin and Larkin, 1910; Maegraith and Carleton, 1939; Miles, 1907). Although the exact cause of the arterial lesion remains obscure, there is ample evidence that genetic factors play an important role in its expression. Lesions have been observed in practically all domestic breeds and even in wild rabbits. However, among the breeds there are distinct differences in incidence. The Dutch breed in the United States (Gaman et al., 1967) and the "Danish Country" strain of albino rabbit in Europe (Garbarsch et al., 1970) have low (10%) incidences of aortic arteriosclerosis, but incidences of 40% or greater are common in many contemporary breeds including laboratory stocks of the New Zealand White breed (Gaman et al., 1967). Diseased aortas have been observed as early as 6 weeks of age. It has been suggested that the early lesions also may have a non-genetic basis, as induction has been attributed to one or more factors in milk from some does rather than to the genotype of the young (Feigenbaum and Gaman, 1967).

In the typical case, lesions can occur in all major arteries but predominate in the ascending aorta and

aortic arch (Schenk et al., 1966). Grossly, the appearance of the intimal surface is quite variable, with longitudinal striations and furrows, raised plaques with or without central depressions, intimal nodules, or an admixture of the above. Schenk et al. (1966) characterized the lesions into two categories: type A lesions were nodular, located in the inner one-third to one-half of the media, and primarily consisted of fragmentation and disruption of the elastic lamina with the intercellular accumulation of acid mucopolysaccharide. Type B lesions affected the outer one-half of the media, and featured massive calcification and focal areas of cartilaginous metaplasia. The type B lesion was usually seen throughout the length of the aorta and in advanced cases gave the aortic wall an egg shell consistency. This extensive mineralization results in what appears to be an equivalent of Mönckeberg's medial sclerosis in humans. These lesions in the rabbit are free of lipid substances such as those occurring in human atherosclerosis (Feigenbaum and Gaman, 1967; Haust and Geer, 1970; Haust and More, 1965; Schenk et al., 1966) and the hyperlipidemic rabbit mentioned later in this chapter (Watanabe et al., 1985).

Many questions remain unanswered about the specificity of the anatomic lesions seen in so-called aortic arteriosclerosis of rabbits. For example, the lesions in this disease and vitamin D toxicity are remarkably similar, raising the question of dietary factors in aortic arteriosclerosis. The need for clear delineation of etiologies and types of vascular lesions in the rabbit is further supported by the sizable number of instances in which naturally occurring arteriosclerotic lesions of rabbits reportedly have been erroneously interpreted to be the result of experimental procedures (Gaman et al., 1967).

Blood and Vascular Mutants

ATROPINE ESTERASES ($Est-2^F/Est-2^F$; $Est-2^F/Est-2^{f1}$; $Est-2^{f1}/Est-2^{f1}$)

Some rabbits have a circulating atropine esterase that is capable of hydrolyzing atropine and other tropine esters (Glick, 1940; Glick and Glaubach, 1941; Lévy and Michel, 1938), thus explaining the astonishing reports going back to the mid-19th century that rabbits can thrive on belladonna leaves (Kalow, 1968). The presence of serum atropine esterase is controlled by a semidominant gene, $Est-2^F$, which was originally designated as *As* (Sawin and Glick, 1943; Zutphen, 1974b). Three genotypes are recognizable: $Est-2^F/Est-2^F$ with a high level of enzyme; $Est-2^F/Est-2^{f1}$ with a reduced level of enzyme; and $Est-2^{f1}/Est-2^{f1}$ in which the enzyme is absent (Lévy, 1946; Sawin and Glick, 1943). The actual absence of the enzyme in homozygous recessive animals, rather than the presence of an inactive enzyme, has been supported by failure to demonstrate an immunologically related

protein in sera of such rabbits (Margolis and Feigelson, 1964). In animals homozygous or heterozygous for the $Est-2^F$ gene, the enzyme first appears in the serum at about 1 month of age, and it occurs at higher levels in females than males. Atropine esterase is linked with the gene *E* for extension of black pigment in the coat (Fox and Zutphen, 1979; Sawin and Glick, 1943).

There are no known harmful effects of an absence of serum atropine esterase on the health of rabbits. However, the presence or absence of the enzyme makes a significant difference in the response to the administration of atropine for pharmacotherapeutic purposes, regardless of whether it is given by injection or applied topically to the eye (Werner, 1965). The rabbit has been studied extensively to provide information on the classification of esterase enzyme systems and on the genetic and phenotypic variations associated with atropine esterase and cocaine esterase (van Zutphen, 1972, 1974a, 1974b; van Zutphen and den Bieman, 1975; van Zutphen et al., 1977).

PELGER (Pg/Pg) AND SUPER-PELGER (Pg/Pg)

In 1928, Dutch physician Karel Pelger noted two patients who had leukocytes with much reduced nuclear segmentation. Later, G.J. Huët disproved Pelger's mistaken idea that this was associated with tuberculosis and showed that the abnormality was inherited in humans as an autosomal dominant trait. Subsequently, the condition has been referred to as either the Pelger or Pelger-Huët anomaly and has been recognized as an inherited trait in many animals including rabbits, dogs, and mice (Nachtsheim, 1958). In humans the disorder is caused by a mutation in the lamin B receptor (LBR) on the nuclear membrane (Hoffmann et al., 2002).

In rabbits (Robinson, 1958) and humans (Hoffmann et al., 2002) the condition is now recognized to be a partial dominant, i.e., heterozygotes have an intermediate phenotype between those seen with homozygous dominant or recessive genotypes. The Pelger anomaly is fairly well known in rabbits because of studies by the Swiss hematologist Undritz on a single stock of animals established in 1939 (Nachtsheim, 1950). The heterozygotes (Pg/pg), designated as "Pelgers," are normal except that the nuclei of their neutrophils have a reduced number of lobes (usually one or two, possibly three). In the homozygotes (Pg/Pg), referred to as "super-Pelgers," all leukocytes have round, hyperchromatic nuclei which actually appear pyknotic with usual stains. Because of fetal death, only about 18% of theoretically expected super-Pelgers are born, and most of these die during or shortly after birth. Super-Pelgers are smaller than normal at birth due to a marked reduction in the length of long bones. When placed in an upright position, these rabbits rest on their sternums with the forelimbs extending laterally; the deformed limbs

can only make paddling movements. Very rarely do super-Pelgers live to maturity. One super-Pelger buck survived to be used successfully in breeding trials, which helped to confirm the pattern of partial dominant inheritance in the rabbit.

The skeletal abnormality of the super-Pelger is considered a chondrodystrophy. This is particularly noticeable in the ribs, which are reported to be thickened at the costochondral junction. The rib deformity is considered to be responsible for death from asphyxiation in most live-born super-Pelgers. In older animals the epiphyses remain thickened, and often there is fusion of the radius and ulna.

LYMPHOSARCOMA (*LS/LS*)

In general lymphosarcoma has been considered rare in rabbits (Van Kampen, 1969), however it was found to be a common occurrence in strain WH rabbits (Fox et al., 1970a, 1970b, 1971c) with a reported pattern of transmission as an autosomal recessive gene (*ls*), compatible with concepts of both genetic susceptibility to the neoplasm and vertical transmission of a virus.

The disease manifests at an average age of 8 months (range: 2–27.5 months) with the appearance of anorexia, lethargy, and weight loss. Most affected animals die between 5–13 months of age. Terminally, there is severe anemia, with total erythrocytes (RBCs) as low as 2×10^6 and hematocrit as low as 10%. Hematological examination of rabbits with lymphosarcoma reveals an essentially aleukemic picture. Even though the total white blood cells (WBCs) are only 2000–10000/mm³, a shift in cell types is observed, with 80% or more being lymphoblasts. The lymph nodes are increased in size and have a preponderance of lymphoblasts. Advanced cases often have elevated blood urea nitrogen levels coinciding with occurrence of massive infiltration of malignant lymphoid cells into the kidneys. Other anatomic findings include lymphomatous infiltrates in Peyer's patches, liver, and spleen, and sometimes in adrenal glands, gonads, and thymus. The bone marrow from such animals is similar to that in the Hodgkins-type sarcoma observed in humans (Meier and Fox, 1973). Rabbit lymphoma is described in further detail in the chapter on neoplastic diseases of the rabbit.

HEMOLYTIC ANEMIA (*HA/HA*)

Fox et al. (1970a, 1971d) and Meier and Fox (1973) have reported a hereditary hemolytic anemia occurring in the X strain of rabbits at the Jackson Laboratory. Approximately 60 cases were observed in the stock over a period of years. Pedigree analysis suggested autosomal recessive inheritance, and the gene symbol *ha* was assigned.

Clinically, the disease was characterized by anemia with Coomb's-positive red blood cells (RBCs),

hemoglobinemia, petechial hemorrhages on mucous membranes, and weakness, with death generally occurring between 4 and 5 months of age. Severe icterus was seen in some rabbits. The peripheral blood was characterized by reduced hematocrit, polychromatophilia, anisocytosis, increased nucleated RBCs, and lymphocytosis. Necropsy findings included icterus, visceral hemorrhages, hemoglobinuria, swelling of kidneys, and subcutaneous edema. Histologically there was widespread erythrophagocytosis, edema, and accumulated iron pigment in visceral organs. Some animals also had visceral lymphadenopathy and thymoma. No lesions suggestive of glomerulonephritis or arteritis were observed.

It is particularly interesting that the X strain of rabbits with hemolytic anemia shares a common ancestry with the WH strain known to develop lymphosarcoma (*ls*). Indeed, *ha* and *ls* might be a single gene, with the ascribed lesions merely representing different phenotypic expressions (Fox et al., 1971c). Both lesions are of interest because of analogies to such diseases as systemic lupus erythematosus of humans and dogs, various lesions occurring in NZB mice, and hemolytic anemias of other species.

C3 HYPOCOMPLEMENTEMIA (*C3^L/C3^L*)

Serum levels of complement component 3 in a colony of New Zealand White rabbits were separable into three distinct groups: C3 hypocomplementemia (*C3^L/C3^L*), C3 intermediate (*C3^H/C3^L*), and C3 normal (*C3^H/C3^H*) (Komatsu et al., 1988). The hypocomplementemic rabbits (*C3^L/C3^L*) had reduced serum C3 concentrations (6–12% of normal), hemolytic C3 activities (8–13% of normal), and total complement hemolytic activities (27–37% of normal). The C3 hypocomplementemia was transmitted as an autosomal dominant trait with a gene dosage effect. Hypocomplementemic rabbits had reduced survival, with sera showing reduced bactericidal activity.

C6 DEFICIENCY (*C6^D/C6^D*; *C6^N/C6^D*)

Deficiency of the sixth complement component has been recognized in three stocks of rabbits from Freiburg, Germany (Rother et al., 1966), Mexico (Biro and Garcia, 1965), and the United Kingdom (Lachmann, 1970). Thus, they presumably represent three different mutations that may be closely related genetically. The Freiburg and Mexico strains and crosses between the two have been studied most. Homozygous C6-deficient (*C6^D/C6^D*) animals lack detectable C6 antigen in the serum, and heterozygotes (*C6^N/C6^D*) have serum levels approximately half that of normal (*C6^N/C6^N*) animals (Tedesco and Lachmann, 1971). Serum from homozygous C6-deficient rabbits lack hemolytic, bacterial, and leukocyte chemotactic activities. Inheritance is autosomal dominant with a gene dosage effect (Biro and

Garcia, 1965; Lachmann, 1970; Nelson and Biro, 1968; Rother et al., 1966; Tedesco and Lachmann, 1971).

C8 α - γ ^P DEFICIENCY (C8 α - γ ^P/C8 α - γ ^P)

The eighth component of complement (C8) consists of three polypeptide chains, α , β , and γ . The α and γ chains are linked to one another to form the α - γ subunit, which is covalently associated with the β subunit. Genetic deficiency of the α - γ subunit of C8 has been reported in humans and rabbits, including the New Zealand White breed (Komatsu et al., 1985, 1990). Sera lacked both the immunochemical and functional α - γ subunit of C8, and the deficiency was transmitted as an autosomal dominant trait with a gene dosage effect. Double-homozygous rabbits had consistently lower thymus weights but no histological abnormalities. Affected rabbits also had reduced body weight, reduced litter size, and survival up to 3 months of age.

HYPERLIPIDEMIA (Hc/Hc; Hc/+)

Familial hypercholesterolemia (FH) is one of the most common and important inherited diseases affecting humans. It is a "receptor disease" in which a mutation in the gene encoding the receptor for low-density lipoprotein (LDL) causes reduced transport and metabolism of cholesterol. Humans homozygous for this gene mutation have 5–6-fold elevations in plasma cholesterol and develop skin xanthomas as well as coronary, cerebral, and peripheral vascular atherosclerosis early in life. Heterozygotes have 2–3-fold increases in plasma cholesterol and experience premature atherosclerosis in adult life (Cotran et al., 1989). The discovery by Watanabe and colleagues of the Watanabe heritable hyperlipidemic (WHHL) rabbit (Kondo and Watanabe, 1975) provided an important animal model for the study of FH (Aliev et al., 2004; Goldstein and Brown, 1989; Shiomi and Ito, 2009; Tanzawa et al., 1980) as the disease in these rabbits is quite similar to FH in genetic (Watanabe, 1980; Watanabe et al., 1985), clinical, metabolic (Bilheimer et al., 1982; Kita et al., 1981) and biochemical features.

Hyperlipidemia in the WHHL rabbit is due to a nucleotide deletion (the provisional symbol of *Hc* for hypercholesterolemic is used here) in the LDL binding domain of LDL receptor cDNA (Shiomi and Ito, 2009), and is inherited as an autosomal dominant gene with a gene dosage effect. As in FH, homozygotes (*Hc/Hc*) have plasma cholesterol levels of 500–950 mg/dl (90% of which is contained in LDL), marked reduction in the removal of LDL from plasma, virtually no LDL receptors on cell membranes, cholesterol deposits (xanthomas) in tissues, and develop severe atherosclerosis. Heterozygotes (*Hc/+*) have one-half the normal number of LDL receptors and have less severe clinical disease (Atkinson et al., 1989; Bilheimer et al., 1982; Goldstein and Brown, 1989; Havel et al., 1989; Kita et al., 1981;

Tanzawa et al., 1980; Watanabe, 1980; Watanabe et al., 1985). The WHHL rabbit has proved particularly useful in experimental trials to investigate therapeutic modalities prior to use in human patients with FH, including serum cholesterol-lowering drugs (Shiomi et al., 1990), anti-inflammatory drugs (Makheja et al., 1989), antioxidant drugs (Carew, 1989; Carew et al., 1987; Mao et al., 1991) and gene therapy (Chowdhury et al., 1991).

Gastrointestinal

Gastric Trichobezoars

A trichobezoar, or hairball, is a gastric mass composed of hair, fur, or wool. The hair accumulates in the stomach and may mix with ingesta to form round-to-oval masses. A lack of sufficient roughage in the diet and/or boredom has been suggested as reasons for hair ingestion behavior; however, the precise cause for this pica is unknown. The clinical condition associated with these masses was historically termed "wool block." More recently it is referred to as gastrointestinal stasis syndrome (Pilny and Hess, 2004). It is thought that rabbits are predisposed to the gastric accumulation of hair because of both their inability to vomit and the small lumen of the rabbit pylorus (Harkness and Wagner, 1989). Generally the hairballs do not cause an obstruction, and they are often found incidentally during postmortem examination. Leary et al. (1984) found that 48 of 208 (23.1%) clinically healthy New Zealand White rabbits had gastric trichobezoars at necropsy.

Although gastric trichobezoars are typically asymptomatic, they occasionally cause partial or complete obstruction of the digestive tract. Gastric motility can be decreased in rabbits for a number of reasons including a high-carbohydrate, low-fiber diet, dehydration, anorexia, stress, and pain. Decreased gastric motility can cause dehydration and impaction of the normal stomach contents leading to the formation of trichobezoars (Meredith, 2007).

Clinically affected animals may present with anorexia, decreased fecal output, lethargy, and weight loss. The diagnosis of a trichobezoar is typically made with palpation and radiography, though a positive finding on either of these is not always conclusive. A mass in the stomach may be palpable; however, the rabbit stomach remains full even after periods of anorexia (Gillett et al., 1983) and palpable trichobezoars can be an incidental finding, not contributing to the clinical illness. Additionally, Leary et al. (1984) found that the size of the mass does not correlate with the incidence of clinical illness, as small, non-palpable trichobezoars at the pylorus can be clinically significant. Contrast radiography of the stomach has been attempted, but is often non-diagnostic unless the pyloric obstruction completely prevents passage of the liquid barium (Gillett et al., 1983; Wagner et al., 1974).

Radiographic evidence of a large stomach containing gas and ingesta in conjunction with a clinical history of anorexia in a rabbit can support a diagnosis of gastric stasis (Pilny and Hess, 2004); however, in most cases the diagnosis is ultimately made by either response to empiric treatment or exploratory gastrotomy.

Treatment should be aimed primarily at hydration and nutritional support. Quality hay should be made available at all times. Analgesia may be utilized to reduce the pain associated with gaseous distention. The use of enzymatic products, such as papain, is controversial as these do not actually digest the hair, but may help break down the matrix holding the material together (Meredith, 2007). Pro-motility agents such as metoclopramide may be administered, but only if it is certain that there is not a complete obstruction. Rabbits should also be encouraged to exercise to stimulate gastrointestinal motility. Surgery is not recommended as the initial treatment; however if the rabbit does not respond to aggressive medical treatment, surgery is a viable option (Pilny and Hess, 2004). Appropriate peri-operative care and management, including fluid administration, broad-spectrum antibiotics, and use of live *Lactobacillus* culture is critical to the success of surgical management of trichobezoars (Gillett et al., 1983).

Reported complications from trichobezoars include hepatic lipidosis, gastric rupture, and peritonitis (Leary et al., 1984; Lee et al., 1978). For rabbits exhibiting prolonged anorexia, placement of a percutaneous endoscopic gastrostomy (PEG) tube is a feasible and safe option for providing enteral nutrition (Smith et al., 1997).

Pyloric Stenosis

Congenital pyloric stenosis with muscular hypertrophy was first reported in a rabbit by Cardy in 1973. A pre-weanling Flemish Giant male died suddenly, and gastric dilatation with complete closure of the pylorus was observed at postmortem examination. Histologically, the external muscularis layer of the pylorus was extremely thickened. Weisbroth and Scher (1975) reported three cases of hypertrophic pyloric stenosis in rabbits, all of which were older than 12 weeks of age. The rabbits had similar clinical histories of anorexia, listlessness, weight loss, and constipation. Hypertrophy of the muscularis was noted in all three cases, and postmortem measurements indicated that the pylorus was thickened at the expense of the lumen. It could not be determined if the three cases were exacerbations of congenital pyloric stenosis, whether they arose de novo in adulthood, or whether they were secondary to pylorospasm. There have been no further reports of this condition.

Liver Lobe Torsion

Liver lobe torsion has been reported infrequently in *Oryctolagus*. Though it cannot definitively be stated

that French lop and Dwarf lop rabbits are predisposed to this condition, the current literature does present a bias towards these breeds. The clinical presentation of liver lobe torsion in rabbits can vary from an incidental necropsy finding to sudden death. Weisbroth (1975) reported an incidence of three out of 984 rabbits with torsion of the caudate lobe of the liver seen at necropsy in a large research colony. In these cases, no clinical signs were reported, nor were there secondary lesions or sequelae to the torsion.

In rabbits that have been reported with clinical signs, the initial presentation included a sudden onset of lethargy, anorexia, and abdominal pain. Wenger et al. (2009) found heart rate and respiratory rate to be increased and mucous membranes to be pale and slightly jaundiced upon physical examination. Moderate to severe anemia was found consistently on hematologic examination. Clinical chemistry abnormalities included elevations in alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT). Other clinical findings included dehydration, as evidenced by an increase in blood urea nitrogen (BUN) and creatinine, and bilirubinuria (Taylor and Staff, 2007; Wenger et al., 2009).

Diagnostic modalities, such as radiography and ultrasound, have been of limited benefit in diagnosing this condition (Saunders et al., 2009). This is due to extensive gastric stasis and gas accumulation secondary to the liver lobe torsion, which results in reduced abdominal contrast. Additionally, when ultrasound examination has revealed potential hepatic abnormalities, such as enlargement and heterogeneity, fine needle aspiration with cytologic examination has been unremarkable. In all cases histological examination revealed a loss of normal hepatic architecture and extensive hemorrhage of the affected lobe consistent with ischemic necrosis (Wenger et al., 2009). Though the caudate lobe has been reported to be predisposed to torsion in this species, there are reports of torsion involving both the quadrate and the right liver lobes as well (Weisbroth, 1975, Wenger et al., 2009).

Anecdotal reports exist describing diagnosed cases of rabbits with liver lobe torsion that have recovered uneventfully with only supportive care. In these animals the liver enzymes decreased and the hepatic lobe atrophied over time (Wenger et al., 2009). Current literature, however, suggests that rabbits presenting with clinical signs and hematological alterations indicative of liver lobe torsion can be systemically compromised and may die without surgical intervention. Additionally, there are two successful reports of curative surgical hepatic lobectomies in rabbits with this condition (Saunders et al., 2009; Taylor and Staff, 2007).

There may be cases of liver lobe torsion in rabbits that are undiagnosed either because the rabbit exhibited no

clinical signs or because the rabbit died spontaneously without diagnostic evaluation (Wilson et al., 1987). Liver lobe torsion should be considered as a differential diagnosis for rabbits that present clinically with anorexia, lethargy, and abdominal pain. Although radiology and ultrasound may be unremarkable, the presence of anemia and elevations in liver enzymes appear to be the best markers for this condition (Saunders et al., 2009). Despite anecdotal reports of spontaneous resolution with only supportive care, surgical intervention early in the onset of the clinical course is reported to be a promising treatment option for complete recovery.

Urogenital

Renal Cysts

Fox et al. (1971a) have reported the occurrence of multiple small cysts in kidneys of strain III_{VO} rabbits. Cysts were detectable only at necropsy, as there were no adverse effects on health or preliminary renal function tests. Significant numbers of cysts were never found in animals less than 1 month of age, but were found in increasing numbers up to approximately 6 months of age. Pedigree analysis provided evidence consistent with autosomal recessive inheritance with incomplete (76%) penetrance.

Affected kidneys have from one to hundreds of cysts, up to 1 mm diameter and limited in location to the subcapsular region of the cortex. Microdissection studies showed the cysts to be of tubular origin, as was also demonstrated in selected histological sections. A single layer of flat to low cuboidal epithelium surrounded by little or no fibrous tissue usually lined the larger cysts. Elsewhere cysts had the appearance of primitive ductules and tubules in abundant cellular mesenchymatous stroma. The latter areas resembled closely the histological findings of renal cortical dysplasia in humans (Bernstein, 1968; Kissane, 1966). The histologic appearance, the subcapsular location of lesions, and the age at which cysts first appear all tend to point toward an interruption of development in the late stages of renal organogenesis. It is of at least passing interest that similar subcapsular renal cysts have been produced experimentally in the rabbit by a single injection of long-acting adrenal corticosteroids given at birth (Perey et al., 1972). As of 2008, inbred III_{VO}/JU rabbits were maintained by the University of Utrecht (Belgium) from the original Jackson Laboratory stock.

Urolithiasis

Urolithiasis historically has occurred as a sporadic condition in rabbits with no definitive causative etiology. Most reports cite the unique calcium metabolism of rabbits as a potential factor in the development of urolithiasis. Rabbits passively absorb dietary calcium

and serum calcium levels typically are higher than most other species (Buss and Bourdeau, 1984). Calcium regulation occurs primarily via renal excretion or conservation depending upon metabolic needs. High dietary levels of calcium therefore result in high levels of calcium in the urine. Normal rabbit urine is slightly alkaline, with an average pH of 8.2 (Williams, 1976), and has a cloudy appearance owing to the presence of calcium carbonate and triple phosphate crystals (Flatt and Carpenter, 1971). At higher pH (8.5–9.5) the crystals precipitate out of the urine (Leck, 1988). Factors that enhance the development of urolithiasis may include genetic predisposition, metabolic disturbance, nutritional imbalance, infection, and inadequate water intake (Garibaldi et al., 1987).

In the cases reported in the literature, clinical signs if present are usually non-specific and may include weight loss, anorexia, and/or irritability. Radiography is a valuable diagnostic tool in these cases and will detect calculi as well as hypercalcification of soft tissues, which is present in about 26% of affected rabbits in a report by Harcourt-Brown (2007). In most cases, the urolith is composed of calcium carbonate and ammonium magnesium phosphate (triple phosphate) (Garibaldi et al., 1987; Whary and Peper, 1994; White, 2001). Fleming and Carpenter (2000) reported a case of severe bladder distention characterized by about 500 ml of calculi “sand” rather than larger discrete uroliths. Calculi may be located in the kidney, ureters, or bladder with secondary effects such as hydronephrosis present. While pathologic findings include expected trauma to the urinary epithelium, Harcourt-Brown also found a very high incidence of lesions suggestive of *Encephalitozoon cuniculi* infection in the kidneys. The author proposed that inflammatory foci in the kidney in response to *E. cuniculi* may cause obstruction of urine flow as well as provide a nidus for subsequent stone formation. Itatani et al. (1981) proposed that sulfated glycosaminoglycans, which are increased in the urine of rabbits with urolithiasis, may bind calcium crystals together to form a massive aggregate resulting in urolith formation.

Treatment of urolithiasis is surgical removal of the calculus or flushing of the urinary bladder for removal of sand-type calculi. Diazepam for relaxation of the urethralis muscle is useful for catheterization approaches (Keeble, 2007). Prevention is aimed at ensuring adequate water intake and treating any predisposing factors such as excessive dietary calcium intake. Exercise has also been suggested as a preventive measure via encouraging normal urination patterns (Keeble, 2007).

Hydrometra

Hydrometra has historically been reported in rabbits (Bray et al., 1991; Hobbs and Parker, 1990; Morrell, 1989). Coincidentally, all reported cases were rabbits being used experimentally for the production of serum

antibodies. In the report by [Morrell \(1989\)](#), four cases were seen over a 2-year period. Clinical signs consisted of abdominal distention and tachypnea. On gross post-mortem examination, the uterine horns were distended with a transudative fluid. No etiology was determined, although it was proposed that increased progesterone levels could stimulate endometrial gland secretion and a functional cervical closure. [Bray et al. \(1991\)](#) reported on a case in which the abdominal distention resolved with diuretic therapy, but recurred more severely 2 months later. Hydrometra was assumed to be the cause of initial abdominal enlargement as the transudate aspirated during the clinical episode was similar to the intrauterine fluid collected at necropsy. Persistent corpora lutea or follicular cysts were not observed. In the cases reported by [Hobbs and Parker \(1990\)](#), hydrometra was associated with uterine torsion.

Vaginal Prolapse

When compared to other species, rabbits rarely experience maternal injury during vaginal birth, and as a result, spontaneous vaginal prolapse secondary to gestation and parturition is uncommon. This is in part due to differences in the mechanical and anatomical properties of the *Oryctolagus* vagina ([Abramowitch et al., 2009](#)). Pelvic organ collapse in rabbits can occur secondary to reproductive abnormalities such as extrauterine pregnancy, uterine adenocarcinoma, or endometrial venous aneurysms (L. Gallagher, personal observation, 2009).

An increased incidence of prolapsed vagina does occur in the III_{VO}/JU inbred strain of rabbits ([Van Herck et al., 1989](#)). Eight cases were reported over a 5-year period in closely related females. In these cases the prolapse was not associated with gestation, but rather occurred at a time of increased sexual activity when blood supply to the vulva/vestibulum was increased. The authors proposed that an underlying genetic factor may contribute to the incidence in the III_{VO}/JU strain.

Affected rabbits present with a prolapse of engorged vaginal tissue that is bloody ([Figure 17.5](#)) and possibly ulcerated secondary to self-mutilation. Often, the first sign of this condition may be the detection of blood in the cage. Clinically, animals may be lethargic, tachypneic, pale, and have cold extremities consistent with hypovolemic shock. Hematocrit measurements may be as low as 9% ([Van Herck et al., 1989](#)). Treatment including fluid administration and reduction of the prolapsed tissue can be attempted, though there are no reports of successful recovery.

Toxemia of Pregnancy

Pregnancy toxemia is a disease that is seldom recognized in domestic rabbits. Pregnant as well as postparturient and pseudopregnant females may be affected ([Greene, 1937, 1938, 1965](#)). In a large outbreak of



FIGURE 17.5 Vaginal prolapse in a New Zealand White rabbit. Courtesy of The Ohio State University.

pregnancy toxemia involving 72 cases ([Greene, 1937](#)), 60% occurred in pregnant does, 21% in postparturient does, and 19% in resting or pseudopregnant females. The incidence in multiparous does was approximately four times that observed in primiparous does. Affected females were most often obese. [Greene \(1937, 1938\)](#) also found that Dutch, Polish, and English breeds had the highest incidence of pregnancy toxemia. There was also an increased incidence of pregnancy toxemia in carriers of dwarfism in the Polish breed and cretinism in the Dutch breed.

The clinical appearance of pregnancy toxemia varied greatly, with a mild, nearly asymptomatic condition at one end of the spectrum and a severe, rapidly fatal disease at the other end. In the pregnant females, the clinical disease was first detected while nest building was occurring. The most commonly observed clinical signs were depression, dyspnea with acetone odor on the breath in severe cases, and decreased urine production. Abortion, incoordination, convulsions, and coma sometimes preceded death ([Greene, 1937](#)). Recovery occurred in the mild to moderate cases.

Severe cases were accompanied by marked elevation in serum non-protein nitrogen and phosphate and a decrease in serum calcium. Tests for serum acetone were positive. Affected animals that died or were euthanized were characterized by obesity, actively secreting mammary glands (even in the pseudopregnant does), large corpora lutea in the ovaries, and areas of necrosis in the mesenteric fat. The liver was enlarged and pale due to fat deposition ([Figure 17.6](#)); the kidneys and heart were also pale. The thyroid and adrenal glands were small and pale. Enlargement of the pituitary gland was also common. Severe fatty infiltration of the liver and focal hepatic necrosis were seen on histopathological examination of some cases. Fatty infiltration was also observed in



FIGURE 17.6 Hepatic pallor in a rabbit with hepatic lipidosis secondary to pregnancy toxemia. Courtesy of The Ohio State University.

the kidney tubules, heart, and adrenal cortex, especially within the thickened zona fasciculata. Cortical adenomas were often present in the adrenal glands. The thyroid follicles were filled with pale colloid and lined by low cuboidal epithelium. The pars distalis of the pituitary gland contained many acidophils and multiple adenomas, and the pars intermedia was thickened.

Like pregnancy toxemia in other domestic animals, the cause of disease in rabbits is complex. Many factors may have an influence on the development of the syndrome including breed, age, obesity, and the number of previous pregnancies. Harkness (1987) indicated that pregnancy toxemia may be the result of insufficient arterial flow to the uterus, leading to a metabolic derangement. The decreased blood flow could be due to obesity, poor development of the uterine vasculature, or a large fetal mass. On the other hand, Greene (1938) proposed that the disease may be related to abnormal hypophyseal function. The outbreak of pregnancy toxemia in his rabbits was associated with other reproductive problems, including abortions and stillbirths, and an increased incidence of uterine tumors. He postulated that environmental changes might lead to an endocrine imbalance resulting in pregnancy toxemia and other reproductive disturbances. In a more recent case report, it was proposed that there was a possible relationship between pancreatitis and pregnancy toxemia (Leland et al., 1995). No definitive etiology for pregnancy toxemia in rabbits has been determined, however. Successful attempts to treat the disease in rabbits have not been reported.

Extra-Uterine Pregnancy

Extra-uterine pregnancy is a pregnancy that occurs elsewhere than within the uterine lumen. Primary abdominal pregnancy is when an oocyte is fertilized in the abdominal cavity or a fertilized ovum enters the peritoneal cavity and placentation occurs on either

the peritoneal or omental surface. Secondary abdominal pregnancy is when a fetus which has already been implanted enters the abdominal cavity secondary to oviduct or uterine rupture, usually as a result of external trauma or increased internal pressure (Corpa, 2006). Primary abdominal pregnancies are rare, whereas secondary abdominal pregnancies have been reported in several animal species, including rabbits (Corpa, 2006; Eales, 1932; Harper and Ensley, 1982; Segura et al., 2004). Segura et al. (2004) reported 28 abdominal pregnancies out of 550 adult fertile female New Zealand White rabbits from two rabbit farms in Valencia, Spain. Seven of the 28 showed no evidence of uterine rupture, though evidence of intra-abdominal placentation was found in only two of the animals.

Discovery of an ectopic fetus is often an incidental finding as the condition is not generally of great clinical significance to the affected rabbit. There are reports of rabbits exhibiting clinical signs including lethargy and anorexia, though these presenting signs may not have been secondary to the extra-uterine pregnancy (Harper and Ensley, 1982; Segura et al., 2004). Other clinical signs that may be present include vaginal discharge, vaginal prolapse, and mammoplasia, although these are uncommon and not reported in the current literature (L. Gallagher, personal observation, 2009). Additionally, there is no known association between the development of clinical signs and the length of time for which an ectopic fetus has been present (Corpa, 2006).

If a previously normal reproductive doe does not undergo parturition and a firm, non-fluctuant mass (or masses) is palpable in the abdominal cavity, extrauterine pregnancy should be suspected. Diagnosis can be confirmed utilizing radiography and abdominal ultrasonography. The fetuses may appear as dystrophic mineralized abdominal masses upon radiography. Confirmation of fetal tissue versus foreign body can be made via ultrasound which can determine the consistency and texture of the abdominal mass (Eshar and Mayer, 2008).

If the fetuses are expelled from the uterus prior to 21 days of gestation, and there is no implantation within the abdominal cavity, fetal death will occur. After 21 days, incomplete resorption occurs, resulting in mummification of fetuses. However, rabbits have a similar placentation as human beings that permits them to produce a viable extrauterine pregnancy (Segura et al., 2004). Fetuses can implant to the peritoneal or omental surface and establish an extensive blood supply; as such, fetal development can continue until placental perfusion becomes inadequate (Owensby et al., 2001; Segura et al., 2004). It is not uncommon to see multiple fetuses within the abdominal cavity at varying stages of development including term-sized fetuses (Segura et al., 2004; L. Gallagher, personal observation, 2009). Ectopic fetuses typically appear well preserved (Figure 17.7),



FIGURE 17.7 Mummified ectopic fetus in a New Zealand White rabbit (same rabbit as with vaginal prolapse seen in Figure 17.5). Courtesy of The Ohio State University.

though dehydrated, and most commonly they are found encapsulated within a thin, tough connective tissue (Jacobson et al., 1975). Histologically, the fetuses show moderate autolysis, but no active inflammatory response (Segura et al., 2004).

Other Systems

Abnormal Ear Carriage

Rabbits normally carry the ears erect pointing dorsolaterally, or in a relaxed position against the dorsum of the trunk and pointing posterolaterally. Chai and Clark (1967), while studying a number of inbred lines of rabbits, observed a condition designated as “droopy ear” in which the ears drooped downward and antero-ventrally from the base. The condition is thought to be due to more than one gene. Droopy ear is distinguished from the lop ear or flop ear characteristic of certain breeds in which the ears, perhaps due to excessive weight, simply hang downward from their attachments on the head. This trait too is thought to be controlled by multiple factors (Castle and Reed, 1936).

Plant Toxicosis

The poisoning of rabbits by toxic plants has been reported infrequently and generally occurred before commercial feed was widely used (Bergdall and Dysko, 1994). Some of the plants implicated as being toxic to rabbits include the wooly-pod or broad-leafed milkweed, *Asclepias eriocarpa*; ladino clover; comfrey; and a mixture of cock, buttercup, and wild parsnip.

Drug Acetylators – Rapid and Slow

An important metabolic step in the biotransformation of carcinogens called arylamines, and toxins called

hydrazines, is N-acetylation via the cytosolic enzyme N-acetyltransferase (EC 2.3.1.5) located predominantly in the liver and small intestine (Weber, 1973). Differences in N-acetylation rates among humans have been found to be due to a single autosomal locus with two alleles, *R* and *r*. Hein et al. (1982) surveyed a large group of inbred rabbit strains and found, using a variety of substrates, three clear-cut groups: a group of rapid acetylators strains, designated *RR*; an intermediate obligate heterozygous group, *Rr*; and a group of homozygous slow acetylators strains, labeled *rr*. Outbred rabbits studied (NZW) were in the slow acetylators category.

Resistance/Susceptibility to Infection

The hereditary resistance and susceptibility of rabbits to experimental infections was studied in depth by Lurie and colleagues using *Mycobacterium* species as the infectious agent (Lurie, 1964; Lurie and Dannenberg, 1965; Lurie et al., 1952). In general, the studies compared the relative abilities of several families of rabbits to eliminate or suppress standard doses of human or bovine tubercle bacilli. These rabbits were derived from brother-sister matings for approximately ten generations and subsequent line breeding for many generations thereafter. The different families of rabbits varied greatly in response and were classified as resistant, susceptible, or intermediate. Resistance was found to be most directly dependent on the ability of host macrophages to engulf and inhibit the growth of bacilli in their cytoplasm. The family differences in macrophage efficiency were attributable to differences in endocrine balances, involving particularly thyroid and adrenal hormones. The determinants of resistance were considered to be polygenic (Lurie et al., 1952).

Genetic factors also are known to influence the susceptibility of rabbits to certain viral infections. Greene (1935) observed striking breed differences in mortality during an epizootic of rabbit pox. The natural selection of resistance to myxomatosis has been observed in the wild rabbit population since release of the myxoma virus in Australia in 1950 (Fenner, 1953; Marshall and Douglas, 1961; Marshall and Fenner, 1958), and this has been largely duplicated by selective breeding of domestic rabbits (Sobey, 1969). The inheritance of resistance to viral infection is clearly controlled by multiple genes, but the mechanisms by which resistance is conferred remain obscure.

Heat Prostration

Rabbits have limited capability for thermoregulation under elevated ambient temperatures as they cannot sweat, and panting and salivation are inefficient; their primary means of temperature control is the countercurrent heat exchange system provided by the vasculature of the ear (Brewer and Cruise, 1994).

As such, rabbits have been found to be unusually sensitive to elevated ambient temperatures (greater than 28°C or 85°F). In the summer months, losses due to heat prostration may exceed losses due to other diseases in facilities that lack proper environmental control. The *Guide for the Care and Use of Laboratory Animals* recommends that rabbits be housed at temperatures between 16 and 22°C (61–72°F), a range which is noticeably below that listed for other laboratory animal species (ILAR, 2011).

Clinical signs of heat prostration may include hyperthermia, tachypnea, cyanosis, prostration, and blood-tinged fluid from the nose and mouth. Does near kindling are reported to be most susceptible to this condition (Hagen and Lund, 1962). During bouts of especially hot weather, quarters without environmental control may be cooled by such means as water sprays, foggers, and fans. Affected animals may be cooled by placing wet burlap over them (Hagen and Lund, 1962), or by immersion in a cold water bath until the rectal temperature approaches the normal range (March, 1938).

High ambient temperatures can also affect the fertility of male rabbits in breeding colonies. Experimentally, Rathore has shown that New Zealand White bucks exposed to environmental temperature of 36.1°C (96.8°F) at 45% humidity for 1 or 2 days were not as fertile as control (unheated) bucks (Rathore, 1970a) and that abnormal spermatozoa were present in the ejaculate of the heated animals (Rathore, 1970b).

Miscellaneous Abnormalities

Many congenital malformations have been observed in instances where inbreeding of rabbits has been practiced (Chai, 1970; Chai and Degenhardt, 1962; Sawin and Crary, 1964). The abnormalities include conjoined twinning in Y strain rabbits (Chai and Crary, 1971), thoracogastroschisis in Lurie's C line (Crary, 1951; Sawin, 1955), hypospadias in the AX strain (Lindsey and Fox, 1994), and many other conditions that almost certainly have a genetic basis.

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The Rabbit as an Experimental Model

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INTRODUCTION

The total number of rabbits used for research and testing in the United States has declined from a peak value of approximately 550 000 per year in the late 1980s to the current level of approximately 230 000 (Figure 18.1). Of the several factors contributing to this decline, the most important was the rapid expansion of genetically engineered rodent models which largely replaced rabbits for investigation of many diseases such as candidiasis (de Repentigny, 2004) and variola (Smee, 2008). Even for conditions such as herpes simplex keratitis in which rabbits are still a classic and frequently used model, murine models are being developed to replace rabbits (Neumann et al., 2007). During the same period, refined testing methods for the Draize eye and skin irritation tests diminished the number of rabbits needed for toxicity evaluations. These tests were developed in the 1960s and adopted by the Environmental Protection Agency (EPA) to meet the testing requirements of the Federal Insecticide, Fungicide, and Rodenticide Act and the Toxic Substances Control Act. The albino rabbit remains the preferred species for both of these tests (EPA, 1998). While investigators are actively working to develop and validate in vitro substitutes to evaluate product safety, few alternatives have been accepted for regulatory purposes, requiring the continued use of rabbits (Vinardell and Mitjans, 2008).

The relatively large size, gentle disposition, and ease of maintenance in the laboratory are characteristics that ensure the continued use of rabbits as a valuable and essential animal model for many diseases. A Pubmed search of biomedical publications covering the period

from January 2005 to July 2009 returned over 23 500 references using rabbit as a key phrase. While the rabbit may not have been the only species used in these manuscripts, this simple statistic demonstrates the continued high use of rabbits particularly in the areas of atherosclerosis, ophthalmic disease, infectious disease, orthopedic research, and experimental neoplastic research. This chapter will provide an overview of these conditions, by system. Of necessity we will not attempt to describe every disorder for which rabbits have been evaluated as a model, or to summarize the 23 500 articles that have used rabbits. The utilization of the rabbit for cancer research is described in a separate chapter in this book.

CARDIOVASCULAR DISEASES

Heart disease is the leading cause of human mortality in developed countries. A wide variety of animal models of cardiovascular disease have been used to better understand the mechanisms and potential gene regulation underlying cardiac dysfunction and to develop therapeutic strategies. These models include dogs, pigs, pigeons, non-human primates, and small rodents, especially rats and mice. With the advances in genetic engineering, many novel murine models of cardiovascular disease have been developed in recent years. It should be noted, however, that there are major differences between rodent and human cardiovascular systems and this should be appreciated before extrapolating results from mouse to man. In contrast to other model species, including the mouse and rat, rabbits and humans are similar with respect to chemical composition and

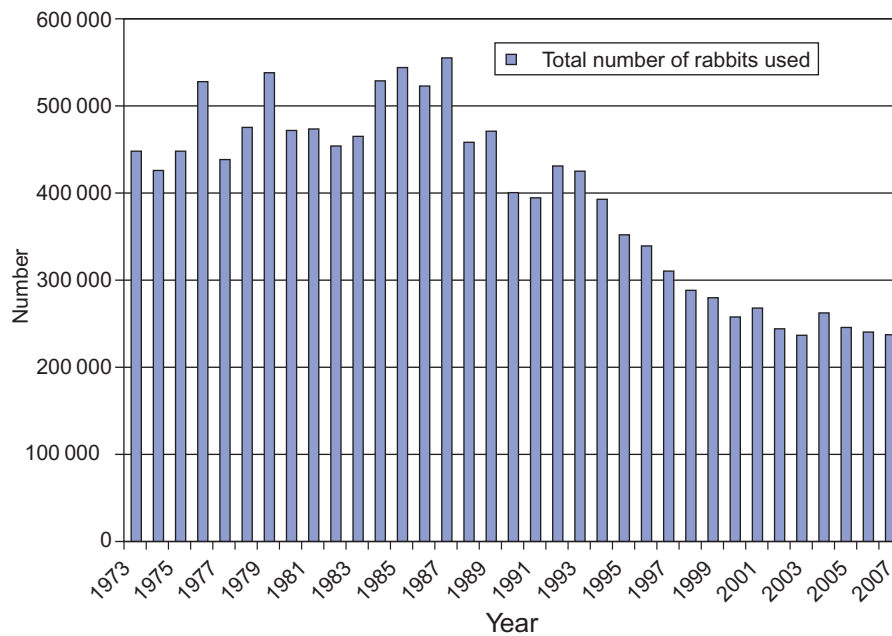


FIGURE 18.1 Total number of rabbits reported to the USDA for use in research and testing in the United States from 1973 to 2007.

content of lipoproteins (including apoB), production of lipoproteins in the liver (including apoB-100), and the presence of cholesteryl ester transfer protein (CETP), a key regulator of lipoprotein metabolism and development of atherosclerosis (Brousseau, 1999; Son and Zilversmit, 1986). The rabbit's cardiac physiology is also more analogous to human cardiac physiology than that of the mouse or rat. One such similarity is related to cellular electrophysiology and Ca^{2+} transport, which is critical when using animal models to study heart failure and arrhythmias due to alterations in ion channel and Ca^{2+} transporter function (Bers, 2001, 2006; Pogwizd 2004).

Although the laboratory rabbit has been used extensively for nearly one hundred years to study the pathophysiology of atherosclerosis, it has also been used to model many other manifestations of cardiac disease. Table 18.1 provides an overview of a number of rabbit models used in cardiovascular research (Pogwizd and Bers, 2008). The conditions for which rabbits have been used most extensively and have provided the greatest advances are discussed below.

Atherosclerosis

Etiology and Disease in Humans

Elevated blood cholesterol levels ($>249\text{mg/dl}$) and vascular plaque formation (atherosclerosis) is now recognized as a major risk factor for cardiovascular disease (CVD) which has accounted for more deaths in the United States than any other major cause of death

every year since 1900, except 1918 (Lloyd-Jones et al., 2009; Rosamond et al., 2008). The specific events contributing to these deaths include coronary heart disease (CHD, 52%), stroke (17%), and high blood pressure (6%) among others (Lloyd-Jones et al., 2009; Rosamond et al., 2008). Atherosclerosis is a complex, chronic progressive disease that develops in a distinct pattern classified into five distinct types. Briefly, these atheroma lesions are inflammatory sites that progress from proliferation and remodeling of smooth muscles cells with lipid infiltration and endothelial cell-monocyte interactions, to a lesion characterized by a deep intimal lipid-rich necrotic core. During the final stage of development the plaques have deep-intimal necrosis and a lipid-rich necrotic core composed of cholesterol clefts, calcium depositions, and neovascularization (Bocan, 1998). While the number of deaths from CVD has decreased from peak levels in the 1970s, largely due to an increased understanding of the risk factors and improved therapies, ongoing research to minimize atherosclerosis continues to be a major public health focus.

Rabbit Model

CHOLESTEROL-FED RABBIT MODEL

While there is no animal model that completely replicates all of the complexities of human atherosclerosis, rabbits are used extensively to study the pathogenesis of atherosclerosis and to evaluate therapeutic interventions. The cholesterol-fed rabbit in particular is the most common model used to evaluate pharmacologic treatments for atherosclerosis (Bocan, 1998). The

TABLE 18.1 Rabbit Models Used in Cardiovascular Research

Experimental Cardiac Condition	References
ISCHEMIC HEART DISEASE	
Myocardial infarction	
Coronary artery occlusion...	(Chatterjee et al., 2002; Ng et al., 1998)
Myocardial ischemia/reperfusion...	(Minatoguchi et al., 2004)
Hibernating myocardium...	(Dispersyn et al., 2001; Driesen et al., 2007)
HEART FAILURE	
Adriamycin cardiomyopathy...	(Arnolda et al., 1985; Pye 1996; Wanless et al. 1987)
Pacing-induced heart failure...	(Eble et al., 1997; Kawai et al., 2000; Spinale et al., 1994; Yao et al., 1998)
Combined pressure and volume overload	(Leclercq et al., 1978; Pogwizd, 1995; Pogwizd et al., 2001; Vermeulen et al., 1996)
MODELS OF VENTRICULAR HYPERTROPHY	
Left ventricular pressure overload...	(Friehs et al., 1999; Mohammadi et al., 1997; Stamm et al., 2001)
Right ventricular pressure overload...	(Gibbs et al., 1990; Hamrell and Alpert, 1977)
In vivo arrhythmia models...	(Allessie et al., 1977; Nattel et al., 2005; Ravelli and Allessie, 1997)
Hind-limb ischemia/angiogenesis model...	(Korosoglou et al., 2007; Liddell et al., 2005)
Volume overload...	(Borer et al., 2002; Magid et al., 1994; Sugawara et al., 2000)

(adapted from Pogwizd and Bers, 2008)

demonstration, by the young Russian pathologist Antischokow in 1913, that rabbits fed a diet supplemented with purified cholesterol dissolved in sunflower oil developed vascular lesions resembling human atherosclerosis marked the beginning of the modern era of atherosclerosis research (Anitschkow, 1913; Steinberg, 2004). Regrettably, Antischokow's discovery was largely dismissed as a peculiarity of the rabbit for about 40 years because similar studies using rats and dogs, species now known to be very efficient in converting cholesterol to bile acids, were unable to reproduce similar lesions. The cholesterol-fed rabbit model is created by feeding a diet supplemented with 1–3% cholesterol and 4–8% fat for 4–6 weeks (Bocan, 1998). The developing atherosclerotic lesions start as fatty streaks on the aorta and progress into advanced lipid-laden intimal plaques that approximate early human atheromas, which is the stage targeted by therapeutic intervention. In the rabbit, the atheromas localize to the aortic arch and thoracic aorta primarily, including the origins of the intercostal arteries (Yanni, 2004). Wilson et al. (1982) created a rabbit model with atherosclerotic lesions typical of advanced human atherosclerosis including a necrotic core and cholesterol clefts by feeding rabbits a diet containing 19% butter and 1% corn oil for up to 5 years. LeVeen et al. (1982) induced similar lesions in a shorter time using a balloon catheter to mechanically denude the aortas of Flemish

Giant rabbits which were then fed a 2% cholesterol, 6% peanut oil diet for about a month. Hypercholesterolemic rabbits, either with or without mechanical endothelial trauma, are the most widely used model for evaluating anti-atherosclerotic interventions including the 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, the “statins”, which can reduce serum cholesterol by as much as 47% in rabbits (Bocan, 1998; Wei et al., 2003), anti-inflammatory compounds such as terutroban (Worth et al., 2005), antioxidants including dietary flavanoids (Terao, 2009; Terao et al., 2008), and drug-eluting stents (Nakamine et al., 1998) among others. The hypercholesterolemic rabbit also serves as a model of valvular heart disease (Rajamannan, 2006), Alzheimer disease (Woodruff-Pak, 2008), and is being evaluated as a model of non-alcoholic, non-obese, non-insulin-resistant fatty liver disease (Kainuma et al., 2006).

Recognition that atherosclerosis is a chronic inflammatory disease (Ross, 1999) prompted interest into the role of infectious agents in disease progression. Several studies have demonstrated the presence of *Chlamydia pneumoniae* organisms in atherosclerotic lesions of humans (Belland et al., 2004). *C. pneumoniae* is an obligate intracellular prokaryotic pathogen that is extremely common in people, with approximately 80% of the population having serologic evidence of infection by 65 years of age (Belland et al., 2004). Rabbits fed

0.25% cholesterol in their diet and intranasally inoculated with *C. pneumoniae* developed significantly accelerated aortic atherosclerosis compared to uninfected rabbits or rabbits treated with an antibiotic, azithromycin (Muhlestein, 2000). However, rabbits infected with *Mycoplasma pneumoniae* (Fong, 2000), which causes similar lung pathology, failed to develop inflammatory changes or atherosclerotic lesions. While human clinical trials found no evidence that antibiotic treatment of *C. pneumoniae* reduced vascular risk (O'Connor et al., 2003), the rabbit studies helped fulfill Koch's postulate that chronic infectious burdens, perhaps by exacerbating inflammation, are an additional risk factor for development of atherosclerosis and its associated complications (Elkind et al., 2010).

SPONTANEOUS HYPERCHOLESTEROLEMIC RABBITS

In 1973, Japanese veterinarian Yoshio Watanabe discovered a male Japanese White rabbit with elevated serum lipid levels despite being fed a standard rabbit chow (Watanabe, 1977). The hyperlipidemia was characterized by grossly elevated levels of serum cholesterol, phospholipids, and triglycerides (Watanabe, 1980). He confirmed that the hyperlipidemia was an inherited recessive trait and used selective inbreeding strategies to develop what is now known as the Watanabe heritable hyperlipidemic (WHHL) rabbit strain, which is the first animal model with natural endogenous hypercholesterolemia (Watanabe, 1980). WHHL rabbits develop spontaneous atherosclerosis by 3–5 months of age, progressing to moderate to severe aortic atherosclerosis by 16 months of age (Figure 18.2). The atherosclerotic lesions are most extensive in the thoracic aorta, coronary arteries (Figure 18.3), abdominal

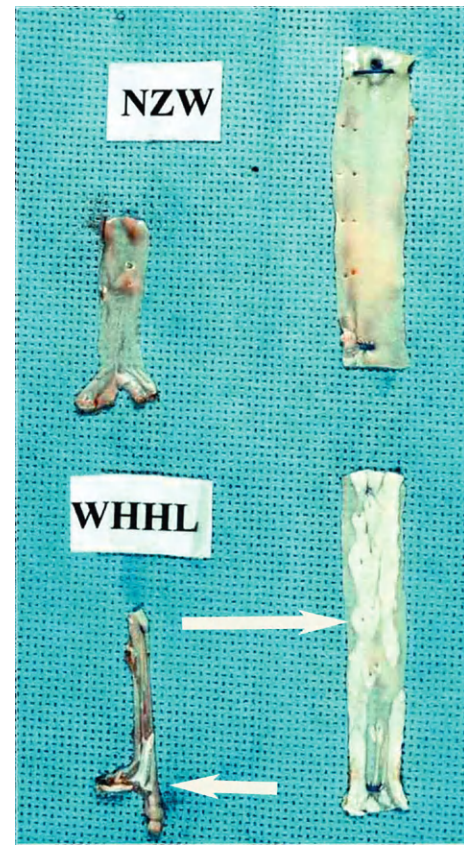


FIGURE 18.2 Gross photographs comparing the aortas in age-matched NZW and WHHL rabbits (>1 year). Note the lack of atherosclerotic plaques in the endothelial areas within the abdominal aorta and at the aortic bifurcation in the NZW (top); and the large amount of raised atherosclerotic plaques present in both the aorta (long arrow) and at the aortic bifurcation (short arrow) of an age-matched WHHL (bottom).

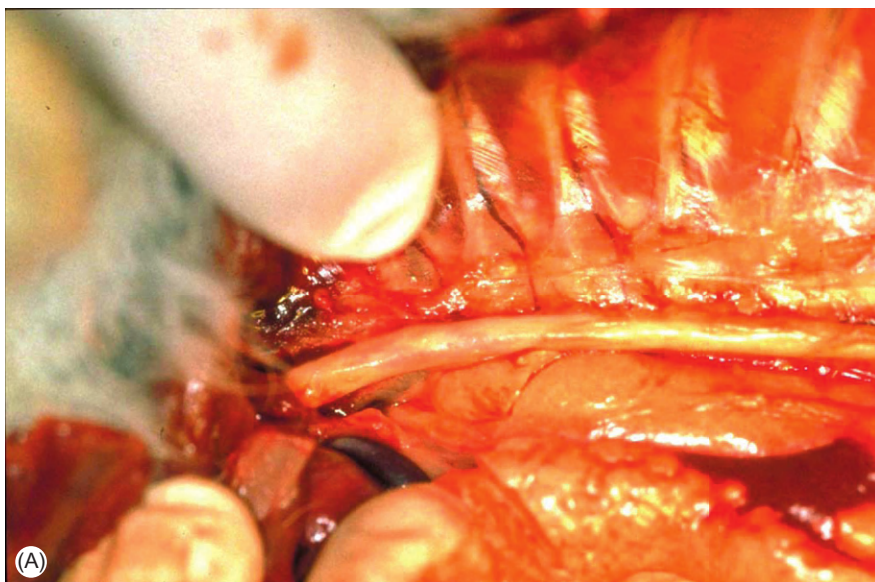
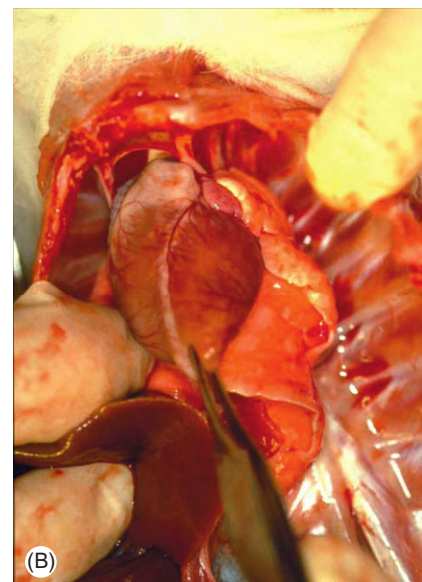


FIGURE 18.3 (A, B) Atherosclerotic plaque in thoracic aorta (A) and in left anterior coronary artery (B) from a 1.5-year-old WHHL rabbit.

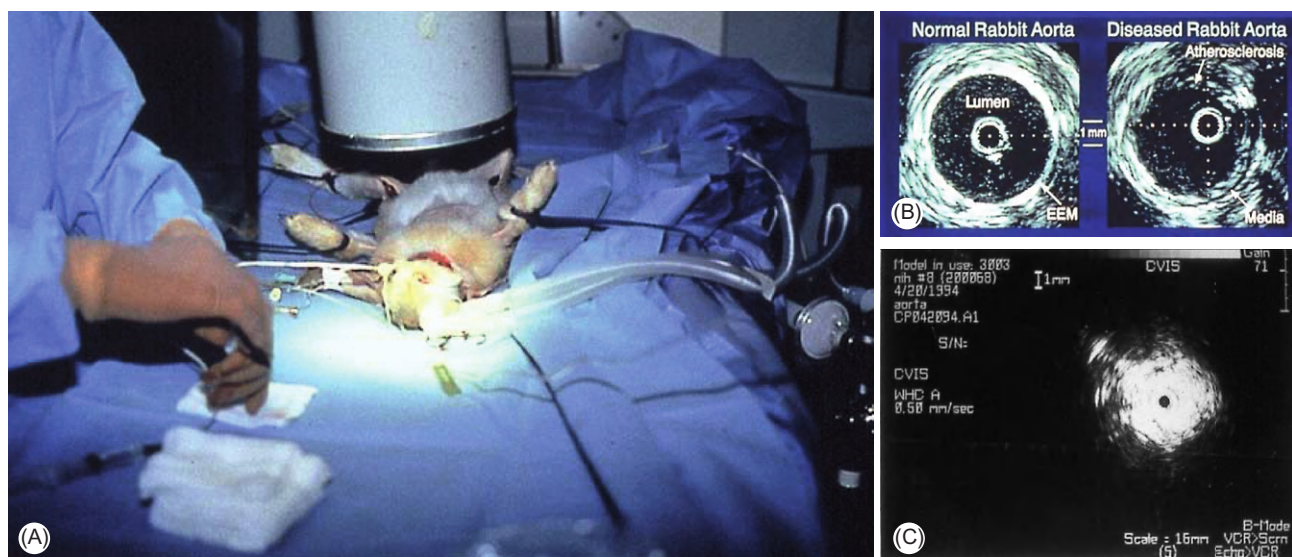


FIGURE 18.4 (A–C) Intravascular ultrasound (IVUS) is often used to assess atherosclerosis within the wall of major blood vessels of rabbits. (A) demonstrates placing the IVUS catheter into the carotid artery of a rabbit. (B) IVUS of a normal NZW rabbit aorta (left) with an aorta of a WHHL rabbit (right) with atherosclerosis. EEM, the external elastic membrane; Media, media adventitia (composed of smooth muscle cells). (C) demonstrates a carotid artery of a WHHL rabbit with severe atherosclerosis.



FIGURE 18.5 Dermal xanthomas in WHHL rabbits. Cholesterol deposits (dermal xanthomas) in the subcutaneous tissue skin of WHHL rabbits are a common finding in animals >1 year of age. These lesions are composed of typical histiocytic foam cells which contain oil-red-O-positive, strongly birefringent lipid deposits.

aorta, and adjacent to branching vessels (Figure 18.4). Approximately 60% of WHHL rabbits also develop cholesterol deposits in the skin (xanthomas) (Figure 18.5), and other tissues, including the cornea (Figure 18.6), and digital joints, by 22 months of age. Homozygous animals have serum cholesterol concentrations 8–14-fold higher than normal control Japanese White rabbits. This phenotype is due to a defective low-density lipoprotein (LDL) receptor, a spontaneous in-frame deletion of 12 nucleotides that eliminates four amino acids from the cysteine-rich,

ligand-binding domain of receptor 7 in exon-4 of the LDL receptor gene (Gross, 2009; Yamamoto et al., 1986). The clinical and subsequent pathologic progression of the hypercholesterolemic trait in WHHL rabbits closely resemble those found in humans afflicted with familial hypercholesterolemia (FH) (Kita et al., 1981).

Like homozygous human hypercholesteroleemics, WHHL rabbits have a severe lack of LDL receptors in the liver, adrenal glands, lymph nodes, and spleen, elevated plasma LDL cholesterol concentrations, reduced plasma high-density lipoprotein (HDL) concentrations, and increased plasma apolipoprotein E concentrations (Buja et al., 1983; Kita et al., 1981). Son and Zilversmit (1986) demonstrated that the activity levels of CTEP are high in homozygous WHHL rabbits. Increased lipid deposition in liver is a common occurrence (Figure 18.7). In contrast to the cholesterol-fed NZW rabbit, the WHHL rabbit does not develop either hepatic cholesterol toxicity or other organ damage (Donnelly et al., 1991).

Several noteworthy differences exist between FH patients and the WHHL rabbit models. Unlike human patients with homozygous FH, WHHL rabbits are also hypertriglyceridemic (300–600 mg/dl) (Havel, 1982; Watanabe, 1980). In addition, the LDL, as well as intermediate-density lipoproteins and very-low-density lipoproteins, contain a substantial amount of triglycerides (Havel et al., 1982). Unlike the WHHL rabbit, the St. Thomas Hospital strain, now referred to as the St. Thomas mixed hyperlipidemic (SMHL) rabbit, develops hypercholesterolemia with normal LDL receptor function and has only mildly elevated levels of triglycerides (La Ville et al., 1987). The SMHL rabbit exhibits an



FIGURE 18.6 Comparison of eyes from aged (>1 year old) NZW rabbit (left) and WHHL rabbit (right). Note the cholesterol deposits in the cornea of the WHHL rabbit: arcus cornea.

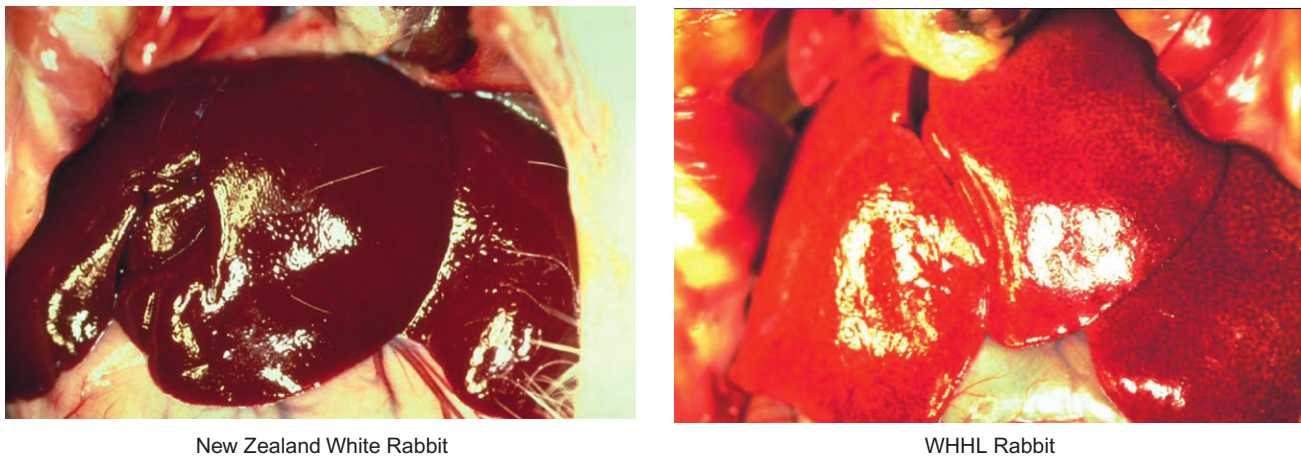


FIGURE 18.7 Gross comparison of livers from NZW rabbit (left) and WHHL rabbit (right). Note the paleness and rounded edges, consistent with a high lipid deposition, in the WHHL rabbit livers.

inherited hyperlipidemia similar to that seen in human familial combined hyperlipidemia and offers a potential valuable animal model system in which to study the relationship between hypertriglyceridemia and insulin resistance (de Roos et al., 2001, 2005).

Many advances in our understanding of the pathogenesis and treatment of atherosclerosis have been made using WHHL rabbits (Shiomi and Ito, 2009). Of particular note, Goldstein and Brown (1983) proved the relationship between LDL receptor activity and atherosclerosis using WHHL rabbits, for which they were awarded the Nobel Prize in 1985. WHHL rabbits have also been used extensively in the development of the anti-atherosclerotic statin drugs (Shiomi and Ito, 2009).

TRANSGENIC RABBITS

The rabbit is an excellent model system for evaluating the metabolic pathways in the pathogenesis of

atherosclerosis. New Zealand White (NZW) rabbits have low plasma total cholesterol concentrations, CTEP activity, hepatic lipase (HL) activity, and lack an analog to human apolipoprotein (hApoA-II). These traits make the NZW an attractive animal model for studying the effects of human transgenes on plasma lipoproteins and atherosclerosis susceptibility (Brousseau and Hoeg, 1999). Details on the generation of transgenic rabbits can be found in Chapter 7.

Utilizing the over-expression of genes relevant to lipoprotein metabolism, researchers have generated a series of transgenic animals, using both the NZW and WHHL rabbits, designed to assess the polygenic nature of the atherosclerotic process. These genes include human transgenes for different apolipoproteins (hapo), the apolipoprotein A-I/C-III/A-IV gene cluster (hapoA-I/C-III/A-IV gene cluster), hepatic lipase (hHL), lecithin:cholesterol acyltransferase (hLCAT), lipoprotein

lipase (hLPL), and scavenger receptor class B type I (hSRB-I) (Table 18.2).

In 1990, Badimon and coworkers first reported that the weekly intravenous administration of HDL protein to cholesterol-fed NZW rabbit models caused regression of previous existing atherosclerotic lesions (Badimon et al., 1990). Duverger and coworkers subsequently generated transgenic New Zealand White (NZW) rabbits that expressed hApoA-1 in the liver and studied its impact on the animal's susceptibility to atherosclerosis (Duverger et al., 1996a). Their results demonstrated that the hApoA-1 transgenic animals developed significantly less atherosclerotic lesions when compared to the control group (Duverger et al., 1996b). The administration of hApoA-1 Milano (a variant of apolipoprotein A-1) was later shown in a clinical trial to significantly regress atherosclerotic lesions in human patients with acute coronary artery syndrome (Nissen, 2003).

Another significant discovery was found in WHHL rabbits over-expressing hLCAT (Hoeg et al., 1996). When fed a high-cholesterol diet these animals showed

a marked reduction in the development of atherosclerosis compared to control rabbits (Hoeg et al., 1996). These results established the importance of LCAT in the metabolism of both HDL and apolipoprotein B-containing lipoprotein particles. The therapeutic impact of over-expressing LCAT was quite surprising. It not only resulted in increased plasma levels of HDL, but was also instrumental in reducing the levels of apolipoprotein B-containing lipoproteins in their transgenic animals (Yanni, 2004). WHHL animals over-expressing hApo(a) on the other hand experienced a more rapid progression in the development of atherosclerotic lesions (Sun et al., 2002).

The generation of genetically engineered rabbit models in recent years has allowed for a better understanding of the functions of genes involved in the pathogenesis of atherosclerosis (Brousseau and Hoeg, 1999; Duverger et al., 1996a, 1996b; Fan et al., 1994, 1995, 2001; Hoeg et al., 1996; Taylor, 1997; Taylor and Fan, 1997). This understanding has led to a number of preclinical discoveries resulting in clinical applications.

TABLE 18.2 Transgenic Rabbit Models Used for Lipoprotein Metabolism and Atherosclerosis Research

Rabbit Strain	References
NEW ZEALAND WHITE (NZW)	
hApoA**...	(Duverger et al. 1996a, 1996b)
hApoB-100...	(Fan et al., 1995; Rouy et al., 1998)
hApoE ₂ ...	(Huang et al., 1997)
hApoE ₃ ...	(Fan and Watanabe, 1998; Taylor, 1997)
hApo(a)++...	(Fan et al., 1998; Fan and Watanabe, 2000; Rouy et al., 1998)
hApoA-I/C-III/A-IV gene cluster...	(Recalde et al., 2004)
hHL**...	(Fan et al., 1994; Taylor, 1997)
hLCAT**...	(Brousseau et al., 2000; Hoeg et al., 1996)
hLPL...	(Araki et al., 2000; Fan et al., 2001; Ichikawa et al., 2005)
hSRB-1...	(Tancevski et al., 2005)
WATANABE HERITABLE HYPERLIPIDEMIC (WHHL)	
hApoA-1**...	(Hoeg et al., 1993)
hLCAT**...	(Brousseau et al., 1998; Hoeg, 1998)
hApo(a)++...	(Fan and Watanabe, 2000; Sun et al., 2002)
hLPL...	(Koike et al., 2004)

Enzymes marked with (**) are anti-atherogenic, and those marked with (++) are pro-atherogenic.

(Adapted from Brousseau and Hoeg, 1999; Fievet et al., 2007.)

Myocardial Infarction and Reperfusion

Etiology and Disease in Humans

Myocardial infarction and reperfusion occurs when plaques rupture and block the arteries of the heart leading to a condition known as acute coronary syndrome (ACS). ACS is the leading cause of morbidity and mortality and according to the World Health Organization will be the major cause of death by the year 2020 (Ostadal et al., 2009).

Rabbit Model

Until recently there were no spontaneous animal models of ACS. To evaluate the cardioprotective potential of different pharmaceuticals and treatments, an induced model of ACS was developed. The advantages of the induced model include predictability in onset of the ischemic event, as well as consistent size and location of the infarcted myocardium. In rabbits, the model is created in anesthetized, mechanically ventilated rabbits via a left thoracotomy. Suture is passed beneath a major branch of the left coronary artery and the ends of the ligature are passed through a short polypropylene tube to form a snare. Myocardial ischemia occurs when the ends of the sutures are pulled through the tubing. The length of the ischemic period varies but is typically maintained for 30–90 min. Reperfusion occurs when the snare is released. In recent years the induced rabbit model has been used to demonstrate the cardioprotective effects of several regimens following infarction which include: rapid cooling of the heart with total liquid ventilation (Chenoune et al., 2010), oral acarbose

(Minatoguchi et al., 2009), high dose of insulin during the infarction period (Wong et al., 2011) and the antidiabetic drug pioglitazone (Yasuda et al., 2009). Acarbose, insulin, and pioglitazone most likely are cardioprotective due to the prevention of hyperglycemia which occurs in response to stress.

Through selective breeding of WHHL rabbits with high plasma cholesterol and severe coronary lesions a colony of myocardial infarct-prone WHHL (WHHLM) rabbits have been developed (Shiomi et al., 2003). In the original WHHL rabbits, atherosclerotic lesions mainly developed in the aorta and the incidence of severe coronary artery disease, including the animals that developed spontaneous myocardial infarctions, was low; approximately 23% cumulative incidence at 30 months of age (Gross, 2009; Nakamura et al., 1997; Rekher, 2002; Shiomi et al., 2003). The cumulative incidence of coronary artery disease WHHLM rabbits approaches 97% by 30 months of age. The tendency of WHHLM rabbits to develop myocardial infarcts is likely due to a change in composition of their coronary plaques from the fibrous lesions described in WHHL rabbits to fibroatheromas, more closely mimicking human disease pathogenesis (Shiomi and Ito, 2009; Shiomi et al., 2003). WHHLM rabbits expressing C-reactive protein and matrix metalloproteinases, both increased in patients with ACS are showing promise in investigating genetic factors that may serve as triggers promoting plaque rupture (Shiomi and Fan, 2008).

Bacterial Endocarditis

Etiology and Disease in Humans

Bacterial endocarditis is characterized by inflammation or infection on the inner surface of the heart. The heart valves and any devices implanted in the heart, such as artificial valves, pacemakers, and implantable defibrillators can be affected, and as a result patients with these devices are at an increased risk of infection (Corey and Lalani, 2008). Patients with vascular access catheters, such as those receiving hemodialysis are also at a higher risk for developing endocarditis (Lafrance et al., 2008). Overall, the disease affects approximately 10 000–20 000 people a year in the United States (Cabell and Fowler, 2003). Histologically, endocarditis lesions are composed of bacteria embedded in a meshwork of fibrin, which protects the bacteria from the body's white blood cells, and leads to poor penetration of antibiotics. Several microorganisms, predominately Gram-positive bacteria, can cause endocarditis in humans (Ge et al., 2008).

Rabbit Model

Infectious endocarditis can be experimentally induced through means such as ventricular catheterization and

intravenous inoculation of bacteria directly onto the valves (Asseray et al., 2002) or surgical exposure of the carotid artery to introduce an indwelling catheter with a subsequent intravenous inoculation (Chambers, 2006). The induced rabbit model is advantageous because it closely simulates the characteristics of the infection in humans, and it allows greater statistical comparisons since the number of bacteria inoculated can be controlled (Carbon, 1994). This model is used to evaluate the kinetics of antimicrobial penetration into vegetations, host–organism interactions, and to evaluate potential therapies (Carbon, 1994). A recent advancement in therapy using the rabbit model includes demonstration of the efficacy of ceftibiprole, a broad-spectrum cephalosporin, for treatment of methicillin-resistant *S. aureus* MRSA (strain 76) and a vancomycin intermediately resistant *S. aureus* induced valvular (aortic) endocarditis (Chambers, 2006).

OPHTHALMIC DISEASES

Laboratory rabbits continue to be one of the most widely used species in experimental ophthalmology research. The large size of the rabbit eye compared to its body size makes rabbits an attractive and economical model for surgical and drug delivery experiments. Rabbits also have few spontaneous ocular diseases. However, the eyes of rabbits share few anatomic characteristics with human eyes. The cornea of rabbits is thinner than humans, and the rabbit's lens is larger (Figure 18.8) causing the iris to bulge forward creating a more curved anterior chamber (Figure 18.9) (Werner et al., 2006). Rabbits also have rod-rich retinas that are very



FIGURE 18.8 Gross photographs of a rabbit (left) and a human (right) natural crystalline lens. Note that the rabbit lens is thicker than the human lens. While both rabbit and human lens are normally clear, the human lens pictured exhibits a yellow discoloration due to cataractogenesis. (courtesy of L. Werner, used with permission of the publisher)

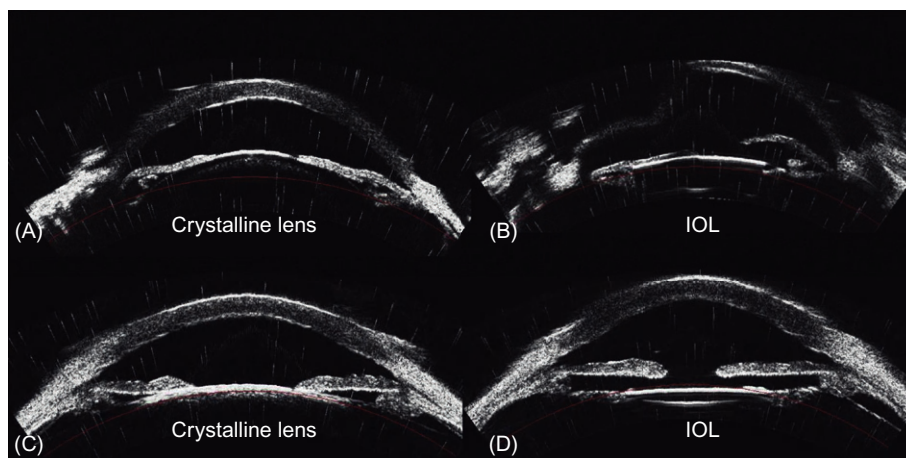


FIGURE 18.9 (A–D) Ultrasound scans obtained from rabbit and human eyes (phakic, containing the natural lens and pseudophakic, containing synthetic intraocular lens (IOL)). The scan from the rabbit phakic eye (A) shows the anterior surface of the natural crystalline lens pushing the iris forward. The anterior chamber of the normal rabbit eye is more curved and shallower than in the human phakic eye (C). The geometry of the anterior chamber of the pseudophakic rabbit eye (B) is much more similar to that of the pseudophakic human eye (D). (*courtesy of L. Werner, used with permission of the publisher*)

poorly vascularized compared to humans (Perlman, 2009). As a result, the effects of drugs on the retina of a rabbit are not easily translated to humans (Perlman, 2009) and rabbits are infrequently used to study the pathogenesis of ocular diseases. Non-human primate eyes share the closest anatomy to the human eye, and of the non-primate species, the eyes of the pig most closely approximate those of humans (Albrecht May, 2008).

Cataract

Etiology and Disease in Humans

Chief among the risk factors for developing cataracts, a clouding of the lens, are chronic ultraviolet light exposure, diabetes, oxidative stress, smoking, and genetic predisposition (West, 2007). Cataracts affect more than 20 million people worldwide, and are a leading cause of blindness, particularly in developing countries (Wong and Hyman, 2008).

Rabbit Model

Rabbits have been used as a model of radiation-induced cataracts since the 1950s when investigators performing radiation studies observed that rabbits developed cataracts similar in structure to human radiation-induced cataracts (Cogan and Donaldson, 1951). The rabbit model is still being used to examine the effects of ultraviolet radiation on the development of cataracts (Hightower and McCready, 1992). While variations in method vary, in general the model is created by exposing the corneas of anesthetized rabbits to ultraviolet light from a distance of 0.05 m for various

frequencies and durations to create different doses of radiation exposure (Fris et al., 2008). The rabbit model is being used to develop treatments for or protection against radiation-induced lens damage (Bigsby et al., 2009; Sasaki et al., 1998).

Despite the overall smaller size of the rabbit eye compared to the human eye (Figure 18.10), the anterior chambers of the two eyes are similar and rabbits can easily accommodate intraocular lenses (IOL) designed for humans (Werner et al., 2006). As a result, the rabbit is a valuable model for training surgeons in the removal of cataracts and replacement with IOL, evaluation of novel IOL designs (Werner et al., 2006), and in the evaluation of new surgical approaches (Gwon, 2008). Rabbits are also used to study the pathogenesis and immune responses to staphylococcal endophthalmitis, one of the most feared complications of cataract surgery (Giese and Mondino, 2001). This model is created by direct inoculation of organisms into the anterior chamber. Two unique characteristics of rabbits warrant modification of standard human protocols when performing surgery on rabbit eyes. Compared to humans, rabbits mount a more pronounced inflammatory reaction following surgery to the eye, which necessitates the use of anti-inflammatories during the post-operative period (Werner et al., 2006). Finally, rabbits, and in particular Dutch belted rabbits, experience a massive protein release into the anterior chamber of the eye in response to disruption of the blood–aqueous barrier. To prevent complications from this protein release, heparin should be injected into the anterior chamber as soon as an incision is made, and heparin should be used in the irrigation solutions (Werner et al., 2006).

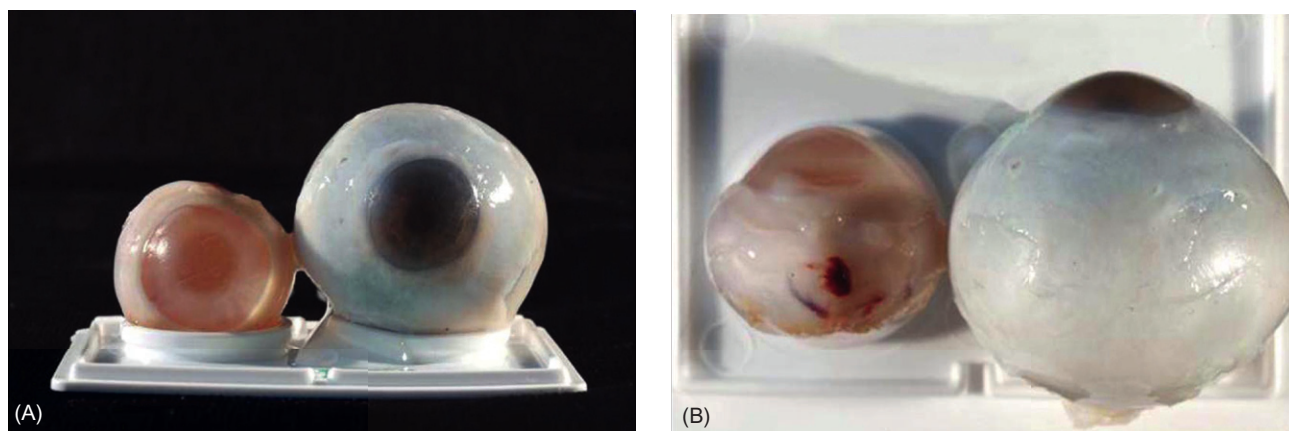


FIGURE 18.10 (A, B) Gross photographs of a rabbit (left) and a human (right) eye from anterior (A) and superior (B) views. The differences in the dimensions of the corneal diameter (white-to-white), equatorial diameter, and anterior-posterior length can be appreciated. (courtesy of L. Werner, used with permission of the publisher)

Herpes Simplex Virus Keratitis

Etiology and Disease in Humans

Herpes simplex virus type 1 (HSV-1) remains a leading cause of unilateral infectious corneal blindness worldwide, with an estimated 59000 new and recurrent cases of disease annually in the USA (Wilson et al., 2009). The virus is acquired by close human contact and spreads to the local sensory ganglia where it either replicates or establishes latency. HSV-1 is ubiquitous, with approximately 90% of the population seropositive by the age of 40 (Pepose et al., 2006). Herpetic stromal keratitis (HSK) occurs when reactivated virus travels down the sensory axon to the cornea leading to tissue destruction, edema, opacification, corneal scarring, and neovascularization (Pepose et al., 2006). Interactions between the recurrent virus and the host's immune response are implicated as the main cause of the corneal scarring (Naito et al., 2005). The aims of current research into HSK include developing improved therapies to target both the virus and the immune system, understanding the complex immune mechanisms responsible for viral reactivation, and using this knowledge to develop a vaccine to prevent primary infection or to minimize recurrent reactivation of the virus (Wilson et al., 2009).

Rabbit Model

Rabbits reliably develop HSK following ocular inoculation with HSV-1 with or without corneal scarification. The disease in rabbits follows a similar course to the human disease including viral reactivation (Nesburn et al., 2007), a characteristic that differs from the murine models (Naito et al., 2005). This characteristic, the large size of the rabbit cornea, and the similarities rabbits and humans share in the conjunctiva-associated lymphoid

tissue has allowed investigators to use rabbits to study the immune mechanisms involved in viral reactivation. Rabbits and humans also have a similar corneal follicular ultrastructure with an abundance of conjunctival lymphoid follicles, unlike rats and mice, which do not have corneal lymphoid follicles. Using rabbits, investigators are characterizing the cellular response to HSV-1 at the mucosal level with the aim of using this information to optimize topical treatments and/or mucosal vaccines (Nesburn et al., 2007). For example, Lambiase et al. (2008) evaluated the role of nerve growth factor (NGF) in modulating the immune response during infection with HSV-1. They discovered that topical NGF was as effective as acyclovir in diminishing the deleterious response to HSV-1. In contrast, 20% of rabbits treated with topical anti-NGF experienced worsening of the clinical outcome and a spreading of HSV to the brain.

Rabbits are also being used to characterize the stromal HSV-1 proteins or antigens in the HSK cornea that incite the inflammatory response. Until recently, investigators had been unable to identify any HSV-1-specific viral proteins in either naturally infected human, or experimentally infected animal, corneas (Naito et al., 2005). However, since some water-soluble corneal proteins diffuse out of the cornea, Naito et al. (2005) evaluated tears from rabbits infected with HSV-1, as well as the buffer soaking solution for corneal sections from these rabbits, for the presence of viral proteins. From both, the authors were able to identify the HSV-1 immediate-early protein ICP0 (Naito et al., 2005). Further studies have confirmed that ICP0 is localized to the anterior stromal layer of infected corneas throughout the course of viral infection. The anterior stromal layer is most affected by corneal scarring caused by epithelial keratitis, suggesting that ICP0 is a likely target of the immune response (Morishige et al., 2006).

Glaucoma

Etiology and Disease in Humans

Open-angle glaucoma is a chronic disease characterized by increased intraocular pressure that results in pathologic changes to the optic nerve, and clinically by loss of vision (Albrecht May, 2008). The disease generally occurs in older humans, and is the second leading cause of visual impairment worldwide, the first among persons of African descent (Wong and Hyman, 2008).

Rabbit Model

While glaucoma occurs as an inherited condition in rabbits, the mode of inheritance differs from that of humans, and rabbits with this condition are not used for glaucoma research. Glaucoma is induced in rabbits using corticosteroids or intraocular injection of alpha-chymotrypsin (Albrecht May, 2008). Recent comparative anatomy studies demonstrate that unlike humans, rabbits have an incompletely vascularized retina restricted to the myelinated portion of the nerve fiber layer (Albrecht May, 2008). As a result of this optic nerve area difference, rabbits are not a useful model of the pathogenic process of glaucoma, and rodent models are increasingly being developed and used (Pang and Clark, 2007). Rabbits continue to be used to evaluate refinements of surgical and medical treatment for glaucoma. Using the established rabbit model of the glaucoma filtration surgery, investigators demonstrated that the combined use of subconjunctival injections of bevacizumab and 5-fluorouracil decreases the aberrant healing and fibrosis that is a major complication of this procedure (How et al., 2009). Rabbits are also used to evaluate novel methods of drug delivery to the eye such as nanoemulsion eye drops containing the drug dorzolamide (Ammar et al., 2009). This formulation had a faster onset of drug action, and prolonged effect compared to the market product allowing a decrease in the number of applications per day and a better patient compliance.

Uveitis

Etiology and Disease in Humans

Uveitis is an inflammation of the highly vascular uveal tract (the portion of the eye including the anterior chamber, iris, and ciliary body). The condition most often occurs in young or middle-aged adults (Smith et al., 1998) and may account for up to 10% of legal blindness (vision that is 20/200 or worse) in Western countries (Suhler et al., 2008). The cause of most cases of uveitis is unknown, however, the disease may occur as a secondary complication of herpes virus infection among others (Suhler et al., 2008).

Rabbit Model

Numerous inflammatory mechanisms have been used to induce uveitis in the rabbit. The classic rabbit model of uveitis, called experimental autoimmune uveitis (EAU), is induced by immunizing rabbits with a soluble bovine retinal extract (Wacher and Lipton, 1968). Acute, bilateral uveitis occurs approximately 2 weeks after immunization in this model. The research utility of the EAU model has largely been replaced by the endotoxin-induced uveitis (EIU) model which is created by intravitreal injection of 10ng *Escherichia coli* endotoxin (Allen et al., 1998). EIU is clinically and histopathologically similar to acute anterior uveitis in humans. The rabbit model is often used in pre-clinical trials for novel therapeutic strategies such as piroxicam nanoparticles for drug delivery to the eye (Adibkia et al., 2007), the successful use of infliximab as a prophylaxis for recurring uveitis (Diaz-Llopis et al., 2007), and the anti-inflammatory properties of topical application of the herbs *Curcuma longa* and *Berberis aristata* (Gupta et al., 2008). The rabbit EIU model is also used to study the role of nitric oxide and peroxynitrite in the pathogenesis of uveitis (Allen et al., 1998). An alternative model is created using two subcutaneous injections, 1 week apart with *Mycobacterium tuberculosis* H37Ra antigen, which creates a severe, non-specific uveitis (Shin et al., 2009). This model is being used to develop a biodegradable intrascleral implant of triamcinolone that is proving effective in suppressing the inflammation of uveitis for at least 4 weeks (Shin et al., 2009).

Proliferative Vitreoretinopathy

Etiology and Disease in Humans

Proliferative vitreoretinopathy (PVR) occurs after retinal detachment and is a major complication of surgical reattachment of the retina, occurring in 3.9–13.7% of human patients (Lei et al., 2009). Disease progression is thought to involve retinal pigment epithelium (RPE), retinal glial cells, fibroblasts, and inflammatory macrophages migrating over the inner and outer surfaces of the retina (Lei et al., 2007). These cells form contractile membranes that can distort the retina or lead to retinal detachment. Retinal detachment leads to blindness if not treated immediately.

Rabbit Model

Rabbits are extensively used as an induced model of PVR using a number of intraocular manipulations to produce contractile membranes. One method of inducing PVR in rabbits, called gas vitrectomy, involves injecting an expanding gas, such as perfluoropropane (C₃F₈), into the eye, thereby compressing the vitreous and creating a void in the posterior segment (Thresher et al. 1984).

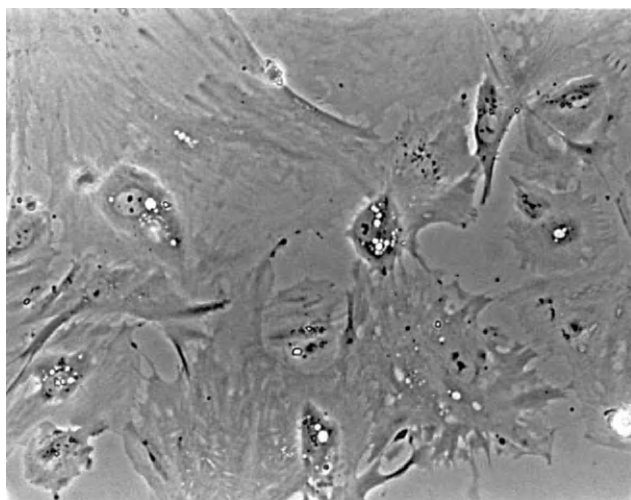


FIGURE 18.11 Unstained rabbit RPE cells in primary culture following adherence to the culture plate. Note the lack of confluency and the spread of their cytoplasm.

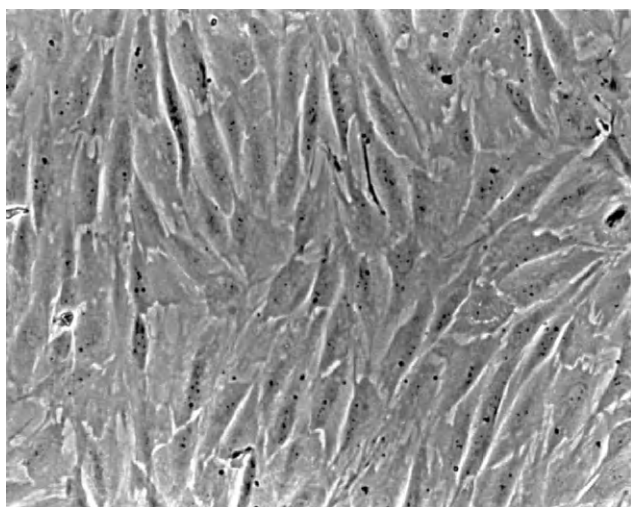


FIGURE 18.12 Rabbit RPE cells in primary culture that have undergone mitogenesis to form myofibroblastic cells.

Thresher et al. (1984) first described this technique as an alternative to mechanized vitrectomy, which cuts and aspirates the vitreous. Fibroblasts or cultured RPE cells are then injected into the voided space and allowed to proliferate to form contractile membranes that model the human condition (Mukherjee and Guidry, 2007). Early in the culture process, RPE cells appear phenotypically normal (Figure 18.11). However, if confluency and cell-to-cell contact is not obtained, these cells will proliferate and undergo mitogenesis to form myofibroblastic RPE cells (Figure 18.12). An analogous process occurs in vivo when retinal tears lead to migration of RPE and other cells into the vitreous. The rabbit PVR model is used both to investigate the pathogenesis of the disorder (Lei et al., 2008) and to evaluate novel treatments for the condition

such as an intravitreal sustained release naproxen and 5-fluorouracil system (Cardillo et al., 2004) and a partial thickness autologous RPE–choroid graft placement (Hu et al., 2008) both of which are showing promise as improved therapies over the current standard of care.

Diabetic Retinopathy, Age-Related Macular Degeneration (AMD), and Other Neovascularization Disorders

Etiology and Disease in Humans

Diabetic retinopathy (DR) and age-related macular degeneration (AMD) differ in their inciting causes. However, both are complicated by choroidal neovascularization (CNV) and edema and, because the two diseases occur in the posterior segment of the eye where drug delivery is difficult, the diseases share many of the same treatment approaches (Doukas et al., 2008; Majumdar and Srirangam, 2009). Hyperglycemia and prolonged duration of diabetes are risk factors in the development of DR, which is characterized by microvascular abnormalities, proliferation of retinal vessels, and increased vascular permeability (Arar et al., 2008). Vision loss with DR can occur due to macular edema or the new blood vessels can distort the retina leading to retinal detachment (Arar et al., 2008). DR is the leading cause of blindness among adults in the United States, affecting approximately 4.1 million people (Arar et al., 2008). Advanced age and cigarette smoking are risk factors for AMD which is characterized by the accumulation of drusen, a whitish yellow deposit under the retinal epithelium (Coleman et al., 2008). More than 8 million Americans are affected by early-stage AMD, making AMD the most common cause of vision impairment in individuals over 55 years of age. The disease will progress to blindness in approximately 1 million of these individuals (Coleman et al., 2008).

Rabbit Model

Because of the size of the rabbit eye, rabbits are one of the most widely used species to investigate novel methods of drug delivery to the posterior segment of the eye for treatment of vitreoretinal diseases (Myles et al., 2005). The outer and inner blood–retinal barriers limit influx of drugs into the retina, therefore simple topical and systemic drug delivery approaches to treat these diseases have limited efficacy. Novel approaches such as iontophoresis, in which a constant electrical field is applied across the conjunctiva, resulting in constant drug flow to the retina, are being studied in rabbits to treat these conditions (Myles et al., 2005).

Yasukawa et al. (2007) developed an AMD rabbit model by introducing glycoxidized microspheres with albumin into the subretinal space via a sclerotomy. In

contrast to previous models which are induced via a non-specific local inflammatory reaction, rabbits in this model developed drusen within 4 weeks. Four out of seven rabbits developed CNV within 12 weeks. Follow-up studies demonstrated that these rabbits have an abnormal fundus autofluorescence similar to the pattern seen in aging human eyes, making this a useful model to elucidate drusen biogenesis and pathogenesis of AMD (Hirata et al., 2009).

CENTRAL NERVOUS SYSTEM DISEASES

Coccidioidomycosis

Etiology and Disease in Humans

Coccidioides is a genus of fungi that causes coccidioidomycosis, an endemic condition in the deserts of the United States (Johnson and Einstein, 2006). *Coccidioides immitis* and *Coccidioides posadasii* are two pathogenic species in this genus, which might become established when a person inhales *Coccidioides* species spores from the environment (Clemons et al., 2007). Typically, the infection is limited, but some cases can lead to fatal meningitis (CM) (Johnson and Einstein, 2006; Williams, 2001; Williams et al., 1992). Treatment can be required for an extended duration, even for the lifespan of the individual, and relapses are a potential complication (Dewsnup et al., 1996; Stevens and Clemons, 2007). Despite prolonged treatment with antifungal oral azole compounds, a cure is not always realized (Dewsnup et al., 1996).

Rabbit Model

Rabbits are particularly valuable to study CM because the induced disease closely mimics the CNS involvement in patients with acute infection. The model is created by inoculating anesthetized rabbits with a viable anthroconidial suspension of *C. immitis*. Rabbits inoculated with 3×10^5 arthroconidia develop a progressive illness of 2–7 weeks' duration before requiring euthanasia (Clemons et al., 2007). Pathological lesions in experimentally infected rabbits are consistent with those found in people and include: hyperproteinemia, progressive pleocytosis, arteritis, granulomatous cerebrospinal meningitis, and infarction (Clemons et al., 2007). Clinical monitoring during infection in experimentally inoculated rabbits includes well-established parameters such as body temperature, hydration, mobility, posture, presence or absence of neurologic signs, and weight to assess the course of the disease (Clemons et al., 2000, 2002; Sorensen et al., 2000a, 2000b; Williams et al., 2002).

The large size of the rabbit brain, compared to the widely used murine models of CM, allows isolation and dissection of large vessels that can be used to

detect the gene expression of inflammatory mediators. Using this technique, metalloproteinase-9 was found to be over-expressed during acute CM. This enzyme is involved in vascular regeneration and may play a role in the vasculitis which is a major complication of CM (Williams, 2001). Recently, the rabbit model was used to show the efficacy of intravenous lipid-complexed amphotericin B and liposomal amphotericin B for treatments of CM (Clemons et al., 2009). The two drugs were similar in their ability to reduce clinical signs and number of fungi, and both provided a 100% survival rate in this model (Clemons et al., 2009).

Listeriosis

Etiology and Disease in Humans

Listeriosis is a rare but potentially fatal food-borne illness caused by *Listeria monocytogenes*, a Gram-positive bacterium found throughout the environment in soil, vegetation, and water supplies. *Listeria* can survive in acidic and salty foods, and continue to multiply at low temperatures, allowing for growth in poorly refrigerated foods (Jacobson, 2008). Raw milk and products made from raw milk are often the source of infection. The bacteria gain access to the body through ingestion and disseminate hematogenously. Pregnant women, neonates, the elderly, and immunocompromised patients are at highest risk for listeriosis due to impaired T-cell function. The most common clinical manifestations of listeriosis are sepsis and meningoencephalitis (Hof et al., 1997; Levidiotou et al., 2004).

Rabbit Model

Listeriosis is induced in rabbits via direct inoculation of into the cistern magna. The use of this model is diminishing, but was recently used to demonstrate that moxifloxacin treatment is similar to ampicillin plus gentamicin for *Listeria monocytogenes* infections when the following parameters (minimum thresholds) for meningitis were assessed: body temperature $>40^{\circ}\text{C}$, cerebrospinal fluid (CSF) bacterial count $>10^3$ CFU/ml, and CSF pleocytosis of >1000 cells, with $>96\%$ polymorphonuclear cells (Sipahi et al., 2008).

RESPIRATORY DISEASES

Bacterial sinusitis

Etiology and Disease in Humans

Sinusitis is inflammation or infection of the nasal passages and is one of the most common conditions treated by family physicians in the United States accounting for 25 million office visits annually (Scheid and Hamm, 2004). Although usually caused by viruses,

rhinosinusitis can be complicated by a bacterial infection, a condition called acute bacterial rhinosinusitis (ABRS). Approximately one in eight patients presenting with upper respiratory symptoms has ABRS (Scheid and Hamm, 2004). Obstruction of the ostia is the most important risk factor predisposing to ABRS, generally caused by inflammation and edema. Patients with septal deviation, nasal polyps, and anatomic variations of the nasal passages or tumors are also at an increased risk (Kara, 2004). *Haemophilus influenzae*, *Moraxella catarrhalis*, *Staphylococcus* spp., and *Streptococcus pneumoniae* are the most common isolates in cases of ABRS (Kara, 2004).

Rabbit Model

Rabbits are a valuable model of ABRS because humans and rabbits share important anatomical and immunologic similarities in their nasal passages (Liang et al., 2008). In both species, the nasal fossa is divided by the septum in the sagittal plane, and the lateral wall of the rabbit has structures similar to the turbinates in humans (Figure 18.13) (Kara, 2004). An ostium on the lateral side of the endoturbinates serves as the connection between the sinuses and the nasal cavity, an anatomical arrangement similar to the middle meatus in humans (Kara, 2004). In the past, ABRS was induced in rabbits via surgical preparations in which the ostium was closed and bacteria were inoculated into the sinuses of rabbits (Kara, 2004). This model reliably produced maxillary sinusitis, but was non-rhinogenic and was criticized for excess surgical manipulation (Liang et al., 2008). More recently, the rabbit model was refined by placing of a polyvinyl acetate absorbent

sponge (impregnated with the bacterium of interest) in the nasal cavity via the nares of the rabbit. This model produces marked mucosal inflammation with purulent exudation by 2 weeks in 83% of rabbits, but the inflammation decreases over time and is generally absent by 10 weeks (Kara, 2004). Surgical preparations are still needed for some studies related to the characterization of topical antimicrobial therapy for sinusitis (Chiu et al., 2007). For example, a technique using surgical placement of an irrigating catheter for delivery of bacteria or saline resulted in a model that could be used for future studies to develop topical therapy guidelines after functional endoscopic sinus surgery (FESS) (Chiu et al., 2007). Rabbits prepared in this fashion and given *Pseudomonas aeruginosa* were successfully treated with topical tobramycin, a commonly used therapy in cystic fibrosis patients because of its anti-pseudomonal effects (Antunes et al., 2007). Rabbits have also been critical in the study of biofilm-forming strains of *Pseudomonas aeruginosa* that can contaminate stents used in frontal sinus surgeries (Palmer, 2006).

Anthrax

Etiology and Disease in Humans

Anthrax is caused by *Bacillus anthracis*, a Gram-positive, facultative anaerobic, spore-forming bacterial pathogen (Skoble et al., 2009). Humoral immunity is the primary defense against anthrax, especially antibodies that neutralize LeTx (Skoble et al., 2009). Depending on the mode of infection, *Bacillus anthracis* can cause lesions in the gastrointestinal tract, skin, or respiratory system (Pile et al., 1998). Edema toxin (EdTx), lethal toxin (LeTx), and the poly- γ -D glutamic (PGA) capsule are the major components responsible for disease in anthrax cases (Skoble et al., 2009). Protective antigen (PA) is a common subunit of EdTx and LeTx, which are classic A-B toxins (Skoble et al., 2009). Edema factor (EF) and lethal factor (LF) are the active moieties that enter the cytoplasm through the action of PA (Skoble et al., 2009). As only PA was used as an antigen in the original vaccine, its modification could thwart vaccine protection in a bioterrorism attack (Skoble et al., 2009). The currently licensed anthrax vaccine uses EF, LF, and PA from *B. anthracis* adsorbed to an adjuvant (aluminum hydroxide gel) (Skoble et al., 2009). The anthrax vaccine-adsorbed (AVA) inoculum is often associated with inflammation at the injection site and sometimes more serious adverse reactions such as neuropathy (Sever et al., 2004). A vaccine composed of purified recombinant PA (rPA) has been developed for humans (Little et al., 2006). Recently, a novel vaccine was developed for protection against anthrax: the psoralen-killed but metabolically active (KBMA) vaccine, which offers the

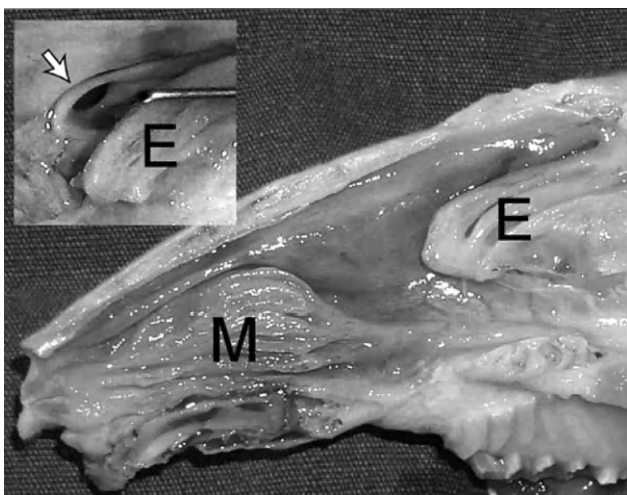


FIGURE 18.13 Lateral wall of the right nasal cavity of the rabbit. Arrow in inset shows the ostium of the right sinuses, which is evident just lateral to the endoturbinates. E, endoturbinates; M, maxilloturbinate. (courtesy of Kara, 2004, used with permission of the publisher)

immunogenicity of attenuated vaccines with the safety of inactivated vaccines (Brockstedt et al., 2005).

Rabbit Model

Rabbits have played an important role in the development and refinement of anthrax vaccines. To address the concern about the conventional PA-based vaccine that relied on one antigen, an experiment using a multiple antigenic peptide with a heptameric structure of PA for immunizations resulted in the *in vitro* neutralization of LeTx (Oscherwitz et al., 2009). Additional promising vaccine research with rabbits includes evidence that the novel KBMA vaccine provides a wider base of humoral immunity than current subunit vaccines (Skoble et al., 2009). Recombinant virus type 1 (rAAV1) vectors that contain genes synthesized from LF or PA produce significant neutralizing antibodies after one administration, indicating a potential use as a vaccine (Liu et al., 2009). Anthrax vaccine safety related to the use of the current vaccine, AVA, in pregnant women has been tested in rabbits, and was found to have no untoward effects on the pregnant does or their kits (Franco et al., 2009). An intranasal rPA vaccine was effective in protecting rabbits against an aerosol inoculum with anthrax spores 9 weeks following a single injection, demonstrating a potentially more practical form of vaccine delivery (Klas et al., 2008). The repertoire of rabbit models for the study of anthrax has been expanded with recent work in the Dutch belted rabbit (Lawrence et al., 2009), which has physiologic parameters comparable to those in humans exposed to anthrax (Lawrence et al., 2009).

Tuberculosis

Etiology and Disease in Humans

Pulmonary tuberculosis (TB) is caused by *Mycobacterium tuberculosis*. *M. tuberculosis* is a facultative intracellular, non-sporeforming, aerobic, acid-fast, Gram-positive organism that can infect and multiply in macrophages and other cells. There are more than two million fatal cases of TB each year (North and Jung, 2004). Most of the fatalities are in Southeast Asia and Africa (North and Jung, 2004). In 2000, there were over eight million new cases of TB globally, and 11% of deaths among adults with AIDS were attributed to TB during the same year (Corbett et al., 2003). Susceptibility to infection with *M. tuberculosis* appears to be related to genetic polymorphism involving genes for mannose-binding protein, natural resistance-associated macrophage protein (NRAMP1), and the receptor for vitamin D (North and Jung, 2004). Infection with *M. tuberculosis* typically presents as a delayed-type hypersensitivity, a result of a Th1 immune response, and most infections are

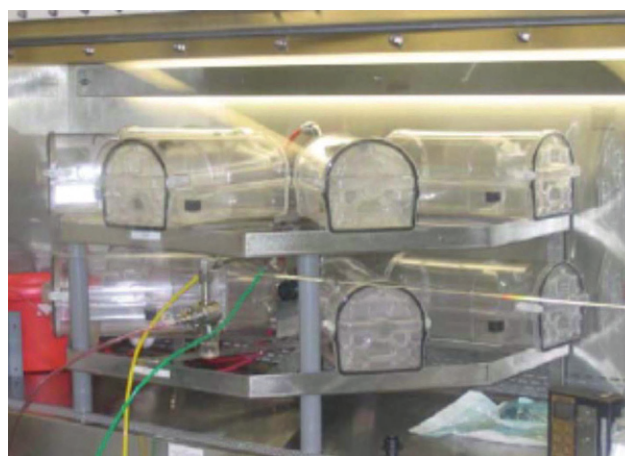


FIGURE 18.14 Aerosolization chamber for infecting rabbits with TB.

confined to the lungs (North and Jung, 2004). However, immunocompromised patients can suffer from systemic disease and infections in many organs (North and Jung, 2004). If infected, pulmonary granulomas can evolve to include caseous necrosis centrally that can cavitate (North and Jung, 2004). Individuals with cavitations can easily spread *M. tuberculosis* (Helke et al., 2006). The cavities are the most highly oxygenated among the lesions related to infection, allowing enhanced multiplication of the *M. tuberculosis* bacteria (Helke et al., 2006; Via, 2008). A hypoxic environment and/or exposure to nitric oxide (NO) from activated macrophages might account for persistent *M. tuberculosis* infections (Converse et al., 2009). Fibrosis within the granuloma and parenchyma results in the characteristic nodules or tubercles. *M. tuberculosis* can also cause serious cases of tuberculous meningitis, especially in children; this form of TB has a 50% mortality rate (Tsenova et al., 2005).

Rabbit Model

While both mice and guinea pigs are used as animal models of TB, the rabbit model is advantageous because rabbits are more resistant, develop a spectrum of disease, and eventually clear the organism, which mimics the clinical course of disease in humans (Manabe et al., 2002). TB is induced in rabbits via aerosolization in a nose-only system in a class II biosafety glove box cabinet under negative pressure (Figure 18.14) (Manabe et al., 2002). The severity of disease produced in rabbits varies with the virulence of the TB strain used, but in general rabbits have visible pulmonary tubercles 5 weeks after infection (Manabe et al., 2002). This clinical feature has made the rabbit a valuable model to examine the relative pathogenesis of different strains of TB. The strain of rabbit used in the experiment also impacts disease expression. When challenged with the Erdman or

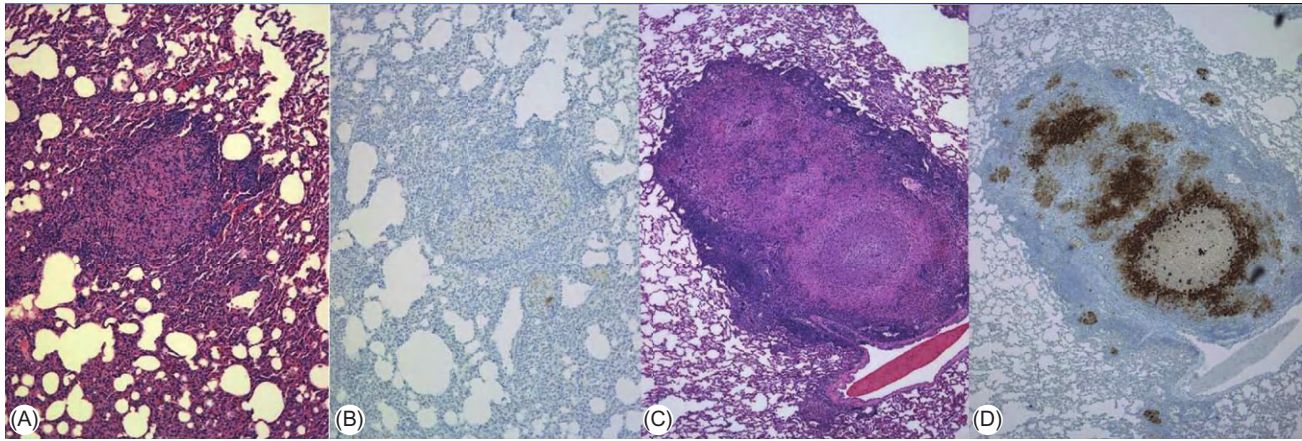


FIGURE 18.15 (A–D) Series of images (10×) showing the comparison of H&E-stained and anti-PIMO adduct-labeled pulmonary sections from rabbits experimentally infected with *M. tuberculosis*. (A) shows granulomas devoid of necrosis. The corresponding image (B) shows weak PIMO staining. (C) is the H&E section for (D) (anti-PIMO adduct-labeled). In (D) the caseous necrotic granuloma has low oxygen tension foci indicated by the brown coloration.

H37Rv strain of TB, inbred NZW rabbits had larger and more tubercles with more colony-forming units in each tubercle than the outbred NZWs (Dorman et al., 2004). The inbred NZWs also had a greater level of caseous necrosis in pulmonary granulomas, a lower number of mature epithelioid cells, lower delayed-type hypersensitivity reaction, more visible *M. tuberculosis* organisms, and a lower amount of tumor necrosis factor alpha from LPS-stimulated peritoneal macrophages than outbred NZWs, indicating a higher susceptibility in inbred NZWs (Dorman et al., 2004). In pulmonary TB cases, lesions that are common to humans and rabbits include granulomas with liquefaction and caseous necrosis, and possibly most significantly, cavitations (Dorman et al., 2004), which is illustrated using pimonidazole hydrochloride (PIMO), a compound that is bioreductive only in hypoxic mammalian tissues (Figure 18.15) (Via et al., 2008). Rabbits have been used for models of central nervous system infections in humans via direct inoculation into the cisterna magna. A similar spectrum of disease based on the virulence of the injected TB strain is observed in this model (Tsenova et al., 2005).

UROGENITAL DISEASES

Papillomatosis

Etiology and Disease in Humans

Human papillomaviruses (HPVs) are double-stranded DNA, non-enveloped, viruses that include more than one hundred different viral “types” (de Villiers et al., 2004). Papillomatosis can include genital warts/cancers, and respiratory lesions (de Villiers et al., 2004; Govan et al., 2008). Depending on their predisposition to

manifest as malignancies, HPVs are classified as “high-risk” or “low-risk” viruses (de Villiers et al., 2004; Munoz et al., 2003). HPV 6 and 11 are the most common “low-risk” HPVs and cause 90% of genital warts (Govan et al., 2008). HPV 16 and 18 are the most prevalent “high-risk” types and the etiologies of 70% of cervical cancers (Govan et al., 2008). Spontaneous resolution of proliferative epithelial lesions can occur over an extended period of time (up to years) in HPV infections (Brandsma et al., 2007). Persistence of the “high-risk” types can last decades, and changes can lead to carcinoma formation in the absence of therapy (Govan et al., 2008). In less developed nations, cervical neoplasia is the most prevalent type of cancer in females, and represents the second most prevalent cancer in this gender globally (Govan et al., 2008). The current L1 virus like particle (VLP) vaccine confers immunity in humans (Hu et al., 2007). Nonetheless, a vaccine that could produce a wider array of protective antibodies would be more beneficial given the diversity of HPV genotypes (Hu et al., 2007). Another shortcoming of the L1 VLP vaccine is that it protects against new HPV infections, but it fails to protect or fully protect the individual if there is an existing infection (Hu et al., 2007).

Rabbit Model

Papillomaviruses are very species-specific (Hu et al., 2007). Thus, the cottontail rabbit papillomavirus (CRPV) has been used to investigate papillomatosis in rabbits (Fausch et al., 2003). Genetically modified rabbits have also been useful in the exploration of HPV biology and pathogenesis (Hu et al., 2007). Researchers developed a transgenic rabbit line that expresses the extensively studied human major histocompatibility complex class I (MHC-I) gene, HLA-A2.1 (Hu et al.,

2007). Germline transfer of this transgene has been confirmed with breeding schemes using outbred NZW rabbits and the EIII/JC inbred strain (Hu et al., 2007). The genome of the CRPV can withstand significant genetic modifications, meaning that it could possibly serve as a mechanism to test HLA-A2.1-specific epitopes (Hu et al., 2007). Promising vaccine study results also include the use of an “ubiquitin-fused” CRPV recombinant DNA vaccine (Brandsma et al., 2007). This “UbE1267” vaccine removed papillomas in rabbits with covert infections and significantly lessened the volume of papillomas in overt cases of papillomatosis (Brandsma et al., 2007). CRPV VLP vaccines reduced the size of papillomas in vaccinated NZW rabbits, which suggests a similar therapy with VLPs vaccines might be possible in humans with low-risk HPVs (Govan et al., 2008).

GASTROINTESTINAL DISEASES

Colibacillosis

Etiology and Disease in Humans

Escherichia coli (*E. coli*) is an important foodborne illness that causes diarrhea particularly in infants and in travelers from developed to underdeveloped countries, affecting approximately 60% of visitors to tropical and subtropical countries (Okoh and Osode, 2008). Traveler’s diarrhea is generally caused by the enterotoxigenic *E. coli* (ETEC) and causes a watery or bloody diarrhea that resolves within a few days. Several serovars of *E. coli* cause more serious clinical conditions: enteroaggregative *E. coli* (EAEC) causes persistent watery diarrhea that has been implicated as a possible cause of irritable bowel syndrome (Kaur et al., 2010), enterohemorrhagic *E. coli* (EHEC) can result in serious hemolytic-uremic syndrome (Kulasekara et al., 2009), enteroinvasive *E. coli* (EIEC), and enteropathogenic *E. coli* (EPEC) (Edelman and Levine, 1983; Levine, 1987; Vial et al., 1988) cause severe diarrhea in infants by attaching and effacing the intestinal epithelia surfaces (Abe et al., 1998). *E. coli* are often involved in foodborne illness outbreaks in the United States affecting adults as well as children (Kulasekara et al., 2009). As a result, *E. coli* remains a significant public health concern.

Rabbit Model

A surgical model designated the RITARD model which stands for removable intestinal tie-adult rabbit diarrhea for producing colibacillosis in rabbits was first described in 1981 (Spira et al., 1981). The model is created via a laparotomy in an anesthetized rabbit. The cecum is ligated and a temporary reversible obstruction (a slipknot tie) of the small bowel is created at the time of challenge and removed 2 hours later. Rabbits reliably develop diarrhea using this technique and this model

continues to be used to understand the pathogenesis of *E. coli* (Fagundes-Neto et al., 1995; Moon et al., 1983; Nobe et al., 2009). The nomenclature of *E. coli* strains in animals often includes a letter in the acronym to identify the natural host: REPEC is the EPEC found in rabbits and used as a model of disease associated with EPEC in humans (Wales et al., 2005). Rabbits, particularly young rabbits are increasingly being inoculated with different strains to *E. coli* via orogastric intubation to more closely mimic the natural course of disease. For example, when 28–56-day-old rabbits were inoculated with *E. coli* serogroup O103 (REPEC O103) 65% developed diarrhea and bacterial shedding, and 23% of the rabbits died between 2 and 10 days after infection (Heczko et al., 2000). This study used scanning electron microscopy to characterize the pathogenesis of these bacteria in vivo, which showed that the organism first adhered to the villi of Peyer’s patches in the ileum forming “fimbriae-like” organelles within the first 24 hours, resulting in attaching-effacing lesions in the ileum, ileal Peyer’s patches, and proximal colon 72h later (Figure 18.16) (Heczko et al., 2000). Various adherence factors cause an initial non-intimate attachment of REPEC organisms in rabbits (Wales et al., 2005). REPEC adhesion-mediated binding was limited to Peyer’s patches of the ileum, which is similar to the condition involving EHEC O157:H7 in humans (Phillips and Frankel, 2000). AAEC bacteria in rabbits are usually associated with a pattern consistent with EPEC bacteria (Wales et al., 2005). However, REPEC strains vary according to serotype, virulence, how they colonize the intestines, and the age of the host (Wales et al., 2005). Attaching-effacing lesions can be diagnosed using a number of methods including microscopy (Wales et al., 2005), murine monoclonal anti-alpha-actinin antibodies (Ismaili et al., 1995), epifluorescent microscopy (Vallance et al., 2002), and laser scanning confocal microscopy (Abe et al., 1998). A naturally occurring rabbit model of EHEC in Dutch belted rabbits was recently described (Garcia et al., 2002). In addition to developing diarrhea, these rabbits develop the clinical and pathological evidence of human hemolytic-renal syndrome and are being further characterized with the goal of establishing an experimental model of this syndrome (Garcia et al., 2002).

Campylobacteriosis

Etiology and Disease in Humans

Campylobacter jejuni is the leading cause of bacterial diarrhea in the world (Stintzi et al., 2005). This agent has been associated with 14.2% of the annual food borne illnesses in the U.S. presenting as mild diarrhea to significant intestinal discomfort (Mead et al., 1999; Stintzi et al., 2005). Guillain-Barré syndrome (GBS), a condition of acute paralysis, can be an uncommon

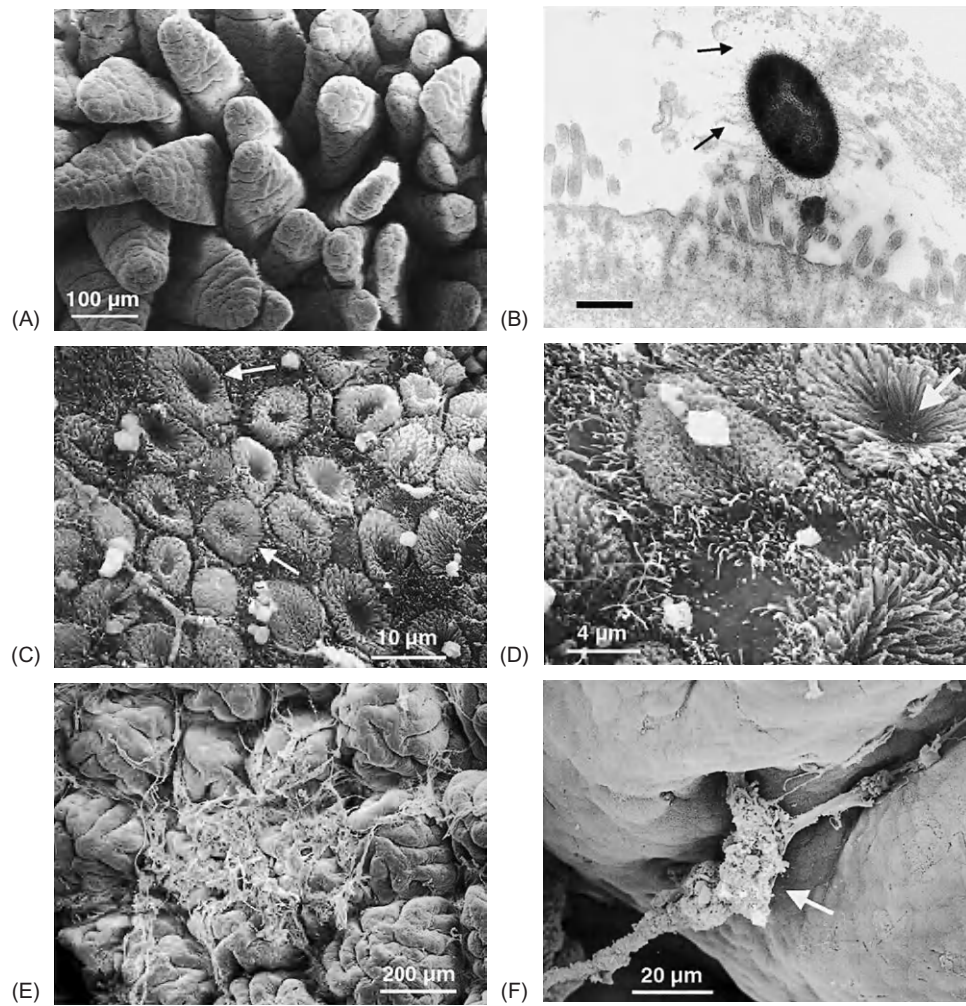


FIGURE 18.16 Scanning (A, C–F) and transmission (B) electron micrographs of rabbit intestinal segments 24 hours after infection with REPEC O103. (A) ileal villi with normal appearance. (B) during early stages of infection bacteria express fimbriae-like structures at their surface (→) resulting in initial non-intimate adherence to epithelial host cells. Bar = 1 μm. (C, D) in response to infection, microvilli on Peyer's patch enterocytes show irregular arrangements resulting in a bowl-like (→) surface appearance. (E, F) a net of aggregates of bacteria (→) closely bound to flocculent or filamentous mucoid material covers part of the colon. (courtesy of Heczko 2000, used with permission of the publisher)

but significant sequella to the infection (van Vliet and Ketley, 2001).

Rabbit Model

The Rabbit Ileal Loop (RIL) model is an established preparation performed prior to inoculating the intestine with an agent of interest. This procedure involves exposure of a section of ileum with a viable mesenteric blood supply and its ligation to inhibit peristalsis, which promotes colonization by the infectious agent (Caldwell et al., 1983; Everest et al., 1993; Stintzi et al., 2005). Using this method to experimentally infect rabbits with *C. jejuni*, it has been shown that the *C. jejuni* transcriptomes were different among infected rabbits, indicating a likely attempt by *C. jejuni* to survive in diverse intestinal conditions (Stintzi et al., 2005).

An up-regulation of the stringent response (microbial stress response to a nutrient-depleted environment) by *C. jejuni* potentially contributes to intestinal colonization (Stintzi et al., 2005).

The neurologic effects of a *C. jejuni* infection are attributed to a "carbohydrate mimicry" involving the lipo-oligosaccharide of *C. jejuni* and GM1 ganglioside in humans, resulting in an autoimmune condition consistent with Guillain-Barré syndrome (Yuki et al., 2004). To illustrate the potential neurologic effects of a *C. jejuni* infection, lipopolysaccharide obtained from *C. jejuni* found in a human with Guillain-Barré syndrome, combined with Freund's adjuvant and keyhole limpet hemocyanin, served as an inoculation for rabbits that subsequently presented with sciatic nerve axonal degeneration and weakness (Caporale et al., 2006).

Endotoxemia

Etiology and Disease in Humans

Sepsis and the systemic inflammatory response resulting from systemic infections are the most common cause of death in adult intensive care units, with the number of deaths increasing over the past two decades (Parker and Watkins, 2001). Despite active research into treatments for septicemia and endotoxemia, management of patients remains largely supportive. Multiple Gram-negative bacteria can produce endotoxins that disrupt the physiology of the gastrointestinal tract including nutrient, water, and electrolyte absorption in humans and animals (Gardiner et al., 1995; Hecht and Koutsouris, 1999). Lipopolysaccharide (LPS), found in the outer bacterial wall, is an example of an endotoxin that induces cellular signaling involving several types of protein kinases (Chow et al., 1995) that result in cytotoxic effects (Amador et al., 2007).

Rabbit Model

Many animal models from mice to non-human primates have been used to study different aspects of endotoxemia (Parker and Watkins, 2001). Large mammals, such as rabbits, are often used in physiological studies requiring invasive monitoring. The rabbit, along with sheep and chimpanzees are more sensitive to the effects of endotoxin and show an enhanced response to lower doses of LPS than other species (Parker and Watkins, 2001). The rabbit endotoxemia model is created by injecting either *E. coli* organisms or LPS into the marginal ear vein (Elmas et al., 2008). This model has been used to demonstrate the poor enteric uptake of D-fructose and L-leucine associated with facilitation of LPS activity by protein kinase C and calmodulin (Amador et al., 2007), and the efficacy of simultaneous treatment with a fluorinated quinolone antibiotic and a non-steroidal anti-inflammatory for the treatment of septicemia (Elmas et al., 2008).

MUSCULOSKELETAL DISEASES

Osteomyelitis and Implant-Related Infection

Etiology and Disease in Humans

Osteomyelitis is an inflammatory process accompanied by bone destruction due to an infectious organism. Infections can result as a complication of bone fracture therapy and joint surgeries or spread hematogenously, particularly in juvenile and elderly patients and in those with diabetes (Lew and Waldvogel, 2004). Approximately 15% of diabetics will develop osteomyelitis as a result of a foot infection during their lifetimes. Some of these patients will eventually require

limb amputation due to non-healing lesions (Lew and Waldvogel, 2004). *Staphylococcus aureus* represents the most common etiology of osteomyelitis (Lew and Waldvogel, 2004). A troubling recent development is the increase in the number of osteomyelitis cases involving gentamicin-resistant or methicillin-resistant *S. aureus* (MRSA) (Giavaresi et al., 2008). Another reason for concern is an increase in the number of *S. aureus* clinical isolates that produce Panton-Valentine leukocidin (PVL), a hetero-oligomeric toxin (with subunits, LukFPV and LukS-PV), that is unrelated to methicillin resistance but often associated with *S. aureus* infectious osteomyelitis or arthritis (Cremieux et al., 2009). An increase in PVL-producing *S. aureus* is attributed to the rising prevalence of community-acquired methicillin-resistant *S. aureus* (CA-MRSA) strains (especially USA300) (King et al., 2006; Tristan et al., 2007). Infections surrounding foreign bodies and internal fixation devices often involve a biofilm on the indwelling object from which *Staphylococcus epidermidis* is most often recovered (Donlan, 2001; Vuong and Otto, 2002). In cases of indwelling medical devices, antimicrobial agents, removal of the device, debridement of necrotic tissue, and potential stabilization with an external device are frequently necessary for a positive outcome (Giavaresi et al., 2008). Local application of antimicrobials to infected osteomyelitis sites has typically involved polymethylmethacrylate for delivery (Jia et al., 2009). Other materials such as bioceramic compounds (silicate-based bioactive glass, synthetic hydroxyapatite, and tricalcium phosphate) have been similarly utilized (Jia et al., 2009). Polymers and composites have also been employed to promote tissue healing and repair (Shi et al., 2009).

Rabbit Model

Anatomically, the tibia of the rabbit lends itself to bone and joint experiments because of the relatively small mass of associated soft tissue, facilitating access and manipulation (An et al., 2006). The femur is more substantial than the tibia, with a greater medullary space which makes it a commonly used site for implantation and internal fixation research (An et al., 2006).

Rabbits are suitable immunologically for studies with microorganisms that infect bones and joints in humans (Cremieux et al., 2009). The significant pathophysiology of a PVL-positive CA-MRSA strain (USA300) has been demonstrated in a rabbit model (Cremieux et al., 2009). Experimental osteomyelitis using *S. aureus* in rabbits was successfully treated when ethyl cellulose microspheres were impregnated with gentamicin for local antimicrobial application (Shi et al., 2009). Additional promising results were seen when a different local therapy regimen with borate glass plus chitosan pellets were filled with the antibiotic, teicoplanin, which is known to have good activity against

most Gram-positive (including MRSA) aerobic and anaerobic bacteria (Jia et al., 2009). Rabbits were also used to evaluate the efficacy of ciprofloxacin-releasing SR-PLGA 80/20 miniscrews to minimize infection in bone fixation devices (Ashammakhi et al., 2006).

Electrical current has been used as part of a novel treatment of fracture non-unions that have been stabilized with indwelling devices (Del Pozo et al., 2009). Rabbit experiments suggested that an electrical current plus doxycycline was more beneficial in treating infections involving foreign bodies (implants) and *S. epidermidis* biofilms, than doxycycline alone (Del Pozo et al., 2009). Extracorporeal shockwave therapy (ESWT), another novel therapy that promotes remodeling in bones and increased vascularity, is becoming more widely used in therapy for fractures and non-unions, and soft tissue conditions such as fasciitis, chronic ulcers, and wound healing (Gollwitzer et al., 2009). ESWT has been withheld for infectious conditions because of an assumed dissemination of bacteria with a potential septicemia (Gollwitzer et al., 2009) secondary to shock-wave-induced neovascularization. However, when applied to rabbits with experimentally induced cases of *S. aureus* osteomyelitis, ESWT did not cause the organisms to spread or an intensification of the infection (Gollwitzer et al., 2009). Instead, ESWT was proven to be beneficial when histologic samples from treated and control groups were compared (Gollwitzer et al., 2009).

Osteoarthritis and Non-Infectious Orthopedic Disease

Etiology and Disease in Humans

Osteoarthritis (OA) is a chronic degenerative disorder characterized pathologically by cartilage loss and clinically by severe pain, joint deformity, and loss of joint motion (Swieszkowski et al., 2007). More than 20 million people in the United States are affected by the disease, making OA one of the major causes of disability among the adult population. Recent studies have demonstrated that OA is a risk factor for early mortality from other systemic diseases, possibly due to poor musculoskeletal function and limited exercise (Hochberg, 2008). Joint trauma, age, obesity, genetic factors, and race are among the many risk factors for acquiring OA (Das and Farooqi, 2008). Articular cartilage and subchondral bone form a load-bearing system that provides joint stability, cushioning, and lubrication to the joint (Swieszkowski et al., 2007). Cartilage is an avascular tissue that has a very limited ability for regeneration. As a result, therapies for early OA including analgesics, intra-articular injections, and acupuncture (Das and Farooqi, 2008) have been largely palliative; with surgery, including partial or total joint replacement, reserved for patients with unrelieved pain.

Rabbit Model

The rabbit is frequently used in orthopedic research to understand the natural history of disease, to evaluate the effects of novel clinical treatments, and to develop new and refined surgical techniques (Pelttari et al., 2008). Gupta and Lee (2007) recently developed a rabbit model to investigate the pathophysiology of rotator cuff injury by creating a tenotomy on the subscapularis tendon complex. Traditional rat models of rotator cuff injuries are made by creating a tenotomy on the supraspinatus tendon (Soslowsky et al., 2002). The supraspinatus tendon is chosen because it is the most common source of injury in human rotator cuff disease. However, the anatomical and biomechanical forces on the shoulder of a quadrupedal animal differ from those in a human, and the supraspinatus tendon is a fairly rudimentary tendon in rats. The subscapularis rabbit model more closely mimics the disease condition in humans and will allow investigation into the natural history of tears and the healing response to surgical correction (Gupta and Lee, 2007).

The rabbit is one of the models used to evaluate the effects of damage to the knee following anterior cruciate ligament (ACL) injuries, one of the most commonly damaged ligaments in the human knee (Zhou et al., 2009). The ACL cannot be naturally repaired due to poor vascularization, rendering the knee unstable and causing damage to the cartilage and meniscus that subsequently results in OA. The classic rabbit model of ACL injury is created by surgically ligating the ACL in an anesthetized rabbit (Kimura et al., 2008). This model is being extensively used to investigate novel therapeutic approaches, such as tissue engineering of the ACL which will allow regeneration of the ACL via integration of cells, a scaffold for cell proliferation and differentiation and growth of trophic factors (Kimura et al., 2008; Petrigliano et al., 2006).

A new rabbit model of traumatic ACL injury was recently described that mimics the high-energy jump landing insult to the knee without bone fracture that often results in ACL rupture (Killian et al., 2009). This model was created in anesthetized Flemish Giant rabbits restrained in a supine position with the femur fixed with Velcro straps, the knee flexed 90° and the foot fixed in a custom-designed boot fixed with Velcro straps. A 1.75-kg mass was dropped from a height of 75 cm to strike the distal femur of the left leg. The tibia was not constrained and subluxated on impact, resulting in rupture of the ACL. In addition to ACL rupture, the left lateral menisci of these animals was also torn, which is a common feature of natural ACL injuries and absent from the surgical model. This is the first ACL injury model to replicate both the acute and chronic meniscal damage following ACL trauma and promises to be an important animal model to investigate the pathogenesis of OA in the knee.

The limited potential for regeneration of cartilage has prompted research into methods to achieve osteochondral regeneration, such as tissue engineering. The rabbit has been one of the primary animal models for these studies (Pelttari et al., 2008). Several investigators demonstrated cartilage growth in the medial condyle of rabbits after transplanting bone marrow progenitor cells that had been seeded on a hyaluronan or collagen matrix (Grigolo et al., 2001; Pelttari et al., 2008). The rabbit, along with mice, goats, pigs, and calves are used to investigate bi-phasic tissue engineering scaffolds for the junction between cartilage and the underlying bone (Keeney and Pandit, 2009). Rabbits are also being used to evaluate the therapeutic potential of bone morphogenetic protein (BMP), particularly osteogenic protein-1 (OP-1), for cartilage repair (Chubinskaya et al., 2007). BMPs are expressed in a variety of tissues and have wide-ranging biological effects including regulation of cell proliferation, apoptosis, and maintenance of tissue homeostasis during adult life (Chubinskaya et al., 2007). OP-1 was the first BMP to receive regulatory approval for bone repair treatment. The encouraging results from multiple rabbit studies may serve as a foundation for approval of the compound for cartilage repair as well (Chubinskaya et al., 2007).

MULTISYSTEMIC DISEASES

Candidiasis

Etiology and Disease in Humans

Most cases of candidiasis in humans are caused by *Candida albicans* (Kao et al., 1999). Typically, this dimorphic fungus is a commensal organism found in the mucosa and gastrointestinal tract. Local infections are common, and it is reported that 75% of all women have had at least one episode of vaginal candidiasis (de Repentigny, 2004). However, *C. albicans* can cause invasive infections as an opportunist. This is particularly true of immunocompromised patients, in whom *C. albicans* is the most frequently isolated fungal pathogen associated with infection in a hospital setting (Bujdakova et al., 2008). Disseminated candidiasis can occur if the organism enters the cardiovascular system from another site, such as the oral mucosa or the gastrointestinal tract, especially in patients with neutropenia or leukemia (Abi-Said et al., 1997; Walsh and Merz, 1986). Infection of the oropharynx can often be a complication of HIV-AIDS (de Repentigny, 2004). Hematogenous infections with fungi, including *Candida*, often involve contaminated indwelling catheters or medical devices that are difficult to treat (Mukherjee et al., 2009).

Rabbit Model

Many animal species have been used as models to evaluate the spectrum of disease caused by *C. albicans*. Transgenic and knockout mice with targeted immune defects have largely replaced rabbits for pathogenesis studies of *C. albicans*, but rabbits are still used to evaluate treatments for systemic, particularly hematogenous *Candida* meningoencephalitis (HCME) (Hope, 2008; Mukherjee et al., 2009) and ocular candidiasis (Suzuki et al., 2008). HCME is induced in rabbits via intravenous inoculation generally through a centrally placed indwelling catheter. Rabbits reliably develop *Candida* positive blood cultures 24h after inoculation, although few have positive cerebral spinal fluid (CSF) cultures despite positive *Candida* cultures from the brain tissue when the rabbits were euthanized (Petraitiene et al., 2008). Patients with HCME likewise rarely have positive CSF fungal cultures which often delays therapy. The rabbit model was used to demonstrate the potential utility of β -glucan, a component of the fungal cell wall in the CSF as a biomarker of *Candida* infection (Petraitiene et al., 2008). Rabbits with HCME has significantly elevated CSF β -glucan levels that reliably fell in response to treatment with the antifungal micafungin (Petraitiene et al., 2008). The rabbit HCME model is also used to evaluate the efficacy of antifungals for systemic disease (Egermann, 2009; Hope et al., 2008; Mukherjee, 2009) as well and *C. albicans* endophthalmitis (Kusbeci et al., 2007; Louie, 1999; Suzuki, 2008).

Variola (Smallpox)

Etiology and Disease in Humans

The potential use of orthopoxviruses, including variola virus (smallpox) and monkey pox, as biological weapons has renewed interest in the development of anti-orthopoxvirus agents (Smee, 2008). The characteristics that make these viruses ideal biological weapons include a high mortality rate (50% or more) (Panchanathan et al., 2008) with low levels of virus, generalized dissemination with secondary lesions, and ease of communicability (Adams et al., 2007). Lesions can range from multiple pustules over much of the body to pneumonia, fatal hypotension, and immune complex disease (Panchanathan et al., 2008).

Rabbit Model

Rabbits inoculated intradermally with either rabbit pox virus (RPV) or the Western Reserve strain of vaccinia virus (VV-WR) develop disease beginning 1 or 2 days post intradermal inoculation with injection site swelling and progressing to systemic disease including a fever and severe respiratory disease requiring euthanasia by 6–9 days post infection (Adams et al., 2007).

RPV and VV-WR infected rabbits are 100% efficient in transmitting lethal disease to co-housed rabbits, and slightly less than 50% efficient in transmitting disease to animals housed 15cm away, suggesting an aerosol route of transmission (Adams et al., 2007). The rabbit is the only small animal model for human smallpox in which natural aerosol transmission has been reported (Adams et al., 2007). This model is being used to evaluate the efficacy of anti-orthopox treatments such as cidofovir and ST-246 which inhibit assembly of the viral envelope and escape of the virus from RPV-infected cells (Smee, 2008) and novel vaccines (Garza et al., 2009).

Syphilis

Etiology and Disease in Humans

Treponema pallidum subspecies *pallidum*, is the causative agent of syphilis. The spiral-shaped bacterium or spirochete belongs to the order, Spirochaetales. In the absence of therapy, this sexually transmitted disease can cause cardiovascular and central nervous system lesions. Antimicrobials, including penicillin, are effective in the treatment of syphilis. However, this disease is a major health issue, especially in less developed countries, in which there are approximately twelve million new cases each year (Brinkman et al., 2008). The disease follows a pattern of three stages over the course of several years. In the initial stage, one or more chancre develop in the genitals approximately 21 days post-infection which may be associated with regional lymphadenopathy (Peeling and Hook, 2006). During this stage, *Treponema* organisms likely penetrate the endothelial intercellular junctions and gain access through the bloodstream to different parts of the body such as the central nervous system (Thomas et al., 1988). The secondary stage generally begins 6–8 weeks later. Depending on the dissemination of the organism, the clinical signs of stage two syphilis can vary widely but often include a generalized maculopapular rash and lymphadenopathy, headache, and malaise (Peeling and Hook, 2006). Individuals with primary or secondary lesions spread the disease through sexual contact. Transplacental infection from an infected mother to the fetus represents the most serious transmission of the organism, and often results in fetal death, or mental retardation and abnormal growth for those who survive to parturition (Peeling and Hook, 2006). Left untreated, about a third of adults infected with *Treponema* will progress to the tertiary stage disease involving neurological and cardiovascular impairment (Peeling and Hook, 2006).

Rabbit Model

While humans are the only natural hosts of *T. pallidum*, rabbits have susceptibility and humoral immune responses similar to those seen during the early stages

of illness in man (Peeling, 2006; Pierce et al., 1983). These characteristics have made rabbits one of the most widely used animal models of syphilis for many years, and the rabbit infectivity test (RIT) in which rabbits are inoculated intradermally with serial dilutions of *T. pallidum* is the “gold standard” laboratory test to determine treponemal viability (Cox et al., 2003). In the RIT test, inoculation with as few as one organism can produce a chancre within 30 days, which is considered a positive test (Cox et al., 2003). Since *T. pallidum* cannot be sustained for prolonged periods using artificial media, the organism is propagated in vivo by inoculating it into the testes of rabbits, which yields substantial numbers of organisms (Peeling and Hook, 2006). Unlike the disease in humans, rabbits inoculated intradermally do not develop stage three neurological manifestations (Peeling and Hook, 2006). To create a model of the late-stage neurosyphilis, rabbits are inoculated either intracisternally (Marra et al., 1991) or intravenously (Tantalo et al., 2005). While the clinical progression varies with the strain inoculated, rabbits develop generalized skin lesions and have *T. pallidum* detectable in the CSF within 2–4 weeks of inoculation. The rabbit has also been used to create a model of experimental congenital syphilis by inoculating pregnant does intravenously with *T. pallidum* (Fitzgerald, 1985). Infected does have a 7–10-fold increase in fetal mortality, and a 49% reduction in birth weight for live births. Extracts of fetal tissue were able to produce syphilis when inoculated into adult rabbits, demonstrating that transuterine infection occurred. The rabbit syphilis model is continuing to be used to understand the pathogenesis (Tantalo et al., 2005) of syphilis and to develop a vaccine to prevent syphilis (Brinkman et al., 2008).

Streptococcal Pneumonia and Meningitis

Etiology and Disease in Humans

The rise in penicillin-resistant *Streptococcus pneumoniae* (pneumococci) is a significant threat to world health. Internationally, these strains rose from 6% in 1997 to 22% in 1999 (Egermann et al., 2009). Pneumococci are the most prevalent infectious agents in cases of adult meningitis involving negative clinical outcomes (Aronin et al., 1998; McMillan et al., 2001). A high dose of vancomycin with or without a third-generation cephalosporin (such as ceftriaxone) is usually prescribed for strains that are significantly resistant to penicillin (Quagliarello and Scheld, 1997). An accepted adjunct for therapy in adults is to include a glucocorticoid in pneumococcal meningitis cases (Egermann et al., 2009). However, vancomycin penetration into the CSF is substantially lower with this regimen (Martinez-Lacasa et al., 2002; Paris et al., 1994). Group

B. streptococcus (GBS) can be found in the genitourinary or gastrointestinal systems of up to 30% of clinically normal females (Hensler et al., 2005). GBS can be vertically transmitted from the mother to an infant when the infant is exposed to microbes from the mother's genitourinary or gastrointestinal tract (Hensler et al., 2005). Newborns infected in such a manner can suffer from pulmonary symptoms within a few hours after birth, and in most cases the organisms escape from the lungs to cause septicemia and meningitis (Hensler et al., 2005; Vollman et al., 1976). In children, *Streptococcus pneumoniae* colonizes the nasopharyngeal cavity, typically reaching a maximum concentration when the child reaches 3 years of age (Bogaert et al., 2004). This colonization provides a means for more invasive dissemination of the bacteria into the lower respiratory tract (Chiavolini et al., 2008). Approximately one million children under the age of five succumb to streptococcal pneumonia in less developed nations annually (Obaro and Adegbola, 2002). Multi-valent vaccines provide effective protection against most serotypes of pneumococci in humans 2 years of age or older and non-geriatric individuals (Chiavolini et al., 2008). Development of protein subunit vaccines could enhance the protection provided by the current polysaccharide vaccines (Barocchi et al., 2007).

Rabbit Model

Induced models of pneumococcal pneumonia in the rabbit include experimental surgeries for intrabronchial, intratracheal, or intrapulmonary inoculation exposures (Chiavolini et al., 2008). The intranasal route is not effective in rabbits (Chiavolini et al., 2008). A widely held tenet is that pneumonia occurs when the dose of the bacteria exceeds the limits of the defenses in the respiratory system (Yershov et al., 2005). Surprisingly, this hypothesis was only recently borne out when an experiment using an inoculum of $>4.60 \log^{10}$ CFU in NZW rabbits demonstrated that lower doses were less likely to cause disease versus a 90% infection rate when the higher dose was used (Yershov et al., 2005). Neonatal rabbits intratracheally inoculated with GBS developed much greater rates of bacteremia, reduced pulmonary compliance under mechanical ventilation, and a 40% mortality rate (vs. 0%) compared to those inoculated with the GBS mutant $\Delta 3h/c$ mutant NCTC:cy/IE Δcat (Hensler et al., 2005). This study was the first to show the importance of the GBS β -hemolysin/cytolysin ($3h/c$) toxin in the damage to pulmonary epithelial barriers that can lead to a hematogenous spread of the organism (Hensler et al., 2005). The rabbit model has also been used to demonstrate that the cyclic lipopeptide, daptomycin, is very effective in treating penicillin-resistant pneumococcal meningitis (Egermann et al., 2009). In related studies, daptomycin alone or in combination with

dexamethasone was much more effective than vancomycin combined with ceftriaxone, or dexamethasone followed by vancomycin and ceftriaxone (Egermann et al., 2009).

Acknowledgments

The authors of this chapter kindly thank Kristina Adams at the Animal Welfare Information Center for providing the information on the number of rabbits used in research presented in Table 18.1, and to Dr. Ginger Tansey for providing editorial assistance with the ophthalmic diseases section. We would also like to thank Dr. Kathy Perdue and Dr. Jeanne Wallace for their careful reading and thoughtful comments on the first draft of this chapter. This review was supported by the Intramural Research Program of the Office of Research Services, National Institutes of Health, and the National Heart, Lung and Blood Institute.

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Taxonomy and History

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OUTLINE

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INTRODUCTION

Guinea pigs (*Cavia porcellus*) occupy a special place in research. This rodent species, with its unique physiology and anatomy, has come to symbolize all experimental subjects (Smithcors, 1963; Wagner, 1976). Since 1913, when a human or animal participates in research or tries something new, they have been called “guinea pigs” to represent that newness of what will be taking place (Oxford English Dictionary, 2007).

This special place in research implies that guinea pigs are one of the most commonly used laboratory animals in research. However, after a peak of usage in the 1960s at an estimated 2.5 million guinea pigs per year, with most animals being used for tuberculosis research, the number of guinea pigs used per year in biomedical research has steadily declined (Kydd, 2010; OTA, 1986; USDA, 1996, 2007) (Figure 19.1). Much of the decline in guinea pig usage in research stems from the growing use of genetically engineered mice and rats for specific disease models plus the acceptance of a replacement animal model, the mouse local

lymph node assay or LLNA, as recommended by the Interagency Coordination Committee on the Validation of Alternative Methods (ICCVAM) for the major allergenic contact dermatitis test for chemicals, i.e., the Klignman guinea pig maximization test (Dean et al., 2001; ICCVAM, 1999; Stokes, 2003).

Even with a decrease in their use in research, guinea pigs have continued to increase in popularity as pets. Their “small size, cleanliness, docileness, and relatively easy maintenance” appeals to pet owners who live in smaller living spaces more common in urbanized societies (Little, 1978; Pritt, 1998). Well over 600 000 guinea pig-owning households exist in the United States (AVMA, 2007). Veterinary medical literature focused on individualized pet guinea pig medicine and surgery can now be found outside of laboratory animal medicine, allowing laboratory animal practitioners to be experts in individual animal treatments as well as herd health medicine (Hillyer, 1997; Pritt, 1998; Richardson, 1992). Veterinary information for pet guinea pigs may be lumped into so-called “pocket pet medicine” or “exotic animal” themed texts and journals (Johnson-Delaney, 1996;

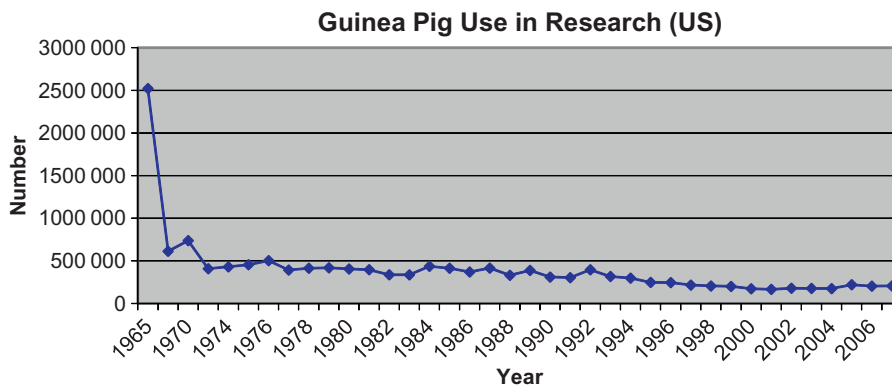


FIGURE 19.1 Guinea pig use in research (US). Data compiled from the U.S. Office of Technology Assessment (OTA, 1986), years 1965, 1967 and 1970; and USDA (2007), years 1973–2006.

Pritt, 1998). This marks a shift from just a few decades ago when most medical information for guinea pig breeders and fanciers came from books intended for children and not from veterinarians (Pritt, 1998).

Some major areas of current research involving guinea pigs include infectious diseases, allergenic assays that cannot be performed via the LLNA method, mechanisms of hearing, reproductive toxicology, and the provision of blood meal sources for blood-feeding insects such as mosquitoes.

TAXONOMY AND GEOGRAPHICAL DISTRIBUTION

Taxonomy

Cavia porcellus, the scientific name for the domesticated guinea pig, was conferred upon the species by the naturalist Erxleben in the late 18th century. The taxonomic outline for domesticated guinea pigs is as follows (Wagner, 1976):

- Kingdom Animalia
- Phylum Chordata
- Class Mammalia
- Order Rodentia
- Suborder Hystricomorpha
- Family Caviidae
- Subfamily Caviinae
- Genus *Cavia*

Guinea pigs are rodents within the hystricomorpha (porcupine-like rodents) suborder while the suborders sciuromorpha (squirrel-like rodents) and myomorpha (rat-like rodents) contain the other rodent species. Within the hystricomorpha suborder are chinchillas, porcupines, capybaras, and other rodents with a zygomatic arch where the “jugal bone forms the center block” (Wagner, 1976). An enlarged infraorbital canal is also present in many hystricomorph rodents, as with the capybara.

Members of the Caviidae family all have four digits on their forefeet and three on their hindfeet, with the soles of the feet being hairless (Harkness et al., 2002). Guinea pigs and others from the *Cavia* genus have one pair of mammae and a vestigial tail (Harkness et al., 2002).

While the tendency to gnaw and the presence of two front incisors (one set of hypsodontic incisors with the enamel being restricted to the buccal surface) were the primary reasons behind the guinea pig being classified as a rodent, several researchers have recently tried to re-classify the species outside the order Rodentia based on molecular studies of evolutionary changes to key proteins (Frye and Hedges, 1995). Accelerated evolutionary rates of change in insulin, as demonstrated by deviating amino acid sequences, led the guinea pig to have an insulin protein that deviates strongly from other mammals starting a controversy (Beintema and Campagne, 1987; Pritt, 1998). It was later noted that the apparent rapid rate of amino acid substitution and other descriptors of unique evolutionary mechanisms for guinea pigs as discussed in the literature stemmed from the species' phylogenetic position (Graur et al., 1991). From there, it was postulated that if the guinea pig was moved to another position, a “separate mammalian order distinct from the Rodentia”, known as rodent polyphyly, that the molecular phylogenetics data would make more sense (Graur et al., 1991, 1992; Ma et al., 1993). Quick refutation of the proposed reclassification of the guinea pig came with the findings that several nucleotide sequences unique and specific to rodents are also found in guinea pigs, but the controversy remained alive for several years with published research supporting both sides (Cao et al., 1994, 1997; D'Erchia et al., 1996; Frye and Hedges, 1995; Harkness et al., 2002; Kuma and Miyata, 1994; Martignetti and Brosius, 1993; Pritt, 1998). Konno and co-authors suggested that depending on which sequences were studied, amino acid versus nucleotide, different phylogenetic position (non-rodent versus rodent, respectively) could be argued (Konno et al., 1999). The controversy was essentially put to rest just a few years ago based on an eventual

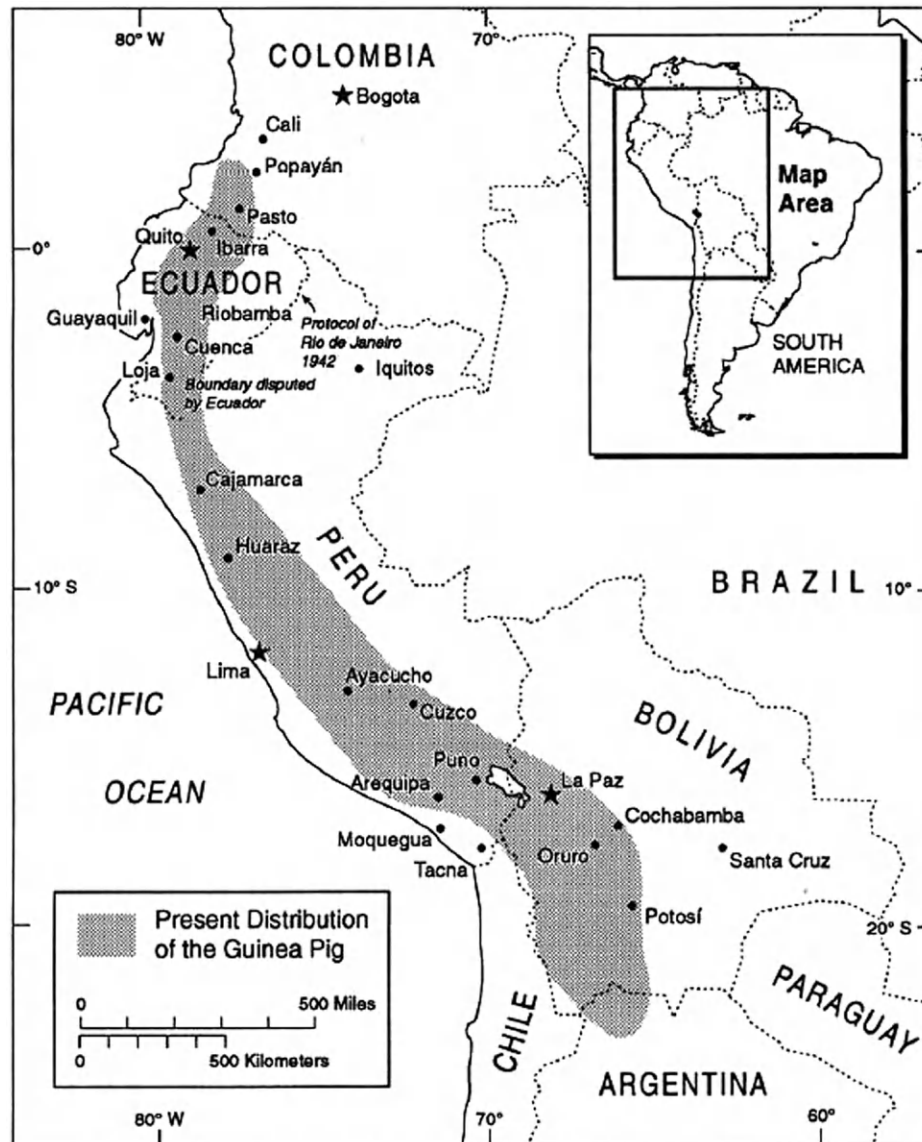


FIGURE 19.2 Current distribution of domestic guinea pigs in South America. From *The Guinea Pig: Healing, Food, and Ritual in the Andes* by Edmundo Morales Copyright © 1995 The Arizona Board of Regents. Reprinted by permission of the University of Arizona Press.

preponderance of data supporting the existing classification scheme. The guinea pig now remains in its historical classification position (Carleton and Musser, 2005).

Geographical Distribution

Peruvian archeologist Luis Lumbreras has proposed that the earliest domestication of guinea pigs occurred around 5000BC in the Altiplano area of the Andes Mountain areas in South America (Morales, 1995). Almost 4500 years later there is archeological evidence that guinea pigs were also being raised in South American coastal areas (Morales, 1995). Modern-day Peru contains the largest populations of both domestic and wild guinea pigs. Population estimates of cuy,

as guinea pigs are known in South America, in Peru put the number at approximately 30 million animals (Morales 1995). Ecuador follows next with over 10 million, then Bolivia with over 3 million, and Colombia with over 700000 (Morales, 1995). Range distribution estimates for wild guinea pigs include Venezuela, from Guiana to Argentina, and from Peru to the state of Pernambuco in Brazil (Caras, 1996; Cooper and Schiller, 1975). In South America, guinea pigs may live very closely with families, roaming in and out of village houses at will (Caras, 1996; Morales, 1995). Morales documented the current distribution of domestic guinea pigs in South America (Figure 19.2).

Movement of guinea pigs throughout the globe did not begin until thousands of years after domestication,

when the colonization of South America by Europeans resulted in an era of active global trade between the 16th and 18th centuries. Guinea pigs were transported on primarily Dutch and Spanish ships traveling from South America to Europe. Their first arrival in Europe occurred in the late 16th century by following established slave trade routes (Caras 1996; Cumberland, 1886; Wagner 1976; Weir, 1974). They might have appeared in West Africa at this time as well (Caras, 1996). Europeans first began documenting the presence of guinea pigs in South America in the literature at this time, noting that they were a common food source for the Incan empire (Caras, 1996). From Europe, guinea pigs traveled to North America with initial arrival probably occurring in the 17th century (Wagner, 1976).

Neither the Europeans nor North Americans utilized guinea pigs as agricultural animals like the South Americans, even though the climates in these diverse geographical areas are able to support efficient guinea pig growth and reproduction (Pritt, 1998; Ragland, 1979; Wagner, 1976). Instead, they became popular as pets and “fancy animals” on these continents and were kept for show, and then eventually used as research animals. By the 19th century, a strong industry for pet and show guinea pigs had emerged in Europe, particularly in England (ARBA, 1991; Cumberland, 1886; Hillyer, 1997).

Therefore, after hundreds of years of traveling throughout the world and thousands of years of domestication, the main population hubs for guinea pigs today remain South America, North America, and Europe. Guinea pigs can be found in other areas of the world typically as research animals and perhaps pets.

ORIGIN AND DOMESTICATION

Historical Considerations

Curiosity surrounds how the name “guinea pig” originated. The name itself is considered a misnomer (Cooper and Schiller, 1975; Cumberland, 1886; Morales, 1995). While the term “cavy” is perhaps more appropriate since it reflects the scientific name and South American origin of the species, the name guinea pig is more widely used in the research, veterinary literature, and popular media (Pritt, 1998; Wagner, 1976). Interestingly, fanciers and show breeders continue to use the term cavy (Wagner, 1976). Theories helping to explain the foundation of the name include (Cumberland, 1886; Morales, 1995; Wagner 1976; Weir, 1974):

- European explorers felt that they looked like suckling pigs
- They were sold for the monetary unit “guinea” in England

- People thought that they came from the country Guinea in Africa due to their travel in ships traveling along slave trade routes
- People mistook the country name Guiana in South America for Guinea, the African country.

Keeping guinea pigs as pets by Europeans and North Americans is documented as early as the very late 16th century when it was observed that Queen Elizabeth I from England owned a pet guinea pig (Morales, 1995). Such notoriety helped to contribute to the species’ popularity (Morales, 1995). Later, guinea pigs were brought to life in popular Beatrix Potter stories and inhabited the White House as pets for presidents Theodore Roosevelt and John F. Kennedy (Anderson, 1996; Comfort, 1994; Pritt, 1998). With recent depictions of guinea pigs in books and movies, they are considered a trendy pet.

Domestication

The domestication of guinea pigs occurred thousands of years ago in the Andes mountain regions of South America, most likely Peru, and since that time the guinea pig continues to be an important part of native culture life (Caras, 1996; Morales, 1995). Domestication efforts resulted in a new species, *Cavia porcellus*, which is closely related to several wild species (Caras, 1996).

Guinea pigs are used as food (called “cuy” in native languages, which also refers to the living animal) as well as in some healing and other rituals in the Andes by indigenous peoples (Morales, 1995). Their manure can be successfully used as fertilizer (Pritt, 1998). Some non-profit groups even provide small herds of guinea pigs to small villages to enable them to build sustainable agricultural programs to bring a good source of a high-protein, low-fat meat to the villagers’ diets.

Cavia porcellus is closely related to several other caviomorph species such as *C. aperea* (the smallest cavy like rodent), *C. cutleri*, *C. fulgida*, and *C. tschudii* (Clutton-Brock and Wilson, 2002; Morales, 1995; Wagner, 1976). Cavies from these related species, but not *C. porcellus*, still exist in the wild in areas of Argentina, Uruguay, Bolivia, Peru, and Brazil (Clutton-Brock and Wilson, 2002; Morales, 1995; Wagner, 1976; Weir, 1970). Wild guinea pigs live in open grasslands or marshy areas in small groups feeding on fresh fruits and resident vegetation (Clutton-Brock and Wilson, 2002; Cooper and Schiller, 1975; Wagner, 1976; Weir, 1970). These guinea pig groups are typically formed around harems, similar to breeding schemes utilized in modern-day breeding operations for research, and seek out burrows created by other animals (Cooper and Schiller, 1975). All guinea pigs are unable to climb and are “strictly terrestrial”, as well as crepuscular, thus they avoid bright sunlight and darkness (Cooper and Schiller, 1975).

Through domestication, guinea pigs became a staple agricultural animal in South America and were used as food along with llamas, shellfish, and fish (Diamond, 2005; Morales, 1995). Besides llamas and alpacas, guinea pigs were the only other animal species domesticated in South America, and the sole animal species domesticated in North, Central, and South America for food (Caras, 1996; Diamond, 2005; Dunlop, 1996). Representation of guinea pigs in ancient art is seen as early as 1400AD, signifying the importance of the species to native peoples (Morales, 1995).

Use in Research

Use of guinea pigs in science is thought to have started with Lavoisier in 1780 who used them in the measurement of heat production, although earlier records exist of the species being dissected for 17th century anatomical studies (Cumberland, 1886; Guerrini 2003). Louis Pasteur, Edward Koch, and others used them in their studies to ascertain various aspects of infectious disease pathogenesis (Guerrini, 2003). Currently, guinea pigs have the distinction of being the only New World rodent commonly used in research (Harkness et al., 2002). Less than two hundred years after Lavoisier, guinea pigs became a major research animal used in the United States with, as previously mentioned, their use peaking in the 1960s (Pritt, 1998).

Space exploration even included guinea pigs as several countries, including the Soviet Union and China, launched them into orbit starting in the 1960s (CNN, 2004; Gray, 1998). Several Nobel prizes have been awarded for research employing guinea pigs. A sampling of such research includes the development of diphtheria antiserum in 1901, the discovery of the antibiotic streptomycin in 1952, the discovery of the mechanism involved in the stimulation of the cochlea in 1961, and the identification of the structure of antibodies in 1971 (AALAS, 2002). Similar to the reasons that their popularity as a pet has grown, their docile nature, availability, ease of maintenance, and historical research database of physiological parameters have contributed to their widespread use in research (Harkness et al., 2002).

It was in North America “that use in scientific research became a defining part of the species” (Pritt, 1998). Dunkin and Hartley established the widely utilized out-bred, albino strain (Figures 19.3 and 19.4) that has been the mainstay of biomedical research for nearly 100 years (Wagner, 1976). Since then, several other strains for research have been developed. Historically, guinea pigs have been used in allergy (including delayed hypersensitivity, anaphylaxis, and asthma), genetic, immunology, infectious disease, nutritional, auditory, and other areas of research (Harkness et al., 2002).



FIGURE 19.3 Dunkin Hartley neonates.



FIGURE 19.4 A Dunkin Hartley young adult in a breeding pen. (Photograph courtesy of Elm Hill Breeding Labs).

Breed Formation

As with other rodents used in animal-based research, the breeds used in laboratory animal facilities were developed to meet a burgeoning need for science starting over 100 years ago (Dunlop and Williams, 1996). Breed formation for guinea pigs traditionally focused on hair coat structure and/or texture, color, and length along with albino versus non-albino features (Castle, 1905). All breeds can intermix (Harkness et al., 2002). Individual animal size generally does not differ between the common breeds. Given the prevalent use of guinea pigs as fancy animals, meaning they are used for show, it was natural that Europeans, and particularly the English, were the first to develop acknowledged breeds in the 19th and very early 20th centuries. Commonly recognized breeds like Abyssinians (Figure 19.5) with their unique cowlicks called “swirls” and “rosettes” in their fur were first identified in England while Peruvians (Figure 19.6) with their long fur brushing the ground were first identified in Paris.

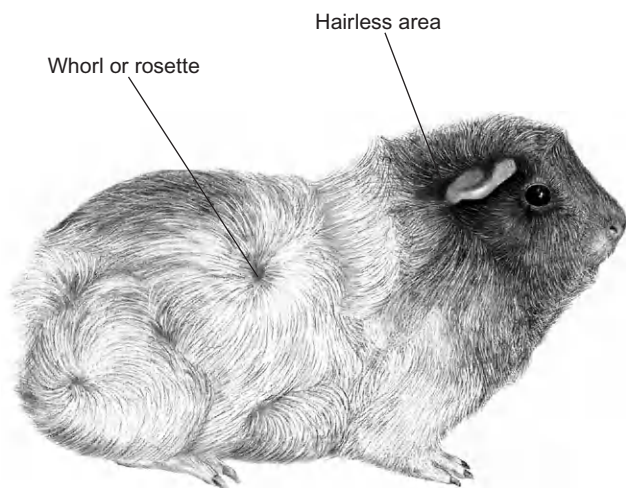


FIGURE 19.5 Rough-haired Abyssinian Guinea Pig (adult). Reprinted from Cooper and Schiller (1975) by permission of Harvard University Press.

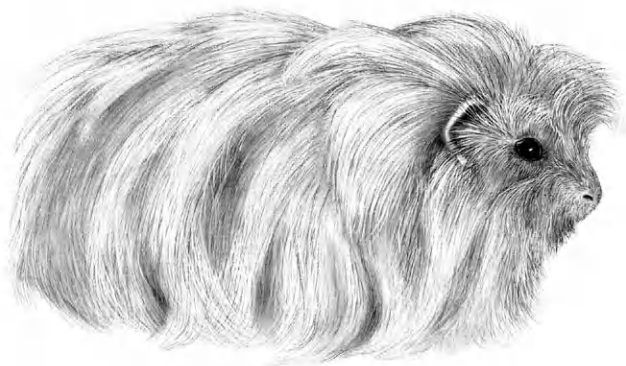


FIGURE 19.6 Long-haired Peruvian Guinea Pig (adult). Reprinted from Cooper and Schiller (1975) by permission of Harvard University Press.

The Zoological Society of London had the responsibility of naming these emerging breeds (Wagner, 1976). For the pet and fancy industries, several breeding groups including the American Cavy Breeders Association (ACBA) and the British Cavy Council as well as other clubs in British commonwealth countries establish contemporary breed standards, of which dozens exist.

American (smooth-coated, short-haired guinea pigs also called English), Abyssinian, and Peruvians are the three most frequently encountered pet guinea pig breeds today. Fancy animal breeders pay special attention to coat color and these breeds can display a wide variety of color combinations with white, dark brown, and gray being the most common and black being the rarest (Morales, 1995).

Laboratory guinea pigs throughout the world are largely derived from the strain begun by Dunkin and Hartley in 1926 (Wagner, 1976). These strains are out-bred, albino, smooth-coated, and usually referred to as “Dunkin-Hartleys” or “Hartleys.” Research animal vendors will sell

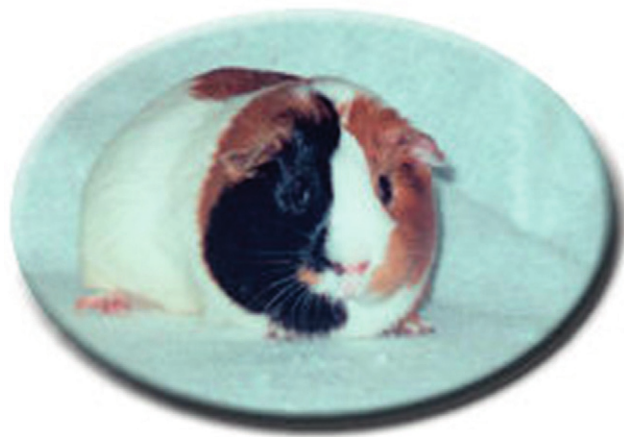


FIGURE 19.7 Tri-color outbred guinea pig for research. (Photograph courtesy of Elm Hill Breeding Labs.).

them as specific pathogen-free (SPF) and/or virus antibody free (VAF). At least one albino, hairless strain that is euthymic and immunocompetent is commercially available for dermatology research (CRL, 2009).

On occasion, multi- or tri-colored Americans (also called “American short-hair” or “English short-hair”) or Abyssinians may be used in the research environment (Figure 19.7). Their pigmented coloration allows for studying certain hearing structures and functions that cannot be studied in the albino breeds available from research-only guinea pig vendors. These breeds may or may not be SPF and/or VAF. Laboratories using these breeds are encouraged to work with the vendor to perform pre-shipment disease testing and/or implement an appropriate health-monitoring program to ensure an acceptable health status to support research.

GENETICS

Genetic Features

Festing (1976) describes two eras of guinea pig genetics, the first era starting in 1900 and extending into the 1960s and the second beginning in the 1960s. The first era was distinguished by studies on phenotypical differences such as coat color and fur characteristics while the second era focused on biochemical, physiological, immunological, and other traits and which could be argued continues today. Festing also noted that mutants described in the literature had not been adequately conserved for future study, a trend that continues today.

Early genetic work in guinea pigs focused on the inheritance of coat color which is complex. Comprehensive reviews of coat color, including post-natal changes in coat color appearance, began in the early 20th century and provide information that is still

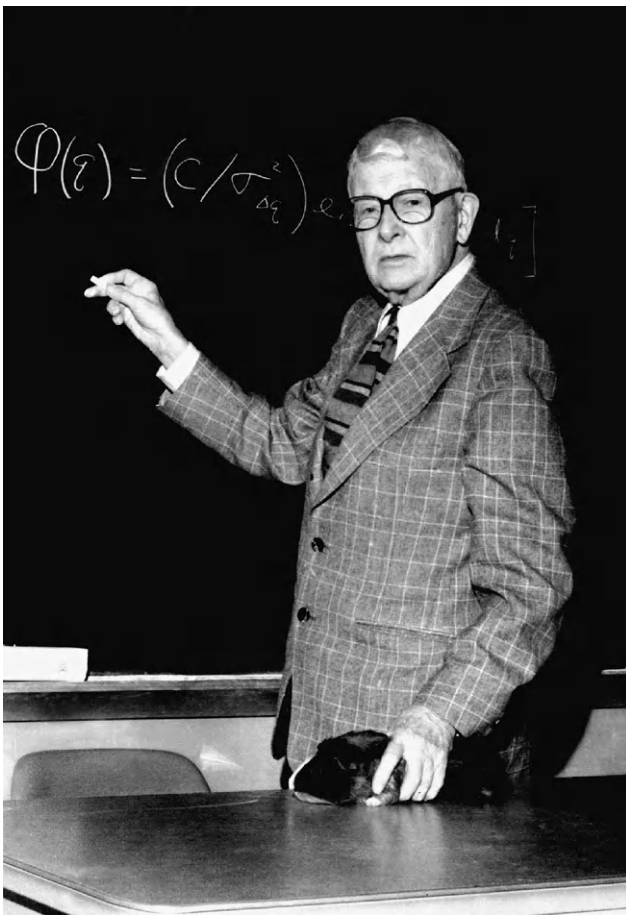


FIGURE 19.8 Dr. Sewall Wright with guinea pig, 1950s (approximate). (Image K9637-1 from the United States Department of Agriculture (USDA) Agricultural Research Service Image Gallery).

used by fancy guinea pig breeders today (Castle, 1905; Chase, 1939; Ginsburg, 1943; Gurney, 2000; Heidenthal, 1939; Wolff, 1954; Wright, 1959, 1960a, 1960b). Several of these articles were authored by Dr. Sewall Wright who is credited with the development of the two most widely used inbred guinea pig strains, strains 2 and 13 (Figure 19.8). A compilation of the genetics involved with coat pigmentation and length can be found in the 1976 publication, *The Biology of the Guinea Pig*. The mode of inheritance for these traits is believed to be controlled by single gene loci although mixed phenotypes resulting from gene interaction do occur, e.g., different pigments or intermediate hair lengths (Festing, 1976).

Later studies focused on genes controlling histocompatibility, delayed hypersensitivity, anaphylaxis, and complement. Several strains of guinea pigs are based on these genetic differences, as described later in this chapter. Various congenital anomalies have been reported by Wright and others with extremely low occurrence rates (Festing, 1976). Overall, there has been a lack of interest in commercializing many of the strains described here.

Outbred Strains

Dunkin-Hartley outbred, smooth-coated, albino guinea pigs as described previously are utilized as the standard strain for biomedical research. Two sublines of the Dunkin-Hartley strain, Hartley A and B, with unique characteristics, were defined and characterized by a research group from the New York State Department of Health laboratories in Albany, New York, and are referenced in several research articles. Rarely, other Hartley strains have been described.

The Hartley A or Albany strain was discovered to have a natural resistance to *Treponema pallidum*, the causative agent of syphilis. Only 10–17% of the Hartley A strain succumb to infection while 80–100% of other strains display susceptibility to infection by the pathogen (Wicher K et al., 1985, 1992; Wicher V et al., 1990; Zhao et al., 1992). This strain arose as a closed colony with a period of inbreeding that was preceded and followed by random breeding, and is reported to be genetically related to inbred strain 2 (Wicher K et al., 1985; Zhao et al., 1992). The Hartley B strain, also described by the Wicher group, arose from the Buckbery Animal Farm in New York (Wicher K et al., 1985). Descriptions of the Hartley A and B strains are scant outside of the syphilis research performed at the Albany laboratory, and their impetus for establishing the strains came from the need for an inbred species as an alternative to the rabbit to study adoptive transfer of cellular immunity in syphilis (Wicher K et al., 1985; Wicher V et al., 1987).

Occasional reports of a multi-colored, outbred, BFA strain (Figure 19.9) from a Charles River Laboratories facility in Germany exist. This strain is no longer available from Charles River Laboratories but can be found in other locations such as a medical center in Leipzig (Charles River Deutschland, n.d.; Giroux, 2010; Medizinisch-Experimentelles Zentrum, 2007). Originating in 1963 from a federal labor office in Tübingen, Germany, it was transferred to the University of Zurich a few years later and then eventually to Charles River. Its name stems from the initials for Germany's federal labor office. A defining characteristic of this strain is its ability to produce large amounts of complement rapidly (Charles River Deutschland, n.d.). Research uses for this strain include parasitology, teratology, pharmacology, and dermatology (Charles River Deutschland, n.d.; Yamashita et al., 2004).

Inbred Strains

Evolutionary theorist and geneticist Sewall Wright began his work with guinea pigs in the early 1900s at the United States Bureau of Animal Industry, the agency that would eventually become the United States Department of Agriculture (USDA) (Banks,

1989; Lescoufflair, n.d.). Wright obtained his Ph.D. from Harvard and his thesis was on the coat colors of guinea pigs (Lescoufflair, n.d.). His prolific scientific research career gave rise to several inbred strains of guinea pigs, two of which are still used, Strains 2 and 13. However, some published reports state that that work on the strain 13 guinea pig began as early as 1906 for histocompatibility (Stone and Amsbaugh, 1984; Wagner, 1976). In addition to noting that inbred strains of guinea pigs had smaller litter sizes, adding to the concept of hybrid vigor, Dr. Wright also founded the concept of genetic drift, which was initially called the Sewall Wright effect (Fisher and Ford, 1950; Joshi, 1999).

Strains 2 and 13 may also appear in the literature as strain 2N or 13N (Trahan, 1987). Although not available from commercial research animal vendors in the United

States at this time, scattered colonies of these strains exist at some university, government, and military facilities. Guinea pigs from non-commercial sources may not be SPF and/or VAF, so health testing and monitoring programs as described elsewhere in this text should be considered. Both inbred strains are multicolored as well as having lower growth rates and less robust physiological parameters compared to outbred Dunkin-Hartley guinea pigs (Liu, 1988).

Several studies document behavioral and physiological differences between strains 2 and 13 guinea pigs. Major differences are provided in Table 19.1.

Strains 2 and 13 are now used in studies pertaining to infectious agents (*Bordetella bronchiseptica*, *Mycobacterium* spp., *Treponema* spp., vaccinia virus, Junin virus, and Pichinde virus) and serve as animal models for experimental allergic encephalomyelitis, leukemia, experimental retinal autoimmunity and hepatic toxicity (Barna et al., 1984; Cohen, 1987; Connolly et al., 1993; Devalle, 2005; Evans and Miller, 1982; Fujiwara et al., 1981; Guo and Liu, 1991; Holt et al., 2002; Huang et al., 1996, 1998; Karlick et al., 1989; Lind et al., 1989; Liu, 1988; Liu and Guo, 1991, 1992; Meyers-Elliott et al., 1983; Preece et al., 1993; Qian et al., 1992, 1993, 1994; Raskin and Arnon, 1981; Sobel and Colvin, 1985; Trahan et al., 1987; Traugott, 1983; Schaeffer and Bitrick, 1993; Stephenson et al., 1989; Wicher et al., 1992; Yun et al., 2008).

Several inbred strains of guinea pigs were created in Japan in the late 1970s based on coat color (Figure 19.10). These strains were designated JY 1, JY 2, JY 3, JY 6, JY 9, and JY 10 (Chiba et al., 1978). Additional strains designated JY 4, JY 5, JY 7, JY 8, and JY G have subsequently been described but only JY 1 and 2 are currently available (NIBIO, 2006). Only scattered reports, mostly regarding histocompatibility complexes and immune responses, about these strains appeared in the literature 30 years ago. The two strains still in use are currently



FIGURE 19.9 BFA guinea pig. (Photograph courtesy of Charles River Laboratories).

TABLE 19.1 Behavioral and Physiological Differences Between Strains 2 and 13 Guinea Pigs

Areas of Difference	Strain 2	Strain 13	References
Lordosis	More ready elicitation of lordosis		Thornton et al., 1987
Dietary insufficiencies		Increased sensitivity	Cohen et al., 1987
Body weight	Higher daily body weight gains		Liu, 1988
Immune Response	Varied differences		Asano et al., 1981; Clark and Shevack, 1982; Clement et al., 1980; Dos Reis and Shevach, 1983; Hasløv et al., 1990
Research models – experimentally induced allergic encephalomyelitis	Resistant	Susceptible	Raskin and Arnon, 1981; Traugott, 1983
Research models – cataracts		Congenital nuclear cataracts due to autosomal dominant trait	Bettelheim et al., 1997; Stone and Amsbaugh, 1984

utilized in superovulation, embryo transfer, and embryo culture research (Chiba and Egashira, 1978; Suzuki et al., 1993, 2000, 2003).

Another inbred strain, Weiser-Maples, was developed in the United States at the Buckshire Corporation (Perkasie, PA, USA) in 1977 but subsequently moved to Japan (Inaba and Wakisaka, 1992; Kitagaki et al., 2005). A “uniform response to experimental tuberculosis”, “radical and vertical growth patterns” of skin melanocytes similar to malignant melanomas in people, strong responses to exposure to UV radiation, and congenital skeletal malformations are features of this strain (Inaba et al., 1992; Kitagaki et al., 2005; Maeda and Naganuma, 1998). Weiser-Maples apparently have a similar appearance to the JY 2 strain pictured here (Suzuki, 2010).

Published Mutations

The complement 4-deficient, also known as C4D or C4D/N, strain was discovered and established at the National Institutes of Health (NIH) from a spontaneous mutation in 1970 (Banks, 1989; Ellman et al., 1970; Wicher K et al., 1985). Described as either outbred or partially inbred, the strain contains a haplotype identical to inbred strain 13 (Wicher K et al., 1985; Zhao et al., 1992). The complete lack of the fourth component of complement is transmitted as an autosomal recessive trait (Ellman et al., 1970). Of the strains studied for susceptibility to syphilis, it is described as the most susceptible strain with up to 100% of the test populations developing disease after exposure to *Treponema pallidum* (Wicher K et al., 1985; Wicher V et al., 1987; Zhao et al., 1992). Many other immunologic reactions in this strain are considered to be normal (Banks, 1989).

Waltzing (Wz) guinea pigs have a mutation resulting in atrophy of the organ of Corti beginning shortly after birth, which results in deafness and “waltzing” or

circling behaviors (Banks, 1989; Festing, 1976). Initially the hair cells begin to atrophy and disappear and then subsequently the other components of the organ of Corti meet the same fate (Festing, 1976). The mutation occurred spontaneously in a guinea pig colony at the NIH in 1953 (Banks, 1989; Festing, 1976). As an autosomal dominant condition, homozygosity leads to high levels of perinatal mortality despite the lack of observable anomalies (Banks, 1989; Festing, 1976).

German waltzing guinea pigs are from a spontaneous mutation found in a guinea pig breeding facility in Bayreuth, Germany in 1996 and were first described in the literature in 1998 (Ernstson and Ulfendahl, 1999; Kawaguchi et al., 2007; Skjõnsberg, 2006). Affected animals display deafness, balance disorders, and the characteristic “waltzing”, from birth (Skjõnsberg, 2006). After identification, the strain was transferred to the Karolinska Institute in Solna, Sweden, where it was subsequently bred (Skjõnsberg, 2006). Unlike the earlier described waltzing guinea pigs, the German origin mutation is a recessive inner ear defect arising from a collapse of the Resinner’s membrane and an absence of the scala media (Kawaguchi et al., 2007). Homozygotes are affected whereas heterozygotes have normal behaviors with an increased resistance to noise-induced trauma and an “augmented” response to the ototoxic drug cisplatin (Skjõnsberg, 2006; Skjõnsberg et al., 2008). Both homozygotes and heterozygotes have albuminuria and sometimes hematuria (Ernstson and Ulfendahl, 1999). German waltzing guinea pigs serve as an animal model of human vestibular atelectasis since the morphology of the mutation and human disease are identical (Kawaguchi et al., 2007).

A hairless and immunodeficient, mutant guinea pig strain was noted from a spontaneous mutation in a closed breeding colony of Dunkin-Hartley guinea pigs at the Eastman Kodak Company in 1979 (Banks, 1989; Reed and O’Donoghue, 1979). Affected animals were described as being smaller at birth, having wrinkled skin, and stunted vibrissae (Banks, 1989; Reed and O’Donoghue, 1979). Hair shaft production was abnormal, resulting in the hairlessness. Animals were either athymic or hypothyroid resulting in immunodeficiencies including agammaglobulinemia). Due to the immunodeficiencies, these mutants experience a shortened lifespan and die from infections common in immunodeficient animals including cytomegalovirus, balanitis, *Pneumocystis carinii* (Banks, 1989; Reed and O’Donoghue, 1979).

Another albino, immunocompetent, hairless mutant strain (Figure 19.11) is commercially available (CRL, 2009). This strain was initially identified in 1978 at the Institute Armand Frappier in Montreal, thus giving the strain the designation of IAF (CRL, 2009). Hairlessness comes from defective hair shafts (Banks, 1989). Charles

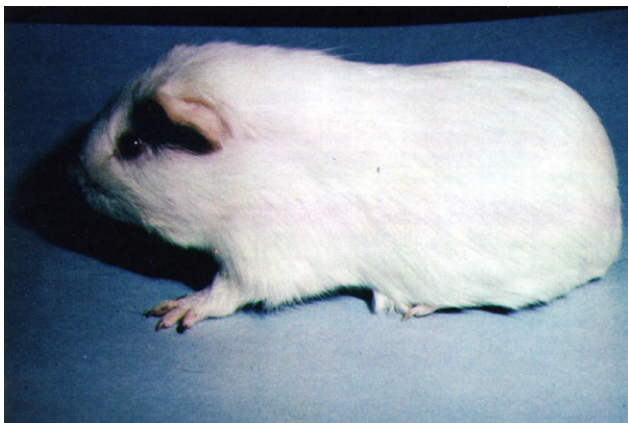


FIGURE 19.10 JY strain guinea pig. (Photograph courtesy of Osamu Suzuki, Laboratory of Experimental Animal Models, National Institute of Biomedical Innovation, Osaka, Japan).



FIGURE 19.11 IAF hairless guinea pig. (Photograph courtesy of Charles River Laboratories).

River Laboratories acquired the strain in 1982. Use of this strain is limited mostly to dermatology research (CRL, 2009).

Polydactyly exists as a heterozygous condition (Festing, 1976). The homozygous condition results in a “polydactylous monster” that typically dies in utero (Wright, 1934, 1935). Festing reported that lines “breeding true” for this condition have been developed (1976). However, there are no reports of such lines in the current veterinary and scientific literature.

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Anatomy, Physiology, and Behavior

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INTRODUCTION

The domestic guinea pig is a descendant of the wild cavy (*Cavia aperea*) which is a common rodent in South America (Kunzl and Sachser, 1999). It is a non-burrowing, herbivorous, crepuscular, hystricomorph (porcupine-like) rodent with a stocky body, short neck and limbs, and with either no tail or a vestigial one. This rodent is considered to be more closely related to chinchillas and porcupines than to mice and rats (North, 1999). The adult male weighs between 900–1200 grams and the smaller female weighs between 700–900 grams, except during pregnancy (Quesenberry et al., 2004). Their lifespan ranges from 2–8 years with the breeding female having the shorter lifespan (North, 1999).

EXTERNAL ANATOMY

Hair

The hair consists of large guard hair and a finer hair undercoat (Quesenberry et al., 2004). Depending on the breed of guinea pig, it can be a variety of colors (mono, bi-, or tricolored): fine or rough; arranged in whorls or rosettes; and long, medium, or short in length (Harkness et al., 2002). Between the sexes, there are no differences in coat patterns or colors (Manning et al., 1984). Color is influenced by the genotype at several minor and six main coat-color loci with some having multiple alleles (North, 1999; Terril and Clemons, 1998). Guinea pigs are typed according to the length, direction, and texture of their hair. Examples of the short smooth-haired variety include the English (Duncan-Hartley and Hartley strains) and American. The Peruvian has long hair, the Abyssinian has short hair that grows in either rosettes or whorls resulting in the rough pattern, and the Silky has medium-length soft hair (Harkness et al., 2002; Quesenberry, 1994).

Each hair follicle has an androgen-dependent sebaceous gland that is most abundant along the dorsum and around the anus (Breazile and Brown, 1976; Quesenberry et al., 2004). Externally, the glandular area is slightly elevated with pigmented skin and greasy fur (Lipkow, 1953). The glands are more developed in the male and are used to secrete a sexual attractant and for marking of territory. In older, overweight animals, overproduction of the glands results in a greasy, matted dorsum.

The hairless areas of the body include the external orifices to the respiratory, digestive, urogenital systems, around the inguinal mammary glands, and caudal to the ear pinna. The hairless area behind the ear is larger in albino guinea pigs. In addition, this area lacks sebaceous and sweat glands (Zackheim and Langs, 1962).

Vibrissae are located around the eyes dorsal to the medial canthus and caudal to the lateral canthus, lateral to the nose, above the upper lip in 5–6 rows, and under the chin (Figure 20.1). These long tactile hairs are known as supraorbital, infraorbital, nasal, buccal or mystacial, and mental vibrissae, respectively (Cooper and Schiller, 1975). Not observed in guinea pigs are interramal and suborbital vibrissae which are present in other rodents (Cooper and Schiller, 1975).

Ear

The ear has cartilaginous and osseous sections. The large external ear (pinna) consists of prominent cartilaginous ridges and folds and an external meatus that leads to the long ear canal. The dorsorostral pinna forms the concha and the caudodorsal border is known as the helix (Cooper and Schiller, 1975). The cartilaginous portion of the guinea pig ear is approximately two to three times the length of the osseous portion of the external auditory meatus. Due to the length and tortuosity of the cartilaginous canal, it is extremely difficult to visualize the tympanic membrane. An important, unique anatomical feature of the external ear canal and bony portion of the guinea pig ear is that the extratemporal portion of the facial nerve lies in close proximity to the caudal and ventral aspects of the cartilaginous ear canal at its junction with the osseous canal (Cooper and Schiller, 1975). This is due to the unusual location of the stylomastoid foramen in the temporal bone which is positioned caudal and dorsal to the external osseous auditory canal.

Nose

This hair-covered, triangular-shaped structure consists of two nares with hairless perimeters. The nares are S-shaped, longitudinal slits and oblique dorsoventrally (Cooper and Schiller, 1975). The shape of each nostril results from the imperfect cartilaginous ring that supports the overlying skin and inner lining of respiratory epithelium (Cooper and Schiller, 1975). The nares come together at the midline dividing the upper lip into two halves. This cleft upper lip, known as a philtrum, is also observed in hamsters and rabbits.

External Reproductive Anatomy

Both the male and female guinea pig have a single pair of mammary nipples located in the inguinal area, however, the sow's nipples are more prominent and longer. Only in the female are mammary glands present.

The female genital region is a Y-shaped depression in the perineal tissues (Figure 20.2). The top branches of the Y point cranially and surround the urethral opening. A paired lateral labia with the vulvar opening lies

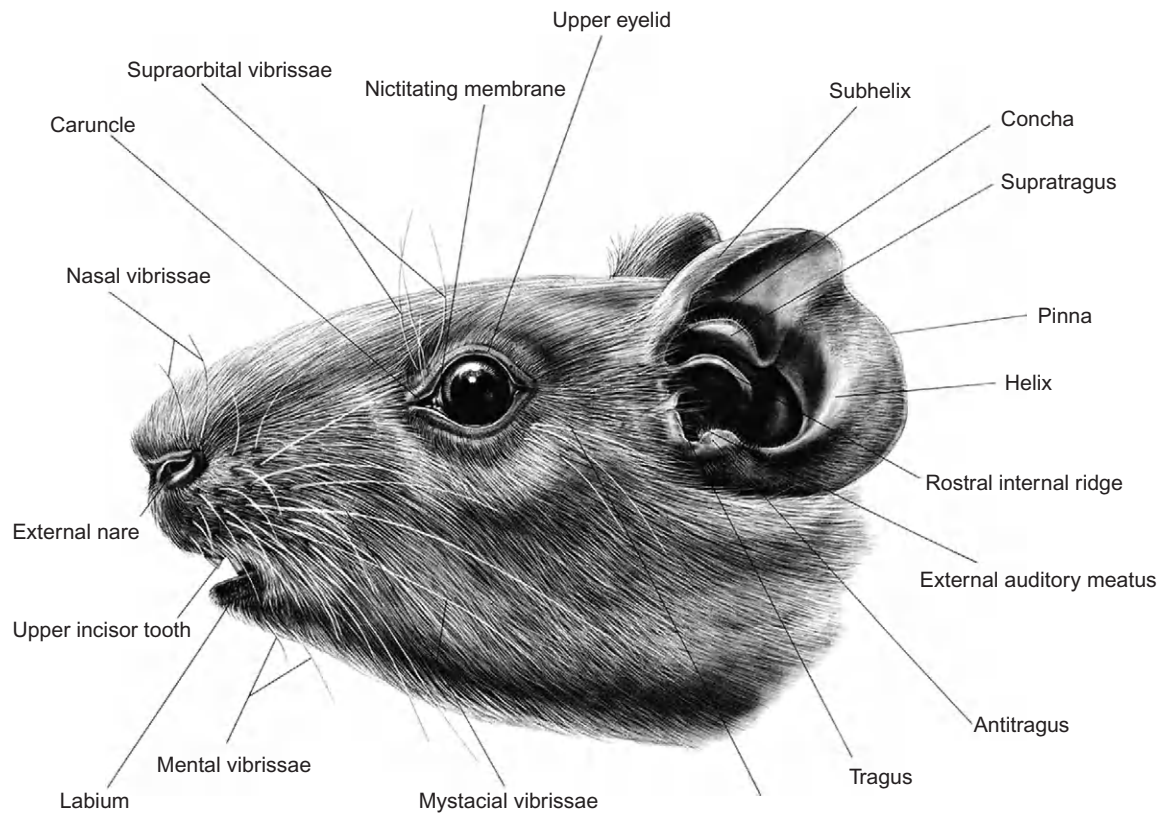


FIGURE 20.1 Lateral aspect of the head showing vibrissae. Reprinted by permission of the publisher from *Anatomy of the Guinea Pig* by Gale Cooper, M.D. and Alan L. Schiller, M.D., Cambridge, Mass.: Harvard University Press, Copyright © 1975 by the President and Fellows of Harvard College.

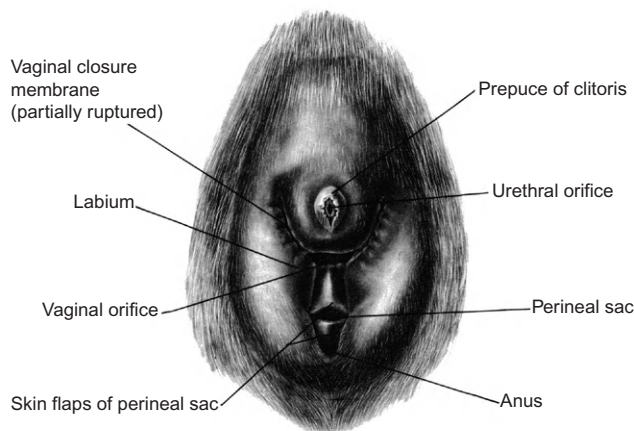


FIGURE 20.2 Female external genitalia. Reprinted by permission of the publisher from *Anatomy of the Guinea Pig* by Gale Cooper, M.D. and Alan L. Schiller, M.D., Cambridge, Mass.: Harvard University Press, Copyright © 1975 by the President and Fellows of Harvard College.

centrally at the intersection of the branches, and the anus is located at the base of the Y (Quesenberry et al., 2004). The perineal sac is a bilateral diverticula from the anal mucocutaneous junction and separates the vagina from the anus. The sac is well developed and surrounded by large sebaceous glands which secrete into the sac. It can be filled with oily fluid, skin debris, and trapped hair (Cooper and Schiller, 1975).

A male guinea pig can be easily identified due to obvious scrotal pouches which are visible as a pair of swellings on either side of the perineum (Figure 20.3). Each pouch contains the testis, epididymis, caudal end of the spermatic cord, and fat bodies (Cooper and Schiller, 1975). The ridge between the pouches is the glans of the penis. Unique to hystricomorphs, the intro-mittent sac is located within the ventral surface of the glans penis (Cooper and Schiller, 1975). This long cylindrical sac opens as a transverse slit caudal to the urethral orifice. During erection, the sac everts and two keratinaceous horny styles (3–5 mm long) which are attached to the caudal end, protrude externally (Cooper and Schiller, 1975). The function of the styles is unknown.

The most cranial orifice is the urethral opening covered by the skin of the prepuce and the most caudal orifice is the anus (Terril and Clemons, 1998). The penis

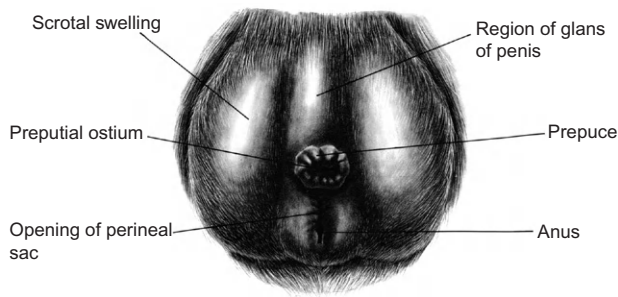


FIGURE 20.3 Male external genitalia. Reprinted by permission of the publisher from *Anatomy of the Guinea Pig* by Gale Cooper, M.D. and Alan L. Schiller, M.D., Cambridge, Mass.: Harvard University Press, Copyright © 1975 by the President and Fellows of Harvard College.

can be everted from the prepuce by placing gentle pressure at its base (Quesenberry et al., 2004).

MUSCULOSKELETAL SYSTEM

The skeletal system consists of the axial portion (head, hyoid apparatus, ribs and sternum, vertebrae); the appendicular portion, consisting of the pectoral and pelvic girdles and limbs; and the heterotopic portion which includes the os penis and sesmoids. The number of bones (256–261) varies in the guinea pig (Cooper and Schiller, 1975).

Axial Skeleton

Head

The major regions of the skull are the cranium, mandible, facial, and palatal (Cooper and Schiller, 1975). The skull has complete, prominent zygomatic arches which are a hystricomorph characteristic with large orbits and deep pterygopalatine fossae (Landry, 1957).

The triangular mouth consists of maxillary bones and the largest bone in the skull, the mandible, leading to a small oral cavity. The mandible is flared caudally (Gannon et al., 1994). The total dental formula is 20 which is I 1/1, C 0/0, P 1/1, M 3/3 (Breazile and Brown, 1976). All 20 teeth are open rooted and erupt continuously (hypsodontic) (Harkness et al., 2002). Only one set of teeth (monophyodont) is present with no primary teeth. In comparison, developing two successive sets of teeth, one deciduous and one permanent is known as diphyodont. The incisors are white and not yellow as observed in other rodents. The lower incisors are longer than the upper incisors (Harkness et al., 2002). The molars lack cusps and possess a groove or deep indentation (Cooper and Schiller, 1975). The toothless space between the incisors and molar teeth is known as the diastema. The upper premolar and molar occlusive surfaces are positioned

towards the buccal mucosa and overgrowth of these maxillary teeth will occur laterally resulting in buccal mucosal ulcerations (Quesenberry, 1994; Quesenberry et al., 2004). The mandibular cheek teeth occlusive surfaces are positioned towards the center of the mouth and overgrowth of these teeth occurs medially, which can cause tongue entrapment (Quesenberry, 1994; Quesenberry et al., 2004). The premolars and molars are the most common to overgrow (Harkness et al., 2002). Incisor overgrowth often occurs secondary to the cheek teeth malocclusion (Quesenberry, 1994). Malocclusion can be due to genetics, trauma, infection, or nutrition.

The muscles of mastication include the masseter (superficial, medial, and lateral layers), temporalis, and pterygoideus (lateral and medial) (Cooper and Schiller, 1975; Myers et al., 2006). The four rodent suborders Myomorpha (mice and rats), Sciuromorpha (squirrels), Hystricomorpha (South American caviomorph rodents and porcupines), and Protogomorpha (mountain beaver) are based on differences in the muscles of mastication and other characteristics of the masticatory apparatus (Druzinsky, 2010; Wood, 1955). The origin of the masseter muscle and the point of insertion on the mandible differ within the rodent suborder. Myers et al. (2006) gives a description and illustrations on the differences. A hystricomorph characteristic is the well-developed masseter muscle (Cooper and Schiller, 1975). Herbivores, including the guinea pig, use the muscles of mastication (Figure 20.4) for gnawing and grinding rather than chewing.

Thorax

Vestigial clavicles are present which attach laterally to the small coracoid processes of the scapula with some references stating the attachment is medially to the sternal manubrium (Breazile and Brown, 1976) or without sternal attachment (Cooper and Schiller, 1975). The sternum consists of the presternum, manubrium, three sternebrae and xyphoid processes. There are 14 pairs of ribs and the first six pairs of true ribs articulate with the sternum. Ribs 7–9 (false ribs) do not directly attach to the sternum, however, they articulate with the sixth rib cartilage. The floating ribs (10–14) do not articulate with the sternum. The vertebral column is composed of 32–36 vertebrae, of which are seven cervical, 13–14 thoracic, six lumbar, 2–3 sacral, and 4–6 caudal (Breazile and Brown, 1976).

Appendicular Skeleton

The suborder, Hystricomorpha, is distinguished by a separate fibula and tibia (Cooper and Schiller, 1975). There are four digits on each front foot (manus; Figure 20.5) and three digits on each hind foot (pes; Figure 20.6). Each digit consists of three phalanges (proximal, middle, and distal) with the distal one arched

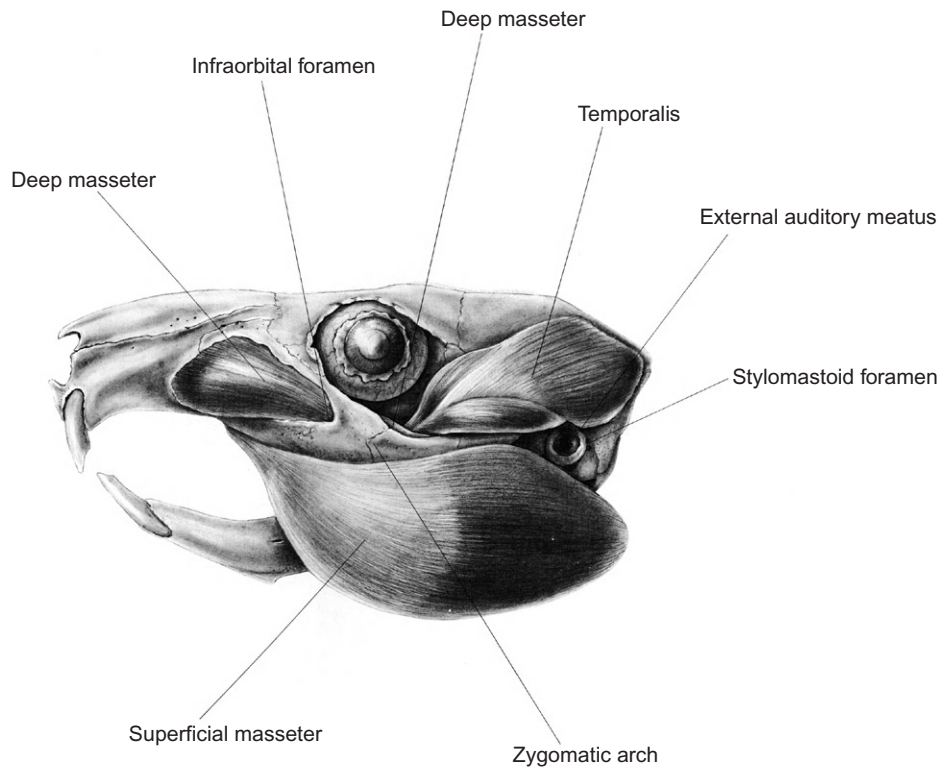


FIGURE 20.4 Lateral aspect of muscles of the head showing a well-developed masseter muscle. Reprinted by permission of the publisher from *Anatomy of the Guinea Pig* by Gale Cooper, M.D. and Alan L. Schiller, M.D., Cambridge, Mass.: Harvard University Press, Copyright © 1975 by the President and Fellows of Harvard College.

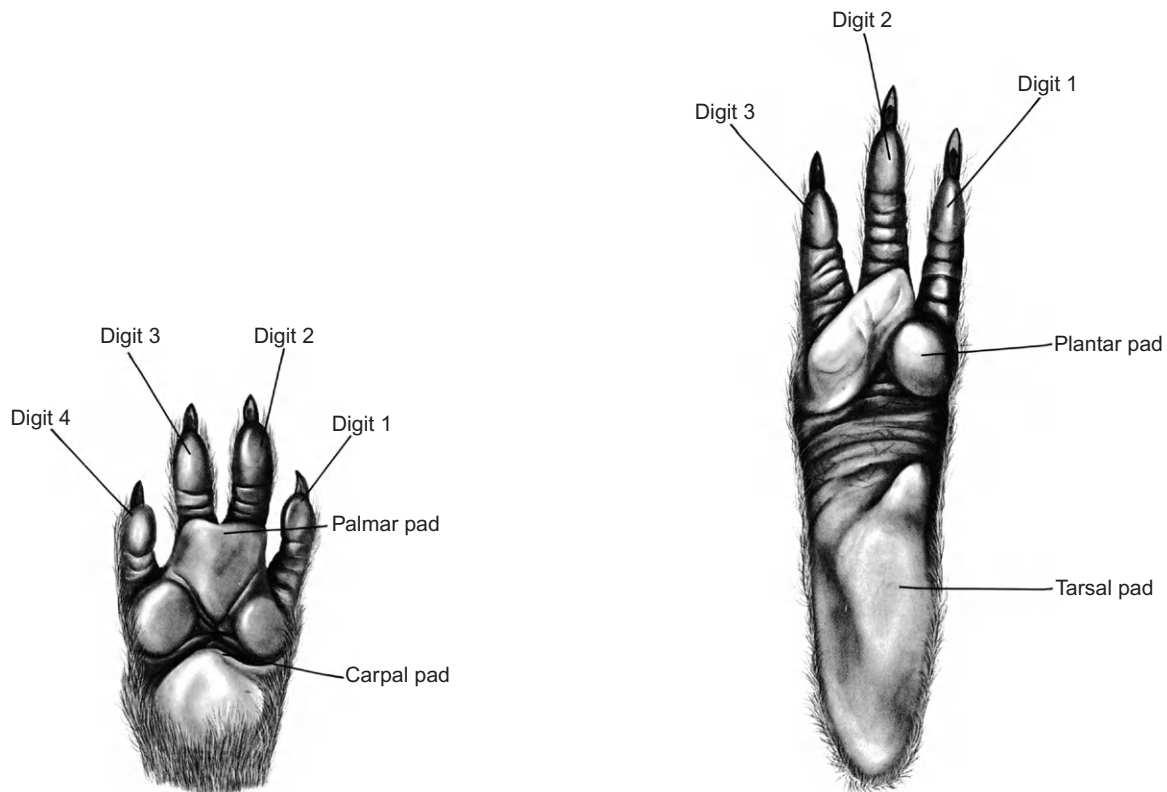


FIGURE 20.5 Palmar aspect of the right manus. Reprinted by permission of the publisher from *Anatomy of the Guinea Pig* by Gale Cooper, M.D. and Alan L. Schiller, M.D., Cambridge, Mass.: Harvard University Press, Copyright © 1975 by the President and Fellows of Harvard College.

FIGURE 20.6 Plantar aspect of the right pes. Reprinted by permission of the publisher from *Anatomy of the Guinea Pig* by Gale Cooper, M.D. and Alan L. Schiller, M.D., Cambridge, Mass.: Harvard University Press, Copyright © 1975 by the President and Fellows of Harvard College.

having a curved nail (Cooper and Schiller, 1975). The nails are longer on the hind digits. The footpads are well developed on the hairless sole of the foot (Teril and Clemons, 1998). Ambulation occurs by alternating all four feet and the animal's weight is distributed on all legs with only the foot pads touching the surface (Bays, 2006). When running, the legs also alternate and when excited or playing, the rear legs occasionally kick out from behind (Bays, 2006).

Pelvis

The pelvic girdle consists of the ilium, ischium, and pubis bone which is known as the os coxae. As the animal matures, the acetabular bone fuses with the three bones to form the acetabulum. Each os coxae is joined at the midline at the pelvic symphysis which consists of symphysis pubis and symphysis ischii and in most

animals, the connection remains cartilaginous (Breazile and Brown, 1976). Ossification of the symphysis may occur in older males. As relaxin production increases in the pregnant guinea pig, the symphysis will soften and the two halves will separate (7–8 mm) in preparation for the delivery of the large fetuses (Hisaw, 1926; Hisaw et al., 1944). The female should be bred before 6 months of age to prevent dystocia that can occur due to pelvic symphysis (Figure 20.7) fusing as the suture line mineralizes (Bays, 2006).

Heterotrophic Skeleton

On the dorsal surface of the glans of the penis is a thin, small bone known as the os penis. This bone is found the entire length of the glans and is approximately 10 mm long.

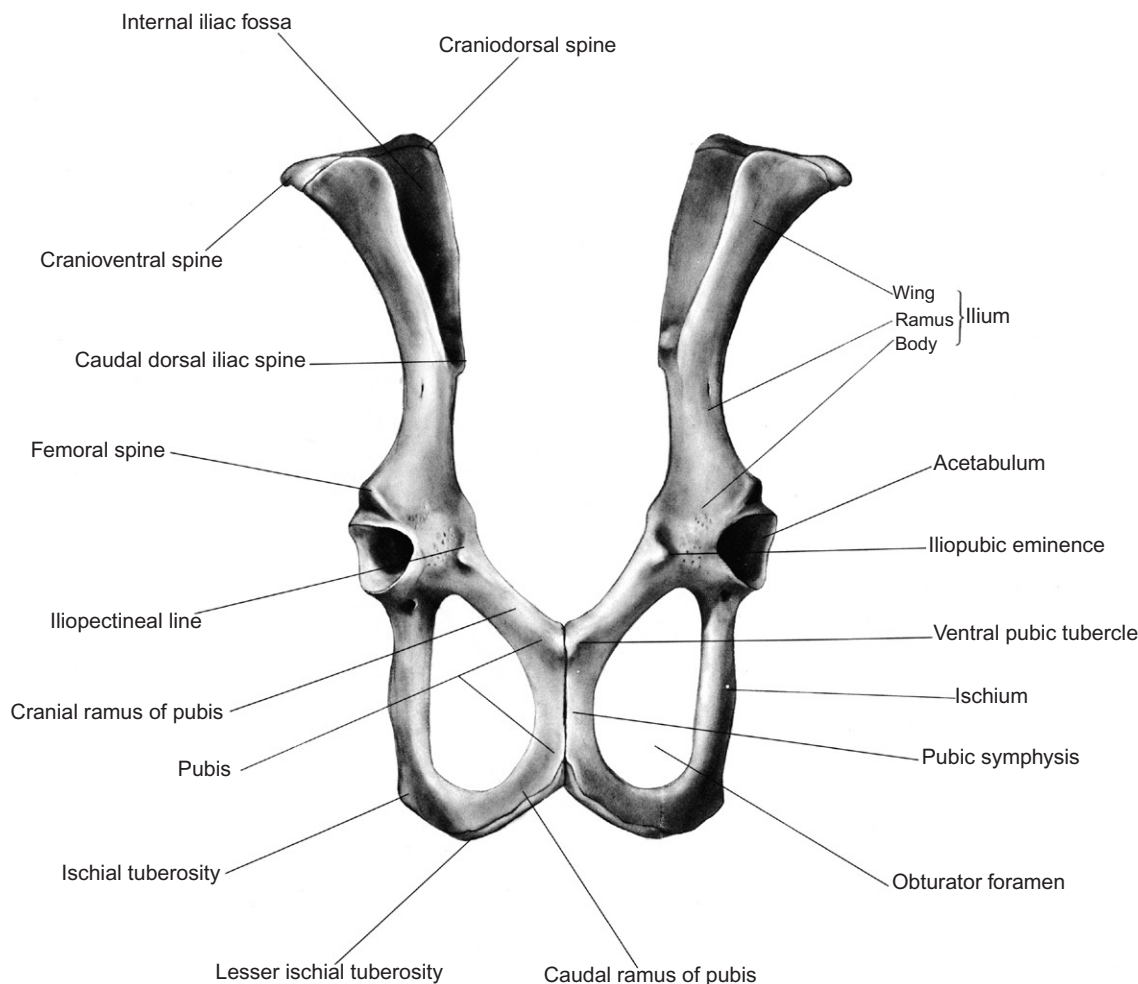


FIGURE 20.7 Pelvic girdle showing the pubic symphysis. Reprinted by permission of the publisher from *Anatomy of the Guinea Pig* by Gale Cooper, M.D. and Alan L. Schiller, M.D., Cambridge, Mass.: Harvard University Press, Copyright © 1975 by the President and Fellows of Harvard College.

DIGESTIVE SYSTEM

The digestive system begins with the oral cavity and ends at the anus. In addition, it contains accessory organs that aid in digestion which include the teeth, salivary glands, pancreas, liver, and gall bladder (Cooper and Schiller, 1975). There are four pairs of salivary glands including the parotid, mandibular, sublingual, and molar (Quesenberry et al., 2004). The glands' ducts empty near the molars (Quesenberry et al., 2004).

Oral Cavity

The lateral walls of the mouth (cheeks) include the buccal pads and bristle areas. The buccal pads are the folded-inward edges of the lips that separate the incisors from the molar teeth. The bristle areas are present in the middle of the diastema and are described as small circular hair-covered flaps (Cooper and Schiller, 1975). Guinea pigs do not have cheek pouches. A large

tongue is present, covering most of the oral cavity and oral pharynx, however, a distinct lingual frenulum is not always present (Breazile and Brown, 1976).

The soft palate is continuous with the base of the tongue covering most of the back of the throat (Figure 20.8). On the median surface there is a small opening, the palatal ostium, from the oropharynx to pharynx (Timm et al., 1987). It has been referenced as the intrapharyngeal ostium and is observed in chinchillas, capybaras, and other hystricomorph rodents. Due to the unique anatomical structure of the pharynx, oral gavage and endotracheal intubation can be challenging.

Abdomen

The adult alimentary tract is 2.3 meters in length from pharynx to anus (Jilge, 1980). The monogastric stomach is located in the left cranial abdomen and is in contact with the left liver lobe and small intestine (Breazile and Brown, 1976). The four regions of the

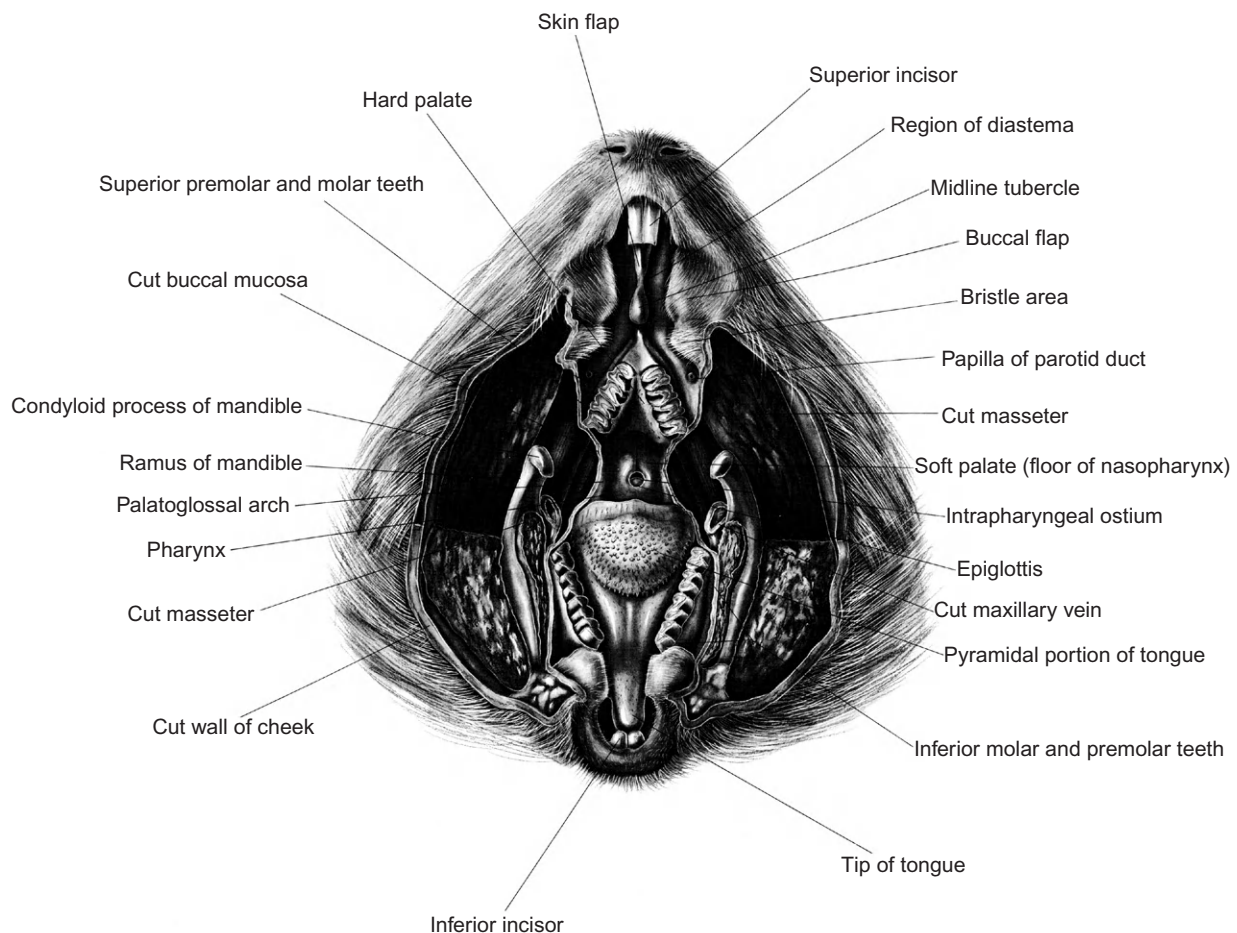


FIGURE 20.8 Pharynx showing the palatal or intrapharyngeal ostium. Reprinted by permission of the publisher from *Anatomy of the Guinea Pig* by Gale Cooper, M.D. and Alan L. Schiller, M.D., Cambridge, Mass.: Harvard University Press, Copyright © 1975 by the President and Fellows of Harvard College.

stomach include the cardia, fundus, body, and pylorus. The esophagus obliquely enters the cardiac region at the lesser curvature of the stomach (Breazile and Brown, 1976). Formation of an angle of the small, lesser curvature of the stomach with the esophagus is termed the angular notch (Cooper and Schiller, 1975). The fundus is a large pouch located cranial and to the left of the cardia. The body of the stomach is located to the right of fundus and leads into the thick-walled pylorus which connects to the small intestine (Cooper and Schiller, 1975). The stomach's inner surface is smooth and the pylorus consists of longitudinal folds or rugae (Breazile and Brown, 1976). Guinea pigs are monogastric with completely glandular stomachs unlike other rodents, there is no non-glandular portion (Harkness et al., 2002).

Grossly there are no features that distinguish the three sections of the small intestine (duodenum, jejunum, and ileum) which is approximately 125cm in length and the longest part of the alimentary tract (Breazile and Brown, 1976). Of the three sections, the duodenum is the shortest (10–12cm long), the jejunum the longest at 95cm and the ileum is about 10cm (Cooper and Schiller, 1975). The ileum exits to the ileocecal papilla to the cecum. In the small intestine, lymph nodes and Peyer's patches increase in number moving distally.

The large intestine does not contain any epiploic appendices, sigmoid colon, or vermiform appendix and it is approximately 70–75cm in length (Cooper and Schiller, 1975). The first section of the large intestine, the cecum, is the largest dilatation of the alimentary tract occupying most of the ventral abdominal cavity and is known for its most unique characteristics. It is 15–20cm in length and contains approximately 65% of the gastrointestinal contents (Manning et al., 1984). It makes up 15% of the body weight and is responsible for the production of large quantities of vitamins. Located on the left side of the abdominal cavity, this large, semicircular thin-walled organ has numerous lateral pouches (haustra) and contains a large source of smooth muscle in three white longitudinal bands: dorsal, ventral, medial taenia coli. The taenia coli run the length of the large intestine. For physiology experiments, the linear smooth muscle fibers are harvested from the taenia coli because they can be obtained without damaging the cecum and large intestine.

The colon can be identified as three sections based on the location in the abdominal cavity; ascending, transverse, and descending. The descending colon terminates at the rectum which appears similar to the colon, located on the midline for approximately 10cm before entering the anal canal (Cooper and Schiller, 1975).

The gastric emptying time is 2 hours and the gastrointestinal transit time is approximately 20 hours (range 8–30) (Jilge, 1980). Due to guinea pigs' high metabolic

rate, cecotrophy or coprophagy is observed several times per day within laboratory settings and animals can be observed eating cecotropes (soft cecal feces) directly from the anus (Ebino, 1993). Obese or pregnant animals may eat feces from the floor and unweaned guinea pigs may eat dam's feces. Coprophagy is a source of vitamin B and protein utilization, however its contribution to guinea pig nutrition has not been fully investigated. There is an increase in the gastrointestinal transit time to 66 hours when coprophagy is included (Jilge, 1980). Prevention of coprophagy results in decreased digestion of fiber, increased excretion of minerals, and weight loss (Cheeke, 1987; Ebino, 1993).

The gastrointestinal flora consists of Gram-positive organisms which are similar to the rabbit's flora (Navia and Hunt, 1976). In the large intestine, anaerobic lactobacilli are the most common organism (Cheeke, 1987). Coliforms, yeasts, and clostridia may be present in small numbers (Harkness and Wagner, 1995). Guinea pigs are sensitive to antibiotics which can disrupt the microbial balance in the gastrointestinal tract. Antibiotic administration leads to a decrease of Gram-positive bacteria and an overgrowth of *Clostridium difficile* which can lead to enterotoxemia and death (Manning, 1984). Antibiotics including erythromycin, clindamycin, lincomycin, penicillins, ampicillin, chlortetracycline, and oxtetracycline should be avoided (Morris, 1995).

Liver, Gall Bladder, Pancreas, Spleen

The firm, smooth, dark red liver is located in the cranial abdomen and consists of six lobes (right lateral, right medial, left lateral, left medial, caudate, and quadrate) that are not well demarcated by connective tissue, instead blend with each other. The guinea pig, humans, non-human primates, and specific bats are unable to produce the hepatic enzyme L-gulonolactone oxidase (Nishikimi et al., 1992). This enzyme catalyzes the terminal step for ascorbic acid biosynthesis and therefore endogenous vitamin C cannot be produced (Hasan et al., 2004). The non-functional L-gulonolactone oxidase gene has been mapped to specific chromosomal regions in pigs and humans (Hasan et al., 1999; Nishikimi et al., 1994).

The gallbladder is a well-developed, thin-walled oval sac attached to a fossa in the quadrate liver lobe and is drained by the cystic duct. The cystic duct and several hepatic ducts join to form the common bile duct. At the end of the common bile duct, there is a unique ampullary swelling from which a small duct drains into the duodenum (Higgins, 1927). When the gall bladder contracts, the bile flows through the ducts into the ampullary swelling. After filling, a sphincter located proximal to the ampullary swelling contracts and bile flows into the duodenum. The sphincter prevents regurgitation of the bile back into the ducts (Higgins, 1927).

The triangular pancreas consists of three lobes extending to the spleen and in contact with the descending duodenum (Breazile and Brown, 1976). The lobes are separated into lobules and lobular ducts drain into a single pancreatic duct which joins the common bile duct before its opening into the duodenum (Breazile and Brown, 1976). Other references do not describe the pancreatic duct joining the common bile duct; in contrast, the pancreatic duct drains separately into the duodenum at approximately 7 cm distal to the common bile duct (Cooper and Schiller, 1975; Mann et al., 1920). The guinea pig lacks an accessory pancreatic duct (Cooper and Schiller, 1975). Similar to other species, the islets of Langerhans are located throughout the pancreas (Breazile and Brown, 1976).

The red-brown spleen is soft to firm and covered by a thin transparent capsule, the tunica serosa. The shape of the spleen is broader than observed in other rodents and rabbits. It is approximately 26 mm in length and at the widest portion, it is 13 mm (Breazile and Brown, 1976). It is located in the left, cranial abdomen along the dorsolateral portion of the stomach and is attached to the stomach's greater curvature by the gastrosplenic (gastrosplenic) ligament. The phrenicosplenic ligament attaches the cranial spleen to the diaphragm.

CARDIOVASCULAR SYSTEM

Circulatory System

Unique features of the circulatory system include the caudal cerebral artery arising between the basilar and internal carotid arteries. In some species, this artery may arise as two separate arteries very close to each other. The internal carotid arteries do not supply a significant amount of blood to the cerebral arterial circle; however, paired internal ophthalmic arteries do provide major collateral input (Shively and Stump, 1974).

The guinea pig has a lower basal coronary blood flow and lower peak coronary blood flow in comparison to the rat (Harkness et al., 2002). With its well-developed collateralization of the coronary arteries, it is very difficult to produce a myocardial infarction by acute coronary artery occlusion compared to humans, cat, dog, pig, and rat (Brewer and Cruise, 1994).

Guinea pig's myocardiocytes are not as "stiff" as the rat's myocardiocytes which makes the rat's heart develop a stronger force at comparable myoplasmic calcium concentrations (Kapelko and Navikova, 1993).

Another unique feature that deviates from the normal mammalian vascular pattern is the bronchoesophageal artery which originates from the right costocervical, the right internal thoracic, or the brachiocephalic trunk instead of from the aorta (Shively and Stump, 1974).

In the guinea pig, the pulmonary trunk differs from the aorta in that it is made up of short irregular branching elastic fibers interspersed with smooth muscle and collagen. The pulmonary artery and its branches are heavily muscled (Kay, 1983). The lumen size of the pulmonary arteries changes abruptly at areas of branching. Arteriovenous anastomoses of the tracheobronchial vasculature in guinea pigs are present (Widdicombe, 1996). The pulmonary capillaries are smaller in the center than in the periphery and pulmonary capillaries are fenestrated (Sekhon et al., 1995). The pulmonary veins also possess muscle swellings in this species (Kay, 1983).

A common incidental finding on histology of guinea pig pulmonary vasculature is a visible aggregation of lymphocytes in the adventitia of the pulmonary vessels. These perivascular nodules are normally seen only in the lung (Percy and Barthold, 2001).

In some guinea pigs and other animals, there may be 2–3 pairs of renal arteries located cranially and caudally to each kidney arising as separate arteries. The testicular artery arises from the aorta supplying the testis and epididymis. When two renal arteries are present bilaterally, the testicular artery may originate from the renal arteries; arise between them from the aorta; or arise caudally to the caudal renal artery (Favre, 1967). In the female, the ovarian artery has a similar origin to the testicular artery. It divides into several branches supplying the oviduct, ovary, and uterus. When two renal arteries are present bilaterally, the ovarian artery may originate from the aorta between the two renal arteries; directly from the caudal renal artery; or caudal to the renal arteries at the aorta with the left ovarian artery originating from a left caudal renal branch (Mattei, 1966).

Thymus

The thymus is located in the cervical region (neck) extending from the mandible to almost the thoracic inlet. It consists of two pink-tan elongated lobes with fat and fascia connecting the lobes (Cooper and Schiller, 1975) and no afferent lymphatic vessels (Ernstrom and Larsson, 1967). Accessory thymic nodules can be located in most animals (Cooper and Schiller, 1975). At birth, the thymus weight is at its maximum. In the immature guinea pig, the functional thymus is located in the ventral cervical area and cranial mediastinum (Breazile and Brown, 1976). As the animal matures and by 12 months of age, the thymus involutes and is replaced by fatty tissue (Ernstrom and Larsson, 1970). The accessible location of the thymus for removal, measurements, and manipulations makes the guinea pig a useful model for immunology studies.

Hematology and Clinical Chemistry Parameters

Guinea pigs lack easily accessible peripheral veins. There is a wide range of blood reference ranges noted

TABLE 20.1 Reference Ranges for Guinea Pig Hematological Values

Parameter	Reference Range
Erythrocytes	$5.4 \times 10^6/\text{mm}^3 \pm 12\%^a$
Hematocrit	$43 \pm 12\%$
Hemoglobin	$13.4\text{ g/dl} \pm 12\%$
MCV	$81\text{ }\mu\text{m}^3$
MCH	25 pg
MCHC	30%
Leukocytes	$9.9 \times 10^3/\text{mm}^3 \pm 30\%$
Neutrophils	28–44%
Lymphocytes	39–72%
Kurloff cells	3–4%
Eosinophils	1–5%
Monocytes	3–12%
Basophils	0–3%
Platelets	$250\text{--}850 \times 10^3/\text{mm}^3$

Quillec, M., Debout, C., and Izard, J. (1977). Red cell and white cell counts in adult female guinea pigs. *Pathol. Biol.* **25**, 443–446.

Laird, C.W. (1974). Clinical pathology: Blood chemistry. In "Handbook of Laboratory Animal Science" (E.C. Melby, Jr., and N.H. Altman, Eds), Vol 2, pp. 345–436. CRC Press, Cleveland.

^aCoefficient of variation.

in the literature. The variables that contribute to these ranges include breed, age, gender, diet, housing conditions, method of collection, anesthesia administered, venipuncture location, and laboratory performing sampling. Refer to [Tables 20.1 and 20.2](#) for a list of reference ranges for hematological and clinical chemistry values.

White Blood Cells

Neutrophils are known as heterophils or pseudoeosinophils due to the eosinophilic cytoplasmic granules (Harkness et al., 2002). These cells are similar to the rabbits' heterophils. Both small and large lymphocytes are the predominant leukocyte in the peripheral blood with the thymus producing many of these circulating cells. Lymphocytes are slightly higher and neutrophils are slightly lower than average human counts (Lewis, 1992). Steroids do not affect the thymic physiology or peripheral lymphocytes of guinea pigs, non-human primates, humans, and ferrets, therefore, these species are considered to be corticosteroid-resistant (Sisk, 1976). Other species such as mice, rats, hamsters, and rabbits respond to steroids with decreased thymus weights and lymphocyte counts (Calman, 1972).

Kurloff cells (Foa-Kurloff) are unique mononuclear leukocytes with natural killer activity that are found in guinea pigs. The cells are found in the circulating blood, splenic sinusoids, bone marrow, and thymus

TABLE 20.2 Reference Ranges for Guinea Pig Clinical Chemistry Values (Serum)

Parameter	Reference Range
Total protein	4.5–5.9 g/dl
Albumin	2.3–3.0 g/dl
Globulin	1.7–2.6 g/dl
Glucose	80–110 mg/dl
Blood urea nitrogen	15.7–31.5 mg/dl
Creatinine	1.0–1.8 mg/dl
Total bilirubin	0.2–0.4 mg/dl
Lipids	95–240 mg/dl
Phospholipids	25–75 mg/dl
Total triglyceride	28–76 mg/dl
Cholesterol	20–43 mg/dl
Calcium	9.0–11.3 mEq/dl
Phosphorus	4.2–6.5 mEq/dl
Magnesium	2.1–2.7 mg/dl
Sodium	121–126 mEq/liter
Potassium	4–6 mEq/liter
Chloride	96–98 mEq/liter
Alanine aminotransferase	31–51 IU/liter
Alanine transaminase	32–51 IU/liter
Alkaline phosphatase	68–71 IU/liter
Aspartate aminotransferase	38–57 IU/liter
Aspartate serum transaminase	38–58 IU/liter
Creatine phosphokinase	80–130 IU/liter
Lactate dehydrogenase	37–63 IU/liter

Altman, P.L., and Dittmer, D.S. (1974). *Biology Data Book*, 2nd edition., Vol 3. Fed Am. Soc. Exp. Biol., Bethesda, Maryland.

Clifford, C.B., and White, W.J. (1999). The guinea pig. In "The Clinical Chemistry of Laboratory Animals," 2nd ed. (W.F. Loeb and F.W. Quimby, eds.), pp. 65–70. Taylor and Francis, Philadelphia.

White, W.J., and Lang, C.M. (1989). The guinea pig. In "The Clinical Chemistry of Laboratory Animals" (W.F. Loeb and F.W. Quimby, eds.), pp. 27–30. Pergamon Press, New York.

stromal tissue (Percy and Barthhold, 2001). This cell is most prominent during pregnancy, as the estrogen level increases, the Kurloff cells increase in number with many migrating to the placental labyrinth (Revell et al., 1971). The cells act as barrier-separating fetal antigens from immunologically competent maternal cells to protect the embryo from IgM and sensitized lymphocytes therefore preventing the maternal rejection of the fetal placenta during pregnancy (Marshall et al., 1971). These cells are usually not observed in newborn guinea pigs and in the fetus (Smith, 1947).

The cells contain a granular structure (Kurloff body) that ranges in size from 1–8 μm within a cytoplasmic

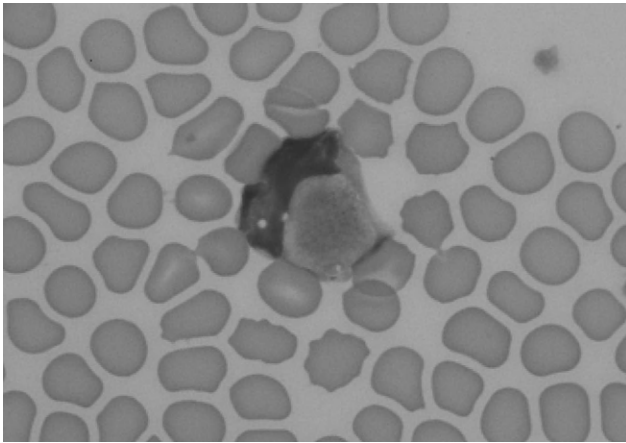


FIGURE 20.9 Photomicrograph of a blood smear showing a Kurloff body (100× objective oil; Wright-Giemsa stain).

vacuole that may exceed the size of the nucleus of the cell in which it is found. Staining of the intracytoplasmic material is positive for periodic acid Schiff and the Lendrum stain which is interpreted as proteoglycans and mucopolysaccharide material (Percy and Barthold, 2001). Figure 20.9 is a photomicrograph of a Kurloff cell.

L2C leukemia (an acute lymphoblastoid B cell leukemia) occurs in the Strain 2 guinea pig and has been used as the animal model for human acute leukemia (Khar et al., 1997). With this disease, the Kurloff cells increase in number and in lysosomal enzyme activity which cause cytolysis of the L2C cells (Debout et al., 1999). Studies have indicated that natural killer cells cause tumor cell death by apoptosis and necrosis, however the process by which natural killer cells bind to the tumor cells is not completely understood but it does include target recognition (Debout et al., 1999; Herbermann et al., 1986).

Erythrocytes

The red blood cell size is 7.1–8.27 μm and its life span is 60–80 days (Terril and Clemons, 1998). Peripheral blood values vary depending on the age of the guinea pig; reticulocytes and hemoglobin decrease with age (Sisk, 1976). Guinea pig erythrocytes, hemoglobin, and packed cell volume are low compared to other laboratory rodents (Manning et al., 1984). The erythrocyte parameters fall within human ranges (Lewis, 1992). Hemoglobin is similar to dog, rat, horse, rabbit, and humans for its high affinity for oxygen yet differs from dog, rabbit, and human hemoglobin by resistance to oxidation by nitrates to methemoglobin (Sisk, 1976). Excess dietary cholesterol causes guinea pigs to develop an acute hemolytic anemia in comparison to other animals which develop cardiovascular lesions. The difference in the species has been attributed to the

TABLE 20.3 Reference Ranges for Guinea Pig Clotting Values

Parameter	Reference Range	Reference
Prothrombin time (PT)	50–100 seconds	a
	20–32 seconds	b
Partial prothrombin time (PTT)	13.0–22.9 seconds	a
	40–49 seconds	c
Thrombin time	27.5–48.0 seconds	a
Factor II	85–150 units/100 ml	a
Factor V	68–140 units/100 ml	a
	55–105 units/100 ml	c
Factor VIII	63–142 units/100 ml	c
Factor IX	67–141 units/100 ml	c
Fibrinogen	263–572 mg/100 ml	c

^aKaspereit, J., Messow, C., and Edel, J. (1988). Blood coagulation studies in guineapigs (*Cavia porcellus*). *Lab. Anim.*, **22**, 206–211.

^bHall, R. J. (2007). Clinical pathology of laboratory animals. In "Animal Models in Toxicology", 2nd ed., (S.C. Gad, Ed.), pp. 819. CRC Press, Boca Raton.

^cHurt, J.P., and Krigman, M. R. (1970). Selected procoagulants in the guinea pig. *American Journal of Physiology*, **218**, 832.

differences in the composition of the red blood cell lipids which can affect the stability of the cell membrane (Ostwald et al., 1970).

Blood Coagulation

The guinea pig's platelet function resembles humans when compared to other species, in particular aggregation. It has the longest prothrombin conversion time of up to 4 minutes when compared to rabbits, rats, dogs, hamsters, cats, and human (Hwang and Wosilait, 1970). The activated partial prothrombin time is short compared to the human (Lewis, 1992). In addition, when compared to other species, it has the least amount of Factor VII and low levels of Factor V (Sisk, 1976). Factors V, VIII, and XII are higher than human levels and Factors II, IX, XI, and fibrinogen are within or close to the human range (Lewis, 1992). Refer to Table 20.3 for a list of reference ranges for clotting values.

Heart

Occupying most of the small thoracic cavity, the four-chambered heart is surrounded by a two-layer pericardium within the mediastinum. The heart is located mostly on the midline and approximately 1 cm cranial to the xiphoid process. Externally, the thin-walled atria are separated from the ventricles by the coronary sulcus that contains the coronary veins and arteries (Breazile and Brown, 1976). The ventricles are separated dorsally and ventrally by two shallow

interventricular sulci (Cooper and Schiller, 1975). Internally, the atria are separated by the intra-atrial septum and externally by the aorta and pulmonary trunk. A thick intraventricular septum separates the ventricles. Usually, the right ventricular lumen contains a single septomarginal trabeculum (moderator band) that is sometimes lacking in the left ventricle (Breazile and Brown, 1976). The larger left ventricle with thicker walls forms the apex of the heart.

The guinea pig electrocardiogram wave forms are similar to humans unlike the mouse, rat, and hamster (Petelenz, 1971). Positive in deflection, the P wave is most distinct in lead II (Lombard, 1952; Peletnz, 1971; Pratt, 1938). The QRS complex is usually diphasic and with most leads, the first deflection is of higher voltage (Mikiskova and Mikiska, 1968). The S-T segment duration does not correlate with the weight of the Cavioidea species (guinea pig, agouti) as it does with animals from the same order (Brewer and Cruise, 1994). For example, many rodents including large species have either shorter or no S-T segment, whereas the guinea pig has a distinct S-T segment (Brewer and Cruise, 1994). Depending on the lead, the T wave can be either positive or negative or diphasic (Pratt, 1938). The time duration of the guinea pig electrocardiogram has been described elsewhere (Lombard, 1952; Petelenz, 1971; Pratt, 1938; Zeman and Wilber, 1965). Reliable measurements of the QT interval makes the guinea pig an excellent model for torsade de pointes predictive studies and for identifying the arrhythmia resulting from the lengthening of the QTc (Eckardt et al., 1998; Hamlin, 2006).

The guinea pig heart is used as an ex vivo model for the Langendorff preparation. Established in 1897 by Oscar Langendorff, this preparation of isolating a mammalian heart for perfusion has been used in research that includes pharmacology, biology, cardiac physiology, pathology, and toxicology (Skrzypiec-Spring et al., 2007). An intact heart contains ion channels, enzymes, receptors, hemodynamic functions, electrophysiology, and action potentials that can be analyzed. The intact guinea pig heart contains receptors, channels, Purkinje M fibers, endocardium, and epicardium which are useful for the testing of a new chemical entity that may impact ventricular repolarization which cannot be evaluated when using the cloned hERG channel, the papillary muscle, or isolated Purkinje fiber (Hamlin et al., 2004). This is especially useful for evaluating new chemical entities and identifying cardiovascular risks early in the drug development program. The guinea pig heart would not be used as the Langendorff model for ischemia studies due to their extensive collaterals in the coronary circulation (Hearse and Sutherland, 2000).

Over the past decade, there has been a withdrawal of marketed efficacious drugs or the revision of drug labels that can be attributed to unexpected, sudden

cardiac death. The events can be associated with QT prolongation due to the inhibition of the rapid potassium current (I_{kr}), which in the human myocardium is the major repolarizing current, and can lead to a rare, but life-threatening ventricular arrhythmia known as torsade de pointes (TdP) (Fermini and Fossa, 2003; Guo et al., 2009). As a consequence, there has been further development of in vivo and in vitro models to evaluate the electrophysiological events and cardiovascular risk assessment of new drug entities to predict TdP in humans. The guinea pig ventricle has potassium channels similar to humans which are delayed, rectifier potassium currents, I_{kr} (r – rapid), I_{ks} (s – slow), and inward rectifier potassium currents (I_{ki}) which make them an interesting animal model to examine cardiac repolarization (Brouillette et al., 2007).

Guinea pig Purkinje fibers are used as an in vitro assay to measure the drug effects on the action potentials and other cardiac channels (Fermini and Fossa, 2003).

PULMONARY SYSTEM

General Anatomy

The guinea pig is an obligate nasal breather (Nixon, 1974). The nasal cavity extends caudal from the nostrils to the choanae and is bounded dorsally by the nasal bones and laterally and ventrally by the incisive and maxillary bones (Cooper and Schiller, 1975). The nasal cavity is rectangular when viewed laterally but T-shaped on cross-section due to its broadness dorsally and extreme narrowness ventrally (Cooper and Schiller, 1975). The stratified squamous epithelium of the skin continues into the external nasal orifices and covers the nasal vestibules (Breazile and Brown, 1976). Nasal hairs measure approximately 1.8×10^{-3} cm in diameter and 35.4×10^{-3} cm in length (Kliment et al., 1972). The lining changes abruptly to ciliated pseudostratified columnar epithelium with goblet cells within the nasal cavity (Breazile and Brown, 1976). There are two recesses in the nasal cavity, the rostral recess and maxillary recess. Multiple sources state that the guinea pig lacks a maxillary, frontal, and sphenoid sinus, although others may refer to the maxillary recess as the maxillary sinus (Cooper and Schiller, 1975). Variations in the number and position of the ethmoturbinates have been documented, with four being the most common (Cooper and Schiller, 1975). The guinea pig has a large nasolacrimal duct which is found in the lateral wall of the nasal cavity (Cooper and Schiller, 1975). The medial or septal wall has a large defect where the two nasal fossa are joined. Just rostral to the defect in the septum is a slight bulge which is the area of the vomeronasal organ (Cooper and Schiller, 1975) associated

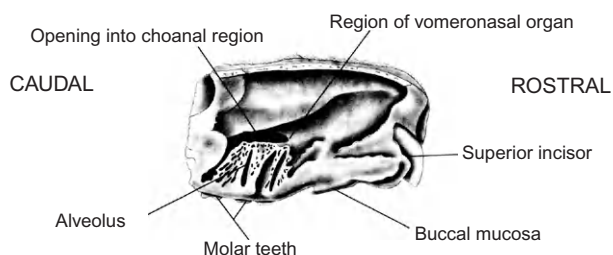


FIGURE 20.10 Septum after removal of the right side of the nasal cavity. Reprinted by permission of the publisher from *Anatomy of the Guinea Pig* by Gale Cooper, M.D. and Alan L. Schiller, M.D., Cambridge, Mass.: Harvard University Press, Copyright © 1975 by the President and Fellows of Harvard College.

with pheromone detection. The lining of the caudodorsal portion of the nasal cavity is olfactory epithelium (Breazile and Brown, 1976). The choanae are the caudal terminations of each nasal fossa and communicate with the nasopharynx (Cooper and Schiller, 1975). Figures 20.10–20.13 show the nasal cavity and positions of the turbinates.

The nasopharynx is relatively short (Breazile and Brown, 1976). The soft palate forms the floor of the nasopharynx (Cooper and Schiller, 1975) and is continuous with the base of the tongue (Brewer and Cruise, 1997). The openings of the Eustachian tube appear as two prominent slit-like openings dorsal to the soft palate on the dorsolateral walls of the nasopharynx.

The larynx is square in shape and is comprised of five cartilages similar to other mammals: epiglottis, thyroid, cricoid, and paired arytenoids (Cooper and Schiller, 1975). The laryngeal cavity is divided into three portions: the vestibule, the glottis, and the infraglottis (Cooper and Schiller, 1975). The laryngeal opening is caudal to the pharynx and is bound laterally by the right and left aryepiglottic folds, ventrally by the epiglottis, and dorsally by the arytenoid cartilages. The aryepiglottic fold extends from the arytenoid cartilage to the dorsolateral border of the epiglottis. The piriform recess is the external depression lateral to each fold (Cooper and Schiller, 1975). The guinea pig has no laryngeal ventricle and the vocal cords are small and poorly developed (Brewer and Cruise, 1997). However, the guinea pig has the ability to vocalize and can produce numerous sounds.

The trachea consists of 35–40 cartilaginous rings joined dorsally by annular ligaments (Cooper and Schiller, 1975). The trachea is lined with ciliated pseudostratified columnar epithelium (Breazile and Brown, 1976). The proportion of ciliated cells decreases distally with only approximately 15% of terminal bronchi cells being ciliated (Rhodi, 1959). The primary bronchi stem from the trachea at the level of the third rib (Breazile and Brown, 1976). The right primary bronchus

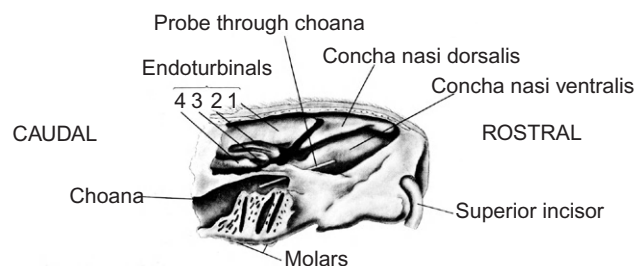


FIGURE 20.11 Left lateral nasal wall after removal of the septum. Reprinted by permission of the publisher from *Anatomy of the Guinea Pig* by Gale Cooper, M.D. and Alan L. Schiller, M.D., Cambridge, Mass.: Harvard University Press, Copyright © 1975 by the President and Fellows of Harvard College.

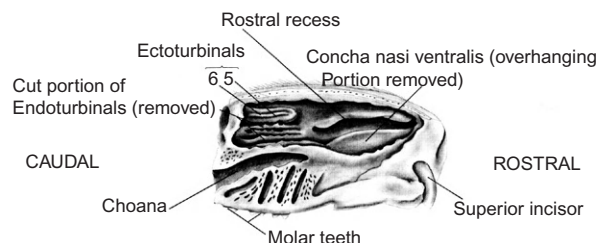


FIGURE 20.12 Nasal chamber after removal of endoturbinates, nasoturbinate, and a portion of the maxilloturbinate. Reprinted by permission of the publisher from *Anatomy of the Guinea Pig* by Gale Cooper, M.D. and Alan L. Schiller, M.D., Cambridge, Mass.: Harvard University Press, Copyright © 1975 by the President and Fellows of Harvard College.

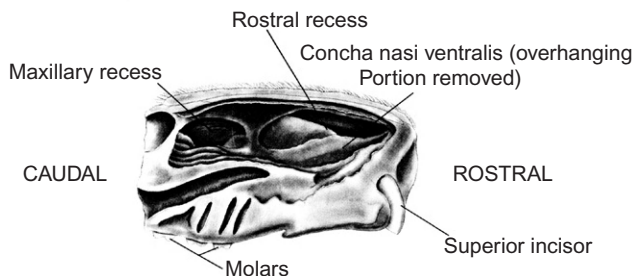


FIGURE 20.13 Nasal chamber after removal of ectoturbinates. Reprinted by permission of the publisher from *Anatomy of the Guinea Pig* by Gale Cooper, M.D. and Alan L. Schiller, M.D., Cambridge, Mass.: Harvard University Press, Copyright © 1975 by the President and Fellows of Harvard College.

is shorter and wider than the left (Cooper and Schiller, 1975). Similar to other rodents, the intralobular airways branch from the primary bronchi laterally and all intrapulmonary airways are therefore bronchioles (Brewer and Cruise, 1997). The bronchioles give rise to respiratory bronchioles which possess alveoli. Compared to other mammals, the guinea pig has the most prominent smooth muscle in the distal bronchi and the muscle is

spirally arranged (Brewer and Cruise, 1997). A detailed morphological description of the guinea pig airways is described by Schneider and Hutchens (1980).

The larger right lung consists of four lobes: cranial, middle, caudal, and accessory each separated by deep fissures. The right cranial lobe is the smallest. The right middle lobe is caudal to the cranial lobe and its medial surface has a concave cardiac impression. The right caudal lung lobe is the largest and has a concave diaphragmatic surface and extends to the eighth rib. The accessory lobe has an irregular shape with a deep notch on its ventral border for the caudal vena cava and a concave diaphragmatic surface (Cooper and Schiller, 1975).

The left lung consists of three lobes: cranial, middle, and caudal. The left cranial lobe has an intralobar fissure that divides it into a smaller cranial segment and larger caudal segment. The medial border is deeply concave to accommodate the heart. The small left middle lobe has a shallow medial notch for the esophagus and a concave diaphragmatic surface. The large left caudal lobe has a concave medial border in contact with the middle lobe (Cooper and Schiller, 1975). There are no intralobular septa in the lung or secondary individual lobulations. Due to the lack of septation between lobules, there exists extensive collateral ventilation. Respiratory bronchioles give rise to terminal sacs with outpockets of alveoli. Alveolar development is almost complete at birth in the guinea pig (Brewer and Cruise, 1997).

Cells of the Respiratory System

There are approximately 40 different cell types in the guinea pig lung (Brewer and Cruise, 1997). Fibroblasts are the most numerous and release cytokines such as interferon β -1, interleukin 6, interleukin 8, monocyte chemotactic factor, colony stimulating factors, and tumor necrosis factor (Brewer and Cruise, 1997; Gauldie et al., 1992). The second most numerous cell type is the pulmonary endothelial cell which makes up part of the pulmonary capillary bed (Brewer and Cruise, 1997). Guinea pigs possess more goblet cells in the airway compared to other rodents but serous cells have not been identified in the guinea pig respiratory system. Clara cells, non-ciliated epithelial cells with a high metabolic rate, make up approximately 73% of the cells lining the bronchioles (Crapo et al., 1983), but have not been identified in the trachea or bronchi in the guinea pig (Brewer and Cruise, 1997). Brush cells are found in the guinea pig, but submucous glands have not been identified, which differs from the murine rodents (Brewer and Cruise, 1997).

The alveoli consist of five major cell types: type I, type II, endothelial cells, interstitial cells, and lung macrophages (Brewer and Cruise, 1997). The type I cells do not divide. The type II cells are the stem cells of the

alveolar epithelium and replace type I cells. The type II cells secrete surfactant and may be involved in defense against oxidative damage. The lung macrophages consist of alveolar, tissue, and intravascular macrophages, but intravascular macrophages are not prominent in the guinea pig or other rodents. In the guinea pig, the neutrophil, not macrophage, captures intravascular foreign bodies up to the size of an erythrocyte (Brewer and Cruise, 1997). The neutrophils also adhere to the endothelial cells, a characteristic of the guinea pig (Terada, 1993).

Mast cells are also present in the lung. In the guinea pig, smooth muscle fibers are prominent in the pleura and mast cells are found in most abundance within the lung along these smooth muscle fibers (Wintrobe and Soter, 1981). Guinea pig mast cells contain no tryptase and only approximately 10% of lung mast cells contain chymase (Brewer and Cruise, 1997).

Eosinophils are also found within the lung. In the guinea pig, there are approximately 300 times more eosinophils in the loose connective tissue under the epithelial surface of the respiratory tract than there are in the circulating blood (Hudson, 1968).

Respiratory Physiology

The guinea pig is a common animal model for studying lung function impairment, asthma, and airway hyperresponsiveness. Some aspects of the normal respiratory physiology of the guinea pig are covered here. A detailed review can be found in Brewer and Cruise (1997) with an emphasis on species differences. Refer to Tables 20.4 and 20.5 for relevant respiratory physiology values in anesthetized and unanesthetized guinea pigs and blood gas values, respectively.

In the guinea pig, sympathetic nerve stimulation of the trachealis muscle causes the muscle to relax. This is in contrast to other species, such as the rat and rabbit (Doidge and Satchell, 1982). Mechanoreceptors are present in the upper airways that respond to touch (e.g. endotracheal tube) with strong laryngeal spasm. The trachea is well innervated but the small airways have little to no innervation (O'Donnel et al., 1978).

Hyperpnea is associated with airway constriction (Nagase et al., 1994). Peptides released from C-fibers, unmyelinated afferent fibers in the vagus nerve, are responsible for bronchoconstriction. On post-mortem, massive bronchoconstriction may be observed in guinea pigs after exsanguination or excision of the lungs (Lai et al., 1984). Contraction of the peribronchial muscle may cause distortion or other artifact of respiratory epithelium on histology (Percy and Barthold, 2001).

In contrast to mice and rats, guinea pig airways are sensitive to histamine and demonstrate early and late allergic reactions. In the late reaction, both eosinophils and neutrophils are present. IgG1 as opposed to IgE

TABLE 20.4 Normal Respiratory Values in Anesthetized and Unanesthetized Adult Guinea Pigs

Respiratory Parameter	Amdur et al. 1958 ^d	Guyton 1947 ^e	North 1999	Lin and Lin 1997 ^{c,f}	Holroyde et al. 1980 ^{c,g}
Respiratory rate (breaths/min)	84+ / -14	90.3 (69–104)	42–150	–	49.3+ / -0.2
Tidal volume (ml)	1.68+ / -0.39	1.75 (1.0–3.9)	1.0–5.3	–	–
Minute ventilation (ml/min)	139+ / -30	155.6 (100–382.2)	–	–	–
FVC ^a (ml)	–	–	–	13.8+ / -2.4	11.2+ / -0.5
FEV _{0.1} ^b (ml)	–	–	–	–	6.5+ / -0.1
Total lung capacity (ml/cmH ₂ O)	–	–	–	0.53+ / -0.5	–

^aForced vital capacity.^bForced expiratory volume in first 0.1 seconds.^cValues obtained in anesthetized state.^dAmdur, M.O. and Mead, J. (1958). Mechanics of respiration in unanesthetized guinea pigs. *Am. J. Physiol.* **192**, 364–368.^eGuyton, A.C. (1947). Measurement of respiratory volumes of laboratory animals. *Am. J. Physiol.* **150**, 70–77.^fLin, C.C. and Lin, C.Y. (1997). Maximal forced expiratory maneuver to measure airway obstruction in anesthetized guinea pigs. *Resp.* **64**, 257–262.^gHolroyde, M.C., Smith, S.Y., and Holme, G. (1980). Evaluation of pulmonary mechanics in guinea pigs during respiratory anaphylaxis. *J. Pharmacol. Exp. Ther.* **212**, 162–166.**TABLE 20.5** Blood Gas Reference Values Obtained by Sanchez-Aparicio et al. (2009) in Guinea Pigs Anesthetized with Ketamine-Xylazine

Parameter	Duncan Hartley ^a	Short Hair English ^a
pH	7.45+ / -0.01	7.46+ / -0.01
pCO ₂ (mmHg)	38.30+ / -0.600	34.25+ / -1.11
pO ₂ (mmHg)	37.80+ / -2.230	47.75+ / -4.34
Na ⁺ (mmol/L)	133.35+ / -0.580	133.95+ / -0.85
K ⁺ (mmol/L)	4.16+ / -0.050	4.17+ / -0.22
Ca ²⁺ (mmol/L)	1.37+ / -0.009	0.97+ / -0.04
Glucose (mg/dl)	143.70+ / -6.690	134.60+ / -8.52
Lactate (mg/dl)	1.86+ / -0.420	2.66+ / -0.25
Hct (%)	43.70+ / -0.620	34.35+ / -0.82
Rectal temp (°C)	37.61+ / -0.190	37.60+ / -0.13
HCO ₃ ⁻	27.22+ / -0.760	24.66+ / -1.01
tCO ₂ ^b	28.39+ / -0.770	25.73+ / -1.03
SO ₂ ^c	71.30+ / -3.310	74.85+ / -5.49
THbc ^d	13.55+ / -0.190	10.66+ / -0.25

Sanchez-Aparicio, P., Mota-Rojas, D., Verduzco-Mendoza, A., Arch-Tirado, E., Hernandez-Gonzalez, R., Alonso-Spilsbury, M., Gonzalez-Lozano, M., Alfaro-Rodriguez, A., Uribe-Escamilla, R., Becerril-Herrera, M., and Trujillo-Ortega, M.E. (2009) Reference values for blood gas analysis, electrolytes and critical biochemical variables for Short-Hair English and Duncan-Hartley guinea pigs anaesthetized with xylazine-ketamine. *J. Anim. Vet. Adv.* **8**, 1893–1899.

^aReference values were determined by a sampling size of n = 20.^bTotal blood calculated CO₂.^cCalculated oxygen saturation.^dConcentration of total hemoglobin.

appears to play a critical role in allergic responses in guinea pigs. Guinea pigs are rather insensitive to cromolyn (Ricciardolo et al., 2008).

Guinea pig bronchi are more sensitive to constriction by acetylcholine compared to other species. In the guinea pig, three of the five muscarinic receptors predominate, M-1, M-2, and M-3 (Brewer and Cruise, 1997). M-1 receptors are located in the sympathetic ganglia in the guinea pig (MacLagen et al., 1989) and enhance acetylcholine reflexes. Pre-synaptic M2 muscarinic receptors modulate M3-induced contraction (Gies et al., 1989). Release of the acetylcholine from the vagus causes airway constriction through M-3 receptor stimulation on the smooth muscle. Activation of the M-2 receptor hinders constriction. Loss of function of M-2 receptors which can occur due to viral infection, ozone exposure, or antigen stimulation may facilitate bronchoconstriction (Gies et al., 1989).

Guinea pig lungs possess β -inhibitory adrenoreceptors, but lack α -excitatory adrenoreceptors. The β -2 subtype predominates in the guinea pig (Zaagsma et al., 1983). β -2 adrenergic agonists inhibit release of mediators from mast cells and basophils, increase movement of chloride ions and water into the bronchial lumen, and increase cilia movement frequency (Brewer and Cruise, 1997).

Adenosine does not cause bronchoconstriction in normal conscious or anesthetized guinea pigs, but in conscious guinea pigs sensitized to ovalbumin, a bronchoconstrictor response to inhaled adenosine is observed (Thorne and Broadley, 1994) and is biphasic (Fozard and Hannon, 2000).

Bradykinin causes intense bronchoconstriction in the guinea pig and the route of administration is important in the response. Intravenous bradykinin causes bronchoconstriction through the release of COX products mediated mainly by thromboxane A₂, whereas aerosolized bradykinin causes bronchoconstriction through cholinergic neuronal activation and the release of neuropeptides (Ellis and Fozard, 2002).

GENITOURINARY SYSTEM

Urinary System

The kidneys in the adult guinea pig are approximately 18–21mm long and 12–14mm in diameter. The cranial border of the right kidney is at the level of the twelfth intercostal space and the caudal border at the third lumbar vertebra. The cranial border of the left kidney is at the thirteenth rib and its caudal border at the fourth lumbar vertebra (Cooper and Schiller, 1975). Both kidneys lie retroperitoneal and lack mesentery (Breazile and Brown, 1976). The renal pelvis is relatively large with a single longitudinal renal papilla (Quesenberry et al., 2004). Ultrasonographic measurements of normal renal parameters in guinea pigs have been published (Beregi et al., 2001), including estimation of renal volume by renal length and area. There is no significant difference in the ratio of short- and long-loop nephrons in guinea pig kidneys, whereas mice and rats have significantly greater short-loop nephrons and hamsters and gerbils, significantly greater long-loop nephrons (Ichii et al., 2006a). Sex differences in the histological and morphometric details of the kidney have been studied and reveal a significantly higher ratio of total kidney weights to body weight in the male guinea pig, no sex differences in the diameter of the renal corpuscle, and significantly higher PAS-positive granules in the proximal tubules of female guinea pigs (Ichii et al., 2006b). The paired ureters are approximately 80mm in length and 2mm in diameter. The urinary bladder is pyriform. When empty, it lies entirely within the pelvis and when distended may extend high above the margin of the pubis. The urinary bladder of the guinea pig lacks a trigone. Urine is alkaline and normally thick and cloudy white or yellow containing many crystals. See Table 20.6 for reference values for guinea pig urine.

Atypical “pacemaker” cells are smooth muscle cells of the upper urinary tract concentrated within sling-like muscle attachments of the pelvic-calyceal junction to the kidney parenchyma. These cells generate pacemaker potentials in the proximal renal pelvis. Cells similar to interstitial cells of Cajal (ICC), termed ICC-like cells, have been found within the lamina propria of the renal pelvis and pelvic-calyceal junction and play

TABLE 20.6 Examples of Guinea Pig Urine Reference Values

Urine Parameter	16-h Collection ^a	Day	Night
Urine volume (ml)	26.6+/-11	–	–
pH	9	–	–
Na (mEq/L)	64.5+/-13	95.6+/-4.8	83.9+/-7.8
K (mEq/L)	203.5+/-12.5	82.2+/-14.5	125.2+/-22.2
Mg (mEq/L)	–	27.2+/-3.0	34.7+/-3.1
Ca (mEq/L)	–	25.0+/-3.5	20.7+/-4.6
Cl (mEq/L)	179.8+/-30.6	77.2+/-4.5	70.9+/-5.5
Osmotic pressure (mOsmol/L)	–	792+/-89	1045+/-93
Osmolality (mOsmol/kg)	934+/-80	–	–
K/Na	–	0.86	1.49
Mg/Na	–	0.28	0.41
Mg/Ca	–	1.09	1.68
Total cations/osmotic pressure	–	0.29	0.25
Reference	Matsuzawa et al. 1981 ^b	Bellamy and Weir 1972 ^c	Bellamy and Weir 1972 ^c

^aCollection overnight from 4pm to 8am.

^bMatsuzawa, T., Nakata, M., and Tsushima, M. (1981). Feeding and excretion in the Afghan pika (*Ochotona rufescens rufescens*), a new laboratory animal. *Lab Anim.* **15**, 319–322.

^cBellamy, D. and Weir, B.J. (1972). Urine composition of some hystricomorph rodents confined to metabolism cages. *Comp. Biochem. Physiol.* **42A**, 759–771.

a role in conducting pacemaker cell signals to smooth muscle cells of the renal pelvis and ureter (Klemm et al., 1999). The guinea pig has intramural ganglia closely associated with the ureters and bladder which are most likely associated with local coordination of the muscular activity involved in continence and micturition. Approximately 2000–2500 neurons are present in the guinea pig bladder (Gabella, 1990). Neurons are either individual or more commonly within ganglia of up to 40 neurons. The ganglia are more numerous on the dorsal wall compared to the ventral wall and are abundant in a radius of 800µm from the entrance of the ureters and urinary arteries into the bladder (Gabella, 1990).

Male Reproduction

The paired testes each measuring approximately 25mm long and 15mm wide lie in obvious scrotal



FIGURE 20.14 Photograph of the horny styles of an adult male guinea pig.

pouches (Cooper and Schiller, 1975). The epididymis lies along the dorsolateral surface of the testis and is composed of head, neck, and tail portions. The tail gives rise to the ductus deferens (Breazile and Brown, 1976). Inguinal canals remain open for life. The penis consists of a body and glans with an associated intromittent sac (Cooper and Schiller, 1975). The S-shaped penis is approximately 45–55 mm in length and 4–6 mm in diameter (Cooper and Schiller, 1975). The glans has the same diameter as the body of the penis and has minute, saw-toothed white scales or spurs along its cranial, lateral, and ventral surface. The spurs are a unique feature of hystricomorphs and are arranged dorsolaterally in two parallel lines extending from the urethral orifice to the glans. The dorsal line is discontinuous, whereas the ventral is continuous. The tip of the glans has ventral and lateral scales. An os penis is present in the guinea pig and is found within the dorsal surface of the entire length of the glans (Cooper and Schiller, 1975).

The intromittent sac is unique to the hystricomorphs and is within the ventral aspect of the glans. The dorsal inner lining has two longitudinal folds continuous with the corpus spongiosus and is covered with tiny scales similar to those of the external glans. There are two retractor tendons attached to the base of the intromittent sac attached to the crura and penile skin. The tendons are mostly elastic with a central artery and vein and during erection, longitudinal folds become engorged. The sac then everts with two horny styles each 3–5 mm in length protruding externally. The function of this anatomical variation remains unknown (Cooper and Schiller, 1975). Figure 20.14 is a photograph of the horny styles of an adult male guinea pig.

The male urethra consists of a pelvic and spongy portion. The pelvic portion extends from the urinary bladder neck through the pelvic canal to the penis.

The pelvic portion is 15–25 mm in length and 4 mm in diameter and has a predominantly straight cranio-caudal course (Man, 2007). The urethral sphincter is incomplete dorsally except in the area of the prostate in which it is complete. The spongy portion is approximately 33–50 mm in length and 3 mm in diameter, and opens externally at the urethral orifice (Cooper and Schiller, 1975). There is no fossa navicularis at the external termination. Studies demonstrate three-dimensional anatomy of the male urogenital system including the urethra, accessory sex glands and corresponding musculature (Neuhaus et al., 2001). Specifically, it is shown that the male guinea pig has a well-developed musculus sphincter vesicae forming the bladder outlet, a tender musculus sphincter urethrae glaber which surrounds the urethra caudally, a strong musculus sphincter urethrae transversostriatus which partially surrounds the urethra with a horse-shoe shape leaving the dorsal surface open, a strong ventral longitudinal urethral muscle, the musculus dilatator urethrae, and a faint musculus ejaculatorius running from the colliculus seminalis. In contrast to humans, there exists a dorsal longitudinal urethral muscle which extends from the bladder outlet to the colliculus seminalis (Neuhaus et al., 2001).

The accessory sex glands of the boar include: seminal vesicles (vesicular glands), prostate gland, coagulating glands, and bulbourethral glands (Quesenberry et al., 2004). The paired seminal vesicles are the largest accessory gland and measure 100 mm in length and 6–9 mm in diameter. They are long, coiled blind sacs that lie ventral to the ureters and enter the urethra by a pair of ducts immediately caudodorsal to the ductus deferens (Cooper and Schiller, 1975). The prostate gland consists of two pairs of lobes, a smaller ventral lobe and larger dorsal lobe connected by a transverse isthmus which lies caudomedial to the coagulating glands and lateral to the base of the seminal vesicles. The paired coagulating glands are pyramidal and lobulated and lie near the seminal vesicles and caudal to the urinary bladder (Cooper and Schiller, 1975). The prostate and coagulation glands are closely related and form an elongate gland which is not encapsulated (Neuhaus et al., 2001). The paired bulbourethral glands are small, lobulated oval masses each connected to the urethra by a single duct. A rudimentary preputial gland may be present (Cooper and Schiller, 1975). Figures 20.15 and 20.16 show the male urogenital system.

Testosterone levels increase in males at 30 days of age (Resko, 1970). The average age of sexual maturity of a male guinea pig is approximately 70 days (Sisk, 1976). Guinea pig spermatozoa cohere in rouleau formation in the epididymis prior to ejaculation. In other species, rouleau formation and dissociation occurs entirely within the male reproductive tract, whereas

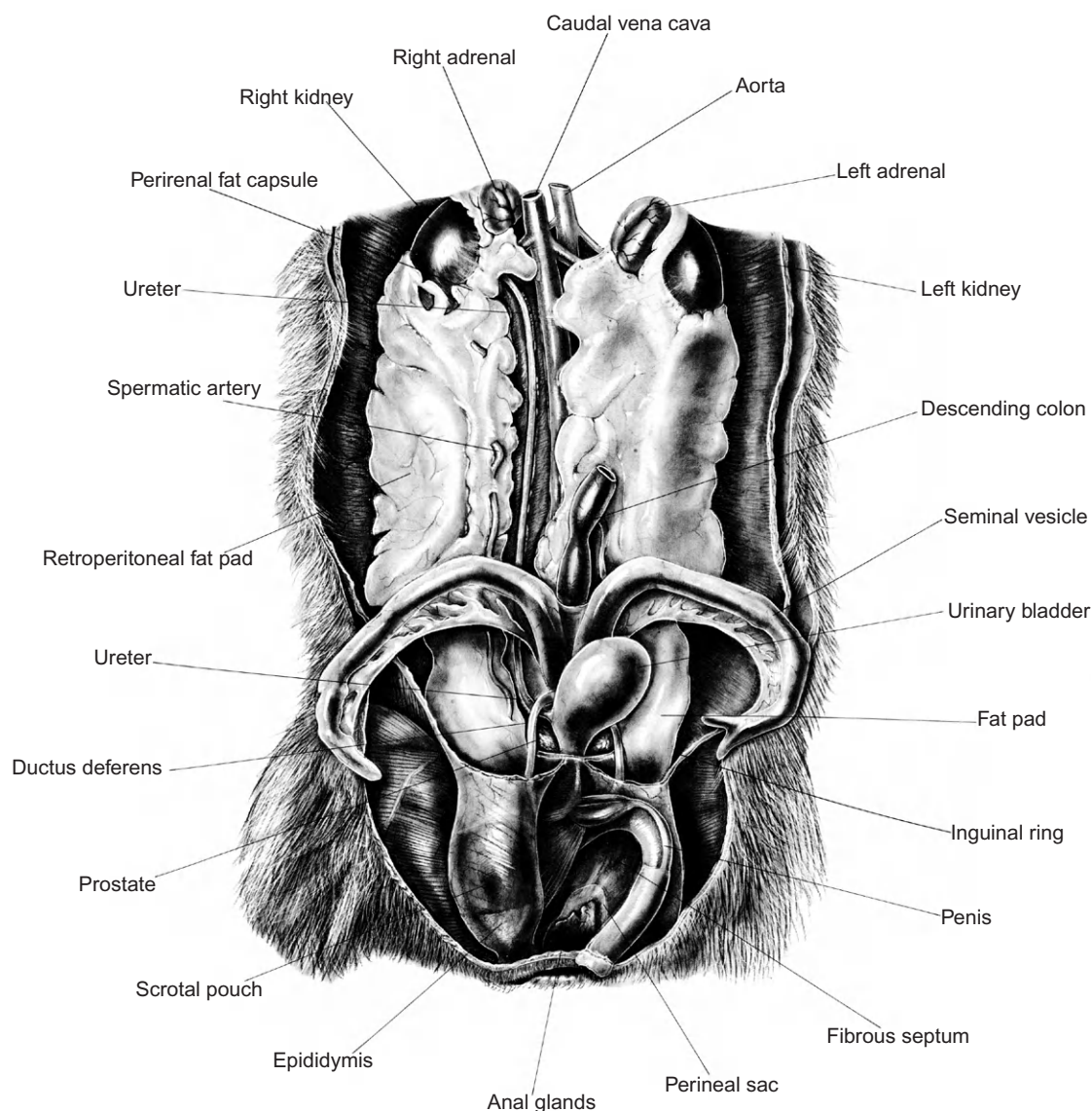


FIGURE 20.15 Male urogenital system in situ. Reprinted by permission of the publisher from *Anatomy of the Guinea Pig* by Gale Cooper, M.D. and Alan L. Schiller, M.D., Cambridge, Mass.: Harvard University Press, Copyright © 1975 by the President and Fellows of Harvard College.

in the guinea pig, it is not until the spermatozoa reach the female's tract after copulation when the complete dissociation of rouleaux occurs (Martan and Sheperd, 1973; Sisk, 1976). Guinea pig sperm have a curvilinear movement with irregular forward progression and the rouleau formation may cause difficulties in rating sperm motility in this species (Sisk, 1976). Ejaculate volume is approximately 0.5 ml (0.1–1.2 ml) with an average of 13.376×10^6 sperm per ejaculate and a transit time from copulation until reaching the oviduct is approximately 15 minutes (Freund, 1969; Martan and Shepherd, 1973).

Female Reproduction

The paired intraperitoneal ovaries measure approximately 3–6 mm by 2–4 mm by 2–3 mm. The right ovary lies caudolateral to the right kidney. The left is cranio-lateral to the left kidney. The oviducts are 50–60 mm long and 1 mm in diameter. The guinea pig uterus is bicornate and consists of paired uterine horns each 30–50 mm long and 3 mm in diameter, a short uterine body approximately 12–20 mm long and 6 mm in diameter, and a single os cervix. The vagina is 30–40 mm long and consists of prominent longitudinal ridges.

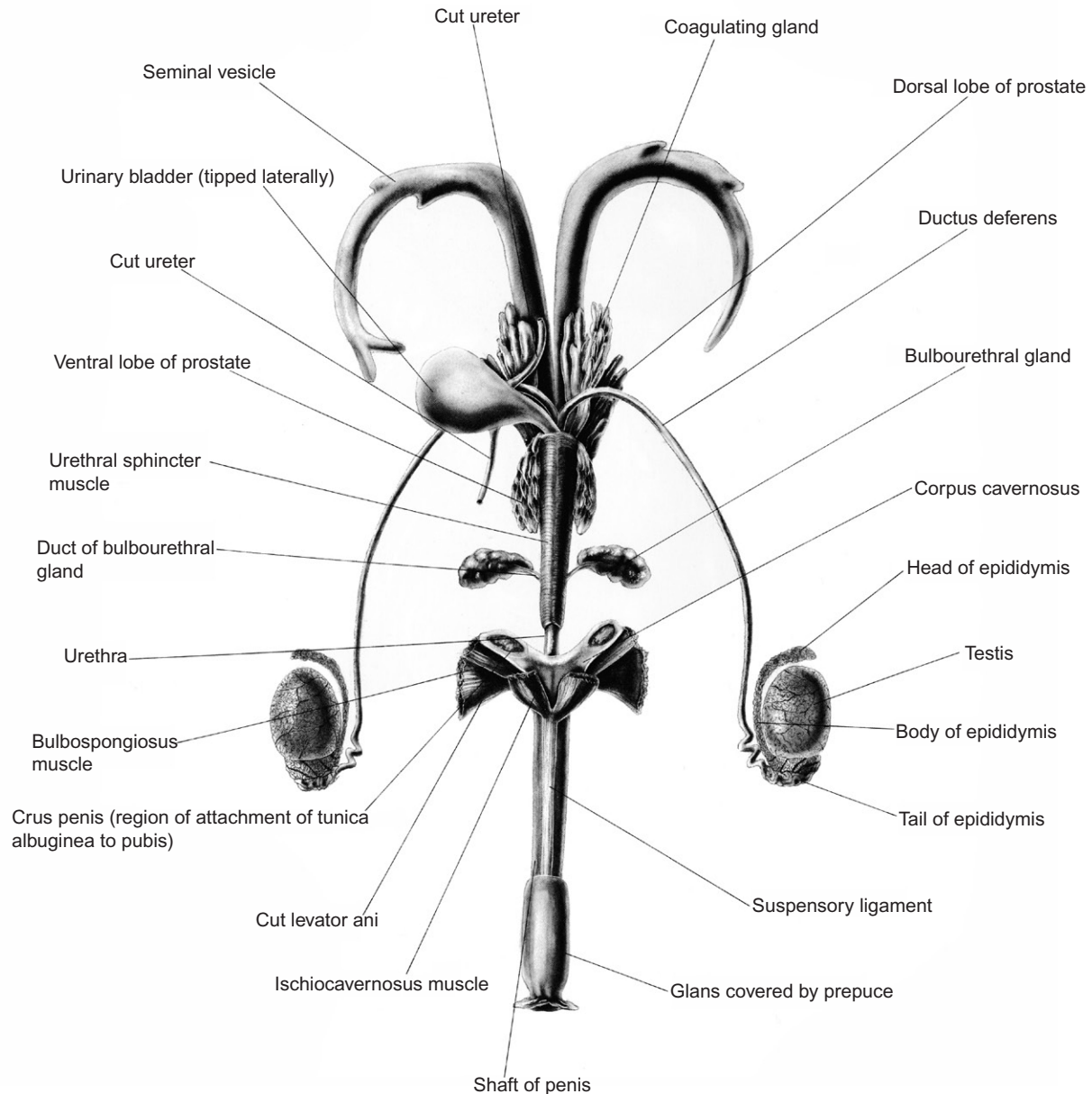


FIGURE 20.16 Male reproductive tract. Reprinted by permission of the publisher from *Anatomy of the Guinea Pig* by Gale Cooper, M.D. and Alan L. Schiller, M.D., Cambridge, Mass.: Harvard University Press, Copyright © 1975 by the President and Fellows of Harvard College.

There is no vestibule between the vagina and the external vaginal orifice (Cooper and Schiller, 1976). The vaginal orifice is U-shaped and is closed by a vaginal closure membrane that opens at estrus, at parturition, and between the 20th and 35th days of gestation, most usually at days 26 or 27 (Weir, 1974). Figures 20.17 and 20.18 show the female reproductive tract.

The estrous cycle is approximately 16 days in length but can range from 13–21 days. Proestrus lasts 1–1.5 days and is characterized by vulvar swelling and rupture of vaginal closure membrane. The vaginal smear

reveals nucleated and cornified epithelial cells. Females show an increase in activity and may be observed chasing their cagemates, swaying their hindquarters and making guttural sounds (Quesenberry et al., 2004). Estrus lasts between 8 and 11 hours and frequently occurs overnight. Estrus is characterized by vaginal swelling, congestion, a mucus secretion, and a perforate vaginal membrane. Females may display lordosis. The vaginal smear contains cornified epithelial cells. Ovulation is spontaneous and usually occurs late in the estrous period, at approximately 10 hours. The number

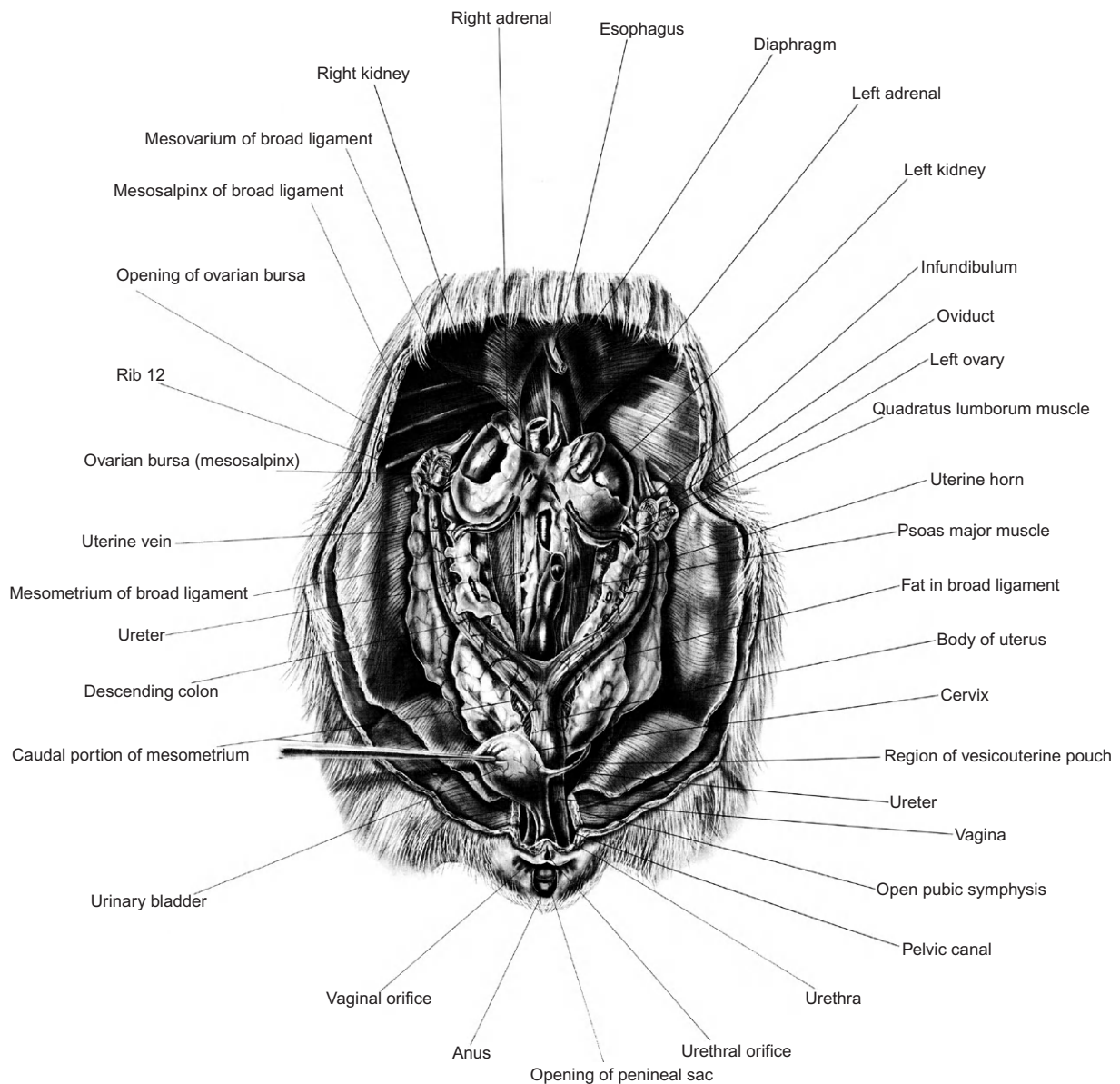


FIGURE 20.17 Female urogenital system *in situ*. Reprinted by permission of the publisher from *Anatomy of the Guinea Pig* by Gale Cooper, M.D. and Alan L. Schiller, M.D., Cambridge, Mass.: Harvard University Press, Copyright © 1975 by the President and Fellows of Harvard College.

of follicles reaching maturity and ovulating is reported as 3.34 and is evenly divided between the two ovaries (Hermreck and Greenwald, 1964). Metestrus lasts approximately 3 days and has three distinct phases: an initial phase in which vaginal secretions become thick and vaginal and uterine vessels are congested, a subsequent phase in which vaginal fluid is serous and vessel congestion markedly increased, and lastly a phase in which the vaginal and uterine epithelium sloughs producing a slight blood secretion. Diestrus lasts for 11–12 days and is characterized by lack of vessel congestion and lack of vaginal secretion.

The hormonal changes during follicular maturation, ovulation, and luteinization have been studied (Reed and Hounslow, 1971). Approximately 13 days after ovulation, the level of progesterone decreases in the corpus luteum and the follicles reach maturity. At this time, follicle stimulating hormone (FSH) increases and stimulates the theca interna which promotes estrogen synthesis by the developing follicle. The estrogen production and basal levels of luteinizing hormone (LH) facilitate an increase in follicle size. An LH surge from the anterior pituitary occurs and ovulation occurs. Plasma progesterone increases rapidly after ovulation (Challis et al., 1971).

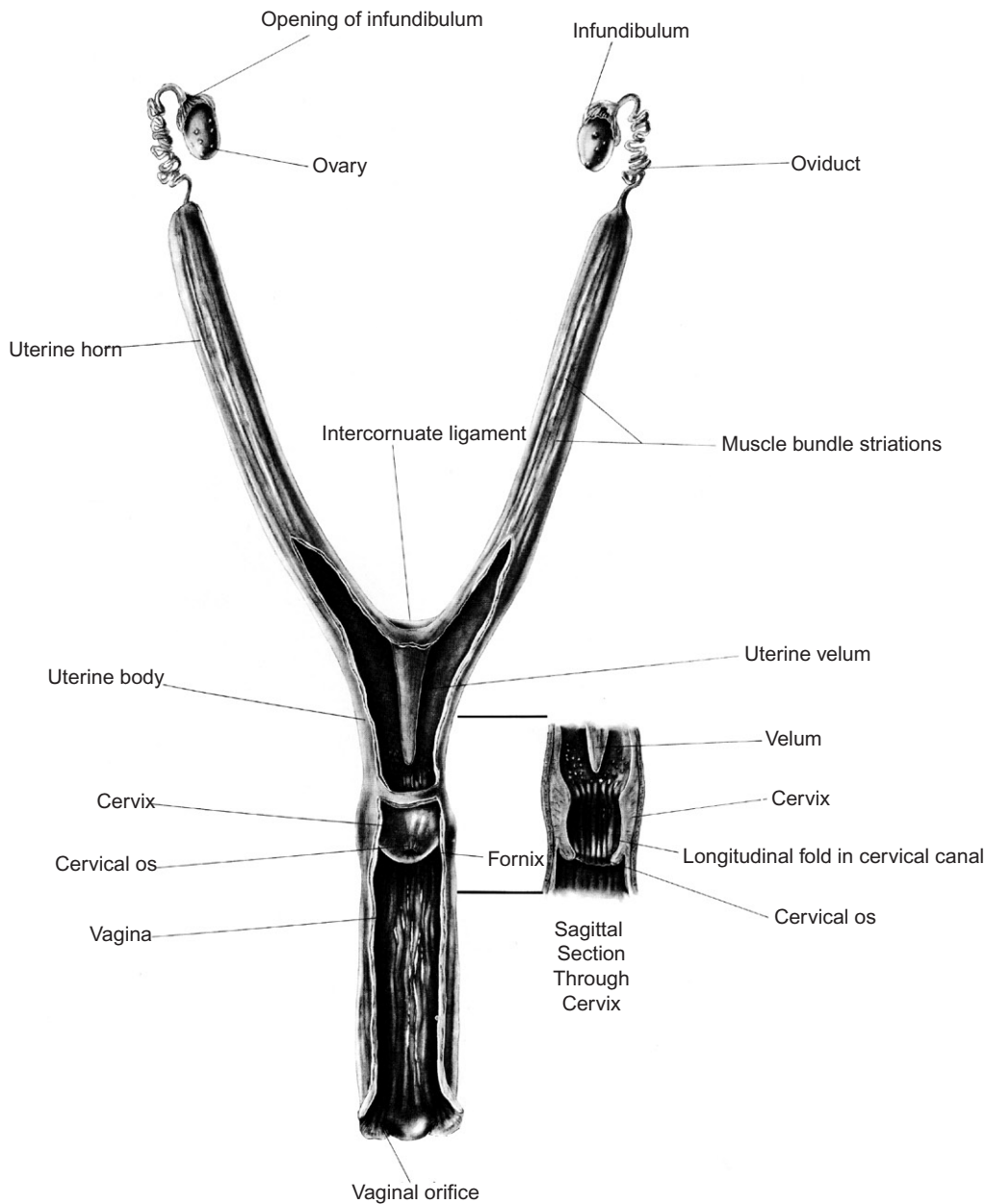


FIGURE 20.18 Female reproductive tract. Reprinted by permission of the publisher from *Anatomy of the Guinea Pig* by Gale Cooper, M.D. and Alan L. Schiller, M.D., Cambridge, Mass.: Harvard University Press, Copyright © 1975 by the President and Fellows of Harvard College.

Approximately 3.5 days post-fertilization, the eight-cell stage embryo reaches the uterus, loses the zona pellucida, and implants at 6 days (Chavatte-Palmer and Guillomot, 2007). The guinea pig has an implantation that differs from the murid rodents. While the blastocyst is in the uterine lumen, it develops an implantation cone at the opposite pole from the inner cell mass. The implantation cone is composed of the syncytial trophoblast and sends processes through the zona pellucida to adhere to the uterine luminal epithelium (Enders and Schlafke, 1969). The entire blastocyst intrudes into

the endometrial stroma beneath the luminal epithelium before a decidual response similar to the murid rodents occurs (Carson et al., 2000).

The guinea pig has a postpartum estrus approximately 2–10 hours after parturition. Guinea pigs are polyestrous breeders in captivity. Gestation is 66–72 days in length. Prominent morphological changes occur during pregnancy at the pubic symphysis. The cartilage joining the two pubic bones begins to dissolve by the thirteenth day of pregnancy. By day 40, only a small cartilaginous bridge extends across the symphysis and by

birth, no cartilage is present. The symphysis widens by 50 times during parturition. Guinea pigs are born precocial. The mammary glands lie just lateral to either side of the midline and become larger during pregnancy. Milk production peaks at 7 days post-partum and regresses at 4 weeks post-partum (Cooper and Schiller, 1976).

NERVOUS SYSTEM

Central Nervous System

The adult guinea pig brain weighs approximately 4g and the cerebral hemispheres are approximately 2.1 cm × 2.25 cm (Cooper and Schiller, 1975). The brain is considered lissencephalic, although a few shallow sulci are evident (Cooper and Schiller, 1975). Most gross anatomical features of the central nervous system including the cerebrum, ventricles, brain stem, cerebellum, and spinal cord are similar to other rodents and mammals. Larsell (1952) described the topographical anatomy of the cerebellum and standardized the nomenclature of the lobules. Larouche et al. (2003) used zebrin II, a widely studied marker of cerebellar homogeneity, to study the topographical anatomy of the guinea pig cerebellum in immunohistochemistry studies. The parasagittal bands of Purkinje cells expressing zebrin II in the guinea pig cerebellum were found to be similar to other animals studied. One characteristic in the guinea pig appears to be at least three levels of expression of zebrin II in the Purkinje cells, whereas other rodents studied either express or do not express zebrin II (Larouche et al., 2003).

As the guinea pig is a common animal model for respiratory studies, it is important to note that respiration control involves the dorsal respiratory group (DRG) and ventral respiratory group (VRG) within the medulla. The guinea pig DRG is located immediately ventral to the tractus solitarius which differs from the rat and cat (Richerson and Getting, 1992).

Peripheral Nervous System

Species differences in the anatomy of the cranial cervical ganglion (CCG) which contain neurons from which post-ganglionic nerve fibers arise providing sympathetic innervation to the smooth muscle, glands, and mucosa of the head have been described (Kabak, 2007). The three major branches of the CCG are the internal and external carotid and jugular nerves. The jugular nerve was found to be a single branch originating from the CCG later splitting into two branches to the vagus and glossopharyngeal nerves near the jugular foramen. No sex differences were noted in studies of the guinea pig CCG (Kabak, 2007). The vascularization of the CCG has also been described (Kabak and Gültiken, 2006).

The guinea pig is a common model of respiratory diseases and literature is published regarding the peripheral nervous system as it relates to the cough reflex in the guinea pig. In the guinea pig, the trachea is well innervated but the small airways have little to no innervation which is similar to humans (O'Donnel et al., 1978; Richardson, 1983). Rapidly adapting and slowly adapting stretch receptors (RARs and SARs, respectively) innervate intrapulmonary airways and lungs which may enhance and facilitate coughing and extrapulmonary C-fibers and RARs can initiate coughing upon activation. However, in the guinea pig, RARs described in some other species like the dog have not been described in the extrapulmonary airways of guinea pigs. Instead, RAR-like receptors in addition to C-fibers, unmyelinated slow-conducting fibers, are present in the extrapulmonary airways of the guinea pig (Canning et al., 2006). Specifically, a distinct class of afferent nerve endings has been identified which stems from the vagus nerve via the recurrent laryngeal nerve to the tracheal muscles that may correspond to slow-adapting stretch receptors (Baluk and Gabella, 1991; Canning et al., 2006). Others describe RAR-like fibers in the guinea pig extrathoracic airways as being derived from nodose and jugular ganglia (Carr, 2004; Undem et al., 2002). Recent interest surrounds the prominent role of transient receptor potential (TRP) ion channels as sensors of airway irritation and initiators of the cough reflex. TRPV1 stimulation results in a robust tissue response in guinea pigs and the TRPA1 channel is being evaluated for its role in the cough reflex in the guinea pig (Geppetti et al., 2010).

SPECIAL SENSES

Hearing

Characteristics of the guinea pig ear such as a large bullae and easy access to the middle and inner ear have caused the guinea pig to be a common animal model for auditory research. Consequently, the anatomy of the guinea pig ear has been detailed in many references (Asarch et al., 1975; Cooper and Schiller, 1975; Goksu et al., 1992; Sanli et al., 2009; Wysocki, 2005). The terminology can differ considerably among references and details of morphology may vary by reference.

The osseous portion of the ear is described as three segments of the temporal bone: the tympanic, bullar, and petrous segments (Cooper and Schiller, 1975) or by others as four elements of the temporal bone: squamous, petrous, tympanic, and mastoid (Wysocki, 2005).

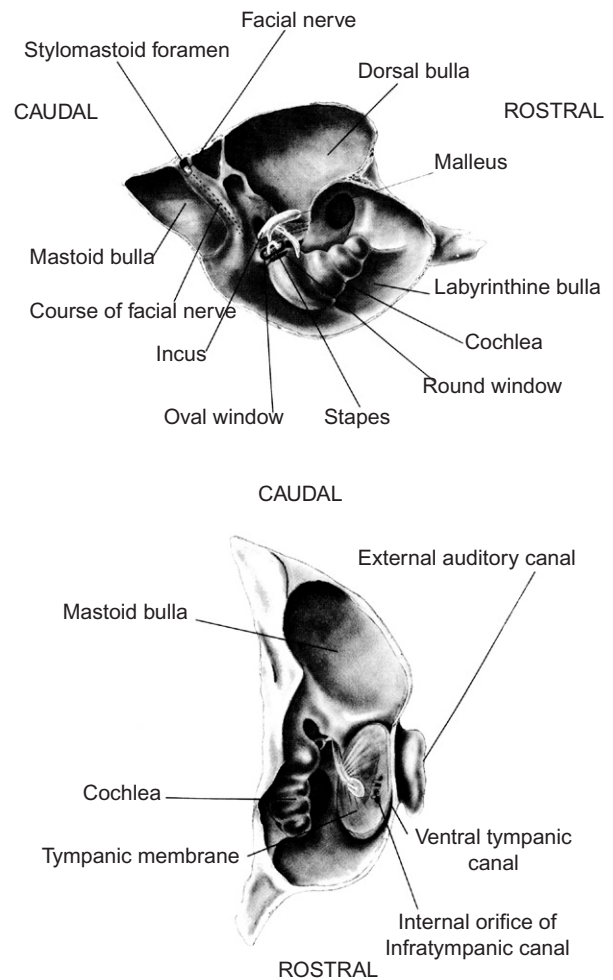
The tympanic portion can be described as three parts: the tympanic ring, tympanic bulla, and dorsal bulla. The tympanic ring forms the walls of the external auditory canal. The tympanic bulla includes the air

spaces of the middle ear and has a trapezoid shape with rounded edges and thin walls. The petrous segment of the temporal bone is the bony capsule of the labyrinth, is pyramid-shaped, and contains three openings for branches of the seventh and eighth cranial nerves. The squamous part adjoins the parietal, frontal, palatine, and ethmoid bones in the area of the acetabulum of the temporo-mandibular joint and is underdeveloped in the guinea pig. The mastoid part forms the mastoid process joined with the occipital bone. The external orifice of the facial nerve canal is in the border of the mastoid and tympanic portions of the temporal bone (Wysocki, 2005). Figures 20.19 and 20.20 show the orientation and internal aspects of the temporal bone.

The inner ear is made of two air spaces termed the tympanic bulla and dorsal bulla, and a bony labyrinth consisting of the cochlea, vestibule, and three semi-circular canals (Wysocki, 2005). Two windows are associated with the cochlea, the oval window placed vertically and the round window placed horizontally (Sanli et al., 2009). In contrast to most mammals, the round window of the guinea pig is directed caudally and dorsally as opposed to ventrally (Cooper and Schiller, 1975). Since this window lacks an overhanging lip of bone, the round window is more prone to injury but also easily accessible to surgical exposure. The round window membrane faces almost directly toward the stapes bone in the oval window. A unique feature of the guinea pig oval window region is the delicate bridge of bone that extends from the fallopian canal through the intercrural space of the stapes bone to the promontory (the basal turn of the cochlea). This may be the remnant of the stepedial artery (Cooper and Schiller, 1975). The guinea pig cochlea lacks a thick bony sheath and consists of 3.5–3.75 turns (Sanli et al., 2009; Wysocki, 2005). The long axis of the cochlea runs almost in a sagittal plane in guinea pigs which differs from humans (Wysocki, 2005).

To analyze solute movements for drug delivery in cochlear fluids, studies have been published detailing the guinea pig round window and its relationship to the scala tympani (Ghiz et al., 2001). The cochlear aqueduct enters the scala tympani at the medial limit of the round window membrane which is approximately 1 mm from the end of the scala when measured along its mid-point (Ghiz et al., 2001). To determine the volume of the aqueduct, the area–distance relationship should be considered (Ghiz et al., 2001) but has been found to average 0.113 mm^3 (Shinomori et al., 2001). The round window in adult guinea pigs has a total surface area of approximately 1.18 mm^2 (Ghiz et al., 2001). The tympanic membrane measures approximately 9–10 mm in diameter and the auditory canal approximately 1 cm in length (Cooper and Schiller, 1975).

Some literature refers to the stylomastoid foramen as the location where the facial nerve exits the medial wall



FIGURES 20.19 and 20.20 Details of the temporal bone. Reprinted by permission of the publisher from *Anatomy of the Guinea Pig* by Gale Cooper, M.D. and Alan L. Schiller, M.D., Cambridge, Mass.: Harvard University Press, Copyright © 1975 by the President and Fellows of Harvard College.

of the temporal bone and courses without protection in a caudal direction towards the cartilaginous external auditory canal (Cooper and Schiller, 1975). Wysocki (2005) gives a detailed description of the course of the facial nerve as it relates to the topographical anatomy of the temporal bone and in his description notes that the guinea pig lacks an internal auditory meatus.

The crista traversa of the internal auditory canal separates the facial and superior vestibular nerves from the other branches of the eighth cranial nerve. The cochlear nerve in the guinea pig consists of approximately 24000 neurons and the vestibular nerve approximately 8000 neurons (Cooper and Schiller, 1975). The cellular anatomy of the guinea pig nuclear complex has been detailed (Hackney et al., 1990).

Rodents can emit and detect acoustical signals between 22 and 85 kHz (Morton, 2002). Data exist

on the arrangement and properties of hair cells in the guinea pig (Pickles, 1985). In addition, comparative studies regarding the number of hair cells, synapses at the hair cell base, and innervation patterns are also published (Nadol, 1988).

Published material is available of intracellular recordings from cochlear hair cells during auditory stimulation. For inner hair cells situated in the high-frequency end of the guinea pig cochlea, resting potentials are in the order of -40 to -45 mV with the response to sound consisting of an alternating potential (Pickles, 1985; Russell and Sellick, 1978, 1983). Others have recorded resting potentials in the low-frequency apical turn of the guinea pig cochlea to be more negative than the inner hair cells at -70 mV (Dallos et al., 1982; Pickles, 1985).

The use of newborn guinea pigs to evaluate hearing began over 100 years ago when Professor Preyer studied sensory function development in neonate humans and animals. The Preyer reflex is a hearing test in which the pinna moves backwards in response to sounds. A normal Preyer reflex may not mean that hearing is normal, however, if the threshold is increased that indicates hearing impairment. This reflex is a value method to evaluate animals used in otologic research and for cochlear function (Bohmer, 1988).

Taste

Interspecies differences in taste preference are most likely due to demonstrated variations in the regional expression of taste receptors among taste regions of the oral cavity and between species (Roper, 2006).

The guinea pig tongue has numerous filiform papillae over the entire surface. Prominent fungiform papillae, four groups of foliate papillae, and two circumvallate papillae are also present (Cooper and Schiller, 1975).

The fine structure of guinea pig taste buds has been characterized and consists of basal, type I, type II, and type III cells. Basal cells are the precursors of the other cell types. The type I cells intervene between other cells to act as structural support. The type II cells are the largest and possess stacks of smooth endoplasmic reticulum above the nucleus. They have a close association with the nervous system and are believed to receive efferent innervation. The type III cells make synaptic contacts with nerves and provide gustatory function (Yoshie et al., 1990).

Vision

The microscopic structure of the cornea is similar to other mammals and has a diameter of approximately 7–7.5 mm (Cooper and Schiller, 1975). The lens is 5.5 mm at its equatorial diameter and 4.0 mm

in its anteroposterior diameter (Cooper and Schiller, 1975). At birth, guinea pigs can visually distinguish between different objects (Gaston et al., 1969). The retina has few vessels clustered around the optic disk (De Schaepdrijver, 1989). The retina is layered similar to other mammals and rods dominate (Peichl and Gonzalez-Soriano, 1994). The guinea pig has dichromatic vision (Howlett and McFadden, 2006; Jacobs and Deegan, 1994) with rods having a peak sensitivity of 494 nm and two classes of cones with peak sensitivities of 429 and 529 nm (Jacobs and Deegan, 1994). The nictitating membrane is a rudimentary structure in this species (Cooper and Schiller, 1975).

Olfaction

On serial coronal sections of the guinea pig nasal cavity, olfactory mucosa is observed lining the posterior roof, the upper nasal septum, and all ethmoturbinates. The olfactory epithelium is avascular. The vomeronasal organ is observed as a paired tubular structure in the lower bony part of the anterior one-third of the nasal septum. It enters into the vestibule of the nasal cavity through a duct lined with stratified squamous epithelium. Its medial wall and upper and lower margins are lined by olfactory neuroepithelium which is pseudostratified columnar and contains intra-epithelial capillaries. The lateral wall of the vomeronasal gland is lined by respiratory epithelium which is avascular. The presence of capillaries in close association with olfactory neurons may be evidence of its neurosecretory activity (Sangari et al., 2002).

Olfaction plays a crucial role in guinea pig behavior, especially regarding reproductive behavior. Additional histological descriptions of the olfactory mucosa of the guinea pig have been published (Sangari et al., 1987).

Tactile

Rodents have a strong tactile sense of touch achieved by well-developed vibrissae which may have a sensitivity of less than 90 μ m (Morton, 2002).

BEHAVIOR

Wild cavies of the genus *Cavia* are social animals that live in small groups of 5–10 in burrows or crevice shelters (Nowak, 1999). In wild cavies neither sex is known to maintain an exclusive territory, but scent marking by secretions of the anal and supracaudal glands and urine to delineate territory has been documented elsewhere in domesticated guinea pigs (Harkness et al., 2002). Males may be seen rubbing their hind quarters along surfaces and may display vocalization, agonistic behavior,

and fighting to define territory. In wild cavies, each sex has its own linear dominance hierarchy. Subordinate animals either retreat or are attacked (Nowak, 1999). Domesticated guinea pigs show less severe aggression but may display aggressive actions such as hair pulling and ear nibbling (Quesenberry et al., 2004). Specifically, the animal may be observed using teeth to pull hair from cagemates and ingesting the hair. This may be used as a means to displace animals from water licks or feed hoppers (Reinhardt, 2005). Mating privileges, space, and food are considered primary stimuli for aggressive interactions (Terril and Clemons, 1998). Attend postures, stand-threats, head-thrusts, and leaping attack lunges are behaviors displayed when guinea pigs respond to a potential threat. In general, though, guinea pigs will seek contact with other guinea pigs when housed together and stand together at rest or at feeding. They do not, however, display social grooming behavior (Quesenberry et al., 2004).

For male guinea pigs, social rearing conditions are crucial for social interactions and endocrine stress responses later in life, as colony-reared animals are found to adjust easily to new social situations as opposed to males raised in pairs which show increased agonistic behavior and shorter latency to escalated aggression with conspecifics (Kaiser et al., 2007). This has not been found to be true in female guinea pigs (Kaiser et al., 2003).

Guinea pigs do not build nests. Approaching parturition, male wild cavies will gather around a pregnant female. The most dominant male will guard the female and will mate her first after parturition (Nowak, 1999). In laboratory settings, within mixed age and sex colonies, male guinea pigs form small harems of females with attachment-like social bonds. These familiar partner males can buffer the cortisol responses of females when exposed to a novel environment (Hennessy et al., 2008).

Under unstable social conditions, the sex ratio of guinea pig pups is significantly more biased towards females than in stable social situations (Kemmer et al., 2009). Preference tests have shown that guinea pig young remain near their mother well beyond weaning, increased time is spent with unrelated females but not males after weaning and littermates spend considerable more time together as opposed to associating with novel animals or potential breeding partners (Hennessy et al., 2003). If guinea pig pups are isolated in a novel environment, they show an initial active phase characterized by vocalizations and locomotor activity followed shortly by a passive stage of crouched stance, eye-closing, and extensive piloerection. This behavior can be alleviated if pups remain with their mother during the introduction to the novel environment (Hennessy et al., 2004). The passive behaviors of pups

during isolation are mediated by proinflammatory cytokines (Perkeybile et al., 2009). Similar behaviors have not been documented if guinea pig pups are left in the home cage when the mother is removed as a familiar socio-spatial environment buffers the endocrine stress response that normally occurs due to maternal separation (Wewers et al., 2003).

Vocal communication is important in precocial species where offspring are actively mobile (Kober et al., 2006) and pups make distinct calls when separated from their mother. Playback studies reveal that mothers respond significantly stronger to calls from their own pups compared to unfamiliar pups, suggesting that mothers are able to use vocal cues in pups recognition (Kober et al., 2006). Mothers of large litters are more responsive to their offspring for a longer period of time than females with smaller litters (Kober et al., 2008).

Vocalizations of guinea pigs have been categorized into 11 call types within five functional categories, specifically – chutt, chatter, whine, tweet, whistle (single or in long bouts), purr, dr, scream, squeal, chirp, and grunt which are used to communicate an increased proximity, greeting and maintaining proximity, regaining proximity, distress, or alarm (Berryman, 1976).

As noted among other rodents, guinea pigs are thigmotactic and will avoid open spaces including middle of cage floors (White et al., 1989). Providing guinea pigs with adequate shelter is an important aspect of their environmental enhancement (Baumans, 2005).

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Management, Husbandry, and Colony Health

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INTRODUCTION

Facility design, environment, and basic husbandry recommendations for the guinea pig are set forth in the *Guide for the Care and Use of Laboratory Animals (Guide)* (NRC, 2011). Since the inception of basic guidelines, standards have become increasingly performance- and “best practices”-based. Although general laboratory animal facility design and principles for rats, mice, and

hamsters have applicability to the guinea pig, characteristics and behavior unique to this species have fostered implementation of new and/or adjunct husbandry practices to improve the care and use of this laboratory animal.

In addition to the natural behavior and physiology of the species, the nature of the research activity to be performed must be considered in planning, design, or modifications of guinea pig holding rooms. Quarantine

facilities, breeding colonies, conventional and infectious disease research have different requirements for housing.

HANDLING

Guinea pigs are generally docile and seldom bite, however they are easily frightened and will try to avoid capture or being held. Guinea pigs not already conditioned to handling will generally run if approached from behind (Bradley, 2001). For instance, guinea pig startling/stampeding may cause abortion or result in orthopedic injuries, particularly in young cavy. It is best to place your hand in the cage in front of the animal to let it become used to it then approach the head, stroke the head and/or top of the nose. Ear scratching and stroking under the chin generally provide positive reinforcement (Bradley, 2001). When picking up a guinea pig, gently place one hand over the dorsum of the thorax and upper abdomen and use the opposite hand to support the hindquarters; or place one hand over the ventrum of the thorax and upper abdomen using the opposite hand to support the hindquarters (Figure 21.1). Care should be taken not to apply too much pressure over the thorax which could result



FIGURE 21.1 Proper method of restraining a guinea pig. (Photo courtesy of the Comparative Medicine Program, Texas A&M University.)

in damage to the viscera or compromise respiration. Pregnant females require additional care in supporting the hindquarters due to significant weight gain in late pregnancy. Never restrain a guinea pig by solely grasping or “scruffing” the skin. The lack of loose skin in the cavy may result in hair depilation using this technique. Due to their precocious development, neonates may be handled from day of birth (University of Miami, 2010). Traditional rodent restraint devices used for mice and rats do not easily adapt for use in the guinea pig due to their compact body structure (Harkness et al., 2002). Wrapping guinea pigs in a towel and holding them close to your body helps to deter struggling.

Allergies to guinea pigs are not uncommon and when handling, it is advisable to wear latex gloves. Surgical or N95 masks (note: an N95 respirator must be fit tested) may provide additional protection for more sensitive individuals. Cage changing stations and dirty bedding dumping stations also help to prevent aerosolization of allergens from dander or urine in the bedding. Small animal cage changing stations that provide laminar air-flow-no recirculation are commercially available. These stations provide high-efficiency particulate air (HEPA) filtered intake and exhaust air for protection of both the research animal and the animal research professional. Similarly, commercially available bedding disposal cabinet systems are available. Protection from allergens is provided through an air barrier across the access opening (100 FPM 0.5 m/s) and HEPA filtration (NuAire, Inc.). Bedding disposal unit options may include vacuum or conveyor belt connections to external dumpsters.

In the wild, guinea pigs live in herds of five to ten with a defined social hierarchy having a dominant male/female pair. Due to their social nature, guinea pigs generally do best in bonded pairs or trios (Bradley, 2001; Walker, 1975). Guinea pigs do vocalize and these vocalizations have been divided into 11 call types based on physical structure of the sonograms. Five functional categories have been defined: calls used to increase proximity; greeting; proximity-maintaining calls; proximity-regaining calls; and distress and alarm calls. These vocalizations include sounds such as whistling (excitement), purring (pleasure), chattering (pursuit), chirping (young wanted to be fed), chattering (agitated), squealing (discontent, danger), rumbling (mating purr), and cooing (mothering sounds) (Berryman, 1976).

HOUSING

Environmental Conditions

Ventilation

Ventilation for laboratory animals should balance air quality, animal comfort, and energy efficiency to

provide cage environments that will optimize animal welfare and research results (Memarzadeh, 1998).

Ten to 15 fresh-air changes per hour have been used as the guideline for secondary enclosures in laboratory animal indoor housing. Drafts should be avoided. Use of performance-based standards as guidance will accommodate a range of mitigating factors including individual species, body size, the number of animals, the type of bedding, type of caging and the frequency of cage changing (primary enclosure factors) and number of cages, room dimension, and air distribution efficiency between these enclosures (secondary enclosure factors). Over-ventilation of the secondary enclosure is not energy-efficient and may create a problematic draft, but under ventilation may inadequately dissipate heat, and allow excessive odor and pollutant accumulation (NRC, 2011).

Historically, air change requirements have been calculated by determining the ventilation requirements (NRC, 1996). The minimal ventilation rate (generally reported in cubic feet per minute) to accommodate the heat loads generated by animals can be calculated with the average-total-heat-gain formula as published by the American Society of Heating, Refrigeration, and Air-Conditioning Engineers (ASHRAE, 1993). This formula is species-independent and is applicable to any heat-generating animal. The minimal required ventilation is determined by calculating the amount of cooling required (total cooling load) to control the heat load expected to be generated by the largest number of animals to be housed in the enclosure in question, plus any heat expected to be produced by non-animal sources and heat transfer through room surfaces. The total-cooling-load calculation methods can also be used to determine the maximal number of animals (based on total animal mass) that can be housed in a fixed ventilation rate space. Odor control, allergen control, and particle generation must also be considered when using minimal required ventilation rates (often calculated below ten air changes per hour) and may necessitate ventilation beyond the determined rate. Conversely, calculated minimal required ventilation may exceed 15 air changes per hour, in which case, increased frequency of cage changes or reduction in animal numbers may be warranted (ASHRAE, 2007a; NRC 1996). Modern heating, ventilation, and air conditioning (HVAC) systems (e.g., variable air volume, or VAV, systems) allow ventilation rates to be set in accordance with heat load and other variables (NRC, 2011).

Individually ventilated cages that utilize an active air supply of filtered room air, or an active independent air supply, reduce the ventilation capacity required of the macroenvironment when compared to passive ventilated caging systems. Passive ventilated systems may need more frequent cage changes than active systems. Non-recycled air is preferred, but not mandatory, in

animal use and holding rooms. The use of recycled air in animal facilities entails risks, particularly for cross-contamination between rooms with animal pathogens that can be airborne or travel on fomites. It is imperative that recycled air should be filtered with 85–95% (at a minimum) ASHRAE-efficient filters to remove airborne particles (NAFA, 1996). More extensive discussion in regards to use of recycled air in animal facilities may be found in the *Guide* (NRC, 2011).

Illumination

Guidelines for illumination take into consideration the physiology, morphology, and behavior of an animal species (Brainard et al., 1989; Erkert and Grober, 1986; Tucker et al., 1984). Factors include; light intensity, duration of exposure, wavelength of light, light history of the animal, pigmentation of the animal, time of light exposure during the circadian cycle, body temperature, hormonal status, age, species, sex, and stock or strain of animal (Brainard et al., 1986). Light in animal holding areas should provide sufficient illumination to allow good housekeeping practices, adequate inspection of animals including cages housed on the bottom of racks, safe working conditions, and general well-being of the animals (NRC, 2011).

The light/dark activity of guinea pigs was first reviewed by Nicholls (1922). Nicholls noted that guinea pigs did not have a true diurnal cycle of activity like traditional laboratory rodents; rather they were active throughout the day and less at night and did not have periods of prolonged sleep. Wild guinea pigs are crepuscular, feeding at dawn and dusk. In the laboratory setting (12 hour: 12 hour light/dark cycle) they feed during the day and night with rest periods between meals (White et al., 1989). One commercial producer of laboratory guinea pigs reports using a 12 hour: 12 hour (light/dark) light cycle for their production colony and 14 hour: 10 hour (light/dark) cycle for their stock colony (Harlan Laboratories, 2010).

Albino animals (including albino guinea pigs) are generally thought to be susceptible to phototoxic retinopathy, although the albino rat is considered the most sensitive to these effects (Beaumont, 2002; Bellhorn, 1980). Recommended light levels are based on levels considered safe for albino rats; 325lux (30 ft-candles) at approximately 1.0m (3.3ft) above the floor with levels between 130–325lux at the cage level (Bellhorn, 1980). This intensity appears to be sufficient for animal care and still safe for albino animals. Very low light intensity has been shown to have a deleterious effect on reproductive physiology, fecundity, and estrus cycles in some rodents (Weihe et al., 1969). For example, light intensity has been reported to affect vaginal opening time, ovarian and uterine weights, estrous cycle length, and preweaning mortality (Donnelly and Saibaba, 1993;

Hautzinger and Piacsek., 1973; Porter et al., 1963; Weihe et al., 1969; Williams, 1971).

Temperature and Humidity

An ambient temperature of 20–26°C (68–79°F), with a relative humidity of 30–70% is recommended for guinea pig housing (NRC, 2011). Ambient temperature recommendations take into consideration the animal's thermoneutral zone (TNZ). The TNZ is the temperature range of a homeothermic animal in which minimal energy exertion is needed to maintain core body temperature. The TNZ is bound by the upper (UCT) and lower critical temperature (LCT) (Gordon, 1993). Ambient temperature outside of an animal's TNZ requires behavioral and physiologic adjustments to ensure homeostasis. These adjustments include postural adjustments, huddling, piloerection, peripheral vascular dilatation or constriction, alterations in respiratory rate, and food consumption. Continued alterations in the ambient temperature for a species combined with inadequate adaptive adjustments result in changes in basal metabolic rate (Clough, 1982; Newton, 1978). Small mammals (i.e. rodents) are generally impacted to a greater degree by alterations in ambient temperature due their large surface area: body weight ratios (Lipman et al., 2002). Even subtle changes in ambient temperature have resulted in changes in the biological effects of insulin, drug effects in toxicity studies, hormonal changes resulting in deleterious effects in breeding, lactation, and offspring survivability (Jans et al., 1987; Yagil et al., 1976). Ideally, a temperature control system should be capable of adjustment in dry-bulb temperature of $\pm 1^\circ\text{C}$ ($\pm 2^\circ\text{F}$) (NRC, 1996). LCT for the guinea pig has been reported as 20–21°C or 29–30°C. and the UCT ranges between 29–34°C; in comparison to the rat with most references in the range of 28–30°C and 30–34°C, respectively (Gordon, 1993). It is recommended that the dry-bulb temperature in animal rooms be set below the animals' LCT to avoid heat stress (NRC, 2011).

Relative humidity (RH) values that trend low or high may also impact research. Elevations in RH affect thermoregulation ability, impact on food consumption (higher RH has shown decreases in consumption) and may potentiate the transmission of infectious organisms (Weihe et al., 1961).

The RH of animal rooms can affect the survival of airborne pathogens and the settling rate of aerosols. Settling rates are a function of air movement and aerosol diameters (for aerosols with a diameter less than 100 μm) (Arundel et al., 1986). High settling rates decrease the amount of aerosols and therefore, reduce the probability of contact with aerosols contaminated with pathogenic substances. High humidity (80–90%) generally leads to higher settling rates by increasing the absorption and therefore size of the aerosol (Hanel, 1977). Conversely, low RH may increase the amount

of infective aerosols produced by exhalation or sneezing by limiting the size of the aerosol (Smith, 1983). Infectious organisms vary on survivability rates in relation to RH. Fungi and mites have increased survivability at RH greater than 70% (Arundel et al., 1986). Viruses composed entirely of nucleic acids and proteins have improved survival in high RH versus lipid-containing viruses which prefer low RH (Knight, 1980). Some bacterial organisms have increased survivability at both low and high RH (<40%, >60%) but decreased survivability at mid-range humidity (Fitzgerald, 1975). The optimum RH range for minimizing adverse health effects appears to be 40–60% (Arundel et al., 1986).

Ammonia production in rodent cages is directly related to the RH of the room (Hasenau et al., 1993; Memarzadeh, 1998). A RH range of 30–50% may be more desirable when static microisolator caging is in use (Hessler and Leary, 2002). Low humidity may slow sexual maturity in rodents (Drickamer, 1990).

Noise

Noise control is a major consideration in the design and operation of animal facilities. Changes in patterns of sound exposure have varied effects on different animals (Armario et al., 1985; Muchnik et al., 1992). Therefore, personnel should try to minimize the production of unnecessary noise, and radios, alarms, and other devices producing sound should not be used in animals' rooms unless they are included as part of a research or enrichment protocol (Lipman and Perkins, 2002). Like other rodents, guinea pigs are susceptible to noise stress (Anthony and Harclerode, 1959). Animals exposed to sounds louder than 85 dB (decibels) can have both auditory and non-auditory effects including eosinopenia, increased adrenal weight, reduced fertility, audiogenic seizures, and reduced locomotor activity (Fletcher, 1976; Geber, 1966; Nayfield and Besch, 1981; Peterson, 1968, 1980; Zondek and Tamari, 1964).

The normal human hearing range is generally noted to be 0.02–20 kHz at 60 dB (Jackson et al., 1999). Wever (1959) reported the hearing range of guinea pigs to be 0.1–40 kHz, with the most sensitive range being between 0.2–2 kHz at 97 dB. More recently, the guinea pig hearing range has been reported as 0.32–50 kHz at 60 dB and 4–28 kHz at 10 dB (Heffner and Heffner, 2007). Rodents (including guinea pigs) and other animals can detect ultrasounds (>20 kHz) that are outside the human hearing range and this ultrasound range may be very important in rodent intraspecies communication, particularly between dam and pup, dominance hierarchies, and mating (Hofer and Shair, 1978; Sales and Pye, 1974; Sales and Smith, 1980; White and Barfield, 1987). Low frequencies (<20 kHz) have also been shown to affect behavior and physiology of laboratory animals. Altered water consumption,

reproductive performance, body weight, learning ability, eosinophil counts, immune responsiveness, corticosteroid blood levels, glucose and insulin concentrations have been impacted in rodents exposed to these low-frequency noises (Anthony, 1959, 1962; Armario et al., 1985; Buckley and Smookler, 1970; Fay, 1988; Lockett, 1970; Wolstenholme and O'Conner, 1967; Zondek and Tamari, 1967).

Due to concern for deleterious effects of noise produced by fire alarm systems, the "silent fire alarm" was developed. The "silent fire alarm" produces a noise intensely irritating to humans but does not awaken rats and mice, or appear to induce a startle response or ear twitching in awake rats and mice. Rabbits and guinea pigs show only "mild disturbance". This alarm produces pure tones alternating between 430 and 470 Hz, below the optimal hearing range for mice and rats (Clough and Fashsam, 1975). Some exotic species of rodents have been reported to still display adverse effects from these "silent alarms". As another alternative, voice alarms have also been used to replace traditional siren alarms.

Vibration has also been associated with biochemical and reproductive changes in laboratory animals (Briese et al., 1984; Carman et al., 2007). Internal sources of vibration include housing systems with moving components (ventilated caging), mechanical equipment, and human activities. Groundborne vibration is related to traffic (subways, trains, trucks). Vibration suppression systems are available (ASHRAE, 2007b).

Sanitation

The *Guide* defines sanitation as "the maintenance of environmental conditions conducive to health and well-being – involves bedding change (as appropriate, cleaning, and disinfection". It further defines cleaning as the removal of excessive amounts of dirt and debris, and disinfection as the reducing or elimination of unacceptable concentrations of microorganisms (NRC, 2011). The Animal Welfare Act (9 CFR Ch. 1 Subchapter A, 1993) requires that "primary enclosures for guinea pigs shall be cleaned and sanitized often enough to prevent an accumulation of excreta or debris, and such enclosures shall be sanitized at least once every 2 weeks by washing them with hot water (180°F) and soap or detergent in a cage washer, or washing followed by a safe, effective disinfectant or live steam". Sanitation schedules and methodology will vary with the type and size of the primary enclosures, the bedding materials utilized, as well as the number and size/age of guinea pigs housed. Protocol-specific requirements related to husbandry may also impact the sanitation schedule. Removal of soiled bedding should be performed as often as necessary to keep guinea pigs clean and dry and frequency is based on professional judgment.

Typical changing schedules vary from daily to weekly. Consideration must be given to the guinea pigs' propensity to "play" with water sippers which may lead to frequent wetting of the cage bedding and fouling of water if water bottles are used. Guinea pigs on water bottles may need daily change outs. Frequent bedding changes may be contraindicated pre- or post-partum and interfere with successful reproduction (NRC, 2011).

Guinea pigs, like hamsters and rabbits, produce urine with high concentrations of proteins and minerals which often adhere to the cage surface and may necessitate acid solution treatment prior to washing. Cages may be disinfected with chemical, hot water, or both. If hot water is used alone, it is the "cumulative heat factor" applied to the surface which provides disinfection; i.e., length of time at a given temperature (Wardrip, et al., 1994); 82.2°C (180°F) is the standard temperature requirement for rinse water. Effective disinfection can be achieved with wash and rinse water at 143–180°F based on time and temperature (1s at 82.2°C/180°F, 15s at 71.6°C/161°F, 30min at 61.7°C/143°F) however, many washers do not allow the machine to complete a cycle that does not reach 82.2°C (180°F) (Hessler and Leary, 2002). When detergents and chemical disinfectants are used, caging, water bottles, sipper tubes, etc., must be thoroughly rinsed prior to reuse. The selected washing time and conditions should be sufficient to kill vegetative forms of common bacteria and other organisms presumed to be controllable by a sanitation program (NRC, 2011).

Automatic watering systems require some mechanism to prevent build up of microorganisms and/or debris. Periodic flushing with large volumes of water or appropriate chemical agent(s) followed by thorough rinsing with water are methods that may be used.

Animal holding rooms, hallways, and support areas should have regular cleaning and disinfection schedules appropriate for the facility.

Sanitation practices should be routinely monitored for compliance and effectiveness. Monitoring may include visual inspection, cage cleaning water temperature monitoring, and/or microbiologic monitoring with swabs and culture, direct agar contact plates, or ATP (adenosine triphosphate) detection systems.

Enrichment

Enrichment of laboratory animals has typically been categorized as social or physical. Social enrichment includes contact and non-contact methods (Baumans, 2005). Guinea pigs are social animals and housing conspecifics may provide the primary valued enrichment, although detailed investigation into their social structure has not been performed. Physical enrichment includes enclosure complexity, nesting material, sensory enrichment, and nutritional enrichment (Baumans,



FIGURE 21.2 Example of enrichment ball and extruded wire flooring with wood shaving bedding. (Photo courtesy of Comparative Medicine Program Texas A&M University.)

2005). Although multiple sources (Bradley, 2001; White et al., 1989) indicate that guinea pigs do not utilize the whole cage space; preferring the periphery, Scharmann noted that when hay or straw was added to the cage, guinea pigs burrowed and hid in it (Scharmann, 1991). Gray et al. (1988) found guinea pigs seek out intracaging shelter when it is available particularly when startled or in breeding situations. Toys such as commercially available ferret roll-about balls stuffed with foraging material, balls with bells inside, bedding bags, hide boxes, PVC piping, foraging materials such as hay and corn husks, and varied food treats have been used (Figure 21.2). Scharmann (1991) noted chewing on bars and hoppers by guinea pigs ceased if wood was added to the cage. Others have noted that hay reduces chewing episodes (Reinhardt, 2007; Sutherland and Festing, 1989). Toys should be made of easily sanitizable materials and be changed out/sanitized, in conjunction with the cage changing schedule. When natural materials such as hay are incorporated into the enrichment program, they should be autoclaved prior to use to prevent microbial contamination (Banjanin et al., 2004).

The potential impact of the cage environment on experimental outcomes must be considered when adding or changing enrichment methodology. “The most significant unexpected impact occurs when an item intended to enhance the animal’s welfare results in harming the animal in some way” (Bayne, 2005).

Caging and Space Requirements

Cage space requirements for the guinea pig are set forth in the *Guide* (see Table 21.1) and are identical

TABLE 21.1 Space Recommendations for the Guinea Pig^a

Weight (g)	Floor Area	
	Per Animal (c ²)	Height (cm)
≤350	387 (60 in ²)	17.8
>50 ^b	≥651.5 (>101 in ²)	17.8

^aFrom the *Guide for the Care and Use of Laboratory Animals* (NRC, 2011).

^bLarger animals may require more space to assure performance standards are met.

to the requirements set forth by the Animal Welfare Regulations (NRC, 2011; 9 CFR, 2002). Cage floor space recommendations generally reflect several times the resting area of the animal. Space requirements for communal species such as guinea pigs have been adjusted to accommodate their communal structure (White et al., 1989). Whenever possible, social animals such as guinea pigs should be housed in pairs or groups as long as group housing is not contraindicated by the protocol in question or causes undue risk to other animals (Brain and Bention, 1979). The cage space should also consider enrichment, individual animal behavior, group housing, and the goals of the housing situation and research protocol (NRC, 2011).

Actual floor space usage was evaluated in groups of breeding guinea pigs. That study found that breeding guinea pigs utilize the periphery of the cage almost to the total exclusion of the center of the cage; approximately 75–85% of day and night time occupancy occurred in 47% of the cage floor periphery (White et al., 1989). In communal groups with pups, the adults align along the perimeter of the cage, end to end, with the pups near the end of the line (Harkness et al., 2002; White et al., 1989).

Additional considerations for housing guinea pigs include their heavy body build. Adults may weigh 1 kg and are prone to developing pressure sores and pododermatitis on wire mesh floors (Fullerton and Gilliatt, 1967). Wire-mesh flooring may also potentiate injuries to feet and legs of smaller, younger guinea pigs which may catch their smaller limbs in spaces between wires. Reduced production in breeding animals, and weight and hair loss in the young, have been reported in guinea pigs housed on these floors (Eidger, 1976).

In the research setting, guinea pigs are commonly held in solid-sided, wire-mesh or solid-floored cages on multi-level racks; individual shoebox style microisolator caging, solid-bottom plastic caging, or solid-bottom plastic caging on ventilated rack systems. Solid-bottom caging is usually bedded with commercially available bedding materials (Harkness et al., 2002). Some institutions place wire bottoms within plastic caging but also use bedding materials. Traditional drawer style rack caging for guinea pigs is available with solid or perforated extruded plastic flooring which allows feces to

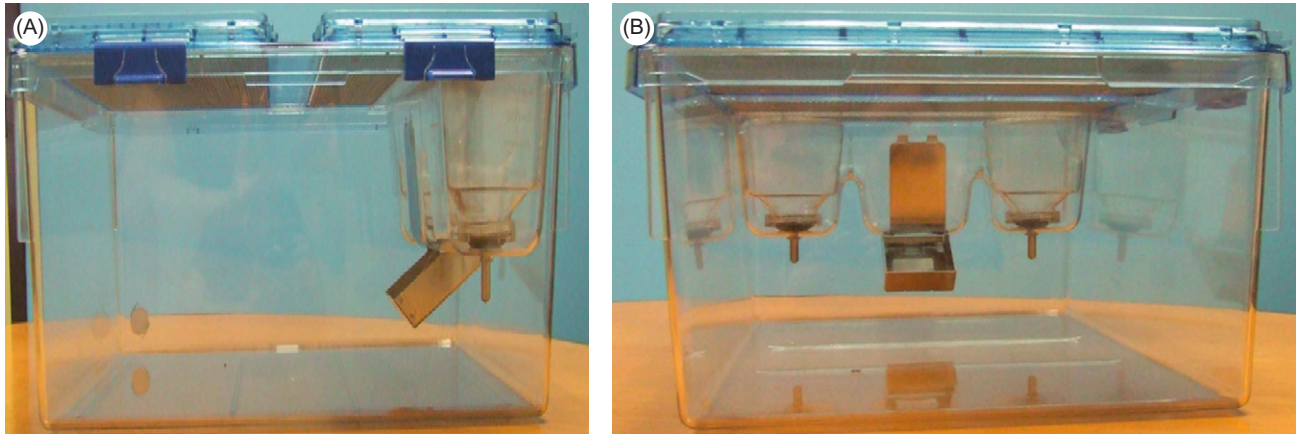


FIGURE 21.3 (A, B) Examples of plastic shoebox style microislator caging, dual water bottle system, and J-feeder for guinea pig housing from the side (A) and end (B). (Photos courtesy of the Comparative Medicine Program, Texas A&M University.)

fall through to a catch pan, and provides a comfortable walking surface without the need for bedding. Others have used mouse cages filled with hay or bedding to house guinea pigs, although use of this method requires attention to assure that internal cage height is sufficient for the needs of the animal(s) and to meet regulatory requirements (Banjanin et al., 2004).

In addition to the safety and comfort of the animal, caging durability and resistance to rust and chemicals should be considered. Stainless steel is the most common metal used and is very durable. Polycarbonate caging (molded plastic) has been used since the 1960s. This caging has high-impact strength and holds up well in 82.2°C wash water. Although polycarbonate withstands autoclaving at 120°C, repeated autoclaving does shorten the life of the cage (opaque changes, cracking). For this reason, various thermoplastics (polyphthalate, polyetherimide, polysulfone, polypenylsulfone) resistant to the detrimental effects of autoclaving were introduced in the 1980s and 1990s (Hessler and Leary, 2002).

When water bottles are used, guinea pigs may have a propensity to push or pile up bedding material which results in wicking of water from the sipper tube, subsequently flooding the cage. The combination of wire flooring (preferably extruded wire) and bedding helps to prevent this problem. Shoebox-style caging often incorporates the use of wire-bar or mesh tops, or plastic mesh tops with filters. Modification of traditional shoebox cages including HEPA filtration of air (passive or active) has been made to accommodate infectious disease work. In non-breeding situations, guinea pig housing typically accommodates one or two animals. Racks with drawer-style caging have been used for breeding colonies (Figures 21.3–21.5).

The Animal Welfare Regulations (9 CFR, Subpart B, section 3.36 parts a–h, 2002) detail the requirements for transport caging of guinea pigs. Briefly summarized,



FIGURE 21.4 Example of microislator filter lids with plastic mesh. (Photos courtesy of Comparative Medicine Program Texas A&M University.)

key requirements include: primary enclosures shall have solid bottoms to prevent leakage, clean litter of a suitable absorbent material which is safe and non-toxic in a sufficient quantity to absorb and cover excreta (unless non-solid floor is also used within the primary enclosure), labeling on the top and one or more sides with “Live Animals” (unless the primary enclosure is permanently affixed to the cargo space of the primary conveyance), and appropriate shipping documents attached (9 CFR, 2002). Guinea pigs from commercial production colonies are usually shipped in standard heavy-duty cardboard shipping boxes with filter-lined air openings, commercial bedding, food and water source (such as gel packs). Air transport must also comply with IATA (International Air Transport Association) rules and regulations.

Transport of guinea pigs within research facilities is generally accomplished using standard solid-bottom, solid-sided microislator caging with filter tops. During



FIGURE 21.5 (A, B) Examples of a microisolator caging system using room supply air as one way flow through. The oval filtered openings on the back of the cage (A) connect to room air supply through manifolds built into the rack (B). (Photos courtesy of University of Texas Health Science Center, San Antonio, Department of Laboratory Animal Resources.)

transport, particularly through common corridors in academic or public settings, cages or racks are generally covered with solid drapes or similar material. This helps to prevent additional stress to the animal as well as limit public view of the animals.

Bedding

Bedding materials traditionally consist of corncob, hardwood chips, shavings, and paper products (Figure 21.6). Some bedding materials may interfere with vitamin C depletion studies due to the presence of low levels of vitamin C in the bedding (Dunhan et al., 1994). Bedding is commonly placed directly onto the cage floor allowing the absorption of urine and providing

material for the animal to burrow into. Sawdust is not recommended as it may lodge in the perineum or prepuce (Plank and Irwin, 1966; Harkness and Wagner, 1995). The preference of guinea pigs for bedding material was reviewed by Kawakami et al. (2003). They concluded that guinea pigs preferred wood shavings to paper cutting sheets (OMEGA-Dri®, Omni Bio Resources, Inc., New Jersey; 6.5–8.0 mm² in size) during light hours and paper bedding during the dark period. It was postulated that the wood shavings provided better nest-building material and shelter from overexposure to light (Lynch et al., 1972). More eating and drinking, and evacuation behavior was noted in the dark period when the paper bedding was preferred (Kawakami et al., 2003).



FIGURE 21.6 Guinea pig housed on wood chip bedding. (Photo courtesy of University of Texas Health Science Center Department of Laboratory Animal Resources.)

Several authors and sources have indicated that high-quality hay should be included as part of the diet and to serve as bedding material. Although guinea pigs were not originally thought to be burrowers, recent references have indicated they do use hay or straw for burrowing, and these products also decrease gnawing on cage components. Hay has also been shown to increase reproductive rates in guinea pig breeding colonies (Reinhart, 2002; Sutherland, 1989).

Room Configuration

Room configurations within a facility incorporate many factors, including security; species to be housed; biological, chemical, and radiation safety; animal health; occupational health; and access to support areas and animal procedure laboratories to name a few. The animal housing area for guinea pigs, as for other rodent species, may include conventional housing, quarantine, biocontainment, and barrier housing (Hessler and Leary, 2002).

The animal room (secondary holding) size should allow easy access to and manipulation of racks for change outs, daily observations, and should include space for support equipment such as changing stations, feed bins, and PPE (personal protective equipment) carts or holders. PPE for conventionally held guinea pigs (gloves, masks, coveralls or lab coats, and booties) can be placed in anteroom areas just outside or just inside the primary animal holding room. PPE and standard operating procedures (SOPs) for barrier facilities (production colonies, biocontainment rooms) will

vary in accordance with the requirements and design of the production facility, or biosafety policies. In general, larger individual animal rooms decrease construction costs but several smaller rooms may make it easier to control infectious disease. Additional safety equipment and specialized housing for containment may require larger room sizes (Ediger, 1976; Hessler and Leary, 2002).

Animal rooms should be close or contiguous with research support areas such as procedure rooms and necropsy (NRC, 2011).

REPRODUCTION AND BREEDING

Guinea pigs are precocious in nature. Male guinea pigs will begin mounting behavior as early as 4 weeks of age, with intromissions occurring around 6 weeks, but are usually not fertile until 8–10 weeks of age. Generally, boars are first used for breeding at 3–4 months, or when they reach 600–700 grams in body weight. Sows may be bred as early as 2–3 months of age, or 350–450 grams body weight and the first breeding should take place prior to 6–7 months of age. After this age, the fibrocartilaginous joint of the pubic symphysis may not separate, resulting in dystocia.

The estrus cycle in the sow is approximately 16 days (13–21-day range) with proestrus lasting 1–1.5 days. Proestrus is characterized by vaginal swelling, rupture of the vaginal membrane, nucleated and cornified epithelial cells on vaginal smear and increased activity (Harkness, 1986; Stockard and Papanicolaou, 1917; Young et al., 1935). Vaginal swelling and congestion, lordosis, perforate vaginal membrane, and cornified epithelial cells signal the onset of estrus which lasts 8–11 hours. The estrus period is followed by metestrus and diestrus lasting 3 and 11–12 days, respectively. In the guinea pig, a fertile postpartum estrus occurs 2–10 hours after parturition (Harper, 1968; Phoenix, 1970; Sisk, 1976). During estrus, the boar will circle the sow, sniff, lick, nibble, and mount the sow. One to two intromissions usually precede ejaculation. Post coitus, the boar may scoot (perianal marking) and groom (Manning et al., 1984). A copulatory or vaginal plug is formed post mating and may be found in the bedding. Mating of guinea pigs typically occurs in the dark (Young, 1969). Generally, 60–85% of all matings are fertile (Harkness, 1986).

Pregnancy can be detected through gentle abdominal palpation. Firm, oval swellings approximately 5 mm in diameter can be felt at day 15 of gestation. By day 25, these swellings measure approximately 7–15 mm in diameter and by day 35 approximately 25 mm in diameter. Individual body parts may be palpated after 35 days of gestation. Obvious abdominal distention

is noted in late pregnancy. In response to relaxin produced by the placenta, the fibrocartilaginous pubic symphysis begins to loosen around day 30 of gestation and this separation grows to approximately 3cm during the last week of gestation (Zarrow, 1947). Pregnancy toxemia or pre-eclampsia in guinea pigs may occur, especially in obese, stressed, multiparous sows with large fetal load (Ganaway and Allen, 1971; Golden et al., 1980). Placentation is hemochorial and strikingly similar to the human (Carter, 2007; Oliveria et al., 2008). Gestation lasts 59–72 days with the gestation length being inversely proportional to the size of the litter. Sows do not build nests. Litters generally consist of 2–5 pups, delivered every 3–7 minutes. The guinea pig is a precocious species, whose pups are born fully haired, with teeth and open eyes and ears. They are fully mobile at birth, with weights ranging from 45–115g and wean at 180g or 14–28 days. Pups begin eating solid food and drinking water a few days after birth, although without milk from the sow during the first 3–4 days of life, pup mortality may reach 50%. Voluntary micturition occurs between 1–2 weeks of age and weaning at 21 days of age (Harkness et al., 2002).

Guinea pig breeding systems incorporate both monogamous (one male, one female) and polygamous (one male, several females) pairings. Males may be left with the sow(s) throughout the gestation and parturition process to take advantage of the sow's fertile postpartum estrus. Continuous cohabitation can result in five litters per year per sow. Heavily bred sows may cease hair growth, resulting in partial alopecia. If the boar is removed prior to parturition, the average number of litters per year is reduced to 3.5 (Harkness et al., 2002). Evidence of estrus cycle synchronization among group-housed sows has not been noted (Donovan and Kopriva, 1965; Harned and Casida 1972). An extended estrus (2–3 days) has been induced in the sow by administration of 1000µg of estradiol for 6 days. The estrus was induced for non-breeding experiments and details on how or if it differs from natural estrus have yet to be determined (Lilley et al., 1997).

Naguib et al. (2009) has shown that concurrent pregnancy reduces lactating guinea pigs' responsiveness to pup calls. Non-pregnant lactating females responded to pup calls whereas most pregnant lactating females did not respond at all.

Hennessy et al. (2003) demonstrated that continuous housing of the pups with the mother suppresses maternal and female sibling-directed sexual behavior in periadolescent (approximately 35 days of age) guinea pig males. If males were housed apart from the mother for as little as 24 hours prior to re-cohabitation, significantly more sexual behavior was observed. This interest (anogenital investigation and other courtship behaviors) was proportional to the time away from

the mother. Interest in strange/new females was not affected. This phenomenon may be the result of pheromones in the home cage or an avoidance of breeding with familiar females as a potential vehicle to reduce inbreeding is not known. In a related study, female novelty in the courtship behavior of male guinea pigs was assessed. This study investigated the sexual behavior of sexually satiated males when placed in contact with a novel mate. The results indicated the existence in male guinea pigs of a process of habituation/recovery of courtship responses under the control of female novelty; i.e., less interest with increased contact of same female(s) and increased interest in new females (Cohn et al., 2004).

Of interest, is a recently identified monogamous wild guinea pig, the "Monogamous Cavy", *Galea monasteriensis*. Mated pairs of this species live harmoniously with each other but are aggressive with other conspecifics, both male and female (Adrian et al., 2007; Hohoff et al., 2002; Solmsdorff et al., 2004). The males of this species exhibit increased levels of paternal care and suppressed reproductive capacity. The testes weight of the males of this species is lower than other wild guinea pigs (Adrian et al., 2005).

NUTRITION

Guinea pigs, like rabbits, are strict herbivores and hind-gut (cecal) fermenters. Unlike rabbits, they do possess lactobacilli and produce propionic acid as the primary fatty acid (Smith, 1965). Guinea pigs require vitamin C in their diet and should be fed chow specifically prepared for this species and supplemented with vitamin C (ascorbic acid). Guinea pigs are coprophagic, usually ingesting fecal pellets directly from the anus (King, 1956; Navia and Hunt, 1976). Pregnant or obese guinea pigs may ingest feces from the cage floor and young animals inoculate their intestines with normal flora by ingesting maternal feces (Harper, 1976; Hintz, 1969).

Feed

Commercial laboratory guinea pig chow is pelleted and contains approximately 18–20% crude protein and 9–18% fiber. Due to the vitamin C supplementation in guinea pig chows, the chow should not be used beyond the manufacturer's recommended shelf life. The use of encapsulated or stabilized vitamin C in the chow has extended the shelf-life of most feeds from 90 to 180 days post milling date, although many institutions still choose the 90-day limitation. Feed should be stored in a cool, dry, dark area that is enclosed to prevent entry of pests. Feed should be stored off the floor on pallets, racks, or carts to allow easy sanitation of the room.

Opened bags of food should be stored in vermin-proof containers to minimize contamination and to avoid potential spread of pathogens (NRC, 2011). Containers of opened feed should be labeled with the milling date and type of feed. Pelleted feed is generally fed in J-type feeders which hang inside the cage suspended from the lid or side; or attached to the cage doors.

Nutritional Requirements

Comprehensive reviews of guinea pig nutrition are available (Mannering, 1949; Navia and Hunt, 1976; Reid and Bieri, 1972). Guinea pigs consume approximately 6 grams of food per 100 grams body weight per day, and require approximately 35% fiber in their diet (Harkness, et al., 2002; Smith 1990). The estimated nutritional requirements for the guinea pig have been summarized elsewhere (Harkness et al., 2002). Guinea pigs will alternate periods of eating with resting through the day and night. As previously noted, several references believe the inclusion of hay for dietary supplementation and enrichment is prudent if not necessary. Timothy hay is preferred (Reinhardt, 2002; Sutherland, 1989). Hay may be autoclaved or irradiated to kill microorganisms. In vitro studies have indicated that autoclaving hay may inhibit fiber degradation (Lancaster and Patterson, 1988). Prepackaged “hay bricks” suitable for autoclaving or irradiated (25 kGy) are commercially available (Lillico Biotechnology, 2009).

Supplementation with vegetables may be incorporated into the diet and enrichment program if not contraindicated by the research, however, vegetable matter may provide a source of bacterial contamination. Supplementation with vegetables and fruit is not commonly done in the research setting for this reason, but if it is included in the diet, vegetables and fruit should be thoroughly cleaned prior to feeding, fed in a separate container from pelleted feed, and leftover supplements removed on a daily basis. Guinea pigs are fastidious eaters and may not readily adapt to novel food items.

Water

Guinea pigs will consume approximately 10 ml of water per 100 grams of body weight per day. Importantly, guinea pigs have a tendency to play with their water bottles which leads to drainage of water into the cage and spitting, reflux, or fouling of the water bottles with saliva and feed particles; thus, water bottles may need to be changed more frequently than with other rodents. If automatic watering systems are used in solid-bottom caging, the valves should be located outside of the cage to lessen the chance of flooding (Harkness et al., 2002). If the water source is changed from water bottles to automatic watering or

from automatic watering to bottles, guinea pigs should be observed for water and food consumption. Abrupt changes in the water delivery system may result in decreased water consumption, and in turn food consumption. One author reported problems with moving guinea pigs from standard caging with water bottle with a 1.4-mm (inner diameter) spout to individual metabolic cages with water bottles with a 0.7-mm spout. This change resulted in decreased fluid intake and subsequent weight loss during a 2-day experimental period. Once the sipper tubes were replaced with the larger size, the animals quickly returned to normal water and feed intake, and regained body weight (Fenske, 1989).

The type of water for guinea pigs ranges from chlorinated tap water, to RO (reverse osmosis) and acidified water sources already in place for rat and mouse housing areas. Consideration must be given to potential effects of the processing and delivery of water. For example, Hall et al. (1980) found that depending on the pH of the water and the acid (hydrochloric vs. sulfuric) used for acidification, there may be a decrease in weight gain, water consumption, and intestinal bacteria isolated from the gut of both immunosuppressed and immunocompetent mice.

Nutritional Diseases

Nutritional diseases are rarely seen in modern research colonies but may be seen as clinical conditions in pet guinea pigs. The following is a brief overview of these conditions.

Hypovitaminosis C (Scurvy)

The primary nutritional disease of concern in the guinea pig is hypovitaminosis C. Like primates and some species of birds, fish, and bats, scurvy in the guinea pig is the result of the inability to produce the hepatic enzyme L-gulonolactone oxidase which converts L-gulonolactone into the isomers L-ascorbate (AH) and 1-dehydroascorbic acid (DHA), thus preventing the conversion of glucose to ascorbic acid (Marcus and Coulston, 1990). Deficiency is often subclinical, and may present as mild conjunctivitis, or non-specific respiratory or intestinal signs. More advanced cases present with weight loss, reluctance to move, and swollen joints (Clarke et al., 1980). The vitamin C deficiency leads to an impaired synthesis of collagen secondary to defective hydroxylase reactions in the formation of hydroxylysine and hydroxyproline amino acids. Treatment consists of supplementation of vitamin C up to 230 mg/kg daily. Recovery is short, often only taking 1–2 weeks (Harkness et al., 2002). Vitamin C deficiency in early postnatal life may result in impaired neuronal development and a functional decrease in spatial

memory in the guinea pig (Tveden-Nyborg, 2009). Because current laboratory guinea pig diets are supplemented with vitamin C, scurvy has essentially been eliminated from the research setting.

Deficiencies of other vitamins

B vitamin deficiencies may occur with symptomology similar to other rodent species. For example, thiamin (B1) deficiency leads to central nervous system problems such as tremors and imbalance. Riboflavin (B2) deficiency leads to corneal vascularization and niacin (nicotinic acid) deficiency in the young may lead to anemia and diarrhea. Pyridoxine (B6) results in folic acid deficiency in young guinea pigs and may progress to terminal convulsions. Pantothenic acid deficiency presents with anorexia, weight loss, and intestinal hemorrhage. In the pregnant sow, it may lead to abortion and possibly death of the sow. Vitamin E and selenium deficiencies may lead to limb weakness (muscular dystrophy), reproductive decline, and even death (Percy and Barthold, 1993).

Pregnancy Toxemia/Pregnancy Ketosis/Pre-eclampsia

Pregnancy toxemia in the guinea pig is recognized to consist of two distinct patterns of disease; a fasting or metabolic (nutritional) form and a circulatory or toxic form. Both forms normally occur in late pregnancy and present with similar clinical signs; depression, acidosis, ketosis, proteinuria, ketonuria, and a lowered urinary pH (5–6).

The metabolic form of pregnancy toxemia occurs in obese sows during the last 2–3 weeks of a sow's first or second pregnancy. The weight of the uterine contents of affected guinea pigs may represent as much as 50% of the total non-pregnant weight of the sow. The disease may be induced by the stress of shipping or changes in feeding routine. Hypoglycemia, glucose, ketosis, and hyperlipidemia are typical findings, with coma and death following 5–6 days after the onset of disease. Necropsy generally reveals abundant fat reserves, fatty infiltration of the liver with microscopic fatty changes in the liver kidneys and adrenals (Percy and Bartold, 2008; Seidl et al., 1979).

The circulatory form of pregnancy toxemia (pre-eclampsia) occurs secondary to vascular changes that lead to ischemia of the uterus and placenta via compression of the aorta (caudal to the renal vessels) by the gravid uterus. The resulting ischemia of the uterus and placenta leads to hemorrhage, necrosis, thrombocytopenia, ketosis, and death. Histological changes include uterine and placental hemorrhage, necrosis and leukocytic infiltration, liver necrosis, nephrosis, and adrenocortical hemorrhage (Percy and Bartold, 2008; Seidl et al., 1979).

Deficiencies of Amino Acids

Restricted diets may result in protein and fatty acid deficiencies. Reproductive complications are a common result. The limiting amino acid for guinea pigs is arginine, followed by methionine and tryptophan (Reid et al., 1960).

Metastatic Calcification

Metastatic calcification may present as muscle stiffness and unthriftiness in the guinea pig. Mineralization may be focal (elbow and rib soft tissue) or diffuse (lung, trachea, heart, aorta, liver, kidney, stomach, uterus, and sclera) and is most often seen in guinea pigs over 1 year of age. It appears this condition is due to dietary imbalances such as low magnesium and high phosphorus, but the exact etiology has not yet been defined (Percy et al., 1993).

Malocclusion

Malocclusion, although not a nutritional disease, may result in nutritional problems in the guinea pig. The hypsodont molars of the guinea pig are prone to overgrowth if the dental surfaces become malaligned. This may result in lingual entrapment, buccal and lingual lacerations, inability to masticate food with resulting weight loss and malnutrition. Drooling or “slobbers” is a classic sign of this condition (Wagner, 1976).

COLONY HEALTH

Preventive Medical Strategies

Preventive medicine strategies should incorporate a multi-faceted program which includes biosecurity, health surveillance, quarantine programs, pest control, well-defined SOPs for cleaning and disinfection, monitoring for compliance of these SOPs, and sound technical and investigative staff training. Detailed descriptions of incorporation of such programs can be found elsewhere (Fox et al., 2002). Biosecurity focuses on preventing transference of infection between animals, from biological and mechanical vectors, and fomites that may contaminate bedding, water, or food (Brachman, 1996; Cohen, 1998; Prince et al., 1991; Waggle et al., 1994).

Today, most laboratory guinea pigs are specific pathogen-free (SPF) animals bred and raised by commercial vendors under barrier conditions. Generally, acclimatization periods for SPF rodents varies from 3–7 days post-shipment. During this time, the animals should be observed by husbandry staff to assure normal activity, ambulation, appetite, and fecal production; and any abnormalities in these parameters or other signs of disease, such as abnormal swellings or masses,

presence of discharges or exudates, or wounds should be reported to the veterinary staff. Quarantine periods for animals transferred from other institutions vary from weeks to months and are based on the animal health histories from the source institution, the receiving institution, housing availability, and research goals.

Once it has been established that an infectious disease is present in the colony, a determination must be made on how to best contain and eradicate the problem. If SPF animals can be obtained for replacement, then depopulation and disinfection is standard practice. Contamination with a zoonotic disease usually warrants euthanasia and disposal of carcasses as biohazardous waste, and decontamination of the premises and support equipment.

Separation of species and animals of different health status can be a very important aspect of preventive medicine. For example, guinea pigs are very susceptible to *Bordetella* infection. Laboratory rabbits, even SPF rabbits, often harbor *Bordetella* without showing any signs of disease. Thus, it is important to separate guinea pigs from rabbits and other species (e. g., dogs, cats, and swine) that could potentially carry *Bordetella*, and husbandry tasks should be assigned and performed in a manner that minimizes the potential for transmission of pathogens such as *Bordetella*. Although vaccination of guinea pigs for *Bordetella bronchiseptica* has been reported, it is not a common practice for the preventive medical care of guinea pigs (Ganaway et al., 1965; Nikkels et al., 1999).

Colony Health Monitoring

SPF guinea pigs are available through established commercial laboratory animal vendors. Current breeding and housing conditions of commercial vendors have provided generally healthy stock which are not typically quarantined, but may need to undergo a period of acclimatization (Harkness et al., 2002; Hessler et al., 2002). Microbiological surveillance programs for guinea pig colonies should be tailored to meet the needs and reflect the concerns of individual programs. Research programs which use limited numbers of SPF guinea pigs for acute or short-duration studies do not necessarily require quarantine or extensive monitoring. Institutions which receive or ship non-SPF, i.e., “conventional” guinea pigs between facilities should incorporate quarantine health-screening procedures (microbiological serologic, and parasite testing). The length of quarantine would reflect the primary infectious disease concerns of the individual institution. Sentinel programs that incorporate euthanasia and harvesting of animals exposed to dirty bedding similar to those used for rats and mice may be cost-prohibitive in guinea pig colonies. Alternatively, surveillance

programs may incorporate the use of retired breeders or necropsy and culture of animals found dead. Screening for ecto- and endoparasites may be performed without sacrifice of the animal.

Barrier facilities are not typically required for most research with guinea pigs, but if the need should arise, the principles and procedures would be based on traditional rodent barrier practices. More commonly, it may be necessary to house guinea pigs in biocontainment areas for hazardous infectious disease work. Practices for laboratory safety, techniques, safety equipment, and facility design are described in detail elsewhere (CDC-NIH 2009; Hessler et al., 2002).

RECORD KEEPING

Identification of Animals

Typically, cage cards are used for identification of guinea pigs. Cards should include the strain of guinea pig, sex, number, principal investigator, and research protocol identification. Breeding group cards may also contain mating and birth records. Individual animal identification may be accomplished by several methods. Temporary identification can be accomplished by dyeing, marking, or clipping of the fur. Dyes such as trypan blue, picric acid, fuschin, or methyl violet as well as permanent marking pens have also been used. These methods generally last only a few weeks. Traditional metal ear tags provide more permanent identification, but may be pulled out by trauma, particularly in group housing situations. Colored guinea pigs have also been identified by coloration patterns (University of Miami, 2010). Microchips provide reliable means of identification. Toe clipping is not a recommended form of identification.

Breeding Records

For many small- to moderate-sized projects involving breeding, notations on breeding, copulatory plug confirmation, pregnancy confirmation, and parturition have been included on the cage card for easy reference. At institutions where cage cards are used as part of colony census and billing, there is a potential for loss of information as cards are turned in. A back-up log, hard copy or computerized format should also be maintained. Room husbandry logs may incorporate breeding records into their database. For institutions using microchip technology, some breeding data may be linked to the ID chip. Automated information systems to aid in the record keeping of rodent breeding colonies have been in use since the 1970s. These systems often have keyboard terminals in each breeding colony room linked to a local

computer for data collection, editing, and short-term storage; with a larger computer network for permanent storage and report generation. Technicians record and enter all breeding activities (births, weaning, disposition of animals, and health observations). Additional information such as pedigrees may be incorporated into these programs (Kalback et al., 1977).

Husbandry and Health Records

Husbandry logs may be kept as hard copy in notebooks, or as data entries for institutions with computer network support within their animal facilities. For individual health checks, many institutions have incorporated “sick call” cards with multiple carbon copies that husbandry staff can place in cage card holders to identify animals requiring veterinary examination or animals that are currently under treatment. With multiple copies, one copy is placed in the cage card holder, one copy is sent to the principal investigator, and another copy is sent to the veterinary staff. These sick call cards include room, cage, animal identification, nature of the problem, space for diagnosis and treatment, dates for reporting, and examination and resolution. Veterinary copies may be filed in the appropriate animal or colony record.

For computerized systems, it is imperative that backup data recovery systems are in place. For institutions that must comply with Good Laboratory Practices (GLP) regulations, additional record-keeping requirements and computer/data security systems may need to be in place as part of software validation requirements (CFR 21, 2003).

The type of colony management software chosen will be based on the needs of the individual institution; the types and numbers of species, type of research involved, regulatory requirements such as GLP, etc. For small organizations with limited numbers of animals, spreadsheets may prove adequate. The upfront costs are low and little training is required to implement a spreadsheet system. However, incorporation of accounting, reports, and statistics into this system requires significant setup time. Data backup requires manual intervention, and accessibility to the information may be slow due to the need for multiple spreadsheets to accommodate broad-based colony information (Duperon, 2010).

Relational databases are more effective methods of storing animal colony data. Whereas spreadsheets are excellent for time-series data (i.e. each row contains a date and then one or more values) and experimental results (treatment data versus outcome), it is difficult to link these different types of information into an easily accessible, interrelated knowledge base. Relational databases are the most effective solution for storing animal colony data, although their use is still inconsistent

through the industry. The key to relational databases is the ability to link together multiple tables or spreadsheets with each other (Tyeus, 2006).

Colony management software systems may be (1) developed in-house, (2) professional client/server database systems, or (3) professional web-based database systems. Solutions developed in-house provide for better backup and data acquisition for reporting, statistics and accounting, and multiple people can usually access those data at one time. However, such systems often have mushrooming expenses, as the systems are often modified to meet additional needs or resolve problems. Other limitations of these systems include the length of time to learn to use these systems, they may not be ACID (Atomicity, Consistency, Isolation, Durability)-compliant with other commonly used databases (Oracle, MySQL, MSSQL, PostgreSQL), and frequently are under-designed leading to system crashes and data corruption and/or loss.

Professional client/server database systems and web-based database systems involve the installation of software on one machine which acts as a server for satellite stations throughout the facility. Advantages of this system include centralization of data; automated backups; capability for simultaneous access by multiple users; and, if using an ACID-compliant database, corruption of the database caused by crash of multi-user accessing is avoided. The company providing the database service typically maintains the software and thus can “fix bugs”, provide support, and install new features. Disadvantages include expense and requiring multiple installations of software throughout the facility, significant training time, and the difficulty to change to a new system if a “transient vendor” goes out of business (Duperon, 2010).

Professional web-based database systems involve the installation of a server which is then accessed via the web on any machine with a browser. The advantages of these systems include: features similar to client/server database systems (i.e., centralization with data matching that in the facility); automated backups; multi-user access; data security with ACID-compliant, reporting ability; and readily available professional support and maintenance. Additionally, there is no client software to install. Crashes generally do not involve the entire application. Disadvantages include expense, training time, and difficulty in transferring to another system.

Benefits of database systems includes real-time census capability, easier budgeting calculations, work tracking, breeding management, and automatic reporting. Pitfalls include the potential for poor implementation and hidden costs. For GLP studies due consideration must be given for how “original data” (handwritten anesthesia sheets, radiographs, and/or lab work that is not digitized) are captured into the computerized data system.

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Basic Experimental Methods

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OUTLINE

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INTRODUCTION

Extensive use of the guinea pig in a variety of experimental procedures ranging from inhalation studies to hearing research has given rise to common biotechnology and the introduction of specialized experimental techniques. All surgical techniques described in this chapter should be performed using aseptic technique, and with the administration of appropriate anesthesia and analgesia.

HANDLING AND RESTRAINT

The handling and restraint of guinea pigs has been previously discussed (Donnelly and Brown, 2004; Terril

and Clemons, 1998). Guinea pigs are easily handled and are docile animals; and they should be approached slowly as they are easily startled and will stampede when being caught. Guinea pigs can squeal and struggle if not handled gently. Most simple procedures can be performed on guinea pigs under manual restraint and chemical restraint is rarely required. When holding the guinea pig, use both hands to grasp it around the chest with one hand placed beneath the thorax and abdomen to provide support while holding (Figure 22.1). Use care not to squeeze too firmly or for too long. For a large guinea pig or pregnant sow, the rear quarters should be supported with one hand while being held to prevent injury. The animal can be held firmly against the body or with the head held in the crook of the elbow for minor procedures. Another restraint



FIGURE 22.1 Manual restraint of the guinea pig with one hand grasping the chest and the other hand supporting the rear legs.

technique involves holding up the anterior portion of the animal while its rear legs remain on the surface supporting the animal, or by holding the chest and forelegs in one hand and the pelvis and hind legs in the other hand. With repeated handling, guinea pigs become accustomed to being held and struggle less.

SAMPLING TECHNIQUES

Blood Sampling

Obtaining blood samples from guinea pigs is difficult. The site for percutaneous blood sampling should be chosen based on the size of the guinea pig, the volume of blood needed, and the expertise of the person performing the phlebotomy. The volume of blood collected depends upon the size of the guinea pig and the time interval between collections. As a general rule, 10% of the circulating blood volume can be collected every 3–4 weeks. For daily sampling, no more than 1% of blood volume should be collected without adverse effects to



FIGURE 22.2 Blood collection from the ear vein. The needle is used to nick the vessel for collection of small amounts of blood.

the animal. The average blood volume of the guinea pig is 75 ml/kg, therefore 7.5 ml/kg body weight could be removed every 3–4 weeks (Terril and Clemons, 1998).

Methods employed to obtain small-volume samples of blood include cutting the nail bed (Hochman and Blanchard, 1983), puncture of the lateral saphenous vein (Hem et al., 1998), the digital veins (Keino et al., 2002), ear vein (Bullock, 1983) (Figure 22.2), the lateral metatarsal vein (Dolence and Jones, 1975), and puncture of the orbital sinus (Sorg and Buckner, 1964). The size of the needle tip or lancet used for nicking the blood vessel should be the smallest possible to enable rapid blood flow. Pressure should be applied after collection to stop the bleeding. For collection of larger volumes of blood, cardiac puncture has been used; however, this technique is always traumatic, must be performed under anesthesia, and is potentially fatal. Indwelling cannula placement in the femoral vein or external jugular vein requires surgical cutdown (Shrader and Everson, 1968), while cannulation of the lateral saphenous vein can be performed percutaneously (Nau and Schunck, 1993). Venipuncture of the cranial vena cava (Reuter, 1987), femoral vein or artery (Palumbo et al., 1975), and medial saphenous vein (Figure 22.3) (Carraway and Gray, 1989) has yielded up to 3 ml per sample. The gauge of needle used for venipuncture should be slightly smaller than the size of the vessel. Stone et al. (1961) reported that carbon dioxide anesthesia increased the yields of blood and serum particularly when exanguinating the animal.

Cerebrospinal Fluid Collection

Up to 300 µl of cerebrospinal fluid (CSF) may be collected by tapping the cisterna magna through the atlanto-occipital space in anesthetized guinea pigs



FIGURE 22.3 Venipuncture of the saphenous vein. This location can also be used for intravenous injections.

using 23-gauge hypodermic needles (Reiber and Schunck, 1983; Suckling and Reiber, 1984). CSF samples (100 μ l) were obtained two times per week from anesthetized guinea pigs by inserting a cannula attached to a 1 ml syringe between the spinous process of the first and second cervical vertebrae (Fassbender et al., 2001). By restraining the head of anesthetized animals using a stereotaxic instrument with ear bars, the tip of the puncture needle can be maintained stably within the cisterna magna for 6–12 hours. This method allows for repeated collections with a total volume as large as 1 ml of CSF (Liu and Guo, 1991). Chronic CSF collection techniques involve surgically fixing a guide cannula secured to a silicone rubber disc to the atlanto-occipital membrane, allowing for daily CSF samples to be collected from conscious, unrestrained guinea pigs (Jones and Robinson, 1981).

Milk Collection

Milk can be collected from guinea pigs by hand milking or by a mechanical milking apparatus. A miniature milking machine using a mechanical vacuum pump has been described for collecting between 1.0–9.0 ml from a lactating sow in one milking (Gupta et al., 1970; Nelson et al., 1951). To eliminate the need for a mechanical pump system, decreasing noise and allowing for collection in animal holding rooms, the operator can serve as the vacuum source using a mouth piece (Anderson, 1990; McKenzie and Anderson, 1979), however the practice of mouth pipetting is discouraged. For satisfactory milking, the level of vacuum should be about 10 inches of mercury and the vacuum pulsations at about 25 per minute (Nelson et al., 1951). It is also possible to

aspirate mammary gland ducts with a 30-gauge needle, if required for experimental procedures (Nonnecke and Targowski, 1984).

Semen Collection

Semen can be collected by singly housing adult male guinea pigs in open-bottom cages and observing service pans for spontaneous ejaculate. One study showed the average number of spontaneous ejaculates ranged from 1–5 over a 14-day period (Shepherd and Martan, 1983). Guinea pig semen can be collected by electroejaculation using an electrode in the lumbar area and an anal electrode. The mean ejaculate volume using this technique was 0.5 ml (Freund, 1958). This technique was detailed further in a report of the characteristics of guinea pig semen (Freund, 1969).

Urine

Urine collection can be performed using a number of techniques (Terril and Clemons, 1998). The urinary bladder is palpated in the caudal abdominal area and when gentle pressure is applied, a stream of urine should be produced. Care should be taken to not apply too much pressure and rupture the bladder. Cystocentesis may be used to collect uncontaminated urine using a needle inserted into the urinary bladder through the abdominal wall. Cystocentesis should be performed following a surgical preparation of the caudal abdomen. If bacterial contamination of the urine is acceptable, urine may be collected from the cage floor or in the pan beneath a suspended cage or using a metabolism cage which can separate urine from feces. A surgical method of complete and continuous urine collection from conscious unrestrained animals via an exteriorized catheter and tether has been described (Mandavilli et al., 1991).

Bile, Gastric Juice, and Feces

The portal vein can be cannulated for blood collection or compound administration by advancing a catheter from a mesenteric tributary vein. Bile can be collected from a catheter inserted in the gall bladder to avoid the difficulty of cannulating the friable bile duct. Both cannulae can be exteriorized via stab incisions in the flank for intermittent sampling (Talbot and Hynd, 1985).

Gastric juice can be collected from conscious restrained guinea pigs. The animals are fasted and housed on wire-bottom cages to prevent consumption of bedding and feces. The guinea pigs are restrained in a vertical position with a gauze loop around the upper incisors maintaining its head in extreme dorsoflexion. A 5- or 6-F infant feeding tube is inserted down the throat, being careful not to

deviate to either side of the buccal cavity. The tube is slid directly down the esophagus, and gastric juice is withdrawn, using a 5-ml syringe attached to the feeding tube. Once the desired volume of juice is collected, the tube is pinched off as it is withdrawn from the esophagus, to prevent backflow of gastric juice (Shomer et al., 1999).

Feces can be easily collected from the floor of the guinea pig's cage. Bauer et al. (2008) measured fecal steroids using enzyme immunoassays to eliminate the profound effect of restraint and the blood sampling procedure on serum cortisol levels. This non-invasive method was validated for the assessment of fecal cortisol, progesterone, and estrogen metabolites, however, fecal and plasma testosterone levels did not correlate significantly.

COMPOUND ADMINISTRATION

Liquids or solids, particularly materials which create problems of contamination such as radioisotopes or microorganisms, may be administered in a gelatin capsule with a small version of a large animal balling gun (Nelson and Hoar, 1969) or through an administration tube inserted into the stomach via the oropharynx (De Brant and Remon, 1991). A simple mouth gag may be used to enable intragastric intubation of the guinea pig by one person without the use of sedation or anesthesia (Smith et al., 1993). Administering compounds by adding them to the drinking water does not provide precise dosing, and due to the guinea pig's aversion to novel tastes, is usually not effective. Solutions or suspensions may be introduced into the bronchopulmonary circulation by occluding the esophagus with a special probe and allowing normal respiratory movements to aspirate liquids placed in the animal's oral pharynx (Vlad et al., 1970). The most common procedure for the introduction of materials in various forms is by injection, either subcutaneous (Figure 22.4), intramuscular (Figure 22.5), intraperitoneal, intrathoracic, intracardiac, or intravenous. Intravenous injections have been administered to guinea pigs by way of the dorsal metatarsal vein (Nobunaga et al., 1966), lateral metatarsal vein (Dolence and Jones, 1975; Grice, 1964), dorsolateral vein of the penis (Grice, 1964; Karlson, 1959), superficial veins of the ventral abdominal wall (Johnson, 1950), the marginal ear veins (Grice, 1964; Karlson and Feldman, 1953; Markham and Kent, 1951), sublingual vein (Waynforth and Parkin, 1969), medial saphenous vein (Hochman and Blanchard, 1983), or the cranial vena cava (Reuter, 1987). Choice of injection site will depend on the size of the guinea pig, amount of compound administered, and the experience and preference of the handler. An indwelling cannula was used in guinea pigs for both repeated injections and blood sampling (Nau and Schunck, 1993; Shrader and

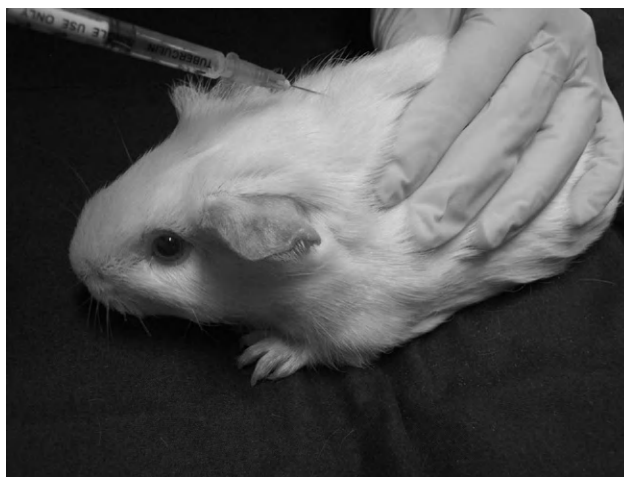


FIGURE 22.4 Technique for subcutaneous injection in the guinea pig. Creating a skin fold aids insertion of the needle under the skin.

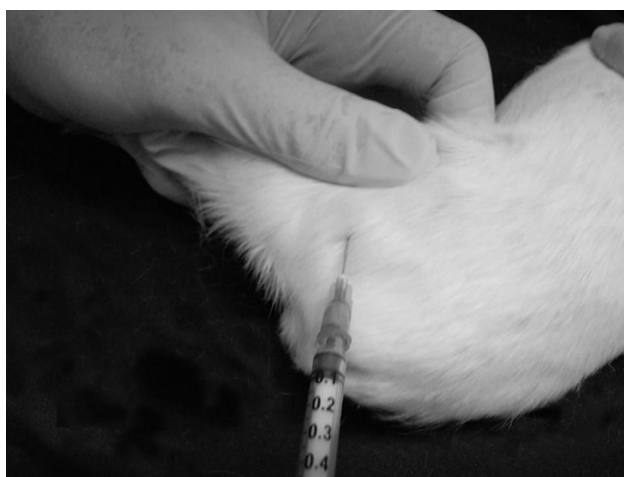


FIGURE 22.5 Technique for intramuscular injection into the gluteal muscles of the guinea pig. The volume of injection should be limited to 0.3ml in adults and care should be taken to avoid the sciatic nerve.

Everson, 1968). Implantable osmotic pumps can be used for continuous administration of compounds into the subcutaneous, peritoneal, or vascular spaces.

SPECIALIZED RESEARCH TECHNIQUES

Endotracheal Intubation

Endotracheal intubation of the guinea pig is difficult due to the narrow oral cavity and the anatomy of the larynx and upper airway. A modified commercially available laryngoscope blade (Kujime and Natelson, 1981; Turner et al., 1992) or pediatric blade using a catheter can be used for direct laryngeal intubation

(Blouin and Cormier, 1987; Tremblay et al., 1990). Kramer et al. (1998) utilized a specially constructed restraint device which assisted in the positioning of the guinea pig for intubation using an endotracheal tube with an outside diameter of 2 mm.

Blouin and Cormier (1987) described a technique for endotracheal intubation without the assistance of another person. The anesthetized guinea pig was placed in a supine position on a surgery board with the upper jaw attached to the surface of the board by an elastic band over the incisor teeth. By proper positioning, the operator could look directly into the opening of the trachea. A small laryngoscope with a modified Wisconsin pediatric blade #0 was used. The blade was narrowed laterally by filing both sides near its extremity, permitting the blade to advance deeper into the laryngeal region without causing trauma while still retaining the tongue and soft palate up and out of the visual path. A cotton tip applicator was used to clean secretions as required. A tracheal cannula was made from an intravenous catheter (14 gauge, 60 mm). The catheter stylet was filed down to remove the sharpened point. Under direct vision, the sterile catheter, with the modified stylet, was advanced into the epiglottis which was lifted with the tip of the catheter. The catheter was then lifted slightly and pushed gently forward into the curved trachea. The laryngoscope and stylet were withdrawn. Formation of water vapor on a clean mirror, with each breath of the animal, confirmed that the endotracheal tube was in place.

Electrophysiology

The guinea pig is preferred by many investigators for study of pre- and neonatal cardiac or cortical activities under various maternal conditions. The technique of Flexner et al. (1950) for determining fetal EEG involved delivery by cesarian section into a bath of warm Ringer's solution while maintaining an intact umbilical circulation. Following craniotomy, electrodes were placed on the cerebral cortex. This procedure was modified by pushing hook electrodes directly through the fetal skull where they would rest on or perhaps 1–2 mm into the fetal cortex (Bergstrom et al., 1961). The technique was further modified in order to prevent loss of amniotic fluid and avoid the trauma of hysterotomy by compressing the fetal vertex against the uterine wall and then pushing the needle electrodes through the myometrium, scalp, and skull to reach the cerebral cortex. A third electrode was sutured to the myometrium over the fetal thorax to record the fetal ECG (Rosen and McLaughlin, 1966a, 1966b; Singer et al., 1973). A chronic model was developed to measure long-term uterine activity for up to 4 weeks in pregnant guinea pigs using a uterine EMG array, intrauterine and intra-abdominal

balloon catheters and vascular catheters (Schellenberg et al., 1995).

Electrical activity has been recorded from the brain in adult guinea pigs by implanting electrodes on the surface of the skull, the epidural tissue, or by stereotactically implanting bipolar electrodes into specific regions of the brain. These techniques have been used for repeated EEG recording for studying central control of heart rate (Pedemonte et al., 2003), sleep–wake cycles (Kato et al., 2007), treatments for epilepsy (Dorandeu et al., 2005), and auditory and visual evoked potentials in response to noise and light (Goksoy et al., 2005) in the guinea pig.

EMG activity was measured to determine musculophysiologic activity. The cutaneous trunci muscle reflex, initiated by brief tactile or electrical stimulation of the skin was shown to be useful as a measure of spinal tract function following spinal cord injury (Blight et al., 1990). Apomorphine-induced jaw movement in ketamine-anesthetized guinea pigs was shown to be similar to lapping jaw movements observed during drinking in awake animals by recording electrical activity of the left anterior digastric and the genioglossus muscles (Gerstner and Goldberg, 1991). To study how work load on a treadmill affects the overall muscle activity and assists in training induced muscle biochemical alterations, EMG fine wire electrodes were chronically implanted in fast-twitch and slow-twitch hind limb muscles (Gardiner et al., 1982). The bladder cooling reflex in response to infusions of cold saline and its effect on external urethral sphincter activity in both the anesthetized and awake guinea pig was studied using electromyographic recordings (Jiang et al., 2008). Avan et al. (1992) studied the protective role of the middle ear muscle in response to loud sounds and active self-vocalization using implanted EMG electrodes.

Cardiovascular Studies

The early drug development process has put great emphasis on the potential of the effect of the compound on QT interval prolongation and other cardiac abnormalities. QT and RR intervals were measured in 6-week and 23-month female conscious guinea pigs to study age-related characteristics on cardiac repolarization (Shiotani et al., 2008). The electrical activity of the hearts of newborn guinea pigs was validated using two different lead systems (Schwartz and Thoss, 1981). Cardiovascular parameters were measured in guinea pigs anesthetized with urethane (1.5 g/kg, i.p.). A polyethylene catheter was inserted into the carotid artery and attached to a pressure transducer. A Millar microtip catheter pressure transducer was inserted into the contralateral carotid artery and advanced to the left ventricle, allowing contractile properties to be measured,

and ECG was recorded by inserting needle electrodes according to standard limb leads. These animals were found to be good models for the hemodynamic effects of repeated intravenous administrations of compounds for safety pharmacology studies, allowing for the measurement of heart rate, blood pressure, left ventricular pressure and ECG (Hauser et al., 2005).

Telemetry has been used to collect cardiovascular measurements from conscious freely moving guinea pigs. Using sterile technique, an abdominal incision was made and a blood pressure sensor was placed in the descending aorta below the renal arteries, pointing upstream. Bipolar electrodes were implanted in the right thoracic ventral serratus muscle and external oblique abdominal muscle to measure ECG. The telemetry transmitter was implanted in the peritoneal cavity and sutured to the abdominal wall. ECG intervals and arterial blood pressure were monitored in these animals for up to 8 months (Hess et al., 2007; Stemkens-Sevens et al., 2009). Implantation of the telemetry system was also accomplished without opening the abdominal cavity. Telemetry transducers were implanted subcutaneously into the back of the neck with the leads for detecting the lead II ECG fixed on the right shoulder and left lower thorax, and a blood pressure catheter introduced into the carotid artery. This subcutaneous approach allowed for longitudinal measurement of cardiac parameters for over 28 days (Provan et al., 2005). It has been difficult to instrument small rodents for multi-lead ECG recording to fully assess cardiac risks of new pharmaceutical agents. By comparing cardiac electrical activity to the magnetic field generated by cardiac activation currents measured by contactless magnetocardiography, Brisinda et al. (2007), showed that multisite recordings could be used for surface cardiac mapping and replace the need for surgical implantation of telemetric devices for long-term longitudinal studies.

A method for inducing prolonged (30s or more) cardiac tachyarrhythmias was developed after rapidly pacing the heart prior to a single 200-V transthoracic stimulus, establishing a small animal model for evaluating short-term pharmacologic effects of electrical therapies (Malkin et al., 1998). Ventricular arrhythmias were also induced in halothane-anesthetized guinea pigs receiving a continuous infusion of adrenaline (epinephrine) (12.5 µg/kg/min). Arrhythmogenicity was significantly increased with vagotomy and higher concentrations of halothane (Noda and Hashimoto, 2004).

A reproducible technique for creating congestive heart failure by aortic banding was detailed by Ling and Bold (1976). Briefly, guinea pigs were anesthetized and ventilated with a small animal respirator. An incision was made in the skin and a thoracotomy was performed in the left third intracostal space. The lung was pushed dorsally to expose the left ventricle and atrium

and the pericardium was opened. Blunt dissection separated the tissue connecting the ascending aorta and the pulmonary trunk. An aortic constrictor (clip bored with a 1.99-mm diameter drill bit) was placed around the ascending aorta, the muscles and skin were closed, and the animal was allowed to recover. This technique resulted in a 74% survival rate, and animals euthanized at 10 days post-surgery showed marked passive congestion of liver, spleen, kidney, and lung, as well as severe centrilobular fatty change of the liver. This technique was used by Kingsbury et al. (1999) to study chronic left ventricular outflow obstruction. After an average of 149 days of banding, there was marked evidence of cardiac hypertrophy (72% increase in heart to body weight ratio compared to sham operated controls) and heart failure.

Aortic outflow was measured using a 2.5-mm electromagnetic flow probe placed on the ascending aorta following a midline thoracotomy. Aortic flow was also measured using thermodilution cardiac output determined by bolus injection of 0.4 ml of physiologic saline solution (25°C) into the right atrium. The temperature change was registered by a thermistor probe in the superficial saphenous artery and cardiac output was calculated with a dedicated thermodilution cardiac output computer. After comparing the thermodilution method to the instantaneous aortic flow, it was determined to be a useful, reliable, and less invasive means of determining cardiac output in the guinea pig (Hart et al., 1987).

Watanabe et al. (2008) showed that the lower lip of the guinea pig contains parasympathetic fibers originating from the otic parasympathetic ganglion which evoke decreases in lower lip blood flow and systemic arterial blood pressure when electrically stimulated by a bipolar silver electrode.

Reproductive Studies

Guinea pigs have not typically been used for reproductive toxicology studies. Compared to other rodents, the estrous cycle is approximately 18 days long, the vagina is closed by a membrane making evaluation of vaginal cytology difficult to impossible during much of the cycle, and the 64-day gestation period is relatively long. Other disadvantages compared to the traditional rodent species are the variability in pregnancy rates, small and variable litter size, relative maturity at birth, and difficulty in repeat dosing of pregnant females (Rocca and Wehner, 2009).

To determine the time of conception for fertility studies and for obtaining cohorts of pregnant females for developmental studies, guinea pigs were mated on the females' post-partum estrus. Pregnant females were co-housed with males starting approximately 4 weeks prior to expected parturition to decrease female

mortality rates associated with pairing during later gestation (Rocca and Wehner, 2009).

Several techniques have been used to determine the timing of the estrous cycle for timed pregnancy and for early determination of pregnancy. Vaginal smears were taken when the vagina was perforate to time the estrous cycle, with the first day of the cycle taken as the day preceding the post-ovulatory influx of leukocytes when cornification was at a maximum (Poyser, 1987). Electrical impedance of the vaginal mucous membrane was measured in the guinea pig using a probe constructed from two silver ring electrodes set 3 mm apart and firmly attached to a plastic rod, diameter 4–5 mm. The probe was introduced into the vagina and electrical impedance was measured by a transistor indicator with a 1-kHz oscillator (Bartos, 1977). A cyclic increase was found during the proestrus phase, more than at other times of the cycle. The disappearance of this cyclic affect indicated early pregnancy (Bartos and Sedlacek, 1977). For early pregnancy studies, the presence or absence of implantation sites in the uterus could be observed at 8 days of pregnancy (Wisel et al., 1994).

Prenatal growth has been studied in guinea pigs. Several methods have been used to influence fetal growth (Jones et al., 1990). Guinea pigs at gestational days 41–42 were injected four times daily over 4 days with 10 ml of 50% glucose intraperitoneally resulting in increased fetal weight. The withholding of food from guinea pigs at days 43–44 of pregnancy for 48 hours led to a sharp fall in maternal and fetal plasma insulin and thyroid hormone concentration, and elevations in plasma glucagon and cortisol, and also depressed fetal weight by about 10%. A unilateral ligation of the uterine artery at day 30 of gestation caused growth restriction of >50% in fetuses adjacent to the arterial tie when measured 10–15 days after the ligation. This effect of growth was particularly apparent in the visceral tissues and liver, whereas the brain was relatively spared. A modification of the uterine artery ligation technique performed between days 30–35 of gestation, resulted in a similar reduction in fetal growth, but a higher fetal survival rate. Instead of ligating the main uterine artery which supplies the blood to one horn of the uterus, approximately half (typically two of four) of the branches leading from the uterine artery directly to one placenta were ablated using a diathermy unit. The treated fetuses showed a 70% reduction in mean body weight at term and had a 245% increase from normal mean values for the brain/liver weight ratios showing the “brain sparing effect” from the redistribution of blood flow to maintain development of the brain (Turner and Trudinger, 2009). The uterine artery ligation method was used to study uterine and placental blood flow by injecting 15- μ m microspheres labeled with ^{46}Sc and counting radioactivity in dissected tissues

(Jones and Parer, 1983). Jansson (1991) showed that three consecutive radiolabeled-microsphere injections at 10-minute intervals in guinea pigs at 47–52 days gestation had minimal effect on regional blood flows and central hemodynamics in anesthetized and awake animals, and allowed for multiple measurements of placental blood flows.

The signs of estrus, including lordosis, in response to manual touch of the back of the guinea pig, and the opening of the vaginal orifice have been described (Young et al., 1937). The effects on spontaneous ovulation associated with the unilateral or bilateral sectioning of the superior ovarian nerves (SON) were analyzed in guinea pigs at different time intervals of the estrous cycle with day 1 defined as the day when the animal presents complete loss of the vaginal membrane. Sectioning of the right, left or both SONs on day 5 (early luteal phase) resulted in a significant increase in the number of fresh corpora lutea, however, ovulation rates were not affected when unilateral or bilateral sectioning was performed during the late luteal phase (day 12) suggesting that the SON modulates ovulation and the degree of modulation varies along the estrous cycle (Luna et al., 2003). Lordosis has been used to study the noradrenergic system and its effects on this behavior (Thornton et al., 1988) and to investigate a non-luteal source of progesterone secretion in guinea pigs (Feder and Goy, 1985).

Surgical sterilization of guinea pigs has been described by Jenkins (2000). Ovariohysterectomy of guinea pigs is performed aseptically through a midline abdominal incision using care when entering the abdomen to avoid the large thin-walled cecum and bladder. The uterus is located and followed to the oviduct and infundibulum. The blood vessels to the ovary are identified and ligated, taking care not to leave a portion of the oviduct. The uterus and uterine vessels are ligated just cranial to the cervix. The mesometrium of hystricomorph rodents can contain an abundance of fat, so care must be taken when locating and ligating the ovarian and uterine vessels. Abdominal closure is routine. To perform an ovariectomy, the ovaries are accessed through individual incisions in the skin caudal to the kidney continued into the retroperitoneal space. Light pressure placed on the abdomen causes the ovary and oviduct to enter the incision. The ovary is exteriorized, ligated with suture at its base, and resected. The ligated vascular pedicle is returned to the abdomen and the dorsal abdominal wall and skin are closed.

Orchiectomy of the male guinea pig is done aseptically by making a 1-cm incision through the skin and vaginal tunic ventrally on both sides of the scrotum. The testis is removed from the tunic and retracted caudally to expose a section of the vas deferens and the vascular structures of the spermatic cord, which are ligated. The duct and vessels are transected distally to the

ligature and the stump is returned to the inguinal canal. Because of the large inguinal opening, the inguinal canal is closed by suturing the tunic and the skin is closed (Jenkins, 2000).

Hearing Studies

Preyer described for the first time in 1881 a pinna reflex in guinea pigs in response to sound, reporting the reflex movements to tones from 1000–41000 cycles per second (Horton, 1933; Preyer, 1890). Using tone-shock conditioning to specific tones, it was shown that guinea pigs could respond to specific tones rather than just noise (Upton, 1929) and that the auditory sensitivity ranged between 64 and 8192 cycles per second, similar to the perception of sound by humans (Horton, 1933). Early studies using recording electrodes in the cochlea showed that tonal interference has its locus in the hair cells of the organ of Corti (Wever and Lawrence, 1941), described the threshold of auditory action potentials for low tones in the guinea pig (Davis et al., 1950), and developed techniques for recording single-fiber responses from the cochlear nerve of guinea pigs from acoustical stimulation (Tasaki, 1954).

Asarch et al. (1975) presented an excellent description of the anatomy of the guinea pig ear and detailed two surgical approaches to the guinea pig temporal bone and to the inner ear. The superior approach, made by an incision at the superior anterior attachment of the auricle and removing the lateral wall of the epitympanic space, exposed the round window, epitympanum, lateral canal, and external auditory canal, leaving the tympanic membrane intact. The inferior approach through the neck exposes the cochlea, Eustachian canal, horizontal and posterior semicircular canals, tympanic membrane, and ossicles. A description of the surgical approach to the endolymphatic sac and the cochlear aqueduct surgical closure of the endolymphatic sac and duct is described for creation of a model of endolymphatic hydrops in the guinea pig (Andrews and Bohmer, 1989). Efferent cochlear innervation, through the olivo-cochlear bundle, has been postulated to exert a protective mechanism on the inner ear. A technique for vestibular neurectomy of both ipsi- and contralateral cochlear efferent fibers in the guinea pig involving a suboccipital route to the posteromedial aspect of the temporal bone allowed for minimal removal and retraction of cerebellar tissue (Barbara et al., 1999).

The guinea pig has been used to study the progression and treatment of deafness by employing aminoglycoside antibiotics to eliminate hair-cell function in the organ of Corti. Bilateral destruction of the organ of Corti was produced by administering 14 subcutaneous injections of 450mg/kg of amikacin with one daily injection 5 days a week (Cazals et al., 1983). A single

subcutaneous dose of kanamycin (400mg/kg) followed in 2 hours by an intravenous injection of the potent diuretic, ethacrynic acid (40mg/kg), caused the permanent loss of hearing and death of cochlear hair cells in guinea pigs for both acute and chronic studies (Nourski et al., 2004; West et al., 1973). Deafness was also induced by administering the loop diuretic, furosemide (100mg/kg) intravenously, followed by kanamycin (400 or 500mg/kg) subcutaneously. Loop diuretics increase the concentration of the aminoglycoside in the scala media causing the loss of the mitochondrial membrane which results in apoptosis of the hair cells (Hildebrand et al., 2005). Conlon and Smith (1998) showed that the co-administration of gentamycin (100mg/kg/day for 30 days) and iron (2mg/kg/day or 6mg/kg/day for 30 days) resulted in a more rapid and profound elevation in compound action potential thresholds compared with animals receiving gentamycin alone. When neomycin is administered systemically at levels high enough to induce ototoxicity, nephrotoxicity also results. To avoid the systemic results and to allow for a contralateral control ear, neomycin was applied by surgical intracochlear infusion. Using a post-auricular approach and exposing the temporal bone in anesthetized guinea pigs, two holes were drilled into the bullae. A 26-gauge needle was used to dispense approximately 0.1ml of 100mg neomycin/ml normal saline solution into one of the holes while the second served as a vent, then the incision was closed. This technique produced significant loss of spiral ganglion cells along the cochlear spiral (Zappia and Altschuler, 1989). Dodson et al. (1994) used this method of cochlear perfusion of neomycin in guinea pigs at ages 1 week and 6 weeks to study the effects of postnatally acquired sensorineural deafness on the maturation of the auditory pathway. The mechanisms for noise-, chemical-, and drug-induced ototoxicity in developing guinea pigs and other mammals are reviewed by Henley and Rybak (1995).

Once deafness is established, therapeutic treatment success can be studied by recording action potentials in the auditory nerve. These potentials can be simulated by both bipolar and monopolar electrical stimulation (Miller et al., 1998) or 4-millisecond tone pips at varying frequencies (Conlon and Smith, 1998). Anatomical changes to the hair cells can be assessed by histological examination of the tissue following fixation with paraformaldehyde (Zappia and Altschuler, 1989). The use of intravital confocal laser scanning microscopy allows the visualization of individual cells inside intact tissue with preserved blood and nerve supply and for the investigation of structural changes caused by toxicity or trauma. A ventral surgical approach to expose the middle ear cavity allowed optical access to the cochlea. Fluorescent vital dyes (calcein AM and RH 795) were administered directly to the cochlea and stained the

cytoplasm and cell membranes of sensory inner and outer hair cells. After exposing the tympanic membrane to 130 dB of sound pressure for 10 minutes, there was a distinct reorganization of the cytoarchitecture and a change in the shape of the visible outer hair cells (Tomo et al., 2007).

The low regenerative capacity of the hair cells of the mammalian inner ear is a major obstacle for functional recovery following sensorineural hearing loss. Mouse embryonic stem cells labeled with green fluorescent protein were transplanted to the aminoglycoside-damaged organ of Corti of guinea pigs. These cells were injected into the scala media cavity of the inner ear. Nine weeks after the xenotransplant, the cells were shown to localize close to the damaged organ of Corti structure in the scala media, however, there was no evidence of integration (Hildebrand et al., 2005). An adenoviral vector expressing the bacterial *lacZ* reporter gene was injected in the endolymphatic sac of guinea pigs. A large number of transduced cells were found in the endolymphatic sac and duct, the vestibular end organ, the cochlea, and the stapes 4 days after the injection. Gene transfer could be used to substitute specific defective genes for treating hereditary deafness or allow for long-term overexpression of protective tropic factors to protect inner ear cells from death due to ototoxicity (Yamasoba et al., 1999). In order to determine the best technique for delivery of cells or gene therapy to the inner ear cells, Backhouse et al. (2008) assessed three surgical procedures for cell delivery into the cochlea; a cochleostomy into the scala tympani; direct access to Rosenthal's canal via localized fracture of the osseous spiral lamina; and direct access to the auditory nerve via a translabyrinthine surgical approach. Using microspheres to simulate placement, delivery, and dispersion of cells, they found that the technique giving direct access to Rosenthal's canal to offer the most promising approach.

Another approach to improve hearing in patients with residual acoustical hearing is the use of cochlear implants. The guinea pig ear can be utilized to test new implant devices or develop techniques for surgical implantation. To install the implant, a dorsolateral, posterior auricular approach is used to gain access to the bulla and the cochlea. A cochleostomy is made with a 0.7-mm diameter drill over the basal turn at a low speed. The implant electrode is inserted with atraumatic technique to the first point of resistance and the cochleostomy is plugged with fascia to prevent perilymph leakage. The extra-cochlear portion of the electrode is secured to muscle with sutures to prevent extrusion (Chang et al., 2009). Preservation of hearing in the implanted ear is a goal of cochlear implant surgery. Utilizing an application of dexamethasone to the round window at the time of the implant protected the residual hearing of guinea pigs with cochlear implants (Chang et al., 2009).

Respiratory Studies

Guinea pigs are one of the oldest models of allergic airway response in asthma, with studies conducted for over 100 years (Auer and Lewis, 1910; Gay et al., 1909; Zosky and Sly, 2007). Smith and Broadley (2007) determined an optimal sensitization protocol for creating a guinea pig with an early asthmatic response followed by a late-phase bronchoconstrictor response, airway hyperresponsiveness to inhaled histamine and methacholine and inflammatory cell infiltration in the airways, hallmarks of human asthma. The guinea pigs were sensitized by intraperitoneal injection of ovalbumin (OA, 100 µg per animal) and aluminum hydroxide (100 mg) in 1 ml of normal saline, in divided doses bilaterally, with a booster injection of the same solution on day 5. After 14–21 days from the first injection, the animals were placed in a stainless steel chamber and exposed to a nebulized solution of OA (10 µg in saline) for 1 hour with animals showing bronchoconstriction at the time of exposure. For collection of pulmonary inflammatory cell infiltrates, bronchoalveolar lavage can be performed after euthanasia by inserting an intravenous catheter into the trachea, instilling saline into the lung, and collecting the fluid (Mukaiyama et al., 2004; Smith and Broadley, 2007).

Normal average values for tidal volume (1.68 ml), respiratory rate (84/minute), minute volume (139 ml), resistance (0.73 cmH₂O/ml/second), and compliance (0.20 ml/cmH₂O) were reported by Amdur and Mead (1958) following their development of techniques for measurement of intrapleural pressure and ventilatory activity. Intrapleural pressures were measured by a fluid-filled tube inserted into the chest cavity and connected to pressure recorders, while ventilatory measurements were determined by a body plethysmography. The technique of plethysmography improved with the use of computer systems to extract the data and by recording from animals without surgical preparation (Alarie et al., 1970; Douglas et al., 1972).

Various plethysmograph chambers and exposure chambers have been designed by research laboratories to measure airway conductance in guinea pigs for studying asthma and other respiratory diseases. Several plethysmography units were designed with two chambers, one encasing the head/neck cavity and the other containing the thoracic/abdominal cavity with a rubber neck divider ensuring a tight seal around each chamber. These systems are useful in exposing the guinea pig to the aerosol, while measuring respiratory-related changes in pressure within the chamber containing the body (Ball et al., 1991; Danko and Chapman, 1988). A single-chamber plethysmograph chamber was designed which allowed for repeated and long-term evaluation of pulmonary performance since the animal

was not restrained by a neck divider used as a seal between the two-chamber units (Wong and Alarie, 1982). Chong et al. (1998) compared the measurement of histamine-induced bronchoconstriction using a double-chamber plethysmograph to that using a single-chamber body box plethysmograph with a central inlet for aerosol administration. They found that the single-chambered plethysmograph correlated well with the double-chambered unit in measuring pulmonary resistance, and the single chamber eliminated stress from restraint, the potential for non-physiological airway resistance due to the neck collar, and allowed for long-term studies since the animal was unrestrained and able to eat and drink during the study.

To test the pulmonary or toxic effect of various compounds on the lungs, several systems have been designed for aerosol exposure of substances to guinea pigs, both while restrained by a neck collar (Snyder et al., 1988), unrestrained (Lewis et al., 2007), or while unrestrained in permanent housing units allowing for exposure of up to 60 guinea pigs at one time (Brown and Moss, 1981).

Chambers have been developed for hyperbaric exposure (Ederstrom et al., 1971; Giblin et al., 1995; Rose et al., 1970; Shoshani et al., 1998). In one long-term study, adult guinea pigs were exposed to 2.5 atmospheres absolute (22.3 psig) for 2–2.5 hours three times per week on alternate days for up to 50 weeks with no apparent ill effects and a steady gain in body weight (Giblin et al., 1995). To study the effects of hypoxia, guinea pigs have been exposed to inspired gas containing 100% down to 8% O₂ balanced with N₂. Yilmaz et al. (2005) exposed 4-month-old, awake, spontaneously breathing guinea pigs raised at sea level, intermediate altitude (1250 meters), and high altitude (3800 meters), to 100%, 21%, and 12% inspired O₂.

The effect of hypoxia was studied in the developing fetal guinea pig brain by inducing maternal hypoxia in dams at 50 and 60 days gestation. Dams were placed in a chamber with a probe to monitor oxygen tension and exposed to 7% O₂ for 40 minutes. There was a 50% reduction in Na⁺, K⁺, and ATPase activity in brains of the 60-day (near-term) fetuses compared to the 50-day preterm fetuses, indicating a high degree of susceptibility of the near-term brain cell membrane functions to maternal hypoxia (Mishra and Delivoria-Papadopoulos, 1988).

Musculoskeletal Studies

Spontaneous joint degeneration and osteoarthritis occur in the knee of the Dunkin-Hartley guinea pig and appear to provide an excellent model for the human disease. Changes in the cartilage and responses to therapy can be studied histologically and by scanning-electron

microscopy, but is limited to observing the cartilage at single time points. A cohort of 30 animals between 2 and 9 months were imaged using a 2.35-T Oxford Instrument 31-cm bore magnet. High-quality 2-D MR images of the knee of anesthetized guinea pigs were generated in about 30 min showing the articular cartilage, subchondral bone, trabecular bone, triangular menisci, and other facets of joint anatomy in fine detail and allowed for serial studies demonstrating the progression of arthritis (Watson et al., 1997). The imaging technique was improved using three-dimensional Varian 4.7-T MRI system, and anesthetized animals were imaged for less than 2 hours. High-resolution, high signal-to-noise 3-D images of the animals serially generated at 3, 6, 9 and 12 months of age, showed changes in cartilage integrity, and bone and meniscus changes which correlated to macroscopic and histological changes in the animals at 12 months (Tessier et al., 2003). Using the change in medial tibial plateau cartilage volume as a MRI biomarker of osteoarthritis, 3-D MR images of 9-month-old guinea pigs treated with doxycycline (0.6 or 3.0 mg/kg/day) or vehicle for 66 days were acquired 7 days pre- and 66 days post dosing. The medial tibial plateau cartilage loss for vehicle treated was 20.5%, while animals treated with 0.6 mg/kg/day doxycycline lost 8.6%, and those treated with 3.0 mg/kg/day lost 10%, demonstrating the value of data collected by serial MRI (Bowyer et al., 2009).

Endocrine Studies

A technique for bilateral adrenalectomy of guinea pigs in two stages was presented by Hoar (1966). The presentation includes a review of the literature, photographs of the operation, a list of equipment, and comments on anesthetic techniques and post-operative care. Hopcroft (1966) used a transverse abdominal incision to remove both adrenals in a single-stage operation. This approach allowed better exposure of both adrenals and, with the intestines limited within a plastic bag containing normal saline, it also allowed more space in which to maneuver within the abdominal cavity.

Surgical thyroidectomy in guinea pigs is a relatively simple procedure but care must be taken to avoid injury to the recurrent laryngeal nerves. Young et al. (1952) and Peterson et al. (1952) were among the first to describe a thyroidectomy technique while Fabre and Marescaux (1966, 1969) and Fabre et al. (1970) noted and measured the rapid recovery of thyroid activity due to the development of small areas of remaining thyroid tissue. Kromka and Hoar (1975) drew from these published reports and added some procedural refinements in their presentations of an improved technique.

Skin Studies

Historically, the most commonly used safety tests to detect either skin or systemic immune-based hypersensitivity reactions are those using guinea pigs (Landsteiner and Chase, 1939; Ramsdell, 1928; Weaver et al., 2003). Protocols of predictive contact allergy tests can generally be divided into three main phases: the induction phase, which stimulates the first contact with the chemical and during which antigen is presented to lymphocytes in the skin, the rest period without treatment during which sensitized cells are proliferating in the lymph nodes, and the challenge or provocation phase during which skin reactions are elicited after new exposure to the chemical (Maurer et al., 1994). A number of protocols have been described identifying chemicals that possess the ability to cause significant skin sensitization, but ultimately only the guinea pig maximization test of Magnusson and Kligman (1969) and the closed patch test of Buehler (1965) continue to be accepted by regulatory authorities in Europe and the USA (Basketter and Kimber, 2007; Consumer Product Safety Commission, 2009; Organisation for Economic Co-operation and Development, 1981, 1992). The maximization test is the most frequently used test in Europe while the Buehler test is most common in the USA (Maurer et al., 1994). Several reviews have questioned the ability of these tests to predict human systemic hypersensitivity (Weaver et al., 2003) and to identify skin sensitization hazards (Basketter and Kimber, 2007; Kimber et al., 2001). More recently, the local lymph node assay (LLNA) using mice has been described to evaluate skin sensitization potential to chemicals and has been accepted by validation authorities in the United States and Europe (Gerberick et al., 2007). While the maximization test and the Buehler test continue to be used, the LLNA is now the recommended method for new assessments of relative potency, and/or for the investigation of the influence of vehicle or formulation on skin sensitization potency (Basketter et al., 2008; Kimber et al., 2001). The methods for the Magnusson and Buehler tests are described below.

For the Magnusson maximization test (Magnusson and Kligman, 1969), albino outbred guinea pigs weighing 300–500 grams are used, avoiding older animals since they are less sensitive. Groups of 10–25 animals are used per test compound. The fur from the dorsal neck is shaved and three intradermal injections of 0.1 ml each are made with the chemical, the chemical incorporated in Freund's complete adjuvant, and adjuvant alone. These injections are repeated on the other side of the neck. One week after the injections, the same area is clipped. The test agent in petrolatum is spread over a 2 cm by 4 cm patch of filter paper and placed on the guinea pig's neck area and secured with overlapping

adhesive tape and then secured by an elastic adhesive bandage wound around the torso of the animal. The dressing is left in place for 48 hours. The animals are challenged 2 weeks after the topical application with another patch test, this time the patch is secured and remains in place for 24 hours. The challenge is read 24 hours after removing the patch to allow for any irritation from the securing tape to subside, and again at 48 hours to detect any weak, slowly developing reactions. Uncertain tests can be repeated within 3–4 days. The reactions are scored on a 4-point scale, 0 = no reaction, 1 = scattered mild redness, 2 = moderate and diffuse redness, 3 = intense redness and swelling. The important parameter in maximization testing however is the frequency of sensitization not intensity.

The Buehler closed-patch sensitization test (Buehler, 1965; Buehler et al., 1985) used young 250-gram albino outbred either-sex guinea pigs. Prior to testing, the primary irritation threshold of the test agent is determined in a pilot study, and the concentration producing minimal amounts of erythema is used to sensitize. An absorbent pad (patch) approximately 4–6 cm² is wetted with 0.5 ml of the test solution and is applied to the shaved back or flank of a guinea pig and secured with a coverlet. The animal is then placed in a restrainer with adjustments made to ensure the animal is restrained but not immobilized. Patches are left on the restrained animal for 6 hours. The compound is applied in the same area weekly for 3 weeks. Two weeks after the last compound application, the animals are challenged with the highest non-irritating concentration of the same test material applied by the patch for 6 hours on a shaved naïve site. At 24 and 48 hours after application of the challenge patch, the sites are graded for degree of erythema and are assigned numbers of 0 through 3, similar to the scoring described for the Magnusson maximization test. Animals with inconclusive results can be retested within a couple of weeks of the primary challenge.

Guinea pigs have also been used to study skin wound healing and for testing a variety of agents to treat or promote wound repair (Carrel and Hartmann, 1916; Spain and Loeb, 1916). Typically partial- or full-thickness wounds are created by excising approximately 2 × 2 cm skin segments from the dorsum of the anesthetized animal. Biopsy punches can also be used to create the skin lesions (Bernstein et al., 1994). Wounds can be closed by primary intention with simple interrupted sutures (Silverstein and Landsman, 1999) or covered and allowed to heal by contraction (Davidson et al., 1992; Lindenbaum et al., 1995). Burns to the skin have also been studied in the guinea pig. Partial-thickness skin burns can be inflicted by immersing the ventral part of the anesthetized animal in 100°C water for 3 seconds (Nanney, 1982; Sakurai et al., 1997) or bathing small

areas of the guinea pig's shaved back in 75°C water for 10 seconds (Tan et al., 2002). A reproducible method to create deep partial-thickness burns utilizes a cylindrical aluminum template (3.76 cm diameter) heated in a water bath for 2 hours prior to the injury at a constant temperature of 75°C. The heated templates are applied to the skin surface in the mid back area on either side for 5 seconds using minimal pressure creating burn wounds (Kaufman et al., 1990).

A wound model for studying ischemic skin ulcers utilized guinea pigs. A 5-cm trans-scapular incision was made in the anesthetized guinea pigs. Using blunt dissection, a subcutaneous path is opened wide enough to accommodate the rubber tip of the plunger of a 10-ml syringe. The stopper is inserted through the tunnel to a site over the vertebral column and 2 cm posterior to the scapula. The insert and skin overlying it are tightly encircled with a rubber band tourniquet, preventing blood flow to a 5-cm² circle of skin over the insert. After 24 hours, the rubber band tourniquet is released and the insert is removed. These ulcers resemble human ischemic ulcers in their progression from edema and blister development through black eschar formation (Constantine and Bolton, 1986).

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Infectious Diseases

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INTRODUCTION

Although guinea pigs are sensitive and susceptible to the development of lesions from a wide range of viruses, bacteria, protozoa, and parasites, only a small number of organisms cause natural infection and only a portion of that group cause clinical disease. The intent of this chapter is to discuss naturally occurring diseases of guinea pigs, although some data from experimental infections may be covered as they relate to the pathogenesis of the disease.

The material is presented under the following headings: background, etiology, epizootiology/pathogenesis, clinical manifestations, pathology, diagnosis, prevention, and therapy. The diseases are discussed in an alphabetical order based on the taxonomic groups to which the organisms belong and are independent of the order of perceived importance of the various diseases.

In laboratory colonies, routine surveillance of guinea pigs is conducted monthly, quarterly, or yearly as required. The Federation of European Laboratory Animal Science Associations (FELASA) recommendations include monitoring for guinea pig adenovirus, guinea pig cytomegalovirus, *Sendai virus*, ectoparasites, endoparasites, *E. cuniculi*, and a variety of bacteria including *Bordetella bronchiseptica*, *Chlamydia psittaci*, *Corynebacterium kutscheri*, dermatophytes, *Pasteurellaceae*, *Salmonella* spp., *Streptobacillus moniliformis*, *Streptococcus* spp., *Yersinia pseudotuberculosis*, and *Clostridium piliforme*. Most laboratories also monitor for these additional viruses, simian virus-5 (SV5), pneumonia virus of mice (PVM), reovirus, lymphocytic choriomeningitis virus (LCMV), and parainfluenza virus-3 (PIV-3). These viruses are routinely monitored both to minimize the risk of transference to mouse and rat colonies (PVM, Reovirus, *Sendai virus*, LCMV) and to identify infections that are zoonotic (LCMV, PIV-3).

VIRAL INFECTIONS

The viral taxonomy used follows the recommendations of the VIIIth International Committee on Taxonomy of Viruses (Fauquet, 2005), but well-established common names for viruses are used when appropriate. Historically, PVM, parainfluenza viruses, cytomegalovirus, and reovirus have been commonly found in guinea pig colonies. From 1984 to 1988 serologic investigations of laboratory animal colonies originating from ten different European countries found seropositivity for four viral infections in guinea pig stocks; reovirus, PVM, *Sendai virus*, and SV5 (see *Paramyxoviridae* section for discussion of serological specificity of *Sendai virus* and SV5) (Kraft and Meyer, 1990). In a 2004 study guinea pigs reared conventionally were positive serologically for *Sendai virus*, PVM, and reovirus (Park et al., 2006).

DNA Viruses

Adenoviridae: guinea pig adenovirus (GPAdV)

BACKGROUND Virus-associated necrotizing bronchopneumonia in guinea pigs is a spontaneous multifactorial disease that has low morbidity, high mortality, and a worldwide distribution (Percy and Barthold, 2007). After the first description in Germany and experimental reproduction of the disease (Kunstyr et al., 1984; Naumann et al., 1981), sporadic spontaneous cases were reported from the United States, Australia, and Switzerland (Brennecke et al., 1983). A 10-year endemic "in-house" occurrence of adenovirus-associated disease was reported in a British pharmaceutical research facility. In all described spontaneous cases, some additional stress factors were involved (Pring-Akerblom et al., 1997).

ETIOLOGY GPAdV is a member of the *Adenoviridae*, genus *Mastadenovirus* that contains canine and bovine adenoviruses as well (Fauquet, 2005). A portion of the hexon gene was cloned and sequenced and found to be

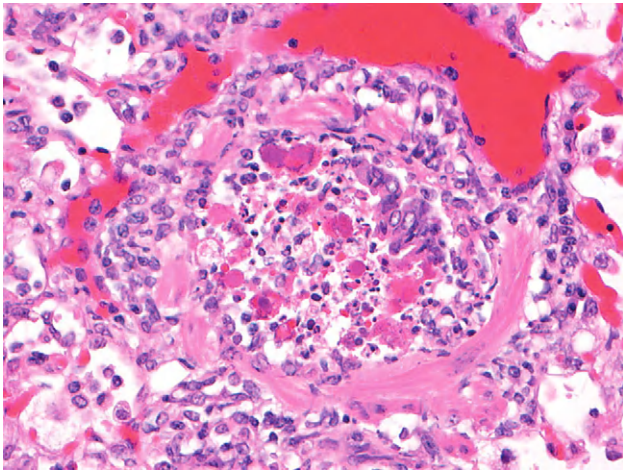


FIGURE 23.1 Section of lung with airway occlusion by necrotic epithelial cell debris and prominent intranuclear inclusions in bronchial epithelium due to adenoviral infection. Photo provided by Cynthia Besch-Williford.

similar to human adenovirus types 2 and 5 in structurally conserved areas, but different in areas associated with type-specific antigenicity (Feldman et al., 2001).

EPIZOOTIOLOGY AND PATHOGENESIS Adenoviral infections in guinea pigs may be more prevalent than are currently recognized as serological tests used to monitor guinea pig colonies use mouse antigens that may not robustly detect serologic responses in guinea pigs (Hankenson et al., 2010; Percy and Barthold, 2007). The virus and disease are reproducible in intranasally inoculated newborn guinea pigs, however, older animals are less susceptible to experimental disease (Kunstyr et al., 1984). The incubation period from experimental infection is 5–10 days.

CLINICAL MANIFESTATIONS GPAdV may cause inapparent infections (Crippa et al., 1997; Hankenson et al., 2010) or severe acute outbreaks with high mortality and low morbidity (Finnie et al., 1999; Naumann et al., 1981; Percy and Barthold, 2007). Dyspnea and tachypnea have been report as well as sudden death. Clinical disease occurs primarily in young animals.

PATHOLOGY Necropsy reveals well-demarcated areas of pulmonary consolidation in the cranial and ventral areas of the lungs (Eckhoff et al., 1998; Finnie et al., 1999; Percy and Barthold, 2007). Histologically, necrotizing bronchitis/bronchiolitis is evident with pronounced desquamation of the epithelium and predominantly mononuclear cell infiltration. Airway occlusion by necrotic epithelial cell debris, leukocytes, and fibrin is seen (see Figure 23.1). In infected epithelial cells of the bronchi, nuclei contain distinctive round to oval basophilic inclusions that are 7–15µm in diameter. Electron microscopy of pneumonic lesions reveals classic intranuclear

adenoviral arrays in epithelial cells. Typical lesions have been observed in the airways of non-symptomatic animals (Crippa et al., 1997; Hankenson et al., 2010).

DIAGNOSIS GPAdV infection can be detected by serology (ELISA, MFIA, or IFA) using antigen from mouse adenovirus (MAV) (Hankenson et al., 2010). Diagnostic labs use both MAV-1 (FL) and MAV-2 (K-87) strains, but unfortunately this method is not specific for GPAdV and infected animals may be only weakly positive using these reagents (Hankenson et al., 2010). Clinically ill animals can be diagnosed by PCR, immunohistochemistry, or characteristic lesions including intranuclear, basophilic inclusion bodies on electron microscopy (Finnie et al., 1999; Hankenson et al., 2010; Percy and Barthold, 2007; Pring-Akerblom et al., 1997).

PREVENTION AND THERAPY Animals with GPAdV are not appropriate for guinea pig pulmonary research projects. Rederivation of infected colonies with aseptic hysterectomy or embryo transfer should eliminate the virus. In addition, there could be a concern that natural infection with GPAdV would interfere with the use of adenovirus gene vectors. This was examined in a study of GPAdV naturally infected guinea pigs that were used as recipients of a human adenoviral vector carrying the gene for green fluorescent protein (Hankenson et al., 2010). In this model system, no difference in transfection-efficiency was seen in guinea pigs naturally infected with GPAdV or GPAdV-free.

Herpesviridae

Three members of the family *Herpesviridae* that specifically infect guinea pigs are catalogued in the Eighth Report of the International Committee on the Taxonomy of Viruses (Fauquet, 2005); *Caviid herpes virus 2* (commonly known as guinea pig cytomegalovirus and in some publications as caviid herpesvirus 1 (Staczek, 1990)), *Caviid herpes virus 1* (also known as guinea pig herpes-like virus or Hsiung-Kaplow herpesvirus, or in some publications as caviid herpesvirus 2 (Staczek, 1990)), and *Caviid herpes virus 3* (also known as guinea pig X virus) (Fauquet, 2005). In addition, *Equid herpes virus 1*, a virus of horses, has been shown to cause severe disease in guinea pigs in one outbreak in a zoo (Wohlsein et al., 2010). Of these viruses, guinea pig cytomegalovirus is the most commonly isolated herpes virus from guinea pigs and serological reactivity to this virus in colonies is not unusual. Guinea pig cytomegalovirus is used extensively in research as an animal model for human cytomegalovirus.

CAVIID HERPES VIRUS 2 (COMMONLY REFERRED TO AS GUINEA PIG CYTOMEGALOVIRUS (GPCMV))

BACKGROUND GPCMV was the first herpes virus identified in guinea pigs and was originally detected by observing intranuclear inclusion bodies in the salivary

glands (Jackson, 1920). Subsequently, virus isolation resulted in the identification of this important animal model of human cytomegalovirus infection (Cole and Kuttner, 1926).

ETIOLOGY GPCMV (species name *Caviid herpes virus 2*) is an unassigned species of the family of *Herpesviridae*, subfamily *Betaherpesvirinae* (Fauquet, 2005). Cytomegaloviruses are species-specific, large, enveloped, double-stranded, DNA viruses that cause diseases in animals and humans (Fox, 2002). The GPCMV genome has been sequenced, is 233kb in length, and consists of highly conserved areas as well as unique open reading frames (ORFs) (Schleiss et al., 2008). When the sequenced glycoproteins of GPCMV were compared to other cytomegaloviruses, most were found to be more closely related to primate cytomegaloviruses (human, chimpanzee, and rhesus) than rodent cytomegaloviruses (rat and mouse).

EPIZOOTIOLOGY AND PATHOGENESIS GPCMV was once relatively common with 70–80% of guinea pigs of 6 months of age or older having typical salivary gland inclusion (Van Hoosier and Robinette, 1976). Current prevalence of GPCMV in laboratory animal colonies is lower with only six out of 15 institutions in Europe from 2000–2003 reporting positive antibody responses to GPCMV (Schoondermark-van de Ven et al., 2006). The incidence of natural infection in guinea pigs appears to be dependent on breeding conditions and husbandry (Staczek, 1990).

Transmission is transplacental and by contact with infected urine or saliva (Choi and Hsiung, 1978; Percy and Barthold, 2007). Transplacental infection occurs primarily during acute infection as once maternal neutralizing antibodies are present, transplacental infection of fetus declines significantly (Choi and Hsiung, 1978). Transmission to neighboring guinea pigs in the same cage is less efficient than transmission via sexual contact with only six out of nine of the same sex pairs seroconverting in one study, while 100% of male/female pairs seroconverted (Choi and Hsiung, 1978).

The pathogenesis of GPCMV has been studied via experimental infection. The severity of the infection is dependent on the virus type, the strain of guinea pig, and the route of infection (Schleiss, 2002). Generally tissue culture-adapted strains produce milder infections than salivary gland-adapted strains. Outbred strains of guinea pigs, such as Hartley, are less susceptible to severe infection and excrete less virus in the urine than inbred strains such as strain 2 and JY-9 (Bia et al., 1979; Hsiung et al., 1980). Infection by mucosal routes usually results in less severe disease than intravenous, subcutaneous, or intraperitoneal inoculation.

Following experimental infection, two distinct phases of infection, acute and chronic, have been observed (Hsiung et al., 1980). In the acute phase, virus

is consistently recovered from the blood and many other tissues including lung, spleen, and kidney during a 10-day period beginning at day 2 post-inoculation (Hsiung et al., 1978, 1980). Viral clearance from the blood and most tissues occurs by 10 days post-infection, but virus remains consistently detectable in the urine and salivary gland during the chronic phase of infection (Bia et al., 1979; Hsiung et al., 1980). Less consistently, virus is found in splenic macrophages, B lymphocytes, kidneys, spleen, pancreas, and cervix of infected animals during this chronic phase.

Pregnancy can lead to more severe disease and changes in viral load following experimental GPCMV infection (Choi and Hsiung, 1978; Griffith and Hsiung, 1980; Griffith et al., 1983). Pregnant guinea pigs may develop interstitial pneumonia, splenomegaly, and necrosis of the liver, kidney, thymus, pancreas, and bone marrow (Griffith et al., 1983). The mortality rate for pregnant animals during an acute viremic episode following experimental infection is significantly greater than for non-pregnant females.

Although the fetus is susceptible to GPCMV infection at any time throughout gestation, transplacental transmission occurs most readily during the acute phase of maternal infection. The frequency of stillbirths and viral infection in neonates is highest in animals infected late in gestation. In neonates, brain lesions are observed and virus can be recovered from the salivary glands and, to a lesser extent, from the brain, lungs, pancreas, and liver of the neonates. In some cases, lesions are detected in the salivary glands of offspring up to 14 weeks postpartum. Placental infection may be detected after maternal clearance of viremia and maintained despite the presence of significant levels of GPCMV neutralizing antibody. The site of infection is localized at the transitional zone between the capillarized labyrinth and the non-capillarized interlobrium of the placenta. Whenever infection of the fetus is demonstrated, the placenta is invariably infected; however, infected placentas may be associated with uninfected fetuses. The status of maternal immunity before pregnancy can favorably affect the outcome of maternal or fetal infections with virulent GPCMV strains (Staczek, 1990).

CLINICAL MANIFESTATIONS Natural infection with GPCMV causes a latent, persistent infection in the salivary gland generally without serious disease in animals in the vivarium (Staczek, 1990; Van Hoosier and Robinette, 1976). However, the death of a sow and fetuses (Motzel and Wagner, 1989) and disseminated infection, including pneumonia, in guinea pigs that were not being manipulated experimentally have been reported (Van Hoosier et al., 1985). Illness is more likely when associated with pre-existent immune suppression or pregnancy (Percy and Barthold, 2007).

PATHOLOGY Natural infection of guinea pigs is characterized by lesions in the salivary glands, kidneys, and liver (Percy and Barthold, 2007; Van Hoosier and Robinette, 1976; Van Hoosier et al., 1985). Infected salivary glands may be enlarged at necropsy. Large, eosinophilic, intranuclear inclusions with karyomegaly and margination of the nuclear chromatin are most commonly seen in the epithelial cells of the salivary duct. Occasional, intracytoplasmic inclusions are seen in ductal epithelial cells as well as mononuclear cell infiltrate around infected ducts. Similar lesions have been described in the epithelium of the proximal and distal convoluted tubules of the kidney. In acute systemic infections, usually associated with experimental infection, interstitial pneumonia with necrosis may be seen with necrosis and inclusions in the liver, kidney, lung, spleen, and lymph node.

DIAGNOSIS Diagnosis has historically been determined by characteristic histopathologic lesions, MFIA, ELISA, or in situ hybridization. A quantitative-competitive PCR (qPCR) assay has been developed, based on the GPCMV homolog of the HCMV UL83 gene, GP83 (Schleiss et al., 2003). The most consistent isolation and highest quantities of viral DNA were observed in liver and spleen, although viral genome could be readily identified in brain, lung, and salivary gland (McGregor et al., 2004; Schleiss et al., 2003).

PREVENTION AND THERAPY Natural infection with GPCMV in laboratory animals likely confounds a variety of research models (Fox, 2002; Percy and Barthold, 2007; Van Hoosier and Robinette, 1976). There is no effective treatment and persistence has been seen. Appropriate screening for the agent prior to studies is suggested. Pet guinea pigs will likely show no clinical signs and treatment will likely not be required.

CAVIID HERPESVIRUS 1 (GUINEA PIG HERPES-LIKE VIRUS, HSIUNG AND KAPOW VIRUS)

BACKGROUND AND ETIOLOGY Guinea pig herpes-like virus (GPHLV) is an unassigned species in the family *Herpesviridae* (Fauquet, 2005). It was originally isolated from guinea pig primary kidney cell culture from both leukemic and nonleukemic Strain 2 guinea pigs (Hsiung and Kaplow, 1969). It does not produce disease in the natural host.

EPIZOOTIOLOGY GPHLV was found primarily in inbred guinea pigs such as strain 2 or strain 13. In one study, GPHLV was isolated from 80% of the guinea pigs of two inbred strains (Hsiung et al., 1980).

CLINICAL MANIFESTATIONS, PATHOLOGY, AND DIAGNOSIS GPHLV does not cause clinical disease in natural infection. Following experimental infection, a

transient increase in mononuclear cells is seen as well as histopathological evidence of lymphoid hyperplasia of the spleen in both the T cell and B cell zones, although no changes were noted in the cervical and mesenteric lymph nodes (Dowler et al., 1984). Intranuclear inclusions in tissues have not been noted in vivo, although they are evident in tissue culture cells (Dowler et al., 1984; Hsiung et al., 1971a, 1971b; Van Hoosier and Robinette, 1976). Virus can be recovered from the lymph nodes and spleen of infected animals well after clinicopathological signs of infection end. Serologically, this virus is distinct from GPCMV and GPXV and can be differentiated from these viruses both morphologically and serologically (Hsiung et al., 1980).

PREVENTION AND THERAPY GPHLV is not a clinically important disease of guinea pigs, but could be a complicating factor in research either through interaction with other viruses or cytopathological effects on cultured cells.

CAVIID HERPESVIRUS 3 (GUINEA PIG X-VIRUS)

BACKGROUND AND ETIOLOGY Guinea Pig X-Virus (GPXV) is an unassigned species in the family *Herpesviridae* (Fauquet, 2005). It was originally isolated from leukocytes of strain 2 guinea pigs that were free of GPHLV and GPCMV (Bia et al., 1980). In one study of 112 Hartley and strain 2 guinea pigs tested serologically for GPXV, 38% were found to be positive.

CLINICAL MANIFESTATIONS AND PATHOLOGY Inoculation of GPXV into Hartley guinea pigs caused viremia which persisted for at least 3 weeks (Bia et al., 1980). Virus could be recovered 5–6 months after inoculation suggesting a persistent infection. In this study, 50% of the animals died between 6 and 11 weeks post-inoculation. Hepatic necrosis was the only consistent lesion seen. GPXV is both morphologically and serologically distinct from GPCMV and GPHLV.

PREVENTION AND THERAPY Natural disease from GPXV is unknown, but similar to GPHLV, this virus may be a complicating factor in research.

EQUID HERPESVIRUS 1 (EHV1) (ALSO KNOWN AS EQUINE HERPES VIRUS 1 AND EQUINE ABORTION VIRUS)

BACKGROUND AND ETIOLOGY EHV1 is an equine pathogen that causes a persistent infection in horses with a variety of clinical presentations including respiratory disease, abortion, neonatal death, and neurologic disease (Reed and Toribio, 2004). EHV1 is a species in the genus *Varicellovirus* of the *Alphaherpesvirinae* subfamily (Fauquet, 2005). Other members of this genus include

the type species *Human herpesvirus 3* (varicella-zoster virus or chickenpox) and *Bovine herpesvirus 1* (infectious bovine rhinotracheitis virus).

PATHOGENESIS AND CLINICAL MANIFESTATIONS

One recent report describes infection with EHV1 resulting in hindlimb paralysis, ataxia, abortion, or stillbirth in 18 of 80 guinea pigs at a European zoo (Wohlsein et al., 2010). In this outbreak, Thomson gazelles (*Equus thomsoni*) kept in the same building as the guinea pigs were first affected and suffered a short course of fatal neurologic disease. The source of the virus was unclear, although a similar strain of virus was isolated 6 months earlier from affected black bears (*Ursus americanus*) and clinically unaffected onagers (*Equus hemionus kulan*). In the second outbreak, a similar strain of virus was also recovered from clinically unaffected zebra (*Equus quagga boehmi*) housed in the same building as the guinea pigs and gazelles.

PATHOLOGY Lympho-histiocytic meningoencephalitis was seen predominantly in the olfactory bulb and the frontal cortical regions of the brain with neuronal and glial necrosis, gliosis, and intranuclear inclusion bodies (Wohlsein et al., 2010). EHV1 antigen was demonstrated in the neurons, neuronal processes and glial cells by immunohistochemistry and encapsulated herpes viral particles of 120–150 nm were detected by electron microscopy.

DIAGNOSIS Differentiation from other members of the *Herpesviridae* is important for diagnosis. Immunohistochemistry, virus isolation, PCR, and DNA sequencing were used in this report to identify the virus involved (Wohlsein et al., 2010).

PREVENTION AND THERAPY EHV1 infection of guinea pigs is an example of the severe disease seen when members of the *Alphaherpesvirinae* infect unusual hosts similar to *Human herpes virus 1* (herpes simplex virus) which has been shown to infect guinea pigs experimentally (Wohlsein et al., 2010) and has also been reported to cause naturally acquired clinical disease in rabbits (Weissenböck et al., 1997) and chinchillas (Wohlsein et al., 2002). Separation of species, control of fomites, and use of appropriate personal protective equipment can be used to prevent transmission of members of the *Alphaherpesvirinae* subfamily of viruses to aberrant hosts such as guinea pigs.

Pox-Like Viruses

There has been one report of pox-like virus particles obtained from tissue culture samples of fibrovascular proliferations involving the rear limb of half of a colony of guinea pigs (Hampton et al., 1968). Particles resembling pox viruses were observed by electron microscopy.

RNA Viruses

Arenaviridae: Lymphocytic Choriomeningitis Virus (LCMV)

BACKGROUND The first identified arenavirus, LCMV, was isolated in 1933 and shown to cause aseptic meningitis in humans and was named for its ability to cause lymphocytic choriomeningitis in mice and monkeys upon intracerebral injection (Amman et al., 2007; Charrel and de Lamballerie, 2010; Percy and Barthold, 2007; Van Hoosier and Robinette, 1976). LCMV is a rodent-borne zoonotic arenavirus endemic in housemice (*Mus musculus*) worldwide. Infection by LCMV in people is known to cause acute central nervous system disease and congenital malformations.

ETIOLOGY The viral genus *Arenavirus* includes 22 viral species, the type species is LCMV (Fauquet, 2005). Like other *Arenaviruses*, LCMV is a non-cytopathic, enveloped, single-stranded RNA virus.

EPIZOOTIOLOGY AND PATHOGENESIS LCMV is uncommon to rare in guinea pigs (Fox, 2002; Percy and Barthold, 2007). In May 2005, the Centers for Disease Control and Prevention reported a cluster of LCMV infections among four solid organ recipients in Rhode Island and Massachusetts who received organs from a single apparently asymptomatic donor (Centers for Disease Control and Prevention [CDC], 2005a). Recipients became gravely ill shortly after transplantation; three subsequently died. LCMV was identified as the etiologic agent. Viral sequences from the organ recipients were identical to those from a pet hamster acquired by the donor's household 17 days before organ donation. Sequence and phylogenetic data provided strong support for the presence of the same LCMV lineage in hamsters and guinea pigs in the Rhode Island pet store and the Ohio distribution center (Amman et al., 2007; CDC, 2005b).

CLINICAL MANIFESTATIONS Clinical signs of LCMV are uncommon in the guinea pig, however meningitis with hind limb paralysis has been reported (Van Hoosier and Robinette, 1976).

PATHOLOGY LCMV in guinea pigs results in lesions involving the brain including lymphocytic infiltrates in meninges, choroid plexus, and ependyma (Percy and Barthold, 2007). Lymphocytic infiltrates are also seen in the liver, adrenal, and lungs. Experimentally, neutrophilic destruction of the splenic red pulp, focal bone marrow necrosis, lymphopenia, and death occur following infection with the more virulent (WE) strain (Djavani et al., 1998).

DIAGNOSIS Diagnosis is typically by PCR, IFA, ELISA, or MFIA (Charrel and de Lamballerie, 2010; Fox,

2002; Percy and Barthold, 2007). Detection of viral antigen in tissue or section via immunohistochemistry is also feasible. Immunity to LCMV is primarily cellular, but virus-specific antibody is produced.

PREVENTION AND THERAPY Lymphocytic choriomeningitis is a zoonotic disease of concern with rodents kept as pets (Pickering et al., 2008). Human infection occurs most commonly through exposure to secretions or excretions of infected animals. In addition, LCMV can contaminate transplantable tumor or cultured cell lines from mouse, hamster, and guinea pig, and viral stocks (ILAR, 1991; Percy and Barthold, 2007). In mice, LCMV has been shown to limit tumor induction by polyomavirus, mouse mammary tumor virus, and transplantable guinea pig leukemia, delay tumor rejection, and alter sensitivity to endotoxins (ILAR, 1991). Natural infections would be expected to interfere with research studies involving enterohepatic, lymphoid, musculoskeletal, nervous, respiratory, and urinary systems.

Coronavirus-Like Infection

Coronavirus-like particles were found in the feces of young guinea pigs that developed wasting, anorexia, and diarrhea with low morbidity and mortality (Jaax et al., 1990). At necropsy, there were large amounts of mucoid material throughout the small intestine. Histologically, the primary lesion was acute to subacute necrotizing enteritis involving the distal ileum with blunting and fusion of the affected villi, necrosis and loss of enterocytes, and mucosal syncytial cells were evident. By electron microscopy, viral particles consistent with coronavirus were demonstrated in fecal matter from affected animals. Coronavirus shedding was also noted in the feces of clinically normal guinea pigs (Marshall and Doultree, 1996). The virus has not been isolated.

Paramyxoviridae

Paramyxoviridae is composed of pleomorphic, enveloped, linear, negative-sense single-stranded RNA viruses occurring worldwide in animals and humans, often associated with subclinical infections of the respiratory tract (Fauquet, 2005; Simmons et al., 2002). The family is split into two subfamilies, *Paramyxovirinae* and *Pneumovirinae* (Fauquet, 2005). In guinea pigs, three distinct members of the *Paramyxovirinae* have been described; *Human parainfluenza virus 3* (including *Human parainfluenza virus 3*, *Cavian parainfluenza virus 3*, and *Guinea pig parainfluenza virus 3*), *Sendai virus*, and *Simian virus 5* (SV5). In addition, murine pneumonia virus, a member of the *Pneumovirinae*, has been described in guinea pigs.

HUMAN PARAINFLUENZA VIRUS 3, CAVIAN PARAINFLUENZA VIRUS 3, GUINEA PIG PARAINFLUENZA VIRUS 3

ETIOLOGY *Human parainfluenza virus 3* is a member of the genus *Respirovirus*, whose type species is *Sendai virus* (Fauquet, 2005). Sequence analysis of a Guinea-pig parainfluenza virus 3 isolated in 1998 from a colony of guinea pigs in Japan indicates that it has 95.6–97.9% nucleotide identity to *Human parainfluenza 3 virus* and 71.0–79.6% to *Bovine parainfluenza virus 3* suggesting this virus was introduced into guinea pigs from a human with human parainfluenza virus 3 (Ohsawa et al., 1998). A novel paramyxovirus (cavian parainfluenza virus 3) was isolated in 2002 from a colony of guinea pigs (Simmons et al., 2002). This virus has 94% nucleotide identity to guinea-pig parainfluenza virus 3 and *Human parainfluenza virus 3* and 80% nucleotide identity to *Bovine parainfluenza virus 3*.

EPIZOOTIOLOGY AND PATHOGENESIS Seroconversion in endemic parainfluenza-3-positive breeding colony was studied in 2002 (Blomqvist et al., 2002) and indicated that pups become infected from 2 weeks to 8 weeks of age. The virus did not persist as sentinels exposed to 4–5-month-old seropositive animals did not seroconvert. Experimental infections indicate that seroconversion in naïve animals occurs approximately 10 days following infection (Graziano et al., 1989). In an infection study guinea pigs inoculated with cavian parainfluenza virus 3, *Sendai virus*, simian virus 5 (SV-5), murine pneumonia virus, or bovine parainfluenza virus developed no clinical signs of disease or lesions during the eight-week course of the study although they developed robust homologous or heterologous serologic responses to the majority of the antigens (Simmons et al., 2002). The serologic response, using an ELISA, of those inoculated with SV-5 was modest or equivocal. In addition, SV-5 ELISA resulted in the highest degree of non-specific reactivity among all of the paramyxovirus assays.

CLINICAL MANIFESTATIONS AND PATHOLOGY In one study, no clinical signs or lesions were seen when guinea pigs were infected with Cavian parainfluenza virus 3 (Simmons et al., 2002). In a separate study, guinea pigs were shown to be susceptible to human parainfluenza virus 3, developing peribronchiolar and interstitial lesions (Clyde, 1980).

DIAGNOSIS Identification of the virus via PCR and sequencing is ideal as cross-reactive serological results make interpretation of serology difficult (Simmons et al., 2002).

PREVENTION AND THERAPY It is apparent that Human parainfluenza virus 3 can be transmitted to

guinea pigs and infection-control measures such as the use of face masks, gloves, and laminar flow hoods to prevent transmission in laboratory settings is prudent. It isn't known whether human or cavian parainfluenza virus 3 can be transmitted to humans from guinea pigs.

SENDAI VIRUS

ETIOLOGY *Sendai virus*, also known as Murine parainfluenza virus, is the type species in the genus *Respirovirus*, which also contains the species *Human parainfluenza virus 3*, *Bovine parainfluenza virus 3*, and *Human parainfluenza virus 1* (Fauquet, 2005).

EPIZOOTIOLOGY AND PATHOGENESIS Numerous studies have reported seropositivity to *Sendai virus*, including a publication in 1978 which describes seropositive reactions by HI or CF in seven out of 16 colonies tested over a 13-year period in the United States (Parker et al., 1978). In addition, there is one report of isolation of *Sendai virus* from guinea pigs associated with mice (Van Hoosier and Robinette, 1976), although no clinical signs were reported. More recently, five of 15 European guinea pig colonies were seropositive to either *Sendai virus*, *Human parainfluenza virus 3*, or both (Schoondermark-van de Ven et al., 2006). Inoculation of guinea pigs with *Sendai virus* in one report resulted in no clinical signs of disease or lesions (Simmons et al., 2002).

DIAGNOSIS As with the other *Paramyxoviridae*, lack of specificity can result in false-positive results with routine serological testing (Simmons et al., 2002). In one study, Sendai ELISA-positive samples were seen in guinea pigs infected with Pneumonia virus of mice and Bovine parainfluenzavirus 3. Guinea pigs were also positive by IFAT in the case of Bovine parainfluenza virus 3 infection.

PREVENTION AND THERAPY The importance of *Sendai virus* infections in guinea pigs is unclear. However since it is a significant disease of mice housed in laboratory animal colonies, guinea pigs should be maintained free of *Sendai virus*. In addition, *Sendai virus* is used as a vector in research and natural infection or sero-reactivity may interfere with such uses (Baker, 1998).

SIMIAN VIRUS 5 (SV5)

SV5 is a species of the genus *Rubulavirus* which also contains mumps virus and human parainfluenza virus 4 (Fauquet, 2005). SV5 was initially isolated from simian kidney cell cultures and has also been shown to infect guinea pig cells in vitro (Zakstelskaya et al., 1976). Serological reactivity to SV5 has been reported in guinea pig colonies (Kraft and Meyer, 1990). Seropositive guinea pigs show no clinical signs or lesions and inoculation with SV5 results in seroconversion, but not clinical signs

of disease (Simmons et al., 2002). Infection of guinea pigs with *Sendai virus*, pneumonia virus of mice, or *human parainfluenza virus 3* can result in positive SV5 ELISA results which were negative by indirect fluorescent antibody test, indicating low specificity of the SV5 ELISA (Simmons et al., 2002).

MURINE PNEUMONIA VIRUS

(FORMERLY PNEUMONIA VIRUS OF MICE (PVM))

ETIOLOGY PVM is a member of the genus *Pneumovirus*, species *Murine pneumonia virus* (Fauquet, 2005). Other members of the genus *Pneumovirus* include the human and bovine respiratory syncytial viruses. The complete genomes of two strains of this virus were sequenced in 2005 (Thorpe and Easton, 2005). PVM is most often subclinical in immunocompetent mice although in immunodeficient mice, it can cause chronic wasting with cyanosis and dyspnea (Percy and Barthold, 2007).

CLINICAL MANIFESTATIONS AND DIAGNOSIS

Seropositive reactions to PVM have been seen historically in guinea pig colonies in animals without apparent disease (Van Hoosier and Robinette, 1976). Inoculation of guinea pigs with a strain of PVM that caused mortality in immunocompetent and immunodeficient mice resulted in no clinical signs, although the guinea pigs seroconverted within 11 days after inoculation (Griffith et al., 1997).

PREVENTION AND THERAPY Transmission of PVM from guinea pigs to mice, or vice versa, appears to be possible and similar in this regard to *Sendai virus*. In laboratory colonies, it is important to keep guinea pigs free of PVM in order to minimize potential transmission to mice.

Picornaviridae (Poliovirus, Theiler's Murine Encephalomyelitis Virus)

It is unclear whether laboratory guinea pigs may harbor a poliovirus which, in 1911, was described as the cause of a disease called guinea pig lameness (Hansen et al., 1997; Van Hoosier and Robinette, 1976). A similar disease was described by Rohrer in 1958 (Van Hoosier and Robinette, 1976). In these investigations, sera from affected guinea pigs reacted positively to Theiler's murine encephalomyelitis virus (TMEV) antigens. Clinical signs involved a flaccid paralysis and weight loss with an incubation period of 9–23 days, and a duration of 1–2 weeks. Affected animals had meningoencephalitis. More recently, two pet shop guinea pigs were reported to suffer from rear limb paresis and generalized debilitation. The guinea pigs had extremely high titers against "poliovirus", while healthy guinea pigs from the same pet shop were negative (Hansen et al., 1997). The diseased guinea pigs recovered

fully after treatment with vitamin C in the drinking water. Further testing of other guinea pig colonies by the same authors demonstrated antibody responses to TMEV in 35 out of 152 laboratory guinea pig sera. Positive results were found in two out of six breeding centers, and in three out of three experimental units, all of which purchased guinea pigs from one of the seropositive breeding colonies. Guinea pig sera from some of the breeding units were also positive serologically for other infections; adenovirus, PVM, reovirus type 3, *Sendai virus*, SV5 and *Encephalitozoon cuniculi*. In experimental studies of susceptibility of laboratory animals to six strains of human poliovirus, newborn guinea pigs were the only species of laboratory animals in which multiplication of any of the viruses could not be detected (Koroleva et al., 1975).

Reoviridae: Reovirus

Naturally occurring antibodies to reovirus have been detected in laboratory guinea pig colonies by IFA, ELISA, and MFIA (Percy and Barthold, 2007; Van Hoosier and Robinette, 1976). No clinical signs or lesions have been described. Experimentally guinea pigs have been exposed to a reovirus associated with the SARS coronavirus (Liang et al., 2005).

Retroviridae: Cavian Leukemia

BACKGROUND Cavian leukemia was first described in 1954 by Congdon and Lorenz as being caused by a retrovirus, however herpes viral inclusions have also been seen in leukemic guinea pigs (Jungeblut and Opler, 1967; Opler, 1969; Percy and Barthold, 2007; Van Hoosier and Robinette, 1976).

ETIOLOGY Type C retrovirus particles have been seen in cases of cavian leukemia (Hsiung et al., 1980; Van Hoosier and Robinette, 1976). An endogenous guinea pig retrovirus was induced by bromodeoxyuridine (Michalides et al., 1975; Nayak, 1974). Molecular hybridization techniques were used to show that guinea pig virus nucleotide sequences are endogenous to both domestic (*Cavia porcellus*) and indigenous (*Cavia aperea*) guinea pigs, but cannot be detected in the DNA of other mammals tested (Dahlberg et al., 1980).

In 1982, a transplantable leukemia (KSL) of unknown etiology, that had the characteristics of a B cell tumor, was found in a female Sewall-Wright strain 2 guinea pig. KSL leukemia was also shown to be distinct from another guinea pig lymphatic leukemia (L2C) with respect to cell morphology, antigenicity, and in vivo growth rate (Key, et al., 1983). A C-type virus was reported in the urine of diabetic guinea pigs (Lee et al., 1978).

CLINICAL MANIFESTATIONS Clinical signs of cavian leukemia include lethargy, pale mucous membranes, secondary infections, peripheral lymph node enlargement,

and death (Van Hoosier and Robinette, 1976). Leukocyte counts may vary from 25000–250000, with the preponderance of cells being lymphoblastic. Peripheral lymphadenopathy is generally evident.

PATHOLOGY Enlarged lymph nodes in the cervical, axillary, mediastinal, retroperitoneal, and inguinal areas are seen in cavian leukemia with splenomegaly and hepatomegaly (Van Hoosier and Robinette, 1976). There is a moderate infiltration of leukemic cells in all organs including the spleen, liver, bone marrow, lung, thymus, gastrointestinal associated lymphoid tissue (GALT), heart, eyes, and adrenals (Opler, 1969).

TREATMENT AND CONTROL There is no described treatment.

Rhabdoviridae: Rabies

Rabies has been reported in a pet guinea pig associated with a bite from a raccoon (Eidson et al., 2005).

BACTERIAL INFECTIONS

Gram-Positive Organisms

Clostridia

Most clostridia are large, spore-forming, Gram-positive, anaerobic rods. The one exception is *C. piliforme* which is Gram negative and is discussed in the section covering Gram-negative organisms below. Two species of clostridia are discussed here, *C. difficile* and *C. perfringens*.

CLOSTRIDIUM DIFFICILE (ANTIBIOTIC-ASSOCIATED TYPHILITIS)

BACKGROUND Severe profuse diarrhea, associated with *Clostridium difficile* in guinea pigs treated with antibiotics, is an important and preventable disease process.

ETIOLOGY *Clostridium difficile* is most commonly associated with antibiotic-induced typhilitis in guinea pigs, although other organisms including *Escherichia coli*, *Clostridium perfringens*, *C. sordelli*, *C. histolyticum*, and *Bacillus pumilis* have been implicated in different studies (Boot et al., 1989; Brophy and Knoop, 1982; Knoop, 1979; Lowe et al., 1980; Rehg and Pakes, 1981, 1982; Rehg et al., 1980; 1982). *C. difficile* is a common anaerobic, spore-forming, fecal-borne, Gram-positive, rod-shaped organism that generally produces both an enterotoxin (toxin A) and a cytotoxin (toxin B) that are crucial in the pathogenesis of disease (Greenwood, 2007).

EPIZOOTIOLOGY AND PATHOGENESIS Normal guinea pigs may carry low resident populations of *C. difficile* in the intestines and toxins can be identified

in normal cecal contents (Boot et al., 1989). In one study, *C. difficile* was recovered by culture in one out of eight normal guinea pigs and 18 out of 34 guinea pigs with typhilitis. Oral or parenteral antibiotic administration, including penicillin, ampicillin, streptomycin, clindamycin, lincomycin, spiramycin, aureomycin, erythromycin, and bacitracin, have all been implicated in causing antibiotic-associated typhilitis by permitting an overgrowth of primarily Gram-negative organisms including toxin-producing *Clostridium* such as *C. difficile* (Young et al., 1987). The enteric flora of guinea pigs is primarily Gram-positive and those antibiotics that target Gram-positive organisms preferentially are of greatest risk to cause this disease (Farrar and Kent, 1965). One study demonstrated that in the 24–48 hours post-administration of penicillin, there was a significant increase in Gram-negative anaerobic and aerobic (coliforms predominantly) bacteria bringing the levels of coliform bacteria from less than 100 organisms per gram to 10^8 – 10^9 organisms per gram. This shift in the normal gut flora allows bacteria such as *C. difficile*, which is generally nearly undetectable, to predominate (Farrar and Kent, 1965; Fox, 2002; Percy and Barthold, 2007). Other Gram-negative organisms, *E. coli* in particular, and other *Clostridium* spp. also can contribute to disease.

Clostridium difficile (toxin)-associated typhilitis has also been diagnosed in guinea pigs not treated with antibiotics that had been rederived by caesarian section and were exposed to mice as a source of anaerobic flora (Boot et al., 1989). Two years after rederivation, an outbreak of typhilitis was investigated. Young guinea pigs 2–4 weeks old and a few adults were affected. *Clostridium difficile* was isolated from half of the animals tested and the cytotoxin was identified in cecal contents from 95% of the affected animals. Co-infection with *C. perfringens* and *Encephalitozoon cuniculi* was present. The authors speculate that the anaerobic flora that the animals were exposed to was not sufficient to protect guinea pigs from *C. difficile* infection. This same flora has been shown to be unsuitable for rats and rabbits.

CLINICAL MANIFESTATIONS Generally, affected guinea pigs have weight loss, rough hair coats, anorexia, dehydration, decreased activity, and hypothermia beginning 24–48 hours after antibiotic administration (Farrar and Kent, 1965; Fox, 2002; Knoop, 1979; Wasson et al., 2000). Some animals have no stool, while others have diarrhea and acute death has been reported.

PATHOLOGY At necropsy, the cecum is often gas-filled and dilated with hemorrhage evident in the cecal mucosa. Histopathologically, there is usually hyperplasia of the ileal mucosa as well as ulceration, edema, and degeneration of the cecal epithelium with leukocytic infiltration.

DIAGNOSIS *C. difficile* can be isolated from cecal contents via anaerobic culture (Boot et al., 1989; Greenwood, 2007). Detection of toxin in cecal contents, tissue, or feces via an enzyme immunoassay, cytotoxin activity in cell culture, serum neutralization assay, or PCR are considered the gold standard, however, false positives and false negatives are not uncommon (Greenwood, 2007; Houser et al., 2010; Songer, 1996).

PREVENTION AND THERAPY Antibiotic-associated diarrhea is usually treated symptomatically. In humans, administration of metronidazole or vancomycin is standard (Greenwood, 2007; Rupnik et al., 2009; Songer, 1996). Anecdotal information suggests that administration of yogurt or other *Lactobacillus*-containing products in conjunction with antimicrobial agents will prevent or minimize the effects of antibiotic-associated enteritis. A recent study attempted to induce antibiotic-associated enteritis by a single subcutaneous injection of clindamycin (Wasson et al., 2000). *C. difficile* and other enteric pathogens were not isolated, however, several guinea pigs that received clindamycin developed enteritis. In affected animals, administration of antibiotics with the oral *Lactobacillus* preparation did not result in any clinical improvement over those animals receiving antibiotics alone.

Certain PCR ribotypes of *C. difficile* are found in both human and animal populations, suggesting that this can be a zoonotic disease, although most of these studies are concerned with *C. difficile* found in food animals rather than guinea pigs (Rupnik et al., 2009).

CLOSTRIDIUM PERFRINGENS (ENTEROTOXEMIA)

Clostridium perfringens is a ubiquitous Gram-positive anaerobic spore-forming bacterium that is the primary cause of enterotoxemia in domestic animals. *C. perfringens* strains produce a variety of toxins, as many as 17 exotoxins, although four major toxins are associated with typing of the bacteria; alpha, beta, epsilon, and iota. Infections typically occur via contaminated food, water, bedding, or soil and primarily affect sheep, pigs, and cattle.

Confirmed *C. perfringens* infections have been reported in germ-free guinea pigs in 1970 and, more recently, in a conventionally housed guinea pig (Feldman et al., 1997; Madden et al., 1970; Songer, 1996). Clinical signs seen in the conventionally housed guinea pig were lethargy and anorexia. At necropsy, there were fibrinous adhesions involving the small intestine and liver (Feldman et al., 1997). The ileum and cecum had ecchymotic hemorrhages. Histopathology revealed acute, multifocal, severe, necrotizing, ulcerative typhilitis and ileitis as well as focal, severe, necrotizing, centrilobular vascular thrombosis of the liver with infarction and coagulative necrosis of the adjacent hepatocytes. PCR was used

to identify the toxins of *C. perfringens* both in the intestine and the liver.

Corynebacterium

ETIOLOGY *Corynebacterium* spp., members of the family *Corynebacteriaceae*, are Gram-positive, non-spore-forming, non-motile, aerobic, pleomorphic rods with coccoid or club-shaped appearance that are catalase-positive and non-acid-fast (Boone et al., 2001; Greenwood, 2007).

PATHOGENESIS, CLINICAL MANIFESTATIONS, AND PATHOLOGY *Corynebacterium* spp. can cause disease in guinea pigs, but are also part of the normal flora. Specifically, they are commonly isolated from guinea pig conjunctiva. In one study, 27 out of 30 clinically normal pet guinea pigs grew *Corynebacterium* spp. from conjunctival cultures (Coster et al., 2008).

Spontaneous disease of guinea pigs associated with corynebacterial infections has rarely been reported, but have involved numerous different species of *Corynebacterium*. In one report, *Corynebacterium pyogenes* was isolated from a guinea pig with septicemia (Ganaway, 1976). Further research with that agent demonstrated it was pathogenic when administered IP to other guinea pigs, but not if it was given orally or intranasally. In another report, *C. kutscheri* was isolated from the lung of a guinea pig during an epizootic of group C, beta-hemolytic streptococcal disease. Further research with this isolate was similar to *C. pyogenes*, in that intraperitoneal administration resulted in disease while intranasal administration did not. The organism was also given intravenously in this case and was rapidly fatal. In a retrospective study of urinary bladder calculi in guinea pigs, it was reported that *Corynebacterium renale* was the most common isolate from urine and bladder samples (Hawkins et al., 2009). Of the 44 cases submitted for bacterial culture, 77% were negative. The most common pure culture isolate was *C. renale*, which was also commonly found in mixed infections with *Staphylococcus* and *Streptococcus* spp.

DIAGNOSIS Diagnosis is made by recovering the organism using aerobic culture conditions, generally on blood agar plates with standard biochemical tests to differentiate corynebacterium from other species (Greenwood, 2007). PCR and sequencing has been used for further identification (Greenwood, 2007; Hawkins et al., 2009).

PREVENTION AND THERAPY Many *Corynebacterium* spp. have significant resistance to a number of antibiotics and this may be true of some organisms in guinea pigs as well (Greenwood, 2007). In the study isolating *C. renale* from the urine and bladder wall, many of those animals were already on antibiotics when those isolates were obtained (Hawkins et al., 2009). In addition, the

Corynebacterium spp. recovered from the normal conjunctiva of guinea pigs, demonstrated some bacterial resistance with six out of nine isolates resistant to oxy-tetracycline, four out of nine resistant to polymyxin B, two out of nine resistant to bacitracin, and one out of nine resistant to erythromycin, gentamicin, amikacin, and ciprofloxacin (Coster et al., 2008).

Mycobacterium tuberculosis

Guinea pigs are highly susceptible to *M. tuberculosis*. Natural infection has rarely been reported in the literature, but in those cases, the sources of the infections were believed to be from contact with human cases (Van Hoosier and Robinette, 1976).

Staphylococcus aureus

BACKGROUND Markham and Markham (1966) studied the prevalence of staphylococcal organisms in various animal species. A 42% incidence of coagulase-positive *Staphylococcus* was noted in the nasal passages of guinea pigs. This was the greatest incidence of staphylococcal infection of any species tested, including humans. All of the guinea pigs cultured appeared clinically normal.

ETIOLOGY The *Staphylococcus* genus, members of the *Staphylococcaceae* family, are common inhabitants of the skin, oral cavity, respiratory system, and intestine, and cause suppurative lesions and septicemia in all species (Songer and Post, 2005). These Gram-positive cocci are coagulase-positive, catalase-positive, oxidase-negative, non-spore-forming and facultative anaerobic organisms (Harkness and Wagner, 1995; Songer and Post, 2005). In guinea pigs, *Staphylococcus aureus* has been isolated from cases of ulcerative pododermatitis and exfoliative dermatitis (Percy and Barthold, 2007).

PATHOGENESIS Despite the existence of species-specific serotypes and biotypes, human *Staphylococcus aureus* phage types have been recovered from animals, and human-animal contact is one means of transmission of *S. aureus* (Harkness and Wagner, 1995). Additionally, direct contact with an infected animal or contaminated surface, as well as spread via aerosol can result in the dissemination of infection.

Ulcerative pododermatitis, or dermatitis of the footpad, is also known as "bumblefoot". Trauma to the footpad from fighting, a sharp cage edge, or abrasive flooring creates a site of entry for bacteria (Harkness and Wagner, 1995). Obese guinea pigs, as well as any other natural or experimental cause that results in a sedentary lifestyle, can predispose an animal to bumblefoot, since perfusion is decreased and the pressure on the feet is increased (Brown and Donnelly, 2008; Harkness and Wagner, 1995).

Exfoliative, or acute staphylococcal, dermatitis has also been associated with *S. aureus* in guinea pigs. Strain 13 guinea pigs are reported with this condition more frequently than other strains, and mortality rates due to this condition are higher in young animals, especially if born to an affected dam that is too painful to nurse (Ishihara, 1980; Percy and Barthold, 2007).

CLINICAL MANIFESTATIONS Swollen, erythemic paws and lameness are noted with bumblefoot (Brown and Donnelly, 2008). Paws will often have erosions or ulcerations on the palmar or plantar surfaces. Osteoarthritis or osteomyelitis may result, and animals that develop such sequela have a poorer prognosis. Alopecia and erythema of the ventral abdomen, leading to cracking and scabbing of the epidermis, are often observed with exfoliative dermatitis (Fox, 2002; Ishihara, 1980; Percy and Barthold, 2007). In addition to its association with ulcerative pododermatitis and exfoliative dermatitis, pneumonia, mastitis, conjunctivitis, cheilitis, and osteoarthritis have been attributed to *S. aureus* infection in guinea pigs (Fox, 2002). Systemic amyloidosis can develop secondary to chronic *S. aureus* infection (Brown and Donnelly, 2008; Fox, 2002; Percy and Barthold, 2007).

PATHOLOGY Hypertrophy of the stratum corneum with a minimal inflammatory response is noted histologically in cases of dermatitis associated with *S. aureus* (Percy and Barthold, 2007). Diffuse cellulitis is the primary lesion associated with ulcerative pododermatitis (Brown and Donnelly, 2008). In cases that progress to amyloidosis, amyloid may be noted in the liver, spleen, adrenal glands, and pancreatic islets (Ganaway, 1976).

DIAGNOSIS Stained impression smears of lesions can demonstrate clumps of Gram-positive cocci (Harkness and Wagner, 1995). *S. aureus* can often be cultured directly from affected tissues and from the upper respiratory tract and pharynx of affected animals (Percy and Barthold, 2007). Blood agar or selective mediums, such as mannitol salt or Staph 110 agar, can be used for primary culture (Songer and Post, 2005). *S. aureus* colonies often present with a golden pigmentation.

PREVENTION AND THERAPY Prevention of ulcerative pododermatitis and exfoliative dermatitis is easier than treatment. Preventing cutaneous wounds by providing sturdy, smooth flooring to guinea pigs, offering non-abrasive feedstuff, and preventing obesity are highly effective in minimizing infection with *S. aureus*. Pododermatitis and exfoliative dermatitis are not usually conditions conducive to lesion drainage and surgical removal (Brown and Donnelly, 2008). Lesions can be cleaned, but the possibility of causing greater trauma to the affected area must be considered. Also,

disinfectants that dry out the skin or agents that are cytotoxic to fibroblasts and reduce white blood cell viability and phagocytic efficiency, such as povidone-iodine or chlorhexidine, should be avoided. Long-term antibiotic treatment is necessary, and enrofloxacin or ciprofloxacin administered twice a day for 2–6 months has been reported in the treatment of ulcerative pododermatitis. Use of an analgesic agent, especially a non-steroidal anti-inflammatory drug such as meloxicam, is also recommended. Low-level laser therapy, or phototherapy, has been reported as successful in treating bumblefoot by accelerating angiogenesis and stimulating vasodilatation, although such therapy can only be used after the infection is first controlled.

Streptococcus

Two strains of *Streptococcus* cause disease in guinea pigs. *Streptococcus pneumoniae* can lead to severe pneumonia and *Streptococcus equi* subsp. *zooepidemicus* is associated with the development of chronic lymphadenitis (Wagner et al., 1976). Infections due to *S. equi* subsp. *zooepidemicus* are more prevalent in guinea pigs than are pneumococcal infections. Members of the *Streptococcus* genus are part of the *Streptococcaceae* family and consist of Gram-positive, catalase-negative, facultative anaerobic bacteria that are non-spore-forming and non-motile (Songer and Post, 2005).

STREPTOCOCCUS PNEUMONIAE

BACKGROUND *Streptococcus pneumoniae* was first recognized in guinea pigs in Europe in the late 1800s (Wagner et al., 1976). Homberger et al. (1945) are credited with the first report of this organism in guinea pigs in the United States; the animals were from a colony in Massachusetts and presented with signs of respiratory infection.

ETIOLOGY *Streptococcus pneumoniae* is an oval- to lancet-shaped, encapsulated coccus that presents in pairs (diplococcus) or short chains (Fox, 2002; Percy and Barthold, 2007). *S. pneumoniae* is an α -hemolytic streptococcus that ferments trehalose and is optochin-sensitive (Harkness and Wagner, 1995; Keyhani and Naghshineh, 1974; Songer and Post, 2005). Additionally, it is not categorized according to one of the Lancefield groupings (Songer and Post, 2005). Serovars of pneumococci are differentiated according to their capsular polysaccharide content, and serovars 4 and 19 are the two most often recovered from guinea pigs (Fox, 2002; Percy and Barthold, 2007).

EPIZOOTIOLOGY AND PATHOGENESIS Asymptomatic guinea pigs can carry pneumococci in the upper respiratory passages; the carrier rate for laboratory colonies can be as high as 50–55% (Fox, 2002; Percy and Barthold, 2007). While

pneumonia epidemics due to *S. pneumoniae* seldom occur in well-managed facilities, stressors which may predispose to infection include poor husbandry, pregnancy, sudden or prolonged changes in temperature, poor ventilation, shipping, inadequate nutrition, concurrent infections, and experimental procedures (Nakagawa et al., 1986; Percy and Barthold, 2007). Transmission of *S. pneumoniae* is by aerosol or via direct contact with abraded skin or oral mucosa (Fox, 2002; Percy and Barthold, 2007; Wagner et al., 1976). Infection can also be passed at the time of parturition by an infected dam. Abortions and stillbirths can accompany a high mortality rate during an epizootic outbreak in a guinea pig colony (Percy and Barthold, 2007).

CLINICAL MANIFESTATIONS In 1974, a spontaneous epizootic of respiratory infection involving 2400 guinea pigs was caused by *S. pneumoniae* type 19 (Keyhani and Naghshineh, 1974). During this outbreak, 450 guinea pigs died within 33 days of infection, and a short course of listlessness and failure to thrive were the only noted clinical signs prior to death. In less acute cases of *S. pneumoniae*, guinea pigs display depression and lethargy, anorexia, and have ruffled fur. They may have a wet nose, nasal or ocular discharge (rhinitis, conjunctivitis), sneezing, coughing, dyspnea, or torticollis (head tilt due to otitis media) (Fox, 2002; Wagner et al., 1976). Wagner, Owens, Kusewitt, and Corley reported that otitis media occurred in 177 of 1373 guinea pigs necropsied during a 6-year period in the 1970s (Wagner et al., 1976). *Streptococcus pneumoniae* was noted in 20% of these cases, and was the most common bacteria isolated. Gupta et al. (1970), reported mastitis caused by *S. pneumoniae* in two guinea pigs.

PATHOLOGY Acute bronchopneumonia is most often associated with *S. pneumoniae* infections. Fibrinous exudate, a polymorphonuclear cell infiltrate and thrombosis of the pulmonary vessels may be observed in the lung (Fox, 2002; Keyhani and Naghshineh, 1974; Percy and Barthold, 2007). Other pyogenic processes noted in infected animals include pericarditis, peritonitis, otitis media, arthritis, endometritis, and suppurative meningitis (Fox, 2002; Percy and Barthold, 2007). Additionally, splenitis, fibrinopurulent meningitis, metritis, and lymphadenitis with focal hepatic and ovarian abscessation have been reported (Percy and Barthold, 2007). Pneumococci are readily demonstrated on impression smears and in stained tissue sections (Wagner et al., 1976).

DIAGNOSIS Culture of nasal washings is an ante-mortem means of diagnosing *S. pneumoniae* (Homburger et al., 1945), as is culture of the nasal passages (Harkness and Wagner, 1995). Post-mortem, direct smears and culture of inflammatory exudates can be performed.

S. pneumoniae is a fastidious bacterium that grows best when incubated under 5–10% CO₂ on blood agar or enrichment media (Fox, 2002; Percy and Barthold, 2007). Serological diagnosis via ELISA has been reported (Matsubara et al., 1988).

PREVENTION AND THERAPY Antibiotic treatment of a clinical animal usually results in reversion to a sub-clinical carrier state, so elimination of clinical animals is recommended whenever possible (Fox, 2002). Chloramphenicol, trimethoprim-sulfa combinations, and tetracycline have been used during epizootic outbreaks (Harkness and Wagner, 1995; Homburger et al., 1945; Keyhani and Naghshineh, 1974). Prevention should focus on good husbandry, adequate diet, early isolation of streptococcal carrier animals, and reduction of environmental stressors.

The same *S. pneumoniae* serovars commonly detected in guinea pigs have been isolated from humans (Songer and Post, 2005). *S. pneumoniae* can lead to respiratory and neurological disease in humans, especially in older and immune-compromised individuals. To date, interspecies transmission of *S. pneumoniae* has not been reported (Harkness and Wagner, 1995; Percy and Barthold, 2007).

STREPTOCOCCUS EQUI SUBSP. ZOOEPIDEMICUS

BACKGROUND Charles Boxmeyer (1907) described his findings of epizootic lymphadenitis in over 3000 guinea pigs in 1907. Cunningham (1929) reported on 20 guinea pigs from a colony of 150 that had “lumps,” and from which “the same unusual type of mucoid hemolytic streptococcus was isolated”. Cunningham noted a 3:1 female to male ratio among the animals with chronic lymphadenitis.

ETIOLOGY *Streptococcus equi* subsp. *zooepidemicus* is a Lancefield group C streptococcus that is beta-hemolytic, encapsulated, and ferments sorbitol (Percy and Barthold, 2007; Songer and Post, 2005). *S. equi* subsp. *zooepidemicus* has a high degree of DNA homology with *S. equi* subsp. *equi*. *S. equi* subsp. *zooepidemicus* has been recovered from human infections, while *S. equi* subsp. *equi* has not (Songer and Post, 2005).

EPIZOOTIOLOGY AND PATHOGENESIS Guinea pigs can carry *S. equi* subsp. *zooepidemicus* asymptotically in the nasopharynx and conjunctiva (Percy and Barthold, 2007), but compared to *S. pneumoniae*, clinical disease is more often noted in guinea pigs infected with *S. equi* subsp. *zooepidemicus*. As noted by Cunningham, females are indeed more susceptible to disease than males (Cunningham, 1929; Fox, 2002; Percy and Barthold, 2007). Strain 2 guinea pigs also appear to be more sensitive than other strains (Fox, 2002; Percy and Barthold, 2007;

Wagner et al., 1976). Transmission is via aerosol, as well as direct contact with abraded skin or oral mucosa (Harkness and Wagner 1995; Fox, 2002; Percy and Barthold, 2007). The organism can be transmitted via bite wounds and secondary to abrasion by rough foodstuff (Ganaway, 1976). While this is likely the route of entry for most natural infections, Murphy et al. (1991) showed that the oral mucosa does not need to be abraded in order for entry of the bacterium. The female genital tract can be infected during farrowing (Percy and Barthold, 2007). After initial entrance, the organism travels to the draining lymph node and replicates (Harkness and Wagner, 1995). Cervical lymphadenitis is commonly noted in guinea pigs when the point of entry for infection is via the conjunctiva or nasal mucosa (Harkness and Wagner, 1995).

CLINICAL MANIFESTATIONS Clinical disease associated with *S. equi* subsp. *zooeidemicus* is colloquially referred to as “lumps,” due to the pyogenic nature of the bacteria and its propensity to travel to and replicate in the lymph nodes (Fox, 2002; Percy and Barthold, 2007). While the cervical lymph nodes are the most common site of *S. equi* subsp. *zooeidemicus* replication and abscessation, any lymph node, and practically any organ, can be affected (Harkness and Wagner, 1995). A common presentation for infection with this organism is an animal that appears in good condition except for a swelling in the neck at the position of the cervical lymph node (Fox, 2002). The swelling is often soft to firm, pus-filled, non-fluctuant and freely moveable (Percy and Barthold, 2007). Cunningham’s (1929) observations were that “in some cases, one or two nodes were greatly enlarged; in others, as in one animal with cervical node involvement, several plum-sized encapsulated abscesses formed a collar across the front and side of the neck”. Depending on what additional organs are affected, torticollis (if middle ear involvement), dyspnea and cyanosis or nasal and ocular discharge (if respiratory involvement), or hematuria and hemoglobinuria (if septic) may be noted (Fox, 2002; Harkness and Wagner, 1995). Abortions, stillbirths and unexpected deaths are possible (Fox, 2002). During epizootic outbreaks, septicemia, acute pneumonia, and high mortality can occur (Harkness and Wagner, 1995).

PATHOLOGY The most common finding at necropsy is that of an abscessed, encapsulated cervical lymph node (Fox, 2002). The node architecture is often destroyed by central necrosis and peripheral fibrosis, and a thick pustular exudate is present (Cunningham, 1929; Fox, 2002; Percy and Barthold, 2007). Smears of the exudate reveal numerous Gram-positive cocci in short chains (Cunningham, 1929). A generalized lymphadenitis may be noted, as may retroorbital abscessation, otitis media, pneumonia, pleuritis, pericarditis, or hepatitis (Cunningham, 1929; Fox, 2002; Percy and Barthold, 2007).

DIAGNOSIS *S. equi* subsp. *zooeidemicus* can be cultured from affected tissues or heart blood (Harkness and Wagner, 1995; Percy and Barthold, 2007; Quesenberry and Carpenter, 2004; Wagner et al., 1976). Culture requires blood agar, on which after 24 hours, a clear zone of hemolysis is observed (Harkness and Wagner, 1995).

PREVENTION AND THERAPY Trimming of teeth, elimination of sharp edges to feeders, and feeding non-abrasive feed may assist in prevention of *S. equi* subsp. *zooeidemicus* infection (Fox, 2002). Infected animals should be isolated from the colony. Surgical removal of abscess contents and capsule can be done with creation of drainage and lavage. Antibiotic therapy is required. In epizootic cases, depopulation of affected animals and disinfection of the environment is advised. Enzootic disease represents significant potential for research complications. Disease-free replacement stock should be obtained and is available from commercial vendors. Vaccines are typically type-specific and do not provide reliable protection (Fox, 2002; Mayora et al., 1978).

Gram-Negative Organisms

Actinobacillus

Actinobacillus lignieresii and *Actinobacillus equuli* have been noted in laboratory guinea pigs (Songer and Post, 2005). The genus *Actinobacillus* is a member of the *Pasteurellaceae* family (Boone et al., 2001). These organisms are pleomorphic Gram-negative rods that on Gram stain, can have a “Morse code” appearance (Songer and Post, 2005).

Actinobacillus lignieresii and *Actinobacillus equuli* were cultured from 36 guinea pigs, rats and mice during a 6-month period at the University of Missouri Research Animal Diagnostic and Investigative Laboratory (Lentsch and Wagner, 1980). Organisms grew on blood agar inoculated with swabs from the posterior nasopharynx, conjunctiva, and middle ear. Conjunctivitis was noted in one animal and otitis media was noted in six.

Bordetella

BACKGROUND Tartakowsky is credited with one of the first accounts of *Bordetella* infection in guinea pigs in the late 1880s (McCartney and Olitsky, 1923; Smith, 1913). Woolfrey and Moody (1991) in their review of this organism stated “Ferry, using the name *Bacillus bronchisepticus*; McGowan, using the term *Bacillus bronchicanis*; and Torrey and Rahe, using Ferry’s epithet, independently observed the association of a small gram-negative coccobacillus with outbreaks of “canine distemper” and respiratory tract illness in laboratory animals such as the cat, rabbit, and guinea pig”. In the early 1970s, *B. bronchiseptica* was still considered to be the primary cause of acute pneumonia in guinea pigs and while it is not often

noted in modern laboratory facilities, it continues to be a major cause of respiratory disease in pet guinea pigs and this species is thought to be quite susceptible to infection (Percy and Barthold, 2007; Rigby, 1976).

ETIOLOGY *Bordetella bronchiseptica* is a short, Gram-negative rod or coccobacillus, that is aerobic, motile, and non-spore-forming (Fox, 2002; Percy and Barthold, 2007). *B. bronchiseptica* is oxidase, catalase, and urease positive, as well as positive for citrate utilization and nitrate reduction (Songer and Post, 2005; Woolfrey and Moody, 1991). *B. bronchiseptica* is considered a commensal organism in the guinea pig, as well as many other mammalian species, including humans (Fox, 2002; Percy and Barthold, 2007; Woolfrey and Moody, 1991). The *Bordetella* genus is a member of the *Alcaligenaceae* family (Songer and Post, 2005).

EPIZOOTIOLOGY AND PATHOGENESIS *B. bronchiseptica* is carried by guinea pigs in the nasal cavity and trachea, and up to 20% of non-clinical animals in a colony can be carriers (Ganaway, 1976). Stressful events, such as transport or pregnancy, may trigger an acute fatal pneumonia in carriers or animals with previously inapparent infections. Transmission of *B. bronchiseptica* is via aerosol spread, direct contact, direct contact with respiratory secretions of infected animals, or fomites (Fox, 2002; Ganaway, 1976). *B. bronchiseptica* adheres to ciliated respiratory epithelium and causes ciliary paralysis (Fox, 2002). Pathogenic *B. bronchiseptica* produce adhesion and cytolytic toxins that lead to an inflammatory response, antiphagocytic activity, and dermonecrosis. While not explicitly described in the guinea pig, the impairment in mucociliary clearance caused by the binding of *Bordetella* is known to reduce the clearance of additional respiratory pathogens in other animal species. The incubation period is 5–7 days (Ganaway, 1976). The highest levels of morbidity and mortality due to *B. bronchiseptica* have been noted in young guinea pigs, as well as strain 2 animals (Fox, 2002; Ganaway, 1976). It is possible for infected animals to develop immunity and clear the organism. Yoda et al. (1972), noted that within 6–54 weeks of initial infection, 69 of 79 naturally infected guinea pigs completely recovered from infection.

CLINICAL MANIFESTATIONS Infection with *B. bronchiseptica* is often subclinical in nature (Fox, 2002). It has been suggested that the clinical signs associated with *Bordetella* in the past have actually been due to infections with a secondary invader, such as guinea pig adenovirus, which has not been recognized as an infectious agent of the guinea pig for as long as *B. bronchiseptica*. During an epizootic outbreak, death may follow respiratory disease and septicemia within a 24–72-hour period (Fox, 2002). Abortion and stillbirths can occur in pregnant

dams (Percy and Barthold, 2007). In an enzootically infected colony, sporadic deaths may occur throughout the year, with highest levels during the winter months. Inappetance, depression, nasal discharge, dyspnea, and cyanosis may also be observed (Fox, 2002).

PATHOLOGY At necropsy, cranioventral pulmonary consolidation is seen, and whole lobes or individual lobules will present as dark red to red-gray in color (Ganaway, 1976; Percy and Barthold, 2007). A blood-tinged froth is noted in the trachea. Mucopurulent exudates may be noted in affected airways. Pleuritis and otitis media may be observed. Suppurative bronchopneumonia is noted histologically, with an influx of heterophils and mononuclear cells. Uterine infections may result in dead fetuses or pyosalpinx.

DIAGNOSIS *B. bronchiseptica* can be cultured on blood or MacConkey's agar from nasal or other respiratory secretions (Songer and Post, 2005). In cases of otitis media, *B. bronchiseptica* can be isolated from the middle ear, and in cases of metritis, from the uterus (Fox, 2002; Yoda et al., 1972) at 35–37°C (Songer and Post, 2005). Smith and Baskerville medium selects for *B. bronchiseptica*, and is another option for culture (Songer and Post, 2005). ELISA and IFA tests have been developed for the detection of guinea pig *Bordetella*, and have been noted as equally successful in identifying infected animals as cultures of the respiratory tract (Boot et al., 1993a; Wullenweber and Boot, 1994).

PREVENTION AND THERAPY Purchasing *Bordetella*-free animals is the best means of control in laboratory colonies of guinea pigs. It is recommended that rabbits and guinea pigs not be co-housed since guinea pigs are highly susceptible to disease and rabbits are frequently sub-clinical carriers of *B. bronchiseptica* (Charles River Laboratories, 2011). Rats and other rodents can be additional sources of infection (Rigby, 1976). Antibiotic treatment with fluoroquinolones or trimethoprim-sulfonamides may successfully resolve clinical signs, but elimination of infection is rare, with animals often reverting to the sub-clinical state. If infection is suspected in a colony, restocking, test and cull, or rederivation may be more beneficial than treatment (Fox, 2002). The efficacy of canine, porcine, human, and autogenous *Bordetella* vaccines and bacterins has been evaluated by several individuals; reports suggest that these vaccines do not completely protect guinea pigs from infection, but a decrease in the incidence and severity of clinical disease has been noted in experimentally challenged animals (Matherne et al., 1987; Stephenson et al., 1989).

B. bronchiseptica apparently does not cause epidemic disease in man, but it can cause a whooping cough-like syndrome in immune-competent children or respiratory

tract infections in immune-suppressed individuals (Songer and Post, 2005). Endocarditis, meningitis, and peritonitis have also been associated with *B. bronchiseptica* infections in humans. The ease of transmission between guinea pigs and humans is unknown.

Brachyspira

BACKGROUND AND ETIOLOGY Spirochetes of the genus *Brachyspira* (formerly *Serpulina*) have been associated with diarrhea and colitis in a variety of animals including birds and mammals. *Brachyspira* contains seven distinct species. Of these, two species have been implicated in guinea pigs, either in natural infections or as an animal model; *B. hyodysenteriae*, the primary cause of swine dysentery, and *B. pilosicoli*, a zoonotic agent associated with disease in chickens, pigs, and humans. Spirochetes have been associated with disease in guinea pigs in several publications, although purposeful infection to demonstrate causality has not been done and in many cases, identification of the species involved has also not been accomplished (Duhamel, 2001). Guinea pigs have been used as an animal model for *B. hyodysenteriae* infection.

CLINICAL MANIFESTATIONS AND PATHOLOGY Two reports in the 1970s describing Tyzzer's disease in guinea pigs also report observing spirochetes on histopathology (McLeod et al., 1977; Zwicker et al., 1978). The significance of the spirochete infections in the progression of the disease seen is unclear. A subsequent report describes a series of cases submitted for necropsy between 1992 and 1996 in Belgium (Vanrobaeys et al., 1998). In 29 of 33 cases when spirochetes were identified, the presenting complaints were either sudden death or a short course of diarrhea followed by sudden death. Flagellated parasites were seen in 12 animals and lesions consistent with vitamin C deficiency in six cases. Gross necropsy of affected animals most commonly demonstrated liquid, occasionally mucoid or hemorrhagic large intestinal contents, and dilation of the cecum and colon. Histologically, the cecum was characterized by hyperemia, mucosal infiltration of neutrophils, a mixed inflammatory infiltrate in the lamina propria, and occasionally, necrosis of crypt epithelium. Lesions involving other organs were present in 30 of the 37 animals identified, suggesting multifactorial disease processes. In a report of a single pet guinea pig, clinical signs of tenesmus, tachycardia, pale mucous membranes, and dehydration were associated with acute rectal prolapse (Helie et al., 2000). Histopathology identified superficial mucosal necrosis and ulceration and congestion with hemorrhage in the intussuscepted segment. Although spirochetes and organisms consistent with *Eimeria* spp. were seen in the rest of the intestinal tract, no other lesions were noted. *B. pilosicoli* was identified by PCR.

DIAGNOSIS Diagnosis can be made by identifying typical organisms on histopathology. PCR of intestinal contents is diagnostic (Helie et al., 2000).

PREVENTION AND THERAPY Treatment with ranidazole stopped the spread of the disease in some cases as described by Vanrobaeys et al. (1998), although cessation of treatment resulted in resumption of disease. As the majority of reports describe co-pathogens, controlling co-pathogens may be the most effective prevention.

Brucella

Brucella spp. are zoonotic, Gram-negative, strictly aerobic, intracellular, non-motile coccobacilli, which infect a variety of species, including guinea pigs (Martirosyan et al., 2011; Pappas, 2010; Van Hoosier and Robinette, 1976). Natural infections in guinea pigs have been described likely associated with contaminated food, bedding, or other biological materials (Van Hoosier and Robinette, 1976). In these outbreaks, guinea pigs developed disease similar to that seen in other species including testicular and joint swelling as well as abscesses in the liver and pancreas. Modern husbandry practices should limit transmission of *Brucella* spp. to guinea pigs.

Campylobacter jejuni

Campylobacter jejuni is a species of curved, rod-shaped, non-spore-forming, Gram-negative, microaerophilic, bacteria that naturally colonize the digestive track of birds and numerous other species, including domestic farm animals, dogs, cats, various wildlife, and rodents (Horrocks et al., 2009). It is a common cause of human gastroenteritis worldwide primarily from contamination of food. It was isolated from laboratory animal guinea pigs in one report where the prevalence of *C. jejuni* in a multispecies laboratory animal facility was investigated (Meanger and Marshall, 1989). In this report, no clinical signs were seen in any of the animals involved and pathological analysis was not done. Guinea pigs have been used as an animal model of *C. jejuni* infection (Burrough et al., 2009; Coid et al., 1987).

Chlamydia caviae

BACKGROUND AND ETIOLOGY Guinea pig inclusion conjunctivitis was first described in 1964 associated with an experiment to examine the correlation between vitamin deficiency and the development of trachoma virus (Murray, 1964). The organism was identified as a type of *Chlamydia psittaci*, a member of the psittacosis-lymphogranuloma-trachoma group, first thought to be protozoa, then viruses because they could pass through a 450-nm filter, and finally in 1966 recognized as bacteria (Longbottom and Coulter, 2003; Moulder, 1966; Songer and Post, 2005). As members of the family *Chlamydiaceae*, they are

Gram-negative, obligate intracellular pathogens that infect a variety of animals, including amoeba, birds, sheep, humans, and guinea pigs (Greenwood, 2007). Recently they have been divided into two genera: *Chlamydia* and *Chlamydophila*. *Chlamydophila* species include *C. caviae* as well as *C. pneumoniae* (humans, horses, koalas) and *C. psittaci* (birds) (Everett et al., 1999; Murray, 1964).

Unlike most bacteria, the cell walls of *Chlamydiaceae* do not have a peptidoglycan layer, but they do have a major outer-membrane protein, which is the determinant for serologic classification (Greenwood, 2007; Songer and Post, 2005). They also have a unique life cycle which consists of two forms; a small infectious elementary body which is more similar to a spore in that it is environmentally resistant and not metabolically active and a larger metabolically active, non-infectious reticulate body that is responsible for division within the host cell.

EPIZOOTIOLOGY AND PATHOGENESIS Generally, *C. caviae* infection is thought to be endemic in guinea pig colonies (Percy and Barthold, 2007), although its detection in colonies has been variable. In a survey done in 1964 of five herds of guinea pigs, conjunctival staining revealed the organism in four out of five herds, although not in any guinea pigs less than 4 weeks of age (Murray, 1964). In 2006, two breeding facilities of conventional guinea pigs in Korea were examined by conjunctival staining and no positive organisms were seen (Park et al., 2006). A survey of pet guinea pigs with normal conjunctiva, also revealed no positive staining (Coster et al., 2008), which may reflect the sensitivity of the test used or the actual prevalence of *C. caviae*. In a study using real-time PCR for diagnosis, *C. caviae* was detected in 12 out of 12 guinea pigs tested, four out of four rabbits, and one dog from samples obtained from a diagnostic laboratory (Pantchev et al., 2010). *C. caviae* in a second report was identified via PCR from conjunctival swabs from a rabbit, cat, and human that lived in the same household as infected guinea pigs (Lutz-Wohlgroth et al., 2006). Also utilizing PCR and DNA sequencing, *C. caviae* was detected in foals with conjunctivitis, rhinitis, and coughing, indicating the host range of the species of *Chlamydiaceae* may continue to be refined as new diagnostic tests are utilized (Gaede et al., 2010).

One study identified *Acanthamoebae* spp. via PCR from two guinea pigs with conjunctivitis that were also PCR positive for *C. caviae* (Lutz-Wohlgroth et al., 2006). Their role in the disease process is unclear.

Urogenital disease has been reported in natural infections with *C. caviae* and has been reproduced in experimental models (Lutz-Wohlgroth et al., 2006; Mount et al., 1973). In experimental infections, inoculation with *C. caviae* in the lower genital tract of female guinea pigs causes a self-limiting infection lasting 3–4 weeks. Typically, the infection involves the epithelium of the

cervix with very little inflammation. However, in 80% of the animals, the infection progresses to involve the oviducts, with half of the animals developing pathologic changes in the oviducts including inflammation and fibrosis (Barron et al., 1979; Rank and Sanders, 1992). Infection can be transferred from infected male animals to females during sexual intercourse and vertically from dam to pups (Padilla-Carlin et al., 2008; Miyairi et al., 2010).

In experimental infections, both cell-mediated and humoral immunity are important in recovery and resistance to re-infection (Miyairi et al., 2010). Early infection is characterized by influx of polymorphonuclear cells while later in infection, lymphocytes predominate. T-cell depletion results in chronic infection and delayed clearance of the organisms. Decreased antibody response also correlates with delayed clearance of the organisms (Miyairi et al., 2010; Rank et al., 1979).

CLINICAL MANIFESTATIONS *C. caviae* has been primarily associated with conjunctivitis. Guinea pigs have reddened, swollen conjunctiva with serous to purulent discharge, follicular hypertrophy, and pannus (Deeb et al., 1989; Lutz-Wohlgroth et al., 2006; Murray, 1964; Padilla-Carlin et al., 2008; Strik et al., 2005). Generally the conjunctivitis resolves in 3–4 weeks. Occasionally, conjunctivitis is accompanied by rhinitis. In one study, nasal discharge was present in 31 of 39 of the animals with ocular discharge (Lutz-Wohlgroth et al., 2006). Vaginal discharge was seen in 25% (ten of 39) of the animals although the organism could not be recovered by PCR from either the nose or the vagina. Abortion was recorded in two animals. An early study also reported rhinitis, bronchopneumonia and abortions, although this study was complicated by infection with *Streptococcus pneumoniae* and *Bordetella bronchiseptica* (Schmeer et al., 1985).

PATHOLOGY Histological evaluation of the eyes and surrounding tissue reveals a mixed inflammatory response and congestion beneath the conjunctival epithelium (Deeb et al., 1989; Lutz-Wohlgroth et al., 2006). Histological examination of the urogenital tract has primarily been described following experimental infection and consists of inflammation of the endometrium, mesosalpinx, and oviduct with a mixed inflammatory infiltrate and fibrosis (Barron et al., 1979; Rank and Sanders, 1992).

DIAGNOSIS Diagnosis is by cytology, PCR, culture, tissue assays, and serology (Songer and Post, 2005). Giemsa, Gimenez, or Macchiavello stains on cytologic preparations reveal inclusions in the cytoplasm. Inclusions are a dark purple with Giemsa and red with Gimenez or Macchiavello stains. Cytology can be insensitive hence PCR can be used in addition (Pantchev et al., 2010; Strik et al., 2005). In one study, 31 samples

were tested via cytology and PCR (Lutz-Wohlgroth et al., 2006). Twenty-one were positive by PCR for *C. caviae*, however, no samples were positive by cytology. As *C. caviae* is an obligate intracellular pathogen, it cannot be cultured in cell-free media, but only on specific cell lines (Songer and Post, 2005). This is an insensitive and difficult process that is not typically used for diagnosis. Direct fluorescent antibody test and enzyme immunoassays can be used to detect antigens in cytology samples or tissues (Percy and Barthold, 2007; Songer and Post, 2005).

PREVENTION AND THERAPY Generally, the disease is self-limiting and treatment is not required (Percy and Barthold, 2007; Quesenberry and Carpenter 2004). No current vaccine prevents chlamydial infection, although vaccination studies have demonstrated a decrease in the intensity of the disease following vaccination (Padilla-Carlin et al., 2008). Recognition that zoonotic transmission is a possibility should guide appropriate personal protective equipment choices (Lutz-Wohlgroth et al., 2006).

Citrobacter

BACKGROUND AND ETIOLOGY *Citrobacter* is a genus of Gram-negative coliform bacteria of the family *Enterobacteriaceae* (Boone et al., 2001). In the 1960s and 1970s, a disease eventually named transmissible murine colonic hyperplasia caused by coliform bacteria of the genus *Citrobacter* was recognized (Mundy et al., 2005; Percy and Barthold, 2007; Petty et al., 2010). Originally described as unusual biotypes of *Citrobacter freundii*, these organisms were eventually separated into their own species, *Citrobacter rodentium*, on the basis of DNA sequencing studies (Luperchio et al., 2000; Petty et al., 2010). Whether the organisms that caused severe septicemia and death in guinea pigs in 1988 (Ocholi et al., 1988) and other disease syndromes in hysterectomy-rederived guinea pigs (Boot and Walvoort, 1986) are *C. freundii* as originally indicated or *C. rodentium* is unclear.

PATHOGENESIS, CLINICAL MANIFESTATIONS, AND PATHOLOGY There are two reports of disease associated with *Citrobacter* spp. in guinea pigs (Boot and Walvoort, 1986; Ocholi et al., 1988). First, *Citrobacter* spp. were isolated from germ-free guinea pigs that were exposed to defined flora from gnotobiotic mice (Boot and Walvoort, 1986). *Citrobacter* spp. were isolated from the middle ear, spleen, mammary gland, and kidney of some animals as well as the intestinal tract, suggesting an ability to cause varied disease in guinea pigs that are not populated with normal flora. Second, an epizootic of *Citrobacter* spp. septicemia in a large colony of conventional guinea pigs was reported (Ocholi et al., 1988). Animals presented with diarrhea, dyspnea, and

decreased appetite. Mortality in weanlings and breeders was 115 of 1300 animals. Gross necropsy revealed lung consolidation with pleural adhesions as well as enteritis and thickening of the intestinal wall. Fibrinous pneumonia and septic thrombi in the capillaries of the lung, liver, and spleen were seen histopathologically. *Citrobacter* spp. were consistently isolated in pure culture from the lung, liver, spleen, and intestine.

DIAGNOSIS Culture is the traditional method of detecting *Citrobacter* spp. which grow well on normal media, are aerobic or facultative anaerobic, ferment glucose, produce catalase, but not oxidase, and are generally lactose-negative or later lactose-fermenting (Greenwood, 2007). However, PCR is more sensitive than bacterial culture for detection of *C. rodentium* in feces from mice (McKeel et al., 2002).

PREVENTION AND THERAPY *C. freundii* strains utilize a number of methods to encode resistance to ampicillin and cephalosporins (Pfeifer et al., 2010). Enrofloxacin has been successful in treating mice infected with *C. rodentium* (Maggio-Price et al., 1998). Prevention would include eliminating contact with *C. rodentium*-infected mice and other rodents.

Clostridium piliforme (Tyzzer's Disease)

BACKGROUND *Clostridium piliforme*, first described in 1917 (Tyzzer, 1917), causes severe disease in many laboratory animals as well as other mammals (Percy and Barthold, 2007). Although rare in modern colonies, outbreaks in mice, rats, rabbits, and guinea pigs have been reported and *C. piliforme* is found in both wild (Wobeser et al., 2009) and domestic mammals (Borchers et al., 2006; Ikegami et al., 1999). While Tyzzer's disease is not generally considered zoonotic, infection of an HIV-positive patient has been reported (Smith et al., 1996).

ETIOLOGY *Clostridium piliforme* (formerly *Bacillus piliformis*) is a Gram-negative, spore-forming, anaerobic rod that can survive for as long as five years in the environment (Percy and Barthold, 2007; Songer and Post, 2005).

PATHOGENESIS *C. piliforme* is thought to be transmitted via fecal-oral transmission. In experimental models, oral inoculation was successful when other routes of inoculation did not result in disease (Waggie et al., 1987). Vertical transmission has been reported in one case of hysterectomy-rederived guinea pigs (Boot and Walvoort, 1984). In outbreaks of Tyzzer's disease in other species, usually predisposing factors such as stress, overcrowding, poor sanitation, or immune-modulating treatments contribute to the development of disease (Percy and Barthold, 2007).

CLINICAL MANIFESTATIONS Clinical signs generally consist of diarrhea, unthriftiness, dehydration, cachexia, or acute death (Boot and Walvoort, 1984; McLeod et al., 1977; Zwicker et al., 1978) typically seen in weanlings. Subclinical infections have been noted (Boot and Walvoort, 1984).

PATHOLOGY Lesions are usually found only in the gastrointestinal tract in young guinea pigs, although liver lesions have been reported in some animals (Sparrow, 1978; Waggle et al., 1987). Grossly, pinpoint gray foci occur on the ileum, cecum, and colon as well as enlarged, reddened mesenteric and colonic lymph nodes (McLeod et al., 1977; Sparrow, 1978; Waggle et al., 1987; Zwicker et al., 1978). White to tan pinpoint foci are also observed on the liver. Microscopically, foci of necrosis occur in the mucosa of the ileum, cecum, and colon as well as foci of periportal coagulative hepatocellular necrosis when the liver is involved. Clusters of intracellular organisms can be observed via silver stain or Giemsa stain in affected enterocytes and hepatocytes. In two reports, affected animals have also had large numbers of spirochetes evident at necropsy (McLeod et al., 1977; Zwicker et al., 1978).

DIAGNOSIS *C. piliforme* cannot be cultured using standard media, although it can be isolated by inoculation of cell lines, embryonated hen's eggs, and primary mouse or chick embryo cell cultures (Niepceon and Licoism, 2010; Percy and Barthold, 2007). Therefore, diagnosis typically relies on characteristic lesions and organisms observed on histopathology using a Warthin-Starry silver stain or Giemsa stain (Percy and Barthold, 2007). PCR has been used to detect *C. piliforme* in feces from rats (Furukawa et al., 2002). Nested PCR was developed to increase the specificity of detection in tissues, better separating *C. piliforme* from other closely related organisms used conserved regions of 16S ribosomal RNA (Niepceon and Licois, 2010). Serology to detect antibodies has been used to monitor colonies for this organism (Boot and Walvoort, 1984; Kraft and Meyer, 1990).

TREATMENT AND CONTROL Treatment of individual animals has not been reported. Tyzzer's disease affects a wide variety of animals and immune-compromised people. Prevention entails modern husbandry and sanitation practices, preventing contamination of food and bedding, and separation of species.

Escherichia coli

Escherichia coli are aerobic, straight Gram-negative rods and are part of the normal intestinal flora of most vertebrates, including guinea pigs (Crecelius and Rettger, 1943; Songer and Post, 2005). Several pathotypes have been reported (e.g. EPEC-enteropathogenic strains),

some which produce cytotoxins. Diarrhea, rough hair coats, wasting and death have been reported to be associated with *E. coli* infection in conjunction with other environmental stressors (Ganaway, 1976). In addition, there is one report of a compilation of cases of mastitis in guinea pigs from a diagnostic lab over a four year period (Kinkler et al., 1976). Of the 26 cases where cultures were taken, *E. coli* was found in 17 cases with the next most common isolate being *Klebsiella pneumoniae* in six cases. Diagnosis may be obtained by culture of affected organs on MacConkey agar and differentiation from other enteric Gram-negative bacteria by a number of biochemical reactions such as indole production and utilization of certain sugars (Greenwood, 2007).

Klebsiella pneumoniae

BACKGROUND Perkins (1901) described an epizootic outbreak in 25 laboratory guinea pigs that resulted in the death of all but two animals 18–24 hours after the onset of disease. A short bacillus, often paired, was isolated in the blood, spleen, liver, and peritoneal exudates. Disease was reproduced in naïve guinea pigs subsequently administered the bacterium by the intraperitoneal route. Perkins' findings were similar to those of Howard (1899), who inoculated dogs, rabbits, mice, pigeons, and guinea pigs with a bacillus organism isolated from human patients.

ETIOLOGY *Klebsiella pneumoniae* is a Gram-negative, rod-shaped bacillus from the genus *Klebsiella* and family *Enterobacteriaceae* (Boone et al., 2001). *K. pneumoniae* is facultatively anaerobic, oxidase-negative, and produces acid and gas from lactose. It is an enteric bacterium, noted in the intestinal tract of 5% of healthy humans (Ganaway, 1976). It can also reside in the skin and mouth. *K. pneumoniae* causes pneumonia in guinea pigs of both sexes and all ages, although there are few reports of natural infections of this animal species with this organism.

CLINICAL MANIFESTATIONS In the epizootic outbreak described by Perkins, animals presented as anorexic and ungroomed, and their illness escalated in severity until, in some animals, a comatose condition ensued (Perkins, 1901). Animals usually died 18–24 hours after the onset of clinical signs. In other epizootic outbreaks, dyspnea has been reported prior to reaching the comatose state (Ganaway, 1976). Less severely infected animals may be able to recover from disease and develop immunity to re-infection (Perkins, 1901). Otitis media and other signs consistent with a state of septicemia have been associated with clinical disease (Fox, 2002; Ganaway, 1976; Kohn, 1974).

PATHOLOGY Seropurulent or serofibrinous peritonitis is noted at necropsy (Fox, 2002; Ganaway, 1976;

Perkins, 1901). Additionally, such exudates may be observed in the pleural cavity and pericardial sac. Congestion of the liver, spleen, and kidney has been noted, as has hepatic coagulative necrosis and degeneration of the renal tubule cells. An acute, necrotizing bronchopneumonia may be observed.

DIAGNOSIS Diagnosis of *K. pneumonia* is usually via culture of the upper respiratory tract. The bacterium has been isolated from blood, spleen, liver, peritoneal exudate, and cerebrospinal fluid (Fox, 2002; Ganaway, 1976; Perkins, 1901). *K. pneumoniae* can be differentiated from *K. oxytoca*, another *Klebsiella* spp. frequently recovered in animals, by the fact that it is indole-negative and does not grow at 10°C, while *K. oxytoca* is indole-positive and does grow at 10°C.

PREVENTION AND THERAPY Antibiotic treatment for *Klebsiella* infection should be based on sensitivity of culture isolates. Ceftriaxone and cefodizime were found effective at clearing *K. pneumoniae* in experimentally infected guinea pigs (Drago et al., 1999), although generally, chloramphenicol, enrofloxacin, trimethoprim-sulfa, and aminoglycoside drugs are considered the safest antibiotic choices for use in guinea pigs.

Lawsonia intracellularis

BACKGROUND AND ETIOLOGY Proliferative enteritis associated with *Campylobacter*-like organisms has been reported in many species including pigs, hamsters, rats, rabbits, foals, NHPs, ferrets, and deer (Lawson and Gebhart, 2000). In the early 1990s, molecular techniques resulted in the identification of *Lawsonia intracellularis* as the causative agent of many of these syndromes. Since that time, diagnostics, including PCR, have enabled the diagnosis of *L. intracellularis* infection (Collins et al., 2011).

PATHOGENESIS, CLINICAL MANIFESTATIONS, AND PATHOLOGY There have been two reports of disease in guinea pigs associated with *Campylobacter*-like organisms, both prior to the identification of *L. intracellularis*. In 1981, one 3.5-month-old guinea pig, treated with steroids for 90 days as part of a study, displayed no clinical signs of disease but on necropsy had marked duodenal mucosal hyperplasia with intracellular Warthin-Starry stained argyrophilic bacteria in affected epithelial cells (Elwell et al., 1981). Two cagemates of this guinea pig, that had the same steroid treatment, died at days 13 and 30. One had diarrhea for two days prior to death. Histopathology revealed acute enteritis with mucosal erosions without mucosal hyperplasia in both animals. Bacteria similar to that seen in the first case were found in the epithelial cells. The authors noted this organism was very similar to the organism associated with

transmissible mucosal hyperplasia of hamsters (subsequently identified as *L. intracellularis*).

In 1983, two dams and five young guinea pigs in a laboratory in Japan developed diarrhea, anorexia, weight loss, and dehydration. Two young animals died 5–7 days post-onset of clinical signs. Grossly, there was thickened jejunum and ileum. Histopathologically, there was hyperplasia and proliferation of the epithelial cells and dilation of the glands primarily in the ileum and the distal part of the jejunum. Immature epithelial cells contained various numbers of intracytoplasmic, non-membrane-bound, curved organisms resembling *Campylobacter* spp. bacteria.

DIAGNOSIS Characteristic histopathological lesions, immunohistochemistry, and PCR are diagnostic (Collins et al., 2011; Lawson and Gebhart, 2000).

PREVENTION AND THERAPY Antibiotics have been used to treat a similar proliferative enteritis in swine with success (Lawson and Gebhart, 2000). Care should be taken to avoid penicillins and other antibiotics implicated in antibiotic-associated dysbacteriosis (Percy and Barthold, 2007).

Leptospira

BACKGROUND AND ETIOLOGY *Leptospira* are tightly coiled, aerobic, Gram-negative, flagellated spirochetes belonging to the genera *Leptospira*, family *Leptospiraceae*, phylum, Spirochaetes (Boone et al., 2001). Before 1989, the genus, *Leptospira*, was divided into two species, *L. interrogans* (pathogenic) and *L. biflexa* (non-pathogenic) which each contained strains separated into numerous serovars (Levett, 2001). More recently, DNA analysis has identified 20 species of *Leptospira* and revisions of these taxonomic groups are ongoing (Galloway and Levett, 2010; Levett, 2001).

EPIZOOTIOLOGY AND PATHOGENESIS *Leptospira* are zoonotic organisms with a world-wide distribution that infect a variety of mammals, including mice, rats, carnivores, and ruminants (Ko et al., 2009; Levett, 2001; Percy and Barthold, 2007). Wild guinea pigs in South America (*Cavia porcellus apera*), infected with *Leptospira* spp., have been implicated as the natural reservoir responsible for transmitting the disease to domestic cattle (Blood et al., 1963). In domestic guinea pigs, there have been only a few reports of natural infection, although the guinea pig infection models have been used extensively in *Leptospira* research (Ko et al., 2009; Van Hoosier and Robinette, 1976). Data from research models and infections in other species indicate that transmission occurs from direct contact with infected urine, soil, or water as *Leptospira* are excreted in the urine (Ko et al., 2009; Lourdault et al., 2009).

CLINICAL MANIFESTATIONS AND PATHOLOGY A report from 1937 describes a natural infection where one out of 12 guinea pigs died from leptospirosis (Ganaway, 1976). Pathological findings include jaundice, petechial hemorrhages in the skin, fascia, and muscles, and ecchymosis in the lung. A more recent report of a case of leptospirosis in a 21-year-old man, described 20 out of 50 guinea pigs that died on his farm in Germany. Three of the remaining guinea pigs were tested and were serologically positive for *Leptospira* serovar *bratislava* similar to the man. No necropsies of affected animals were described.

DIAGNOSIS Direct examination of infected tissue, urine, or blood with dark-field microscopy, immunofluorescence, or light microscopy with special stains is useful for diagnosis (Greenwood, 2007; Levett, 2001). Culture and PCR are additional diagnostic tools. Growth in culture can be slow. Generally, antibody detection is used to demonstrate previous infection.

PREVENTION AND THERAPY Antimicrobial treatment of individual animals is generally successful in other species (Pischke et al., 2010; van de Maele et al., 2008), although in guinea pigs, care must be taken to avoid antibiotic-associated dysbacteriosis (Percy and Barthold, 2007). Prevention includes preventing exposure to wild rodents and *Leptospira*-infected food, bedding, soil, or water.

Listeria monocytogenes

BACKGROUND *Listeria monocytogenes* was first discovered in 1926 by Murray et al. (1926), who isolated it from laboratory rabbits and guinea pigs. Since that time, few cases have been reported in guinea pigs, although guinea pigs are very susceptible and have been used extensively to model the pathogenesis of listeriosis (Cossart, 2007; Disson et al., 2009; Fox, 2002).

ETIOLOGY *Listeria monocytogenes* is a facultative intracellular, non-spore-forming, zoonotic, food, soil-, and silage-borne bacterium which causes disease in birds and many mammals.

PATHOGENESIS, CLINICAL MANIFESTATIONS, AND PATHOLOGY In spontaneous infections of guinea pig colonies, *L. monocytogenes* infection has been correlated with acute death, reproductive disorders such as abortions and still births, as well as conjunctivitis (Chukwu et al., 2006; Colgin et al., 1995; Fox, 2002). In an outbreak associated with feeding infected greens to a colony of 80 guinea pigs, the mortality rate was reported to be 80–100% with lung and liver lesions predominating (Chukwu et al., 2006).

Conjunctivitis was reported in one outbreak affecting 11 out of 49 newly arrived guinea pigs (Colgin et al.,

1995). *L. monocytogenes* was cultured from 10 of 12 specimens submitted. The animals presented with unilateral or bilateral serous to purulent discharge with hyperemic conjunctiva. Histologically, there was corneal ulceration, stromal edema, and vascular proliferation. The conjunctiva contained an inflammatory infiltrate and the lacrimal gland had multifocal areas of inflammation and necrosis. Intracytoplasmic Gram-positive rods were seen in the conjunctival epithelium and no chlamydial organisms were identified on conjunctival scrapings or by histopathology. Experimentally, guinea pigs have been shown to be susceptible to *Listeria*-induced conjunctivitis.

PREVENTION AND THERAPY Prevention of contamination of food or bedding is essential to prevent this infection.

Pasteurella multocida

BACKGROUND Pasteurellosis is rare in present-day guinea pig laboratory colonies. Reports of epizootic outbreaks due to Gram-negative coccobacilli were reported in Europe in the late 1800s (Wright, 1936).

ETIOLOGY *Pasteurella multocida*, a Gram-negative, non-spore-forming and non-motile rod is a member of the *Pasteurella* genus and the *Pasteurellaceae* family (Songer and Post, 2005).

EPIZOOTIOLOGY AND PATHOGENESIS *Pasteurella* spp. are mucosal commensal organisms of the oropharynx and gastrointestinal tract of many species, including man (Songer and Post, 2005). When stressed, the animal's immune system can be overwhelmed and, in guinea pigs, pneumonia may result. *P. multocida* can be transmitted to man via contact with infected saliva, which is likely to occur as a result of a bite wound or scratch.

CLINICAL MANIFESTATIONS Wright detailed an outbreak of pasteurellosis that killed 600 guinea pigs over a 1-year period (Wright, 1936). Clinical signs were not noted prior to death. *P. multocida* has been isolated from cases of conjunctivitis (Percy and Barthold, 2007).

PATHOLOGY Fibrinopurulent serositis is present in cases of guinea pig pasteurellosis (Fox, 2002; Ganaway, 1976; Wright, 1936). Such serositis has been noted in the pericardium, pleura, and peritoneum. Lung consolidation may also be observed (Fox, 2002).

DIAGNOSIS *Pasteurella* infection is diagnosed via culture. The bacteria do not reliably grow well on MacConkey agar, therefore blood agar is routinely used for isolation (Ganaway, 1976; Songer and Post, 2005). Plates should be incubated at 3–5% CO₂ at 37°C for

24–48 hours (Songer and Post, 2005). In animal tissue or fluids, *P. multocida* appears as a bipolar rod after aniline dye staining, but in a smear taken from culture, it stains evenly (Ganaway, 1976). Unlike *P. pneumotropica*, *P. multocida* is o-nitrophenyl- β -D-galactoside (ONPG) and urease-negative (Songer and Post, 2005). *P. pneumotropica* is also positive for the fermentation of mannose, while *P. multocida* is negative. An ELISA-based assay has been developed to monitor antibodies to the SP group of *Pasteurellaceae*, which is one of five serologically distinct groups of *Pasteurellaceae* in guinea pigs (Boot et al., 1995).

PREVENTION AND THERAPY Antibiotic treatment of *P. multocida* should be based on culture sensitivities. Antimicrobial therapy will eliminate clinical signs, but will not eliminate the organism from the colony (Songer and Post, 2005).

Pseudomonas* and *Aeromonas

BACKGROUND Scherago (1937) reported on a fatal epizootic septicemia in a colony of young guinea pigs received from a local breeder. Animals died within 5–6 hours after showing clinical signs and “Gram-negative, non-spore-forming encapsulated rods with rounded ends and straight sides appearing singly and occasionally in pairs end to end” were found on smears from the peritoneal cavities of all examined animals. The route of infection was not determined in this colony, but Scherago did complete the required steps to fulfill Koch’s postulates and demonstrated that the infection was due to *Pseudomonas caviae*. *P. caviae* has since been reclassified as *Aeromonas caviae*, of the *Aeromonadaceae* family.

Pseudomonas aeruginosa was noted as the cause of pulmonary botryomycosis in two guinea pigs (Bostrom et al., 1969).

ETIOLOGY *P. aeruginosa*, a member of the genus *Pseudomonas*, is part of the *Pseudomonadaceae* family (Songer and Post, 2005). *Pseudomonas* spp. are Gram-negative rods that are aerobic, non-spore-forming, oxidase-positive, and non-fermentative.

EPIZOOTIOLOGY AND PATHOGENESIS *Pseudomonas* organisms thrive in aqueous environments (Songer and Post, 2005). Additionally, they are ubiquitous in soil, decaying organic matter, and vegetation. *Pseudomonas* spp. are opportunistic pathogens of both humans and animals, yet, there are few reports of infections caused by these agents in guinea pigs (Fox, 2002).

CLINICAL MANIFESTATIONS Bostrum et al. (1969), did not describe the clinical signs associated with the epizootic outbreak of pulmonary botryomycosis. Otitis

media, conjunctivitis, and an inflamed prostate gland are signs that have been associated with *Pseudomonas* in other reports of guinea pig infection (Fox, 2002).

PATHOLOGY A focal, necrotizing bronchopneumonia, along with pulmonary consolidation has been described in cases of guinea pig *Pseudomonas* infection (Bostrom et al., 1969; Fox, 2002; Ganaway, 1976; Percy and Barthold, 2007). Bostrum noted atypical “sulfur granules”, resembling the spherules of *Coccidioides immitis*, in the lungs of affected animals (Bostrom et al., 1969).

DIAGNOSIS *P. aeruginosa* can be cultured from infected tissues (Songer and Post, 2005). *P. aeruginosa* grows well on MacConkey agar and trypticase soy agar. Additionally, Cetrimide agar, a commercial formulation, selectively isolates *P. aeruginosa*.

PREVENTION AND THERAPY The best means of controlling *Pseudomonas* is to reduce possible sources of infection, such as damp bedding or stagnant water. Animal drinking water should be monitored for *P. aeruginosa*, and in some situations, water may be chlorinated to eliminate contamination (Songer and Post, 2005). Reducing stressors, such as overcrowding and concurrent infection, also aids in control. *Pseudomonas* spp. have an inherent resistance to many antimicrobials.

Salmonella

BACKGROUND Some of the earliest reports of salmonellosis in guinea pigs date back to the 1800s (Nelson and Smith, 1927). Multiple outbreaks of natural infections in guinea pig colonies in the United States were documented throughout the early 1900s (Holman, 1916; Nelson and Smith, 1927). Since this period of time, as laboratory standards have grown more stringent and general hygiene and sanitation have improved, the occurrence of salmonellosis in laboratory guinea pigs has declined greatly (Fox, 2002; Harkness and Wagner, 1995; Percy and Barthold, 2007).

ETIOLOGY Members of the genus *Salmonella* are Gram-negative, non-spore-forming bacilli that are facultative anaerobes. *Salmonella* other than *S. typhi* or *S. paratyphi* produce hydrogen sulfide, as well as acid and gas from glucose, but not lactose (Ganaway, 1976; Songer and Post, 2005). *Salmonella* is a member of the *Enterobacteriaceae* family. While multiple serovars have been recovered from guinea pigs, the most frequently isolated are *S. typhimurium* and *S. enteritidis* (Fox, 2002; Ganaway, 1976; Harkness and Wagner, 1995; Quesenberry and Carpenter, 2004; Percy and Barthold, 2007). Humans are susceptible to disease from the same serovars and phage types as guinea pigs (Fish et al., 1968; Percy and Barthold, 2007; Rigby, 1976).

EPIZOOTIOLOGY AND PATHOGENESIS Guinea pigs are extremely susceptible to *Salmonella* (Fox, 2002). Transmission of *Salmonella* is primarily fecal–oral or via ingestion of contaminated feed (contaminated with feces of infected guinea pig or a wild rodent) (Fox, 2002; Harkness and Wagner, 1995; Percy and Barthold, 2007; Quesenberry and Carpenter, 2004). Entry via the conjunctiva has also been reported in guinea pig epizootics (Iijima et al., 1987; Moore, 1957). Additionally, ingestion of contaminated blood or tissue can transmit *Salmonella* (Fox, 2002). Stressed animals, due to conditions such as weaning, parturition, old age or poor nutrition, are more susceptible to infection (Quesenberry and Carpenter, 2004). Recovered animals can develop an asymptomatic carrier state with intermittent shedding in the feces, making elimination of this bacterium from a colony difficult (Fox, 2002; Harkness and Wagner, 1995; Percy and Barthold, 2007). The incubation time for *Salmonella* is 5–7 days and epizootic or enzootic patterns of infection are possible (Fox, 2002; Ganaway 1976).

CLINICAL MANIFESTATIONS Disease due to *Salmonella* occurs as peracute septicemia or acute, subacute, or chronic enteritis (Songer and Post, 2005). Acute disease is characterized by high morbidity and low to moderate mortality. Clinical signs include depression, weakness, and fever. Dehydration and electrolyte imbalance may be severe. Infection spreads rapidly within a colony. Conjunctivitis and abortions have also been noted (Percy and Barthold, 2007). Although weight loss is present, diarrhea may or may not occur in guinea pigs. In an epizootic outbreak, young guinea pigs may die suddenly without prior signs of illness.

PATHOLOGY In acute and peracute cases, there may be no lesions seen at necropsy (Harkness and Wagner, 1995; Percy and Barthold, 2007). Splenomegaly may be observed in subacute and chronic infections. Minute white foci and/or yellowish-white nodules, up to several millimeters in diameter, may occur in the spleen, liver, or other lymph nodes. Similar foci or nodules may also occur in the lung, pleura, peritoneum, and in the wall of the uterus. Rupture of the nodules may result in a suppurative pleuritis, peritonitis, and/or pericarditis. Gas may be observed in the small and large intestine, along with catarrhal enteritis (Fish et al., 1968).

On histopathology, lesions support bacterial invasion, with subsequent necrosis and abscess formation (Ganaway, 1976). Nodules are noted to be “typhoid-like” granulomas, with areas of central necrosis, surrounded by histiocytes. Polymorphonuclear leukocytes and histiocytes are present in the Peyer’s patches of the ileum and jejunum.

DIAGNOSIS *Salmonella* can be isolated from blood, feces, and affected organs (most commonly from the

spleen) (Fox, 2002). Recovery requires special conditions: enrichment in a broth such as selenite-F or tetracycline and culture at 37°C on MacConkey’s or brilliant green agar (Fox, 2002; Songer and Post, 2005).

PREVENTION AND THERAPY Prevention is the best approach (Harkness and Wagner, 1995; Rigby, 1976). All fresh fruit and vegetables should be thoroughly washed and stored in airtight containers. Since so many species of mammals are potential carriers, the exclusion of wild rodents, birds, and other vermin is essential. When increased colony mortality is apparent and a chronic case is recognized at necropsy, *Salmonella* infection is likely widespread and well established in the colony. In situations such as this, it is reasonable to depopulate the colony, sanitize the premises, and restock with *Salmonella*-free guinea pigs.

Treatment on an individual basis will include the appropriate antibiotics with fluid therapy. Animals that become asymptomatic after treatment may become carriers, a problem with zoonotic potential. Antimicrobial resistance is expanding; 90% of *Salmonella* isolates from domestic animals demonstrate multi-drug resistance (Songer and Post, 2005).

Streptobacillus moniliformis

BACKGROUND *Streptobacillus moniliformis* is one of the causative agents of rat bite fever, a significant worldwide, zoonotic, systemic infection of humans associated with contact with rats (Gaastra et al., 2009; Khatchadourian et al., 2010). It was described in early recorded history in India and was recognized in the US as early as 1839 as a syndrome that developed following contact with rats (Gaastra et al., 2009). *S. moniliformis* also causes Haverhill fever, a systemic infection of those who eat food contaminated with rat urine or feces (Abdulaziz et al., 2006; McEvoy et al., 1987; Shanson et al., 1983).

ETIOLOGY *Streptobacillus moniliformis*, a facultative anaerobic, non-motile, non-capsulate, pleomorphic, Gram-negative rod, is the type species of the genera, *Streptobacillus*, family *Leptotrichiaceae* within the phylum *Fusobacteria* (Greenwood, 2007; Nolan et al., 2009). Its genome has been completely sequenced (Nolan et al., 2009).

EPIZOOTIOLOGY AND PATHOGENESIS *S. moniliformis* is commonly found in the nasopharynx of wild and pet rats (Gaastra et al., 2009; Wullenweber, 1995), although typically excluded from modern SPF laboratory animal facilities (Nicklas et al., 2002; Pritchett-Corning et al., 2009). The organism has also been found in carnivores, calves, pigs, turkeys, and a variety of rodents including guinea pigs presumably following contact with infected rats (Gaastra et al., 2009; Wullenweber, 1995).

CLINICAL MANIFESTATIONS AND PATHOLOGY Most commonly, *S. moniliformis* in guinea pigs has been associated with cervical lymphadenitis and other localized abscesses (Aldred et al., 1974; Fleming, 1976; Percy and Barthold, 2007; Van Hoosier and Robinette, 1976; Wullenweber, 1995). Severe necrotizing bronchopneumonia with pyogranuloma formation was reported in one guinea pig by Kirchner et al. (1992). No clinical signs of disease were noted in their report.

DIAGNOSIS Gram stain of smears can be suggestive of infection by demonstrating short bacilli in chains or long filaments followed by bacterial culture and isolation (Greenwood, 2007; Kirchner et al., 1992; Wullenweber, 1995) although because of the fastidious nature of *S. moniliformis*, PCR is more commonly used for detection (Boot et al., 2002). If culture is attempted, samples must be cultured on a media containing serum, blood or ascitic fluid, such as Loeffler's serum medium (Greenwood, 2007). Cross-reactive sequences between *Leptotrichia* spp. and *S. moniliformis* have been demonstrated and can result in false positives when PCR is used for diagnosis (Boot et al., 2008; Wouters et al., 2008). Sequence of the amplicon can differentiate these outcomes. As the entire sequence of *S. moniliformis* has recently been completed (Nolan et al., 2009), new primers for diagnosis may be forthcoming. Antibodies to *S. moniliformis* can be demonstrated by ELISA (Boot et al., 1993b, 2006).

PREVENTION AND THERAPY Because the organism is not spread easily, outbreaks can be controlled by culling affected guinea pigs (Van Hoosier and Robinette, 1976). Treatment of individual animals with antibiotics is effective in other species (Gaastra et al., 2009; Wullenweber, 1995), although treatment should be approached with caution because of the zoonotic potential of this organism. Prevention of contact with infected rats, other animals that contact wild or infected rats, or infected food and/or bedding should eliminate introduction of this organism (Van Hoosier and Robinette, 1976).

Yersinia pseudotuberculosis

BACKGROUND Ganaway states that "one of the earliest recognized bacterial disease of the guinea pig was caused by *Yersinia pseudotuberculosis*" (Ganaway, 1976). In present day colonies of laboratory guinea pigs, natural outbreaks of pseudotuberculosis are rare (Fox, 2002; Percy and Barthold, 2007).

ETIOLOGY *Yersinia pseudotuberculosis*, an ubiquitous enteropathogen, is a Gram-negative, non-spore-forming, facultative anaerobic rod that is oxidase-negative and catalase-positive (Ganaway, 1976). It is non-hemolytic and produces both exotoxin and enzyme (Fox, 2002). Growth is

optimal at 20–30°C (Fox, 2002; Ganaway, 1976). The genus *Yersinia* is part of the family *Enterobacteriaceae* and, in addition to guinea pigs, it causes pseudotuberculosis in other rodents, cats, and turkeys (Songer and Post, 2005).

EPIZOOTIOLOGY AND PATHOGENESIS *Y. pseudotuberculosis* can result in clinical disease or a sub-clinical carrier state. Guinea pigs are very susceptible to infection with this bacterium (Fox, 2002; Ganaway, 1976). *Y. pseudotuberculosis* is shed in feces, therefore ingestion of food contaminated with feces from a carrier guinea pig, wild rodent, or bird can transmit the organism, as can aerosol spread, or entry via a bite wound or skin laceration (Fox, 2002; Ganaway, 1976; Rigby, 1976). Dams can pass infection to their pups (Fox, 2002). Orally absorbed organisms disseminate from the intestinal tract to the mesenteric lymph nodes.

Acute and chronic forms of disease have been described (Ganaway, 1976; Rigby, 1976). An acute septicemic form of infection can lead to mortality in 24–48 hours. A chronic infection that often lasts several weeks to months and ultimately results in death is the more classic pseudotuberculosis presentation.

CLINICAL MANIFESTATIONS The classic clinical presentation associated with *Y. pseudotuberculosis* is that of wasting and lymphadenitis, sometimes with accompanying diarrhea, and frequently resulting in death about a month after initial infection (Obwolo, 1977; Sebesteny, 1976). The acute form of the disease can present with no clinical signs or consist of one to two days of coughing and respiratory distress prior to death (Rigby, 1976).

PATHOLOGY Necropsy of animals chronically or sub-acutely infected with *Yersinia pseudotuberculosis* reveals nodules throughout the body, including the regional lymph nodes, spleen, liver, lung, and bone marrow (Fox, 2002; Percy and Barthold, 2007). The grayish-white spherical nodules vary from miliary pinpoint to 2–3 cm in diameter and may contain creamy to caseous exudate. In acute cases, nodules are noted in the intestinal wall, especially at the location of the terminal ileum and cecum, and often with accompanying mucosal ulceration and acute enteritis (Percy and Barthold, 2007). Lung congestion may be seen in acute cases (Rigby, 1976).

Yersinia nodules often have a central area of necrosis with neutrophilic infiltration; foamy macrophages are often noted in the periphery (Fox, 2002; Ganaway, 1976). Blood vessels within the nodule may contain bacterial emboli. In chronic lesions, fibroblasts and epithelial cells proliferate and nodules may become granulomatous, but do not calcify.

DIAGNOSIS *Y. pseudotuberculosis* can be cultured from abscesses (Rigby, 1976). Direct smears may also

be beneficial. In acute septicemic cases, *Yersinia* may be cultured from the blood (Ganaway, 1976). *Yersinia* spp. can be cultured on standard media and *Y. pseudotuberculosis* can be differentiated from *Y. pestis*, the causative agent of plague, by the fact that it is urease, and rhamnose-positive, while *Y. pestis* is negative for both of these growth conditions (Songer and Post, 2005).

TREATMENT AND CONTROL Euthanasia of infected animals is advised due to the fact that antibiotic treatment can result in a carrier state, which, due to its zoonotic potential, puts human handlers and staff at risk. A killed vaccine has been unsuccessful at protecting guinea pigs from infection, but a more recent live vaccine has produced promising results (Quintard et al., 2010).

FUNGAL INFECTIONS

Dermatophytosis – *Trichophyton mentagrophytes*, *Microsporum canis*, *Microsporum gypseum*

BACKGROUND Dermatophytosis, also known as ringworm or tinea, indicates infection with pathogenic fungi in the phylum Ascomycota that cause the bulk of superficial fungal infections in humans and animals (Ameen, 2010; Howard et al., 2003). The fungi are keratinolytic and keratinophilic and infection is usually limited to cornified layers of the skin, hair, or nails (Howard et al., 2003; Weitzman and Summerbell, 1995). Their importance as pathogens is clear in that the majority of dermatophytes infecting animals are zoonotic (Chermette et al., 2008). Originally, the dermatophytes were organized into three anamorphic, or asexual, genera, *Microsporum*, *Trichophyton*, and *Epidermophyton*. The primary structure associated with asexual reproduction is the conidium and classification into these genera was based on the morphology of the macroconidia in culture. Subsequent studies revealed sexual reproduction in certain *Microsporum* and *Trichophyton* spp. that occurs by means of an ascospore found within an ascus. The dermatophyte sexual states (teleomorphs) are classified in the genus, *Arthroderma* (Weitzman and Summerbell, 1995). Recent efforts at phylogenetic analysis and identification of dermatophytes have been achieved by sequencing 28S ribosomal DNA (rDNA) and internal transcribed spacer (ITS) regions flanking 5.8S rDNA (Drouot et al., 2009; Frealle et al., 2007; Symoens et al., 2011). Dermatophyte species can also be divided into three groups determined by their adaptation to a certain habitat. Human-associated dermatophytes are anthropophilic (e.g. *Trichophyton rubrum*), those primarily found on animals and occasionally infecting humans are zoophilic (e.g.

Trichophyton mentagrophytes), and species persisting in the soil are termed geophilic (e.g. *Microsporum gypseum*) (Weitzman and Summerbell, 1995), however some overlap does occur. Zoophilic and geophilic dermatophytes infecting humans cause a more intense inflammatory reaction than anthropophilic species (Weitzman and Summerbell, 1995).

ETIOLOGY Dermatophytosis in animals is most often caused by the anamorph species *Microsporum* and *Trichophyton*, however the infecting species can vary with the geographic region (Bond, 2010). Of these genera, *Trichophyton mentagrophytes* is the most common cause (Aho, 1980; Balsari et al., 1981; Drouot et al., 2009; Feuerman et al., 1975; Lopez-Martinez et al., 1984; McAleer, 1980a, 1980b; Papini et al., 1997; Pombier and Kim, 1975; Stenwig, 1985; Vangeel et al., 2000). *T. mentagrophytes* is a species complex with several variants including both anthropophilic and zoophilic pathogens. Pollock (2003) considered *T. mentagrophytes* var *mentagrophytes* as the most common variant infecting rodents, however the specific variant is not always reported in the literature. The teleomorphs are known for the *T. mentagrophytes* complex and there are two, *Arthroderma benhamiae* and *A. vanbreuseghemii*. The *A. benhamiae* genome has been sequenced (Burmester et al., 2011). The different variants and sexual states of the *T. mentagrophytes* complex coupled with more recent phylogenetic analyses presents confusion about proper nomenclature and species assignment (Drouot et al., 2009; Fumeaux et al., 2004). Infection with either *M. canis* or *M. gypseum* has also been reported in the guinea pig but less frequently (Feuerman et al., 1975; Papini et al., 1997).

EPIZOOTIOLOGY AND PATHOGENESIS Guinea pigs, and other animals, are infected by direct exposure to an infected animal, contaminated environments, or fomites. Arthroconidium produced by septation of hyphae are infectious and persist in the environment for months to years (Bond, 2010; Weitzman and Summerbell, 1995). Hyphae invade the stratum corneum eventually colonizing the hair follicles. The sequenced genome of *A. benhamiae* revealed numerous predicted protease-encoding genes and their secretion was confirmed experimentally in a keratin digestion assay indicating an important role in invasion and pathogenesis (Burmester et al., 2011). Young guinea pigs appear to be more susceptible to disease and other predisposing and susceptibility factors include pregnancy, high temperature and humidity, health status, and genetics (Pollock, 2003; Pombier and Kim, 1975). Surveys of dermatophytosis in guinea pigs have been reported from around the world, with prevalence ranging from 1.3–56% (Balsari et al., 1981; Drouot et al., 2009; Feuerman et al., 1975; Lopez-Martinez et al., 1984; Papini et al., 1997; Vangeel et al., 2000).



FIGURE 23.2 A well-circumscribed, hairless, scaling lesion on a guinea pig with dermatophytosis. Photo provided by Dr. Martha Hanes.

CLINICAL MANIFESTATIONS Infected guinea pigs are most often asymptomatic but can exhibit lesions typical of dermatophytosis. When present, lesions are most common on the head and appear to begin on the nose or muzzle and then can spread over the body to the trunk and limbs (Figure 23.2). Circumscribed or irregular lesions with combinations of alopecia, scale, crust, erythema, and ulceration develop, with or without pruritis, and spontaneous remission is possible (Drouot et al., 2009; McAleer, 1980a; Pollock, 2003; Pombier and Kim, 1975). Pustules may be associated with the lesions due to secondary bacterial infections. Furthermore, in one outbreak of *T. mentagrophytes*, affected animals were in poor body condition and there was approximately 50% mortality in young guinea pigs (Pombier and Kim, 1975). Onychomycosis is rare in the guinea pig (Drouot et al., 2009).

PATHOLOGY Microscopic analysis of affected epidermis and adnexa can include acanthosis, vascular congestion, dermal lymphocytic infiltration, folliculitis, perivascular, and interstitial dermatitis, and intra-epidermal pustules (Chermette et al., 2008; Pombier and Kim, 1975).

DIAGNOSIS Microscopy and culture are the primary means of diagnosing dermatophytosis. Wood's lamp can be used as a screening tool for *M. canis* and infected hairs will fluoresce with an apple-green color. However, false negatives can occur (Chermette et al., 2008). Hairs are plucked or the skin scraped from the periphery of an active lesion or from the whole lesion

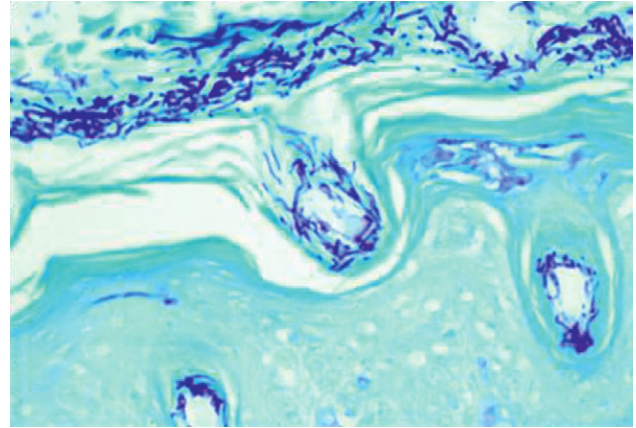


FIGURE 23.3 Skin section stained with periodic acid-Schiff demonstrating dermatophyte arthroconidia and hyphae. Photo provided by Dr. Martha Hanes.

if there is no evidence of inflammation and samples placed in 10% potassium hydroxide to remove keratin. The samples are then examined for hair or skin invasion with hyphae and/or arthroconidia. To increase sensitivity, fluorescent microscopy with Calcofluor white and Congo red can be used (Bond, 2010; Robert and Pihet, 2008). Geimsa staining will also identify arthroconidia. All the dermatophytes infecting guinea pigs will have ectothrix hair shaft involvement. Prior to obtaining a skin or hair sample for culture, the area should be wiped with alcohol to reduce contamination. Saprophytic fungi contamination of the fur may hinder isolation of dermatophytes (Aho, 1983). Hair, skin, or crusts are collected using a sterile toothbrush, swab, forceps, scalpel blade, or surgical scrub brush and placed onto culture medium. Sabouraud's dextrose agar supplemented with cyclohexamide and chloramphenicol is commonly used. Dermatophyte Test Medium is available, however contamination can cause both false-positive and false-negative results (Robert and Pihet, 2008). If the animal is simply a mechanical carrier, results of repeated testing will vary (Chermette et al., 2008). Biopsy samples can be stained with periodic acid-Schiff or silver stains to detect arthroconidia and hyphae (Figure 23.3) (Chermette et al., 2008). As mentioned earlier, polymerase chain reaction (PCR) amplification and sequencing of ITS and 28S ribosomal DNA (Drouot et al., 2009; Fumeaux et al., 2004; Symoens et al., 2011) will identify isolates, and mating experiments can be performed (Hironaga et al., 1981).

PREVENTION AND THERAPY Reducing exposure to infected animals and contaminated environments is critical in preventing dermatophytosis. Assuring a well-managed facility with no overcrowding, parasite control, and proper temperature and humidity regulation are important preventive components (Pollock, 2003;

Pombier and Kim, 1975). Infected animals should be removed, the environment thoroughly decontaminated and any items that cannot be disinfected should be discarded. Animal care staff should wear proper personal protective equipment when handling potentially contaminated items or bedding since zoonosis is a concern (McAleer, 1980a). Dilute bleach, benzalkonium chloride, and glutaraldehyde are effective surface disinfectants (Pollock, 2003). In a laboratory setting, it is doubtful that animals will be treated for ringworm. Readers are referred to other summaries of available topical and systemic treatments (Pollock, 2003).

Encephalitozoonosis/Microsporidiosis

BACKGROUND The Microsporidia (members of the phylum Microspora) are a group of single-celled intracellular spore-forming eukaryotic parasites that infect a wide range of animals, from insects to mammals. The defining and unique feature of the phylum is the presence of a polar filament (tube) in the mature spore (Wasson and Peper, 2000). The microsporidia also have one of the smallest genomes of any eukaryote and lack several key features, such as typical mitochondria. Long thought to be protozoa, certain characteristics indicated that these organisms were fungi-like such as presence of a chitinous spore wall, genes for trehalose metabolism, and intranuclear division (Brosson et al., 2006; Dunn and Smith, 2001). Recently, Lee et al. (2008) concluded that microsporidia are true fungi related to zygomycetes. Of the microsporidial species infecting humans, *Enterocytozoon bieneusi* is the most common with lesser, but still significant, numbers of cases due to the *Encephalitozoon* spp. The majority of patients are immunocompromised as a result of HIV infection, AIDS, or are organ transplant recipients. However, healthy immune-competent persons are also susceptible to disease (Didier and Weiss, 2006; Mathis et al., 2005). Animals are hosts for those common species strongly supporting their zoonotic potential.

ETIOLOGY The primary agent of microsporidiosis in guinea pigs is *Encephalitozoon cuniculi* (syn. *Nosema*). *E. cuniculi* was first reported in the rabbit as the cause of "Infectious Motor Paralysis" (Percy and Barthold, 2007) and subsequently has been identified in a wide range of animal species (Wasson and Peper, 2000). Katinka et al. (2001) have sequenced the 11 chromosomes of the approximately 2.9-Mb genome, highlighting the genome compaction and shortened protein sequences that reflect its intracellular location and host dependence.

EPIZOOTIOLOGY AND PATHOGENESIS Wasson and Peper (2000) provide an informative summary of the mammalian microsporidian life cycle. Environmentally

resistant spores are shed in feces, urine, and mucus and then ingested or inhaled. Transplacental transmission has been documented in the rabbit (Baneux and Pognan, 2003) and has been claimed in the guinea pig (Boot et al., 1988). Predictably, primary sites of infection commonly include the small intestine, respiratory tract, and placenta. An unknown signal triggers the polar filament (tube) to be extruded from the spore and infectious sporoplasm is injected into the host cell cytoplasm. The sporoplasm then divides into meronts (merogony) that differentiate through intermediary forms before becoming mature spores (sporogony). For *E. cuniculi* and other *Encephalitozoon* spp., developing spores are packaged in a parasitophorous vacuole. The host cell ruptures releasing spores that commonly disseminate to the kidney, liver, and brain. Host protection from infection is primarily achieved by cell-mediated immunity with lesser contributions from the humoral immune system (Khan et al., 2001).

From serologic surveys, prevalence of encephalitozoonosis in guinea pig colonies has ranged from 13%–63%. This variability may be due to differences in housing and husbandry for the particular colony and diagnostic methodology (Boot et al., 1988; Gannon, 1980).

CLINICAL MANIFESTATIONS Encephalitozoonosis is subclinical in the guinea pig (Boot et al., 1988; Gannon, 1980; Illanes et al., 1993; Moffatt and Schiefer, 1973; Wan et al., 1996).

PATHOLOGY Gross lesions of encephalitozoonosis in the guinea pig are not consistently found and when present have only been described in the kidney. Infected animals may have pale kidneys and/or pitting of the renal cortex (Gannon, 1980; Moffatt and Schiefer, 1973). Histologic lesions are found primarily in the brain and kidney. Focal to multifocal granulomatous encephalitis may be evident in different regions of the brain, with or without associated necrosis, and perivascular and meningeal mononuclear cell infiltrate (Moffatt and Schiefer, 1973; Wan et al., 1996). Organisms approximately 1–1.5 µm in width × 1.5–2.5 µm in diameter (Illanes et al., 1993; Wasson and Peper, 2000) can be found within or adjacent to lesions or free in the tissue with no associated inflammation. In the kidney, interstitial nephritis and necrosis, fibrosis, perivascular cuffing and tubular ectasia have all been described (Boot et al., 1988; Gannon, 1980; Moffatt and Schiefer, 1973). Here, organisms may occasionally be visualized in renal epithelial cells and collecting tubule lumens. Absence of typical lesions in the brain or kidney in a seropositive animal may be due to the multifocal nature of the lesions and insufficient sections being analyzed (Gannon, 1980; Wan et al., 1996). Other lesions reported include necrotic liver foci and interstitial pneumonia

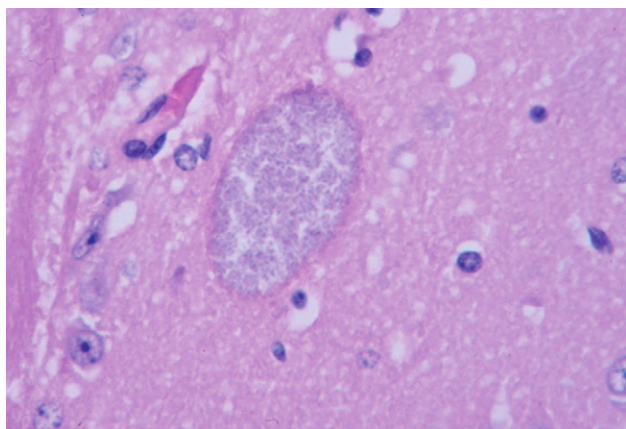


FIGURE 23.4 *Encephalitozoon* spores in a brain section. Note absence of inflammation in the surrounding tissue. Photo provided by Dr. Kim Waggle.

with perivascular and peribronchial lymphoid accumulation.

DIAGNOSIS Serology tests including carbon immunoassay, IFA, ELISA, multiplex fluorescent immunoassay, and Western blot have all been or currently are being used for colony monitoring (Percy and Barthold, 2007; Wasson and Peper, 2000). Presence of typical histopathologic lesions and special stains confirm the infection. While indistinct with hematoxylin and eosin staining, spores are periodic acid-Schiff, Gram, and modified trichrome positive (Figure 23.4). Chemofluorescent agents such as Calcofluor white can also be used (Garcia, 2002) and spores are birefringent (Tiner, 1988). PCR primers are available (Baneux and Pognan, 2003; Ghosh and Weiss, 2009) providing a fast and easily interpretable means of diagnosis and the genome for *E. cuniculi* has recently been sequenced providing further opportunities for novel molecular diagnostic strategies (Katinka et al., 2001). Urine, brain, and kidney are optimal specimens for testing by PCR. Electron microscopy is the gold standard for diagnostic confirmation and offers species identification which is particularly important for human infections (Garcia, 2002).

PREVENTION AND THERAPY *E. cuniculi*-free guinea pigs are commercially available and therefore, protection from contaminated animals or environments is the primary means of prevention (Gannon, 1980). Faced with contamination, eradication may be successful through serological analysis and euthanasia of seropositive animals (Baker, 2007) coupled with environmental decontamination. *E. cuniculi* spores are environmentally stable for days to weeks depending on the temperature and humidity, but are susceptible to common disinfectants (Jordan et al., 2006; Waller, 1979). Vertical transmission is

thought to occur in guinea pigs (Boot et al., 1988), so hysterectomy rederivation should be used with caution and offspring extensively tested. It is unlikely that laboratory guinea pigs would be treated for encephalitozoonosis.

Miscellaneous Diseases

Candida pintolopesii (Formerly Known as *Torulopsis pintolopesii*)

Two of four laboratory-bred guinea pigs were found dead several days to two weeks after adoption (Kunstyr et al., 1980). Diarrhea was noted in one animal prior to death and each had gross evidence of enteritis at necropsy which was confirmed histologically. The yeast *Candida pintolopesii*, a normal inhabitant of the gut, was isolated from the small intestine, lung, and ascitic fluid of one animal. The authors hypothesized that a change in social structure, diet, and environment precipitated disease.

Cryptococcus neoformans

Cryptococcus neoformans is a basidiomycetous yeast causing serious infections of the lungs and central nervous system in immunocompromised persons (Boekhout and Guého, 2003). Betty (1977) reported chronic, subclinical cryptococcal meningitis in laboratory-reared Dunkin Hartley guinea pigs with no evidence of generalized infection. The source of infection was not identified. Van Herck et al. (1988) described dermal cryptococcosis of an adult male pet guinea pig. Histopathologic analysis of a large ulcerative lesion on the dorsal aspect of the nose showed extensive focal ulcerative dermatitis with infiltrating neutrophils, plasma cells, lymphocytes, and macrophages with edema and ovoid organisms. Dermal and subcutaneous tissue also contained large numbers of these thick-walled organisms that were positive by periodic acid-Schiff staining.

Enterocytozoon bieneusi

Enterocytozoon bieneusi is a significant cause of intestinal microsporidiosis in immunocompromised persons and has been identified in many mammalian and non-mammalian hosts (Mathis et al., 2005). Microsporidian spores were found in the feces of a two-year-old male entered into a prospective study of pediatric enteric parasites in Peru. To investigate the source of infection, stool was analyzed from animals in the household, including guinea pigs, chickens, dogs, and cats. Seven of eight asymptomatic guinea pigs were positive for spores, whereas all the other animals were negative. Polymerase chain reaction and sequence analysis confirmed that both the child and guinea pigs were infected with *E. bieneusi* and with the same genotype. Furthermore, this specific genotype was found in guinea pigs from other unrelated

households suggesting that guinea pigs are the natural host and there is zoonotic potential for this organism.

Histoplasma capsulatum

There is a single report from Brazil of a naturally occurring histoplasmosis outbreak in laboratory guinea pigs (Correa and Pacheco, 1967). Clinical signs of disease in adults included progressive emaciation and hindlimb dysfunction before death. Young animals exhibited a hunched back, ruffled fur, and conjunctivitis with discharge before dying at two months of age. Gross and histologic lesions were numerous; however, for these authors a characteristic lesion of colitis with the wall expanded by numerous lymphocytes, macrophages, epithelioid and giant cells with basophilic organisms free or inside macrophages and culture results established the diagnosis. There may have been a link between this outbreak and histoplasmosis diagnosed in a cow on a farm from which the grass used to feed the guinea pigs was sourced.

Hortaea werneckii

Hortaea werneckii, the causative agent of tinea nigra in people, was diagnosed in a household guinea pig in Japan (Sharmin et al., 2002). Lesions included a focal area of ulceration and alopecia on the back and black pigmentation on the palmar aspect of the right forepaw. *H. werneckii* was detected by mycologic culture of the back lesion and DNA sequencing of an amplified region of large subunit ribosomal DNA. The authors hypothesized that the animal contracted the infection from the environment, but could not definitively rule out contamination with the fungus rather than a real infection.

Paecilomyces

Paecilomyces spp. are occasional opportunists in humans and animals (Summerbell, 2003). This fungus was identified during routine monitoring from a single conventionally housed laboratory guinea pig with no apparent clinical signs (Kunstyr et al., 1997). *Paecilomyces* spp. were most commonly identified in specific pathogen-free rats from one particular animal facility with the trachea and lungs being the most common site of infection in all animals analyzed. Mild dermatitis was noted in a group of hairless mice; however, the vast majority of animals had no clinical or histologic evidence of disease.

Pneumocystis carinii

A hairless mutant arose in a closed colony of Hartley guinea pigs. In addition to abnormal haircoat development, affected guinea pigs were smaller than their haired siblings and died early due to infections otherwise associated with an abnormal immune system such as systemic cytomegalovirus and balantidiasis, and *Pneumocystis* pneumonia (Reed and O'Donoghue, 1979). Abnormal

thymic and lymphoid follicle morphology and agammaglobulinemia confirmed the immunodeficiency.

PROTOZOA INFECTIONS

Protozoa rarely cause disease in guinea pigs and intestinal protozoa are often considered part of the normal flora. In this review, the more frequently reported protozoa of domestic guinea pigs will be discussed. The reader is referred to *Parasites of Laboratory Animals* (Baker, 2007) and *Morphology and Taxonomy of the Intestinal Protozoa of the Guinea Pig* (Nie, 1950) for a more detailed description of the protozoa of this species. The nomenclature used in this section is in accordance with the 2007 reference.

Amoebiasis

ETIOLOGY *Endolimax caviae* and *Entamoeba caviae* (syn. *Entamoeba cobayae*) are the causative agents of amoebiasis in guinea pigs (Levine, 1985). Nie (1950) reported the incidence of *Endolimax caviae* to be 18% and the incidence of *Entamoeba caviae* to be 14% in a colony of guinea pigs studied at the University of Pennsylvania.

PATHOGENESIS *Endolimax caviae* and *Entamoeba caviae* are observed in the cecum of the guinea pig (Nie, 1950). Both of these organisms are thought to feed upon the fecal material and microflora of the host intestine, without invasion of tissue or consumption of blood. Transmission is via ingestion of cysts passed in the feces.

CLINICAL MANIFESTATIONS Infection with either organism does not generally result in clinical signs (Levine, 1985).

PATHOLOGY *Entamoeba* and *Endolimax* are usually non-pathogenic (Baker, 2007; Levine, 1985).

DIAGNOSIS Trophozoites can be observed in a smear of intestinal contents (Baker, 2007; Levine, 1985). Cysts are detected by zinc sulfate float. *Endolimax caviae* trophozoites are smaller than those of *Entamoeba caviae*, with an average diameter of 1.6 µm and 14.4 µm, respectively (Nie, 1950). The cyst forms of either organism have rarely been observed, and little detail has been recorded on their appearances.

PREVENTION AND THERAPY Generally, treatment is not necessary for guinea pig amoebiasis.

Balantidia caviae

BACKGROUND Upon initial description, controversy developed over whether *B. caviae* was the same organism as *B. coli* in swine (Vetterling, 1976). Morphological

descriptions and infection studies have since proved that the two species are distinct.

ETIOLOGY *Balantidium caviae* is the causative organism.

PATHOGENESIS *B. caviae* transmission is via ingestion of cysts in the feces (Baker, 2007).

CLINICAL MANIFESTATIONS If the mucosal barrier of the intestine is compromised, *B. caviae* can become invasive and result in enteritis; otherwise infection is non-pathogenic (Baker, 2007).

PATHOLOGY *B. caviae* may be observed in the walls of the cecum and colon, but penetration can occur post-mortem, a consideration that should be made when diagnosing infection at the time of necropsy (Baker, 2007; Nie, 1950). If invasion is ante-mortem, intestinal inflammation should be present; more often, though, *B. caviae* is considered a commensal protozoan.

DIAGNOSIS In addition to diagnosing *B. caviae* in histologic sections of the cecum or colon, ciliated organisms with micro- and macro-nuclei can be observed in fresh fecal smears (Baker, 2007; Fox, 2002).

PREVENTION AND THERAPY Proper hygiene is most important in controlling *B. caviae* infection in guinea pigs (Baker, 2007). Tetracyclines have been used to treat balantidiasis in other species.

Cryptosporidiosis

BACKGROUND *C. wrairi* is named after the Walter Reed Army Institute of Research where the organism was first identified in a colony of laboratory guinea pigs (Vetterling et al., 1971).

ETIOLOGY *Cryptosporidium wrairi* is the causative organism.

PATHOGENESIS The route of infection for *C. wrairi* is likely fecal–oral, with ingestion of oocysts in feces (Baker, 2007; Fox, 2002). Experimental infection has shown that the duration of infection for animals older than 16 weeks is usually short, lasting as little as 1–2 weeks, after which the organism is cleared (Chrisp et al., 1990). Younger animals have a longer duration of infection. Recovered animals appear to be refractory to subsequent infection.

CLINICAL MANIFESTATIONS Clinical signs are more likely to be seen in young animals, with weight loss noted most commonly (Baker, 2007; Fox, 2002). If infection is severe, anorexia, a potbellied appearance and a greasy hair coat may be observed with or without

accompanying diarrhea (Baker, 2007). Morbidity and mortality, especially in young animals, can reach levels of 50% during an outbreak (Harkness and Wagner, 1995; Percy and Barthold, 2007). *Echinococcus coli* has been associated with clinical cases of *C. wrairi*, but the significance of this infection is unknown (Percy and Barthold, 2007). Subclinical infections with *C. wrairi* are thought to be possible (Baker, 2007; Fox, 2002).

PATHOLOGY Erosion, hyperemia, and inflammation of the small intestine have been observed with *C. wrairi* infection (Baker, 2007; Chrisp et al., 1992; Harkness and Wagner, 1995). Edema of the lamina propria and hyperplasia of the crypt epithelium occurs. In chronic infections, villous atrophy and bridging, metaplasia of the mucosal epithelium, and lymphocyte infiltration of the lamina propria have been noted. Developmental stages of *C. wrairi* can be seen throughout the intestine, with highest concentrations of the organism noted in the brush border of the ileum.

DIAGNOSIS Oocysts can be seen in fecal floats (Gressler et al., 2010; Percy and Barthold, 2007). Kinyoun staining of fecal smears and microscopic examination of mucosal scrapings or histological sections are also diagnostic options.

PREVENTION AND THERAPY Prevention is the best means of control for *C. wrairi*. Oocysts are resistant to many disinfectants or require high concentrations and/or long contact times before being rendered non-infectious. Heating above 65°C for greater than 5 minutes has been described to be successful for the destruction of *Cryptosporidium* organisms, as has exposure to temperatures below 0°C (Baker, 2007; Harkness and Wagner, 1995). Successful treatment with sulfonamides has been reported, but the efficacy of this treatment has been questioned (Fox, 2002; Percy and Barthold, 2007; Sebesteny, 1976). Harkness suggests the “extra-label” use of high doses of the coccidiostat, Decoquinat, to treat cryptosporidiosis (Harkness and Wagner, 1995). While *C. wrairi* is not known to infect humans, the genus has a general lack of specificity and precautions should be taken to prevent zoonotic transmission (Baker, 2007; Harkness and Wagner, 1995).

Eimeria

BACKGROUND Reports of coccidia in guinea pigs date back to the late 1800s, although the observed organisms were initially thought to be a variety of rabbit coccidian (Vetterling, 1976). In the early 1920s, Bugge and Heinke showed that coccidia noted in the guinea pig were indeed distinct from those of the rabbit and in 1924 Sheather named the guinea pig coccidia *E. caviae*.

ETIOLOGY *Eimeria caviae* is the causative organism.

PATHOGENESIS Transmission of *E. caviae* is fecal-oral, with ingestion of oocysts in feces (Baker, 2007).

CLINICAL MANIFESTATIONS Clinical signs due to *E. caviae* are noted in severe infections and include diarrhea, anorexia and a rough hair coat (Baker, 2007; Ellis and Wright, 1961; Rigby, 1976). The first appearance of diarrhea is often 10–11 days after initial infection (Baker, 2007; Elsheikha et al., 2009; Percy and Barthold, 2007). Watery, pasty, and hemorrhagic forms of diarrhea have all been reported (Baker, 2007; Elsheikha et al., 2009; Fox, 2002; Percy and Barthold, 2007). Severe infections can result in death (Baker, 2007; Fox, 2002). In mild infections or infections of older animals, diarrhea is likely to resolve in 4–7 days (Baker, 2007; Fox, 2002), after which constipation may follow (Fox, 2002). Stress, such as a diet change or transport, may exacerbate an otherwise non-pathogenic infection of *E. caviae* in the guinea pig (Ellis and Wright, 1961; Elsheikha et al., 2009; Rigby, 1976).

PATHOLOGY The large intestine is most affected by *E. caviae*, with organisms most often settling in the proximal colon (Baker, 2007; Elsheikha et al., 2009). Hyperemia, edema, and petechial hemorrhage of the colonic mucosa may be seen (Baker, 2007). White or yellow plaques may be present in the colon or cecum. Watery intestinal contents can contain blood (Baker, 2007; Percy and Barthold, 2007). Dilated cystic crypts of Liekberkuhn may be observed (Baker, 2007). Developmental stages of *E. caviae* can be seen in intact epithelial cells or free in the intestinal lumen. Enterocytes may slough and an infiltration of polymorphonuclear and mononuclear cells is possible (Baker, 2007; Percy and Barthold, 2007). Microgametocytes and macrogametocytes may be present in the cecal and colonic mucosa (Percy and Barthold, 2007).

DIAGNOSIS Oocysts are seen on fecal float (Baker, 2007; Fox, 2002; Percy and Barthold, 2007). Fox et al. (2002) recommend using a flotation medium of 1.33 specific gravity. The prepatent period of *E. caviae* is 11–12 days, yet diarrhea may occur before day 11; therefore, a fecal float performed at the beginning of diarrhea may result in a false-negative finding. Multiple floats performed every 4 or 5 days for several weeks is recommended (Vetterling, 1976). Organisms may also be identified on mucosal scrapings or histopathology sections (Baker, 2007; Fox, 2002; Percy and Barthold, 2007).

PREVENTION AND THERAPY Sulfonamides are recommended for treatment. Minimizing stress and providing an adequate level of vitamin C help to prevent clinical infection. Oocysts in the feces take 6 days to become infective, therefore regular cleaning of pans will help to

minimize the spread of infection (Rigby, 1976). Ammonia, followed by a thorough rinsing, has been used to clean cages and pans of infected animals (Elsheikha et al., 2009). Steam has also been used to clean enclosures. Calhoon notes that *Eimeria*-free colonies can be achieved via cesarean rederivation (Calhoon and Matthews, 1964).

Giardia duodenalis

ETIOLOGY The causative agent of guinea pig giardiasis is *Giardia duodenalis*, formally referred to as *G. caviae* (Baker, 2007; Vetterling, 1976).

PATHOGENESIS *Giardia duodenalis* is a flagellate of guinea pigs that is transmitted by the fecal-oral route or via ingestion of contaminated food or water (Baker, 2007).

CLINICAL MANIFESTATIONS Overt signs of infection, such as diarrhea, are rare, although some animals are severely infected and may present as weak and moribund (Baker, 2007).

PATHOLOGY The small intestine, primarily the duodenum, is colonized by trophozoites (Baker, 2007). Colonization may result in mild inflammatory lesions, decreased villar height, and cystic enlargement of duodenal crypts.

DIAGNOSIS Diagnosis of *G. duodenalis* is via direct fecal smear, where trophozoites or cysts may be observed (Baker, 2007). Only trophozoites, and not cysts, are found in diarrheic feces (Vetterling, 1976). Shedding of *G. duodenalis* is intermittent, so unless multiple fecal smears are performed, there is a high likelihood of obtaining a false-negative result. FELASA recommends including *Giardia* spp. on guinea pig breeding colony health monitoring reports (Rehbinder et al., 1996).

PREVENTION AND THERAPY Metronidazole and fenbendazole are treatment options for guinea pig giardiasis (Baker, 2007). Infection can be prevented by thorough environmental sanitation using a quaternary ammonium product or sodium hypochlorite. Hot temperatures will destroy cysts. Cysts thrive in moist areas, so environments must be kept dry. The zoonotic potential of *G. duodenalis* of guinea pigs is unknown, but the possibility of transmission between guinea pigs and humans is suspected.

Klossiella cobayae

BACKGROUND *K. cobayae* is also known as *Klassia caviae*, as it was described and named simultaneously by two individuals, Pearce and Sangiori (respectively) working independently of each other (Vetterling, 1976). Occurrence in modern laboratory guinea pigs is rare (Percy and Barthold, 2007).

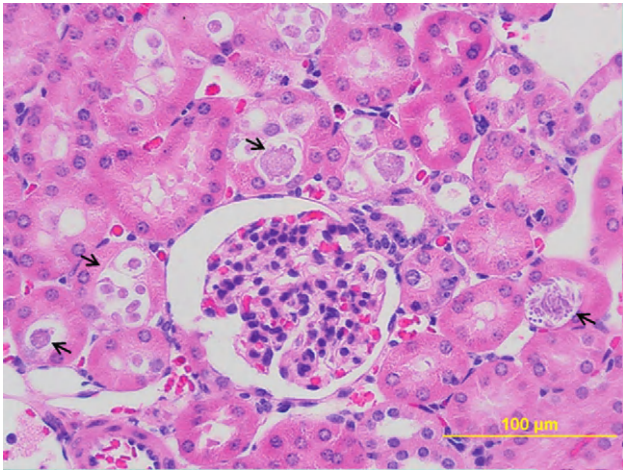


FIGURE 23.5 *Klossiella* coccidial organisms (arrows) in renal epithelial cells. Photo provided by Tammy D. Utteridge.

ETIOLOGY *Klossiella cobayae* is the causative organism.

PATHOGENESIS *K. cobayae* is a parasite of the epithelial cells of the renal tubules, as well as the endothelial cells associated with glomerular capillaries and other organs, such as the spleen and lungs (Griffiths, 1971; Van Andel et al., 1995; Vetterling, 1976). Transmission is via ingestion of sporocysts in urine (Baker, 2007).

CLINICAL MANIFESTATIONS Clinical signs associated with *K. cobayae* are rare. If infection is severe, signs secondary to renal damage may result.

PATHOLOGY Infection can be non-pathogenic or result in nephritis (Figure 23.5) (Baker, 2007; Sebesteny, 1976). Heavy infection can lead to an irregular surface and a gray mottled appearance of the kidney (Baker, 2007; Fox, 2002). Interstitial and perivascular lymphocytic and histiocytic infiltration may occur (Baker, 2007). A large number of interstitial fibroblasts may also be observed.

DIAGNOSIS Sporocysts are passed in the urine, yet observation of this stage in the urine is difficult (Baker, 2007). Most often, diagnosis is made at the time of necropsy by identifying the different developmental stages of *K. cobayae* in glomerular capillaries or in the cytoplasm of epithelial cells lining the renal tubules (Baker, 2007; Percy and Barthold, 2007). FELASA recommends including *Klossiella* spp. as part of a health monitoring report for guinea pig breeding colonies (Rehbinder et al., 1996).

PREVENTION AND THERAPY Control of *K. cobayae* is best achieved through prevention. Contamination of food and bedding with infective urine should be

minimized (Baker, 2007). Sulfonamides might be effective forms of treatment.

Leishmania enrietti

BACKGROUND *Leishmania enrietti* was first noted in laboratory guinea pigs in 1948 in Brazil (Medina, 1946). According to the review by Machado et al., this flagellate was next reported in 1967 in a guinea pig captured from the outskirts of Curitiba (Machado et al., 1994). The sandfly, *Lutzomyia monticola*, which is frequently noted on the trunks of Curitiba pine trees, was proposed as a possible vector.

ETIOLOGY *Leishmania enrietti* is the causative organism.

PATHOGENESIS Little is known about the lifecycle of *L. enrietta*, but other members of the genus *Leishmania* have indirect lifecycles and sandfly vectors (Baker, 1998, 2007; Nie, 1950). *L. enrietta* results in cutaneous infection.

CLINICAL MANIFESTATIONS AND PATHOLOGY An infected guinea pig may present with a cutaneous nodule at the site of entry 1–2 weeks after infection (Baker, 2007). Additionally, ulcers can develop on the feet, ears, nose, and genitalia of infected animals. Ulcers will go through a series of pathologic changes, such as necrosis of surrounding macrophages 4–5 weeks after initial infection (especially in naive animals), followed by an infiltrate of giant cells, plasma cells, and lymphocytes at the periphery of the ulcer. By 7 weeks, giant cells are gone and fibroblasts appear, indicating resolution. Resolution is usually complete by 10 weeks post-infection. Hematogenous spread of the organism can occur and *L. enrietti* may be observed in the lymph nodes.

DIAGNOSIS Detection is via identification of amastigotes in lesion histology or via culture of the organism (Baker, 2007).

PREVENTION AND THERAPY Control is achieved by elimination of vectors. Treatment with meglumine antimoniate has been attempted, with inconsistent success (Thomaz-Soccol et al., 1996). Contact with possible sandfly vectors should be avoided.

Toxoplasma gondii

BACKGROUND This organism was first reported in Brazil by Carini and Migliano (Vetterling, 1976). In early reports, *T. gondii* was mistaken for *Sarcocystis* in the guinea pig (Kean and Grocott, 1945).

ETIOLOGY *Toxoplasma gondii* is the causative organism.

PATHOGENESIS Cats are the definitive hosts of *T. gondii* (Baker, 2007). Oocysts are released in cat feces and are ingested by guinea pigs which act as intermediate hosts for *T. gondii*. Cysts can remain viable in a guinea pig for up to five years before ingestion by the definitive host. In addition to the guinea pig, a large number of mammalian and avian species are intermediate hosts to *T. gondii*, including humans. Ingestion of contaminated biological material and transplacental transmission are alternative routes of transmission for the guinea pig (Baker, 2007; Percy and Barthold, 2007).

CLINICAL MANIFESTATIONS *T. gondii* infection in guinea pigs is often asymptomatic and, therefore, frequently goes undiagnosed (Baker, 2007; Griffiths, 1971; Percy and Barthold, 2007). Infection in pregnant sows can result in vulvar bleeding and abortion (Fox, 2002). In a case of spontaneous toxoplasma encephalitis in a guinea pig, spastic paralysis, opisthotonos, and loss of urethral and anal sphincter control were reported (Markham, 1937).

PATHOLOGY Hepatitis, pneumonia, encephalitis, and uterine infection (possibly blood-filled), have been noted in infected animals, as have cysts in both the myocardium and central nervous system (Baker, 2007; Fox, 2002; Percy and Barthold, 2007). In spontaneously infected animals that develop toxoplasma encephalitis, congested blood vessels of the meninges were noted and were surrounded by mononuclear leukocytes (Markham, 1937).

DIAGNOSIS Serology is often used for diagnosis in guinea pigs (Baker, 2007). Cysts can be observed on histology. Mice or hamsters can be inoculated with tissue homogenate from suspected positives and act as sentinels of infection. FELASA recommends including *Toxoplasma gondii* on guinea pig breeding colony health monitoring reports (Rehbinder et al., 1996).

PREVENTION AND THERAPY Sulfadiazine and pyrimethamine are reported treatment options for clinical cases of *T. gondii*, yet the effectiveness of such treatments is questionable (Baker, 2007; Fox, 2002). Temperatures above 60°C kill oocysts (Baker, 2007). Strict sanitation and periodic colony monitoring are necessary for *T. gondii* control.

Tritrichomonas caviae

BACKGROUND *Tritrichomonas caviae* has also been referred to as *Trichomonas caviae* and *T. flagelliphora* (Tanabe, 1925).

ETIOLOGY *Tritrichomonas caviae* is one of the largest intestinal flagellates of the guinea pig, measuring an average of 16 µm long by 8.5 µm wide (Baker, 2007). No cyst stage is known.

PATHOGENESIS Transmission of *T. caviae* is fecal-oral (Baker, 2007).

CLINICAL MANIFESTATIONS *Tritrichomonas caviae* is a non-pathogenic flagellate of guinea pigs (Baker, 2007).

PATHOLOGY *T. caviae* is most often noted in the cecum, although it has been observed in the duodenum, jejunum and lower ileum as well (Baker, 2007). Although rare, tissue invasion and cecal or colonic ulceration are possible.

DIAGNOSIS Diagnosis of *T. caviae* is via direct fecal smear (Tanabe, 1925).

PREVENTION AND THERAPY *T. caviae* does not usually result in clinical signs or pertinent pathology and does not routinely require treatment (Baker, 2007).

Trypanosoma cruzi

Guinea pigs, among other mammals, are reservoirs for the flagellate *T. cruzi* (Baker, 2007; Vetterling, 1976). Reduviid bugs act as vectors, becoming infected after a blood meal from a positive animal. *T. cruzi* mature and reproduce within the vector. The bugs then go on to deposit *T. cruzi*-containing feces on humans during another blood meal. The organism enters human skin through a wound (potentially the bug bite) or contact with mucous membranes. Chagas disease may result in humans.

In the guinea pig, *T. cruzi* can encyst in multiple organs, including the skin and cardiac muscle. *T. cruzi* has been noted in domesticated guinea pigs of South America exposed to reduviid vectors. Clinical and pathological signs are not described.

PARASITIC INFECTIONS

The reader is once again directed to Flynn's *Parasites of Laboratory Animals* for further discussion on many of the parasites discussed below, including anatomical appearances and detailed life cycles (Baker, 2007). The nomenclature used in this section is also in accordance with this reference.

Arthropods

Note: the common names of arthropod organisms have been used in place of the name of the resulting condition (e.g. Flies instead of Myiasis).

Fleas

Ctenocephalides felis has been noted on pet guinea pigs living in a household with cats and/or dogs.

Infected guinea pigs presented with pruritus, alopecia, dermal crusts, and anemia (White et al., 2003). Aqueous-based pyrethrin sprays have been recommended as the safest treatment option for such infection. *Nosopsyllus fasciatus*, the northern rat flea, can also inhabit guinea pigs, although similarly to *C. felis*, infection of laboratory guinea pigs is rare (Fox, 2002).

Flies

It has been stated that “fatal myiasis involving at least three species of flies, *Lucilia sericata*, *Calliphora vicina* and *Calliphora vomitoris*, have been reported in guinea pigs” (Baker, 2007). Eggs are deposited on the skin of a guinea pig by an adult fly, where larvae will develop and then feed on living or necrotic animal tissue (Baker, 2007).

TABLE 23.1 Other Parasites of Wild Guinea Pigs

Parasite	Location in Host	Method of Infection	Pathologic Effects
Arthropods			
FLEAS			
<i>Hectopsylla</i> spp.	Pelage	Direct contact	Unknown
<i>Leptopsylla seginis</i>	Pelage	Direct contact	Unknown
<i>Nosopsyllus fasciatus</i>	Pelage	Direct contact	Unknown
<i>Pulex irritans</i>	Pelage	Direct contact	Dermatitis
<i>Rhopalosylla clavicola</i>	Pelage	Direct contact	Unknown
<i>Tiamastus cavicola</i>	Pelage	Direct contact	Unknown
LICE			
<i>Polyplax spinulosa</i>	Pelage	Direct contact	Unknown
<i>Pteropthirus</i> spp.	Pelage	Direct contact	Unknown
MITES			
<i>Ornithonyssus bacoti</i>	Skin	Direct contact	Dermatitis
<i>Ornithonyssus braziliensis</i>	Skin	Direct contact	Unknown
<i>Ornithonyssus wernecki</i>	Skin	Direct contact	Unknown
<i>Eutrombicula</i> spp.	Skin	Direct contact	Unknown
CESTODES			
<i>Anoplocephala</i> spp.	Intestine	Unknown	None
<i>Monoecocestus parvitesticulatus</i>	Intestine	Unknown	None
NEMATODES			
<i>Ackertia borgosi</i>	Abdominal cavity	Arthropod host	None
<i>Capillaria hepatica</i>	Liver	Ingestion of embryonated egg	None
<i>Graphidioides mazzai</i>	Small intestine	Ingestion of infective larvae	None
<i>Trichuris gracilis</i>	Large intestine	Ingestion of embryonated egg	None
<i>Viannella travassosi</i>	Small intestine	Ingestion of infective larvae	None
TREMATODES			
<i>Pseudoquinqueserialis caviae</i>	Intestine	Ingestion of metacercaria	None
<i>Taxorchis caviae</i>	Cecum	Ingestion of metacercaria	None
<i>Taxochis ringueleti</i>	Cecum	Ingestion of metacercaria	None

Adapted from Baker (2007).

Lice

ETIOLOGY Biting lice from the order Mallophaga, *Gyropus ovalis*, *Gliricola porcelli*, and *Trimenopon hispidum* (previously *Trimenopon jenningsi*) are often observed in guinea pigs (Baker, 2007). *G. porcelli*, the most commonly observed of the three, is known as the “slender louse” due to its general appearance, especially when compared to *G. ovalis* or the “oval louse” (Baker, 2007; Harkness and Wagner, 1995; Kim et al., 2008). Several species of sucking lice (order Anoplura) have been noted on wild guinea pigs in South America: *Pterophytirus alata* and *Polyplax spinulosa* (Dittmar, 2002). These latter lice are listed in Table 23.1, but not discussed here in detail.

PATHOGENESIS Biting lice attach to hair shafts and abrade the skin to ingest cutaneous fluids (Fox, 2002; Kim et al., 2008). Transmission is via direct contact (Harkness and Wagner, 1995; Kim et al., 2008). The life cycles of guinea pig lice are not specifically described, but in general, biting lice go through the following stages: egg, three nymphal stages, and adult (Baker, 2007).

CLINICAL MANIFESTATIONS *G. ovalis*, *G. porcelli* or *T. hispidum* infection of a guinea pig can be asymptomatic or may result in pruritus and a rough hair coat or alopecia (Baker, 2007; Coman et al., 2009; Harkness and Wagner, 1995; Kim et al., 2008; Percy and Barthold, 2007; Rigby, 1976). Scabs and crusts are sometimes noted secondary to scratching, especially around the ears (Harkness and Wagner, 1995).

PATHOLOGY Signs of louse infection are usually superficial. Dermatitis may be noted grossly.

DIAGNOSIS Lice (adults or nits) can be seen on the haircoat of an infected animal, with or without the aid of a magnifying lens (Figure 23.6) (Baker, 2007; Griffiths,

1971). On a dead animal, lice will migrate to the warm tips of the hair as the skin cools (Griffiths, 1971). Tape tests and flea combing can also be used to diagnose louse infestation (Coman et al., 2009). As stated previously, *G. porcelli* is slender in appearance (1.0–1.5mm × 0.3–0.4mm), compared to *G. ovalis* (1.0–1.2mm × 0.5mm) (Figure 23.7) (Huerkamp et al., 1996). *T. hispidum*’s dimensions are similar to those of *G. ovalis*, yet can be differentiated by the fact that *T. hispidum* has five abdominal segments as compared to *G. ovalis*’ eight.

PREVENTION AND THERAPY In reports of successful control of guinea pig louse infestations, both infected animals and the environment have been treated (Harkness and Wagner, 1995; Kim et al., 2008). Systemic ivermectin (Baker, 2007; White et al., 2003), carbamate or pyrethrin-based powders (Baker, 2007; Griffiths, 1971), Romavermectin B1 (Coman et al., 2009) and a combination of imidacloprid and moxidectin (Kim et al., 2008) have been noted as effective drug choices for treating infected guinea pigs. Hypochlorite bleach has been used for environmental decontamination (Kim et al., 2008).

Mites

Guinea pigs are susceptible to infection with several species of mites, some occurring more commonly than others. *Chirodiscoides caviae* and *Trixacarus caviae* are the most commonly noted infectious mites in guinea pigs, with *Demodex caviae*, *Myocoptes musculus*, *Sarcoptes scabiei* and *Notoedres muris* reported less frequently (Baker, 2007; Fox, 2002; White et al., 2003). Infection with the astigmatic mite, *Acarus farris*, was described in a case report of two guinea pigs (Linek and Bourdeau, 2005). *Psocoptes cuniculi* has been described on a pet guinea pig in close contact with a pet rabbit (Yeatts, 1994). White et al. (2003) note *Cheyletiella parasitivorax* as a cause of pruritus and scaling along the dorsum of guinea pigs. The more commonly noted mites, *C. caviae* and *T. caviae*, will be discussed below.

CHIRODISCOIDES CAVIAE

BACKGROUND The first reports and illustrations of *C. caviae* were by Stanley Hirst in the early 1900s (Hirst 1922). Hirst noted this mite attached to the hairs of the back of the guinea pig.

ETIOLOGY *Chirodiscoides caviae* is the causative organism (Figure 23.8).

PATHOGENESIS *C. caviae* is a species-specific fur mite that tends to concentrate in the lumbar area and the lateral aspects of the hindquarters (Harkness and Wagner, 1995; Percy and Barthold, 2007; Schonfelder et al., 2010). *C. caviae* feed on the scales of hair shafts (Hirsjarvi and Phyla, 1995) and transmission is via direct contact with



FIGURE 23.6 Adult and nymphal stages of *G. porcelli* found in a fur sample. Photo provided by Dr. Susan Szczepanski.

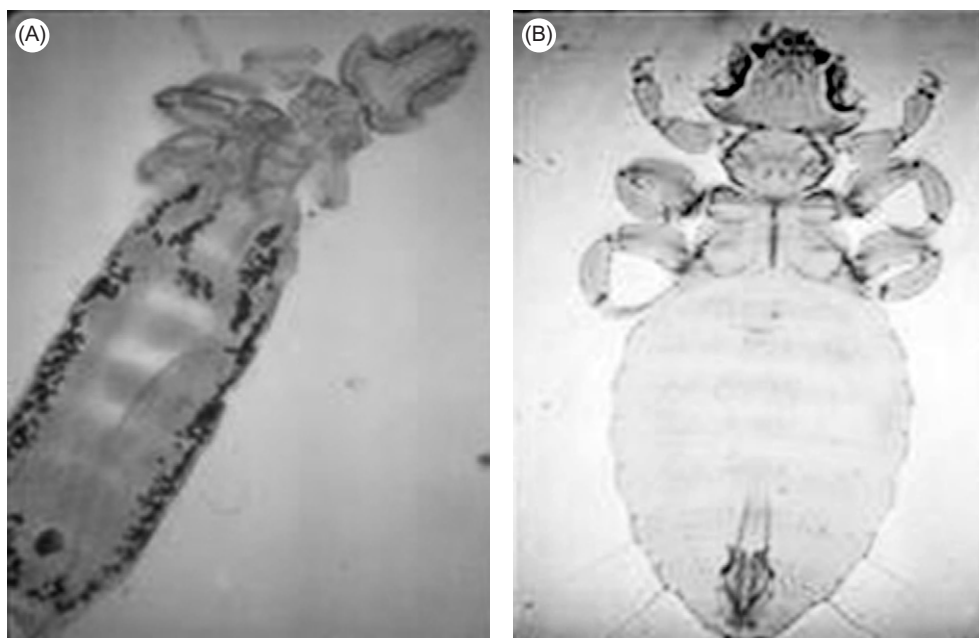


FIGURE 23.7 *Gliricola porcelli* (A), the slender louse, is more commonly noted on guinea pigs than is *Gyropus ovalis* (B), the oval louse. Both are biting lice from the order Mallophaga. Image courtesy of Dr. G. Van Hoosier.

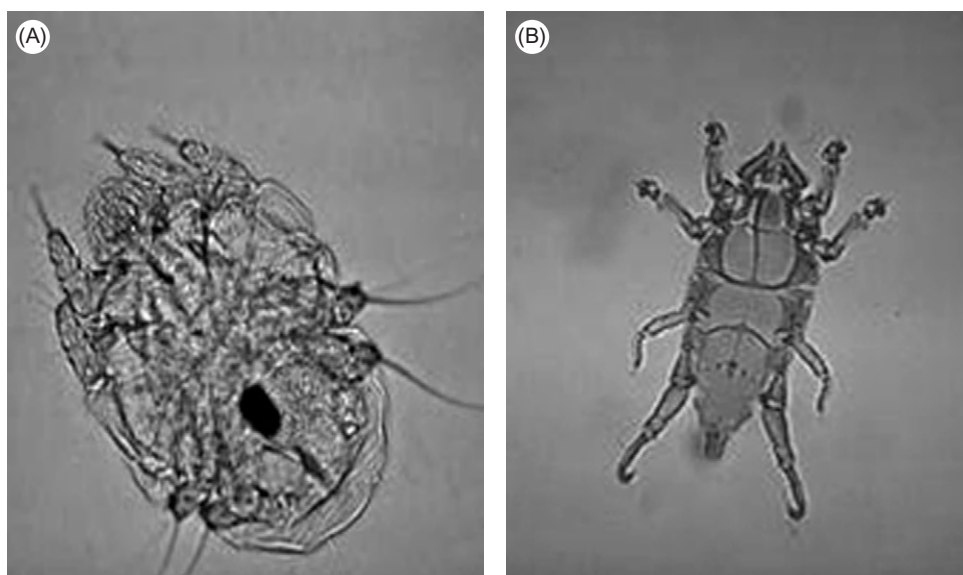


FIGURE 23.8 *Trixacarus caviae* (A) and *Chirodiscoides caviae* (B) are two of the most reported species of mites to infect guinea pigs. *T. caviae* is a common cause of dermatitis in guinea pigs, while infection with *C. caviae* is often asymptomatic. Figure courtesy of Dr. G. Van Hoosier.

an infected animal (Fox, 2002; Schonfelder et al., 2010) or with infected cage debris or bedding (Fox, 2002). The life cycle of *C. caviae* has not been specifically described, although mites in general commonly go through egg, larval, nymphal, and adult stages (Baker, 2007). Concurrent infection with lice is not uncommon (White et al., 2003), and mixed infection with *Demodex caviae* (detected via

deep skin scrapping) has also been reported (Schonfelder et al., 2010).

CLINICAL MANIFESTATIONS Infection with *C. caviae* can be asymptomatic or, if infestation is heavy, pruritis, alopecia, hyperemia, and dermal crusts may be seen (Coman et al., 2009; Fox, 2002; Hirsjarvi and

Phyala, 1995; Lumeij and Cremers, 1986; Schonfelder et al., 2010). Anorexia may result secondary to grooming pruritic areas (Schonfelder et al., 2010).

PATHOLOGY The appearance of *C. caviae* mites themselves is the major pathologic finding (Fox, 2002). Mites and ova are located on the hair shafts of the guinea pigs, not burrowed into the skin.

DIAGNOSIS Mites may be seen with the unaided eye, hand lens or dissecting microscope. *C. caviae* can also be found on a superficial scrape (Harkness and Wagner, 1995; Percy and Barthold, 2007; Schonfelder et al., 2010). Combing, hair plucking, and cellophane tape testing can aid in isolating mites for observation (White et al., 2003).

PREVENTION AND THERAPY Many treatment options have been described in the literature for *C. caviae*. Dilute ivermectin (diluted in aqua/propylene glycol) sprays have been successful, especially for treating large colonies of infected animals (Baker, 2007; Hirsjarvi and Phyala, 1995; White et al., 2003). Selamectin, 12–15 mg/kg, applied twice at 2-week intervals has also eliminated infection (Baker, 2007; Fox, 2002; Schonfelder et al., 2010). Pyrethrin sprays and powders have been used to rid the environment of *C. caviae* (Harkness and Wagner, 1995; Hirsjarvi and Phyala, 1995). 1% Virkon S® was used to treat an automatic watering system (Hirsjarvi and Phyala, 1995). Older successful treatments include the use of dichlorvos vapors (Henderson, 1973) as well as immersion of shaved animals in 0.15% trichlorfon (Lumeij and Cremers, 1986).

TRIXACARUS CAVIAE

BACKGROUND *T. caviae* was discovered in a colony of albino guinea pigs in the early 1970s by Fain and was described as a disease very similar in appearance to sarcoptic mange in other animals (Dorrestein and Vanbronswijk, 1979; Fain et al., 1972). The first report of *T. caviae* in North America was in 1980 at Davis, California (McDonald and Lavoipierre, 1980).

ETIOLOGY *Trixacarus caviae* is the causative organism (Figure 23.8).

EPIZOOTIOLOGY AND PATHOGENESIS *Trixacarus caviae* is a species-specific sarcoptic mite that most commonly affects guinea pigs that are 1–3 years of age (Dorrestein and Vanbronswijk, 1979). If left untreated, the parasite load peaks 1 month post-infection, after which it slowly regresses (Fuentelba and Hanna, 1996). The life cycle of *T. caviae* includes an egg, larval, two nymphal, and a final adult stage, all of which inhabit the host guinea pig (Baker, 2007). Transmission is by direct

contact, with nymphal or larval stages indicated in establishing new infestations (Baker, 2007; Rothwell et al., 1991). Within 72 hours, pups born to an infected guinea pig dam will show clinical signs consistent with *T. caviae* infestation (Beck et al., 2007; Kummel et al., 1980).

Humans have been reported to develop transient pruritic, papulovesicular dermatitis after contact with infected animals, although mites appear to be incapable of persisting on human skin (Fuentelba and Hanna, 1996; Kummel et al., 1980; Mederle and Indre, 2009).

CLINICAL MANIFESTATIONS While infection with *T. caviae* may be asymptomatic and result in non-clinical carrier animals, *T. caviae* has been reported as the most common and important cause of dermatitis in guinea pigs (Dorrestein and Vanbronswijk, 1979; Fuentelba and Hanna, 1996; Percy and Barthold, 2007; White et al., 2003). Severe pruritis and excoriation along with erythema and alopecia can accompany dermatitis in some animals (Ackerman, 1987; Beck et al., 2007; Dorrestein and Vanbronswijk, 1979). In those guinea pigs that are clinical, grayish to yellow or white crusts may be present on the skin and can be dry or slightly greasy (Ackerman, 1987; Dorrestein and Vanbronswijk, 1979). Secondary bacterial infections of infected skin areas are possible (Fuentelba and Hanna, 1996). Infection usually starts on the face and ears, spreading to the lumbar regions and lateral aspects of the legs (Dorrestein and Vanbronswijk, 1979; Percy and Barthold, 2007). The intense pruritic response in some guinea pigs is reported to be due to an initial allergic reaction to mite antigen (Fox, 2002). Extreme restlessness and emaciation have been noted in affected animals, especially in those that seemingly scratch uncontrollably (Beck et al., 2007). This constant scratching can lead to “fits” of muscular spasms or sporadic epileptiform seizures (Ackerman, 1987; Beck et al., 2007; Dorrestein and Vanbronswijk, 1979; Kummel et al., 1980) caused by generalized pruritus-induced hyperesthesia (Baker, 2007).

PATHOLOGY Orthokeratotic hyperkeratosis and acanthosis have been noted in animals showing clinical signs of *T. caviae* infection (Ackerman, 1987; Dorrestein and Vanbronswijk, 1979; Fuentelba and Hanna, 1996; Zenoble and Greve, 1980). A polymorphonuclear leucocytic infiltrate is present in the dermis (Dorrestein and Vanbronswijk, 1979; Percy and Barthold, 2007; Rothwell et al., 1991; Zenoble and Greve, 1980). Adult mites may be noted in “tunnels” or burrows located in the hyperkeratotic areas (Dorrestein and Vanbronswijk, 1979; Zenoble and Greve, 1980). Such tunnels approach the epidermis perpendicularly, with some entering the mouth of hair follicles (Zenoble and Greve, 1980). Eggs may also be present in tunnels (Dorrestein and Vanbronswijk, 1979). *T. caviae* mites have sharp spines on

the cuticle of their dorsums (Zenoble and Greve, 1980), which differentiates them from *Notoedres* (Ackerman, 1987). The anus of the female *T. caviae* mite is located dorsally, while this structure is located terminally on *S. scabiei* (Ackerman, 1987; Baker, 2007; Fuentealba and Hanna, 1996).

DIAGNOSIS Deep skin scrapes of crusted areas with 10% KOH reveal *T. caviae* (Dorrestein and Vanbronswijk, 1979; Fain et al., 1972; Fuentealba and Hanna, 1996; Mederle and Indre, 2009). Zajac et al. (1980) report that live mites could be demonstrated for 5 days postmortem from deep skin scrapings taken from a guinea pig held at 4°C after death.

PREVENTION AND THERAPY Treatment of both affected animals and the environment has been employed to rid guinea pig colonies of *T. caviae*. Multiple treatment regimens have been reported as successful over the years, including lindane baths (Dorrestein and Vanbronswijk, 1979; Zajac et al., 1980), weekly lime sulfur dips (Ackerman, 1987; McDonald and Lavoipierre, 1980; Zenoble and Greve, 1980), ivermectin injections (White et al., 2003), and monthly spot-on treatment with 10% imidacloprid and 1% moxidectin (Beck et al., 2007). Dilute lime sulfur can also be used to treat the environment (White et al., 2003). Valium has been used to control seizure-like activity related to *T. caviae* infection by some, whereas others suggest that treating the mite infection alone eliminates this sequela to infection (Beck et al., 2007; White et al., 2003).

Cestodes

According to Harkness and Wagner (1995), tapeworms rarely infect guinea pigs. *Anoplocephala* spp. and *Monoecocystus parvitesticulatus* (Table 23.1) have been reported in the intestines of South American guinea pigs (Baker, 2007; Sardella and Fugassa, 2009).

Nematodes

Baylisascaris procyonis

ETIOLOGY *Baylisascaris procyonis* is the causative organism.

PATHOGENESIS Guinea pigs are paratenic hosts of *B. procyonis* and contract infection by ingesting eggs in raccoon feces (Baker, 2007). Once ingested, larvae will hatch and penetrate the small intestine of the guinea pig, migrate through the liver to the lungs and disseminate throughout the body via the circulatory system. Larvae then encapsulate where they remain until ingested by a raccoon.

CLINICAL MANIFESTATIONS No signs are present in the guinea pig due to *B. procyonis* unless organisms migrate to the brain. Migration can result in lethargy, head tilt and ataxia, which may progress to cachexia, stupor, hyperexcitability, lateral recumbency, torticollis, or opisthotonos (Van Andel et al., 1995).

PATHOLOGY In cases where *B. procyonis* migrates to the brain, multifocal eosinophilic granulomatous inflammation and neutrophilic infiltration is seen, along with perivascular lymphoid cuffing and malacia (Van Andel et al., 1995). Eosinophilic granulomata have been noted in the lungs of some infected animals.

DIAGNOSIS Histology is diagnostic for *B. procyonis* (Baker, 2007). The Baermann extraction technique can be used to extract organisms from the cerebral tissue of clinical animals (Van Andel et al., 1995).

PREVENTION AND THERAPY Prevention, rather than treatment, is the most logical approach for *B. procyonis* control, as clinical signs are often not noted until the end stage of infection and diagnosis relies on histopathology. Contamination of bedding or food with raccoon feces must be avoided. Ova can be viable for years in soil and weeks to months in straw and are resistant to most disinfectants (Fox, 2002). Removal of contaminated bedding and the autoclaving of caging may be required (Baker, 2007). Humans, similarly to guinea pigs, can contract infection by ingesting raccoon feces, but transmission from guinea pigs to humans is not feasible (Fox, 2002).

Paraspidodera uncinata

ETIOLOGY *Paraspidodera uncinata* is the causative organism.

EPIZOOTIOLOGY AND PATHOGENESIS *P. uncinata* is the cecal worm of guinea pigs and the most common nematode infecting this species (Griffiths, 1971). The prevalence of *P. uncinata* in wild guinea pigs of South America was found to be 37%, while the prevalence was noted as 23.8% in a laboratory colony of guinea pigs (Coman et al., 2009; Dittmar, 2002). Transmission is via ingestion of an embryonated egg in feces (Baker, 2007; Fox, 2002). Eggs become infectious 3–5 days after they are initially shed (Fox, 2002). The life cycle is not well described (Baker, 2007).

CLINICAL MANIFESTATIONS Infections are normally asymptomatic, but with heavy parasitic loads weight loss, diarrhea, and disability are possible (Fox, 2002).

PATHOLOGY Infection can, and often does, occur without pathologic changes (Baker, 2007; Percy and

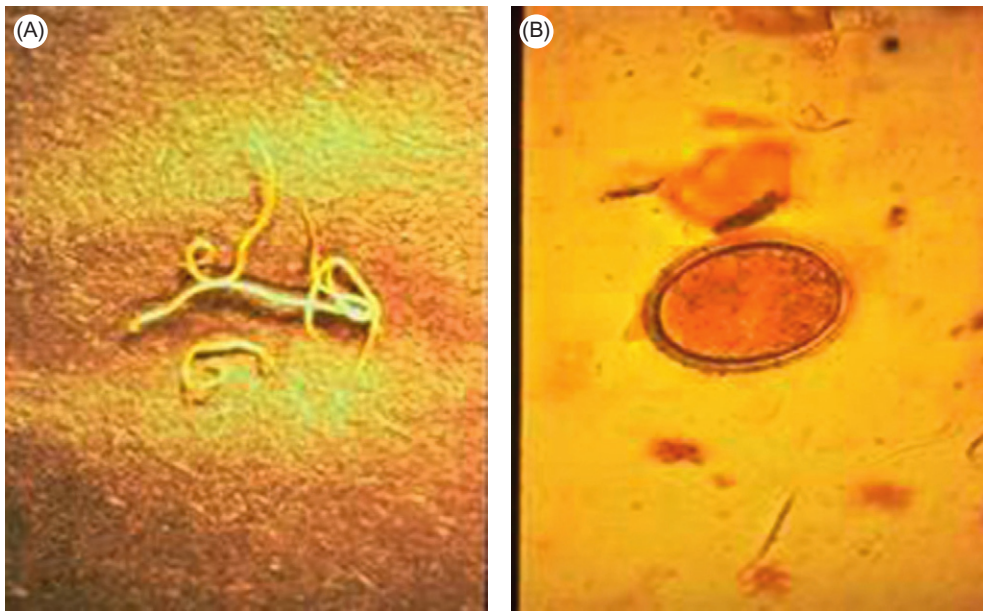


FIGURE 23.9 The cecal worm, *Paraspidodera uncinata*, is the most common nematode of guinea pigs. The adult worm (A) is noted in the cecum, while the eggs (B) are shed in the feces. Figure courtesy of Dr. G. Van Hoosier.

Barthold, 2007; Rigby, 1976). If cecal worms migrate through the intestinal mucosa, a hemorrhagic typhlitis may be seen along with capillary ectasis in the submucosa (Coman et al., 2009).

DIAGNOSIS Adult worms may be seen in the cecum at necropsy and eggs may be observed in feces (Figure 23.9) (Baker, 2007; Rigby 1976).

PREVENTION AND THERAPY Levamisole administered at 25mg/kg, either orally or subcutaneously, has most often been reported as an effective treatment for *P. uncinata* in the guinea pig (Baker, 2007; Eliazian et al., 1975). Other reported treatment regimens include oral mebendazole at 50mg/kg (Baker, 2007) and Romavermectin B1 given subcutaneously at a dose of 0.2mg/kg twice at a three-week interval (Coman et al., 2009).

Pelodera strongyloides

ETIOLOGY *Pelodera strongyloides* is the causative organism.

PATHOGENESIS *P. strongyloides* organisms are usually found in damp soil or vegetation (White et al., 2003). Bedding can be a possible source of infection.

CLINICAL MANIFESTATIONS While typically non-pathogenic, if *P. strongyloides* invade hair follicles dermatitis can result (Baker, 2007; White et al., 2003).

PATHOLOGY Organisms may be found in hair follicles of clinically infected animals.

DIAGNOSIS The observation of larvae in skin scrapings or biopsies signifies infection (White et al., 2003). Adults may be observed in the bedding.

PREVENTION AND THERAPY Cages should be kept dry, as organisms thrive in moist environments (White et al., 2003). The bedding of any positive animal should be replaced. A 1–2% chlorhexadine bath has been recommended for infected animals as a means of preventing secondary infections.

Trichinella spiralis

Trichinellosis due to *Trichinella spiralis* is possible in guinea pigs, but rare, especially in laboratory animals that seldom come in contact with infected material (raw or undercooked meat) (Sebesteny, 1976). Enteritis could develop in infected animals if *T. spiralis* organisms migrate through the intestinal wall.

Trematodes

Fasciola

BACKGROUND Infection in present laboratory-housed guinea pigs is rare, but when *Fasciola* organisms are noted it is often in conjunction with recent diet supplementation of a leafy vegetable carrying encysted trematodes (Baker, 2007; Fox, 2002).

ETIOLOGY Two *Fasciola* species are noted in guinea pigs, *Fasciola hepatica* and *Fasciola gigantica* (Baker, 2007). *F. gigantica* is slightly larger in size than *F. hepatica*, although *F. gigantica* rarely reach patency in guinea pigs.

PATHOGENESIS Guinea pigs ingest encysted *Fasciola* metacercariae on vegetation (Baker, 2007; Fox, 2002). Once ingested, metacercariae hatch in the small intestine and migrate through the wall to the peritoneal cavity and the liver. Juvenile flukes develop in the liver and, once mature, burrow in the bile ducts. Eggs are shed and enter the gut, where they pass in the feces. A snail intermediate host is necessary for maturation from cercariae to infectious metacercariae. Metacercariae encyst on leafy vegetation.

CLINICAL MANIFESTATIONS Emaciation and anemia have been associated with *Fasciola* infection (Sebesteny, 1976). Posterior paresis was noted in animals that developed cysts in the lumbar musculature after aberrant fluke migration (Baker, 2007).

PATHOLOGY Hepatic congestion and hemorrhage, especially around portal vessels, central veins and sinusoids is possible (Baker, 2007). Fibronectin tracks and granulomas may also be observed in the liver. Other pathology due to fluke infection is often the result of aberrant migration, resulting in cysts in areas such as the kidney, the peritoneal cavity and the pelvic cavity. Cysts often contain coffee-brown-colored material.

DIAGNOSIS *Fasciola* spp. can be observed on fecal flotation (Baker, 2007).

PREVENTION AND THERAPY Prevention is best achieved by limiting the amount of leafy vegetation supplemented in a guinea pig's diet; if supplemented, do not feed vegetation from fluke-endemic locations. *Fasciola* in ruminants is treated with albendazole and clorsulon (Baker, 2007).

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Non-Infectious Diseases

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INTRODUCTION

This chapter covers the non-infectious conditions which have been described in guinea pigs, including nutritional, metabolic, congenital, and neoplastic diseases. Since the first edition of this chapter, ably penned by Dr. Joseph Wagner over 30 years ago, the available veterinary literature regarding diseases of guinea pigs has grown remarkably; however, publications of infectious diseases have greatly outnumbered those of non-infectious conditions. In today’s clean laboratory environment, the lab animal practitioner or pathologist is far more likely to encounter conditions described in this chapter, which should stress the continued importance of these “classic” entities in this species. The primary neoplasms of each organ system are included at the end of each respective section.

CARDIOVASCULAR SYSTEM

Rhabdomyomatosis

Over a quarter century after its first description in this species by Hueper (1941), the poorly named lesion, “rhabdomyomatosis,” is a morphologic change in cardiac myocytes whose exact etiology is still to be elucidated. This relatively common finding in the heart of guinea pigs may be grossly or microscopically mistaken for a neoplasm, but it is an incidental finding of little to no clinical significance. Rhabdomyomatosis may be seen in animals of all ages, even animals as young as 3 weeks. Rhabdomyomatosis is characterized by well-defined areas of cardiac muscle in which myocytes are swollen by excessive cytoplasmic glycogen, which gives them a characteristic pale appearance when viewed grossly (Takahashi et al., 1985). These lesions may occur in any region of the heart, but are most commonly seen in the left ventricle, especially subendocardially. The gross lesion may appear as a tan streak in the myocardium which is barely visible to the naked eye, or may incorporate large areas of myocardium. Microscopically, glycogen accumulation within cardiac myocytes results in

the formation of large cleared areas of the cell (resulting from dissolution of glycogen during routine histologic processing) separated by small threads of eosinophilic cytoplasm radiating outward from a centrally placed nucleus, resulting in the colloquial name “spider cells”. While rarely performed, the presence of glycogen may be demonstrated by staining of alcohol-fixed or unfixed fresh-frozen specimens with periodic acid-Schiff (PAS) preparations. Immunohistochemical procedures reveal that affected cells stain positively for desmin and myoglobin (attesting to their muscle origin), and negatively for smooth muscle actin and vimentin (Tanimoto and Ohtsuki, 1995). The origin of this lesion is still unknown in the guinea pig and other species in which it has been reported, such as swine, dogs, and man. These lesions are no longer regarded as neoplasms, current investigations point toward the possibility of hamartoma, glycogen metabolism disorder, or a degenerative condition affecting cardiac myofibers (Hoch-Ligeti et al., 1986). In man and swine, it is considered congenital, as it is often seen in children less than a year of age, and has been reported in certain breeds of swine in individuals as young as 3 weeks (McEwen, 1994). Investigations in humans have shown that rhabdomyomatosis arises as part of a hereditary autosomal-dominant syndrome related to TSC-1 and TSC-2 gene mutation (Vaughan et al., 2001). Even the cell of origin continues to be in dispute; ultrastructural analysis of rhabdomyomas in humans (Silverman et al., 1976) and swine (Tanimoto and Ohtsuki, 1995) have identified Purkinje cells and cardiac myofibers, respectively, as the cell of origin in rhabdomyomatosis. Some authors have hypothesized that “rhabdomyoma cells” may actually arise from pluripotential cells with combinations of the two (Silverman et al., 1976). Clinical signs associated with this lesion are rare; in one case in the dog, congestive heart failure and chylothorax were attributed to the presence of an intra-atrial rhabdomyoma (Mansfield et al., 2000). Clinical disease associated with rhadomyomas in the guinea pig has not been reported.

Cutaneous Vascular Malformation

A cutaneous vascular malformation was reported (Osofsky et al., 2004) in a young adult female guinea pig. The violaceous plaque measured 3×2 cm, was ulcerated and bled intermittently, eventually resulting in fatal blood loss. The lesion consisted of a mass of well-differentiated vessels which were distributed throughout the dermis, subcutis, and skeletal muscle. Vessels were lined by endothelial cells and surrounded by smooth muscle cells; hematopoietic cells were distributed between vessels.

Miscellaneous Cardiac Disease

Documented reports of non-infectious cardiac disease in the guinea pig are rare. A single case of presumptive cardiomyopathy, as evidenced by an enlarged heart, ascites, and pulmonary edema has been reported (Cox and Haworth, 2000); however, the patient was lost to follow-up following successful intervention with furosemide and enalapril therapy. The same hospital reported a case of fatal hemopericardium identified at necropsy of a guinea pig; the cause was not subsequently identified.

Cardiovascular Neoplasia

Reports of primary cardiac tumors are rare in guinea pigs. Mesenchymal tumors have been described (McConnell and Edinger, 1968) in several Hartley guinea pigs from the same colony, suggesting a familial genetic predisposition. These neoplasms were composed of well-differentiated fat, cartilage, bone, hematopoietic tissue, and smooth muscle.

RESPIRATORY SYSTEM

Pulmonary Perivascular and Peribronchiolar Lymphoid Nodules

Bronchiolar-associated lymphoid tissue (BALT) is a common finding in domestic and laboratory animals of all species. In general, the amount of BALT increases with age, exposure to inhaled antigens or any number of infectious agents (especially viruses). Lymphoid aggregates associated with pulmonary blood vessels, however, are uncommon in any animal species, and if present, are usually a sign of significant pulmonary inflammation.

In the guinea pig, nodular aggregates of lymphocytes are a common finding in association with the smaller branches of both pulmonary arteries and veins. These nodules are a peculiar anatomic feature of the

guinea pig which has likely caused great consternation with pulmonary researchers over the years (Thompson et al., 1962). The nodules appear to originate within the adventitia, and larger aggregates may extend into surrounding alveolar septa.

Perivascular lymphoid aggregates may be seen in animals as young as 5 days of age, and are commonly seen in older animals; they are restricted to the lung and present in no other tissue. Grossly, they may appear as pinpoint, white foci in the subpleural areas of the lung. Ultrastructurally, the aggregates are composed of morphologically normal lymphocytes, and studies have failed to identify infectious agents in association with them (Baskerville, 1982).

Thick Pulmonary Artery Musculature

Another common anatomic finding in guinea pigs is the thick muscular wall seen in pulmonary arteries and arterioles. This finding is not considered a true hypertrophy or hyperplasia of the smooth muscle. To investigators unfamiliar with the microscopic anatomy of the guinea pig lung, cross-sections of pulmonary arteries and arterioles show an apparent thickening of the muscular wall and a narrow lumen, especially when compared with that of other species. While these changes might be associated with pulmonary hypertension in other species, they are considered to be a normal finding in the guinea pig.

Osseous Metaplasia of the Lung

This is another common incidental finding in guinea pigs, as well as other lab animal species, including rats and hamsters. The incidence appears to increase with age. Affected animals have spicules of well-differentiated, mineralized lamellar bone within the pulmonary parenchyma, usually associated with little or no inflammation (Kaufmann, 1970). Foci of bone have also been reported in the eye (Donnelly and Brown, 2002) and kidney in a few individuals. While several investigators have commented on the possibility of these bone fragments being inhaled (Innes, 1956), the well-differentiated nature, presence of bone marrow, and lack of inflammation strongly suggest an endogenous origin. Foci of bone should not be considered to be the cause of pneumonia in the guinea pig. If such a histologic picture is seen, testing for common infectious agents, such as *Bordetella* sp., is strongly warranted.

Foreign Body Pneumonia

The presence of plant or food material within alveoli or airways is a direct result of living on contact bedding, the typical environment for most guinea pigs.

The problem is more commonly seen in young guinea pigs, but is rarely of clinical significance. The problem may be incorrectly labeled *pneumoconiosis*, which is more properly applied to the inhalation of mineral dusts, especially those of coal (anthracosis) or silicon (silicosis).

Most foci of plant material are found in smaller airways, as a result of being larger than the 5µm or smaller particle size needed to enter the alveoli (Muto, 1982). Grossly, airway obstruction as well as the inflammatory response to the foreign material leads to localized atelectasis characterized by depressed, reddened areas of the lung. Contaminated plant material usually incites a pyogranulomatous response. Neutrophils respond initially, often due to bacterial contaminants, but over time, a gradual shift in the inflammatory infiltrate to epithelioid macrophages and lymphocytes occurs. In more severe cases, multinucleated macrophages will be seen around plant material. Small airways are most commonly affected, but in severe cases, the inflammatory infiltrate will extend into the surrounding alveoli. Plant material is generally birefringent, and polarization of a lung section with multiple granulomas will usually disclose the location of any plant material (Percy and Barthold, 2007). Although rarely seen today, granulomas associated with the injection of Freund's complete adjuvant should be included in the differential diagnosis for this condition.

Pulmonary Granulomas Due to Injection of Freund's Complete Adjuvant

The injection of Freund's complete adjuvant in guinea pigs, as well as other species, will result in the production of granulomas in a number of organs, including the lung, liver, spleen, lymph nodes, and skeletal and cardiac muscle (Schiefer and Stunzi, 1979). In the lung, multifocal to coalescing areas of granulomatous inflammation, sometimes with granuloma formation, efface airways, alveolar septa, and alveoli. More chronic lesions may include abundant fibrous connective tissue. Vertebral osteolysis and posterior paresis were reported in addition to pulmonary lesions in several guinea pigs receiving epaxial injections of complete Freund's adjuvant in one report (Hill et al., 2006).

Respiratory Neoplasia

Bronchogenic neoplasms are the most common primary pulmonary neoplasms reported in the guinea pig (Greenacre, 2004; Manning, 1976). In guinea pigs over 3 years of age, these tumors are reported to have an incidence of up to 35%, with benign papillary adenomas being the predominant neoplasm. Malignant neoplasms are rare (Manning, 1976).

Microscopically, the papillary adenomas consist of cuboidal epithelial cells which line pre-existent alveolar septa, and are similar to neoplasms which may be caused in other species by infectious agents or other stimuli, but infectious agents have not been isolated. Clinically, these neoplasms, as they attain a large size, mimic pneumonia, but fail to respond to appropriate therapy. Radiography may help to identify single primary neoplasms and to differentiate them from infectious disease (Greenacre, 2004).

A single case of nasal adenocarcinoma was reported by Vannevel and Wilcock (2005). The neoplasm presented as a bleeding pink mass which infiltrated the maxilla, resulting in tooth loss.

GASTROINTESTINAL SYSTEM

Malocclusion

Like other rodents with continually growing hypsodont teeth, malocclusion is a common problem in the guinea pig. With the exception of fluorosis (Hard and Atkinson, 1967), a definitive cause has not been identified for malocclusion, although genetics, diet, and in some cases, trauma and infection may play a significant role. A genetic predisposition has been suggested in guinea pigs including inbred strains 2 (Rest et al., 1982), 2/N, and 13/N, and outbred Dunkin-Harley guinea pigs (Ediger et al., 1975). Scurvy has been suggested as a predisposing factor as movable teeth may predispose to uneven wear (Crossley and Aiken, 2004).

The most common clinical signs associated with malocclusion in the guinea pig include low feed intake, loss of condition, poor coat, low skin elasticity, increased amounts of fluid ingesta in the cecum, and scant feces (Jekl et al., 2008). In Jekl's study, bruxism was noted in only 15% of guinea pigs. Drooling, known colloquially as "slobbers", has also been described as a presenting clinical sign (Wagner, 1976), although it occurs less commonly in the guinea pig than in rabbits or chinchillas with malocclusion (Jekl et al., 2008).

Examination of the teeth, especially the cheek teeth, should be part of a thorough examination of every guinea pig. The narrow oral cavity and large buccal folds make oral examination difficult in this species. A thorough oral examination is facilitated by isoflurane anesthesia. Loops of gauze behind the incisors will allow opening of the jaws, and an otoscope, vaginal speculum, or a small-diameter flexible endoscope will allow visualization of all dental surfaces.

Gross elongation of the coronal surface, especially of the mandibular cheek teeth, is one of the most commonly seen types of malocclusion in the guinea pig. In the guinea pig, the rostral (mesial) mandibular cheek

teeth (especially PM 4) elongate, forcing the mouth open. As the coronal surfaces of the premolars elongate, the mandible is pushed forward, predisposing to even more severe malocclusion. In advanced cases, the cheek teeth may grow medially, entrapping the tongue and impairing the animal's ability to pass a food bolus into the esophagus. In one study, 25% of affected animals had this "bridge-like" malocclusion (Legendre, 2002). For obvious reasons, the medial growth of the mandibular teeth, along with the normal lack of lateral excursion in this species (Legendre, 2002), provides an opportunity for lateral overgrowth of the maxillary cheek teeth and damage to the buccal mucosa.

Treatment of malocclusion involves shortening the coronal surface and restoring a normal occlusal surface. Short-term control is possible, but a long-term prognosis is guarded at best. Clipping of any teeth with scissors or rongeurs is not acceptable as it predisposes to fracture of the crown (Crossley and Aiken, 2004). The use of a low-speed dental handpiece and flat or fissured bar is the easiest way to reshape the teeth; a dental drill with diamond bur may also be used to good effect (Crossley and Aiken, 2004; Legendre 2002). Retrimming should be done every 4–6 weeks.

Gastric Ulcers

Gastric ulcers are relatively common in guinea pigs and this species is often used as test subjects for new anti-ulcer medications. A wide range of infectious agents and chemicals have been used to incite ulcer formation in this species, including *Helicobacter pylori*, non-steroidal anti-inflammatory agents, histamine, and alcohol, among others (Lee, 2000). However, as a clinical condition, most gastric ulcers are secondary to other diseases, and likely a result of stress. The first report of gastric ulceration in a guinea pig occurred over a century ago in association with *Streptococcus pneumoniae* infection (Curtis, 1899). In one study over a 5-year period, one author reported 15 cases of gastric ulcers, mostly secondary to other diseases (Wagner, 1976). In this report, one ulcer perforated, resulting in a septic peritonitis.

Gastric Dilation and Volvulus

Gastric dilation with or without volvulus in guinea pigs has been well-documented. Facility "outbreaks" have been reported (Keith et al., 1992) but underlying dietary or husbandry factors are yet to be identified.

Clinical signs are non-specific, if observed at all. Anorexia, inappetance, dyspnea, and scant feces are sometimes observed. At necropsy, most cases have a 180-degree rotation along the mesenteric axis, and the stomach is dilated with fluid and gas (Lee et al., 1977). In most cases, the cause of death is respiratory insufficiency

due to lung compression from the expanded stomach, as well as concomitant vascular compromise and shock. A single case of omental torsion with partial gastric torsion has been reported in one animal (Shrubsole-Cockwill et al., 2008).

Antibiotic-Associated Typhlocolitis

This condition is well-documented. The first report described it as a penicillin "toxicity" (Hamre et al., 1943), and over the years ampicillin, erythromycin, lincomycin, chlortetracycline, oxytetracycline, streptomycin, and bacitracin were added to this list. However, other antibiotics in common use during this time, such as chloramphenicol (Eyssen et al., 1957), were found not to result in death of treated animals. Newton et al. (1964) discovered that penicillin had no toxicity for germfree animals.

A common hypothesis for this condition lies in the fact that the flora of the intestinal tract in the guinea pig is primarily Gram-positive. Administration of the antibiotic agents listed above, even at appropriate pharmacologic doses, will result in severe perturbations of the intestinal flora and a process known as "dysbiosis" or "dysbacteriosis" (Percy and Barthold, 2007). Moreover, the pharmacokinetics of many of these antibiotics includes biliary excretion, which concentrates them in the intestinal tract.

In the small intestine, cecum, and colon of guinea pigs, streptococci and lactobacilli predominate. Administration of antibiotics with broad efficacy against these bacteria may result in a hundred-fold decrease in "beneficial" Gram-positive bacteria and a ten-million-fold increase in Gram-negatives within only 12 hours. In addition, high levels of clostridia, particularly *Clostridium difficile*, have been recovered from affected animals as well as demonstration of *C. difficile* enterotoxin (Rehg and Pakes, 1981). Occasionally, *E. coli* bacteremia has been reported in affected animals as well (Farrar and Kent, 1965).

Affected animals have profound diarrhea, with up to 100% mortality within 5 days. Most lesions occur in the cecum and colon, occasionally extending into the terminal ileum (Figure 24.1). The primary gross finding is in the cecum, which is massively distended with gas and fluid. There is at least mucosal hemorrhage, which is often transmural and can be seen from the serosa. Due to the extensive necrosis associated with *C. difficile* cytotoxins, the cecal wall may be friable and tear easily with minimal traction. In cases of *C. difficile* intoxication, the toxin produces a very distinct band of coagulation necrosis and hemorrhage, beginning in the superficial mucosa and progressing deeper into the tissue over time. The mucosa is denuded of epithelium, and edema and hemorrhage is seen in the lamina propria and deeper layers. Infiltration of neutrophils and other inflammatory cells



FIGURE 24.1 Marked enlargement of the cecum (arrow) in a guinea pig following administration of broad-spectrum antibiotics and marked alteration of normal flora. (Photo courtesy of the Armed Forces Institute of Pathology.)

is seen only in animals surviving for a length of time. Mucus cell hyperplasia has been reported in the terminal ileum; this reparative change is likely seen only in animals which survive the initial insult.

Diagnosis of this condition includes a careful history, microscopic examination of necropsy samples, anaerobic culture of cecal contents, and ELISA assay for toxin. The cytologic finding of large numbers of robust bacilli in feces or gut contents is non-specific and not diagnostic for this condition. Characteristic histologic findings in the small intestine may yield a provisional diagnosis prior to return of culture and toxicology results.

Appropriate antibiotics for the treatment of bacterial disease in the guinea pig are trimethoprim-sulfa, chloramphenicol, and enrofloxacin. One author recommends supplementing the animals with a *Lactobacillus*-containing probiotic whenever antibiotics are used in this species (O'Rourke, 2004).

Cecal Impaction and Torsion

Impaction of the cecum or obstruction of the cecocolic orifice with bedding, phytobezoars, or inspissated fecal material has been reported (Wagner, 1976). Deaths due to cecal torsion have also been reported with the massively enlarged and hemorrhagic organ, filled with gas and fluid occupying a large part of the abdomen. In such cases, infectious causes such as *Eimeria caviae* and *Clostridium* sp., often associated with the administration of antibiotics, should be considered (Percy and Barthold, 2007).

Rectal Impaction

This condition is well-known in older pet guinea pigs. As a result of muscle wasting in older animals,



FIGURE 24.2 Aged sows and boars may experience rectal impaction as a result of muscle wasting.

the rectum and anus may become plugged with a large fecal mass, which often necessitates manual removal (Figure 24.2). This condition appears to be more common in boars than in females or neutered males. The use of a combination of mineral oil and warm water will soften harder pellets and careful cleaning of the perineum (also called the perineal sac) will prevent scald. Older animals may require assistance on a daily basis. Genetic predisposition and diet may be contributing factors, but evidence is lacking.

Prolapsed Rectum

Rectal prolapse is a non-specific finding in guinea pigs as it is in a number of other species. Any disease resulting in straining may result in prolapse of the rectum; the incidence increases with age and as the muscles of the perineum grow weaker. Common causes include rectal or anal impaction, enterocolitis, or dystocia, and the condition may occur in conjunction with vaginal prolapse at parturition (Wagner, 1976).

Focal Coagulative Hepatic Necrosis

This condition is occasionally seen at necropsy in guinea pigs, as it may be seen in other laboratory rodents. This finding may be seen in control animals as well as in animals afflicted with systemic disease (Cuba-Caparo et al., 1977). These lesions might be mistaken for other conditions causing hepatic necrosis, such as *Salmonella enteritidis* or *Clostridium piliforme* infection.

Grossly, this change appears as sharply demarcated areas of pallor in one or multiple lobes of the liver, usually in subcapsular areas. Microscopically, these areas are composed of regionally extensive coagulative necrosis of hepatocytes with minimal hemorrhage. This

finding lends credence to the current hypothesis that this change is the result of terminal hepatic vascular insufficiency and locally impaired blood flow to certain regions (Percy and Barthold, 2007).

Idiopathic Cholangiofibrosis

In one report, of a colony of 167 animals of the Jena:Alb outbred stock, between the ages of 2 and 47 months, 36 (21.5%) had post-necrotic, multilobular cirrhosis. Affected animals had roughened hair coats, emaciation, weakness, apathy, and occasionally abdominal distention (Guttner and von Zychlinski, 1981). Grossly, all hepatic lobes had a micronodular appearance. Microscopic examination revealed an ongoing necrotic process, with affected livers containing large foci of coagulative necrosis, which was eventually replaced by large bands of mature collagen separating proliferating biliary ductules. While labeled “cirrhosis”, the characteristic lesion of macronodular hepatocellular regeneration was not identified in this report, so the term cholangiofibrosis appears more appropriate (Percy and Barthold, 2007). The etiology of this particular lesion was not elucidated, and the lesion was attributed to an unidentified toxin (Guttner and von Zychlinski, 1981).

More recently, xenobiotics have been used to create a guinea pig model of primary biliary cirrhosis, a condition of autoimmunity in which lymphocytes infiltrate and destroy small interlobular biliary ducts (Leung et al., 2007).

Neoplasia of the Alimentary System

Primary neoplasms of the gastrointestinal tract are rare in the guinea pig. Tumors of the alimentary tract consist primarily of gastric leiomyomas, usually in the region of the pylorus (Papanicolaou and Olcott, 1942). Reports of tumors of the hepatobiliary system are equally rare. A bile duct carcinoma was reported in a guinea pig which also had a nasal adenocarcinoma (Vannevel and Wilcock, 2005). The biliary neoplasm was successfully treated through surgery. A single case of abdominal mesothelioma affecting the serosa of the liver and gastrointestinal tract has been reported in a guinea pig (Wilson and Brigman, 1982).

URINARY SYSTEM

Chronic Interstitial Nephritis

Guinea pigs, like other rodents, develop a syndrome of progressive renal insufficiency with advancing age. Evidence of chronic renal disease is present in almost all guinea pigs 3 years and older. This condition results



FIGURE 24.3 Irregular contour and linear areas of fibrosis in an older guinea pig with chronic interstitial nephritis. (Photo courtesy of Dr. Paul Stromberg.)

in progressive debility in older guinea pigs, and complicates renal research conducted in aging guinea pigs.

Clinical signs include anorexia, cachexia, polyuria, and polydipsia. Clinicopathologic data reveal increased blood urea nitrogen, creatinine, and phosphorus levels, and a non-regenerative anemia associated with decreased erythropoietin levels. The urine specific gravity is low or isosthenuric. Ultrasound can demonstrate shrunken kidneys with areas of hyperechogenicity. At necropsy, kidneys are small with an irregular outline and the cortices are pitted. On cut section, white areas of fibrosis extend downwards from the capsule into the medulla (Figure 24.3). Microscopically, there are multifocal to coalescing areas of roughly linear interstitial fibrosis which entrap tubules and glomeruli (hence the colloquial name “nephrosclerosis”). In these areas, there is loss and marked atrophy of tubules, which worsens toward the renal pelvis. Remaining tubules in these areas are often ectatic and may contain proteinaceous fluid and cellular debris. Rarely, tubules (especially in the deeper cortex) exhibit regenerative or hypertrophic changes. Glomeruli, while generally normal, may have dilated Bowman’s capsules. Aggregates of lymphocytes and plasma cells may be scattered throughout the kidney. In severe cases, renal arterioles and arteries have marked medial thickening.

The cause of chronic interstitial nephritis has not been established in the guinea pig, although hypotheses include vascular disturbance, autoimmune disease, and diet as causative factors. The distribution of fibrosis (both anatomic and temporal) and the presence of arterial lesions suggest the possibility of periodic disturbances in blood flow (Percy and Barthold, 2007). In addition, a mild degree of hypertension has been recorded in affected animals (Takeda and Grollman, 1970). In one study, complement and IgG were demonstrated in the glomerular basement membranes, suggesting that antigen–antibody

disease contributes to the lesion (Steblay and Rudosfsky, 1971). Further investigation has failed to disclose an infectious agent common to affected individuals (Foltz et al., 1994). An increased incidence and severity of this condition has been identified in guinea pigs fed a high-protein diet.

Urolithiasis

Urolithiasis is a common problem in older female guinea pigs, but is occasionally seen in older boars as well. Affected animals may exhibit dysuria, stranguria, hematuria, and pain on caudal abdominal palpation. In a study of 19 cases, older female guinea pigs approaching 3 years of age were most frequently represented (Peng et al., 1990). The majority of uroliths are composed of calcium carbonate (Hawkins et al., 2009). Uroliths may range in size from sand-like crystals to large stones. Bacterial infection has been identified as a major predisposing cause in the guinea pig. In a colony outbreak of *S. pyogenes* in Dunkin-Hartley guinea pigs, 50% of breeders and adults had evidence of urolithiasis; *Streptococcus pyogenes* was often isolated from the urinary bladder (Okewole et al., 1991). *Corynebacterium renale* and *E. coli* have also been isolated. In addition, the anatomy of the female urogenital tract and immunosuppressive diseases such as diabetes mellitus have been purported to be contributing factors.

Diagnosis of urolithiasis is most often made by radiography (O'Rourke, 2004). The majority of successful treatments involve surgical removal. A recent report described cystoscopic removal (Pizzi, 2009). Urinary acidifiers appear to be of little use in guinea pigs as the animals cannot easily remove an acid load (Harkness and Wagner, 1989).

One study (Wood, 1981) reported an incidence of 0.6% of cystitis in parous breeding-colony female guinea pigs with accompanying nephritis and pyelitis; bacteriologic examination performed in six of 19 cases revealed a variety of bacterial agents including alpha-hemolytic streptococci, staphylococci, and one case of *E. coli*.

Nephrotoxicity

Acute renal failure was diagnosed clinically in a pet guinea pig that had ingested peace lily. The diagnosis was made by clinical history, signs of azotemia, hypercreatininemia, hyperphosphatemia, non-regenerative anemia, and isosthenuria, and the finding of calcium oxalate crystals in the urine. Peace lily (*Spathiphyllum* sp.) is a member of oxalate-containing plants that rarely cause toxicity in domestic species due to its propensity for causing oral lesions, salivation, and vomiting. A confirmatory necropsy was not performed in this case (Holowaychuk, 2006).

Urinary Neoplasia

Reports of neoplasia of the urinary system of the guinea pig are uncommon and poorly documented. An early report of osteosarcoma in the urinary bladder described a pleomorphic spindle cell tumor with multinucleated cells interpreted as osteoclasts but did not describe osteoid production (Twort and Twort, 1932). A second report describes four neoplasms of the urinary bladder, including one transitional cell carcinoma, and three pleomorphic tumors, one "anaplastic tumor", one "epidermoid carcinoma", and one "epidermoid papilloma" (Manning, 1976). While being described as "morphologically malignant", metastases were not noted.

A single case of "proliferative urocystica" along with a transitional cell adenoma has been reported and attributed to the long-term feeding of *Christella dentata* fern (Somvanshi and Sharma, 2005). Finally, a mural urinary bladder papilloma was reported in a guinea pig with concurrent urolithiasis and hydroureter (Stieger et al., 2003). Based on the relatively poor descriptions of these neoplasms and the questionable morphologic diagnoses in the literature, it is probably best simply to realize that urinary system neoplasia is rare in the guinea pig.

ENDOCRINE SYSTEM

Diabetes Mellitus

Diabetes mellitus has been reported in the guinea pig (Vannevel, 1998) and this species has been identified as an animal model for juvenile diabetes (Lang et al., 1979). It can be a significant problem in both research colonies and individual pets.

Affected animals have chronic weight loss, cachexia, polydipsia, and polyuria. In a colony setting, glucosuria (>250mg/dl urine), hyperglycemia, ketonuria, and abnormal glucose tolerance were noted as early as 3 months. In pets, diabetes is typically diagnosed at several months of age. Fasting blood glucose and ketonuria are variable, even in glucosuric animals, and measured levels of glucose in blood and urine are often no different from controls (Lang et al., 1979). Affected sows experience a marked decrease in fertility. In some animals, researchers described a subclinical form with transitory clinicopathologic abnormalities and minimal lesions (Lang et al., 1979).

Diabetes occurs in male Abyssinian-Hartley guinea pigs. Affected animals also develop bladder hypertrophy and voiding dysfunction (Belis et al., 1996). Diabetes has also been induced in this species by administration of high levels of glucocorticoids (Herberg, 1982).

In both the colony report and in single animal reports, one common theme is that the condition may be transient and animals may undergo spontaneous remission

(Lang et al., 1979; Vannevel, 1998). Spontaneous remission is defined by a lack of glucosuria and normal blood glucose readings. Another interesting finding in the colony “outbreak” was the predilection for normal animals, once introduced into the colony, to develop hyperglycemia and glucosuria. The cause for this condition was never found.

Pathologic findings in affected animals are similar to those seen in other species and include marked vacuolation of beta cells and fibrosis of islets. In some advanced cases, diabetic glomerulopathy (thickening of glomerular basement membranes and accelerated glomerular senescence) and diabetic cataracts occur (Lang et al., 1979). One diabetic pet guinea pig had dysuria resulting from a bladder infection presumably as a result of high levels of urine glucose which served as a substrate for bacterial growth.

The spontaneous remission of this condition has led some authors to suggest a dietary cause for transient diabetes mellitus and advocate restricting access of affected animals to high-fat dietary components such as seeds, as well as maintaining affected animals on quality hay and dark leafy greens as staples (Vannevel, 1998). Glucosuric animals appear to respond well to neutral protamine Hagedorn (NPH) insulin (O’Rourke, 2004).

Fatty Ingrowth of the Pancreas

With age, the proportion of exocrine tissue within the pancreas decreases, resulting in apparent increases in the amount of adipose tissue and to a less noticeable extent, islet tissue. This should be considered an aging change of no clinical significance (Wagner, 1976).

Hyperadrenocorticism (Cushing’s Disease)

There are currently two reports of Cushing’s disease in the literature and all cases appear to be the result of functional adrenocortical lesions (Gaschen et al., 1998; Zeugsweter et al., 2007). Clinical signs in both affected animals included alopecia, apathy, obesity, and lethargy, and one animal had polydipsia and weight loss. In one animal (Gaschen et al., 1998), hyperadrenocorticism was suspected clinically and enlarged adrenal glands were noted on ultrasound during a pre-surgical workup, however, the animal died during surgery to remove a concurrent urolith. In the second case (Zeugsweter et al., 2007), clinical testing, including the measurement of salivary cortisol levels both before and after ACTH stimulation, was performed. Urine glucose levels were consistently negative; however, urine specific gravity was consistently dilute (around 1.012). Both resting and post-ACTH cortisol levels were consistently elevated. The animal was successfully treated with trilostane (Vetoryl, Arnolds Veterinary Products) at a therapeutic level of 2 mg/lb.

Thyroid Disease

Currently, non-neoplastic thyroid disease has not been reported in the guinea pig. However normal levels of thyroxine have been reported in this species (Muller et al., 2009). Thyroxine levels do not appear to differ significantly between males and females, or intact and neutered animals. Thyroxine concentrations ranged from 14.2–66.9 nmol/L (1.1–5.2 µg/dl).

Endocrine Neoplasia

Insulinoma has been reported in the guinea pig (Vannevel and Wilcock, 2005). In both cases, animals presented with ataxia and head tilt. Hypoglycemia, which responded temporarily to intravenous glucose administration, was noted in both animals. Both animals were euthanized and red pancreatic masses were noted at necropsy. Microscopic examination revealed neoplasms composed of nests and packets of epithelial cells on a vascular stroma. Mitotic figures were not observed. In this case, insulin assays were not performed; normal insulin levels have not been reported in guinea pigs and testing has not been validated in this species.

Adrenal gland neoplasms have been identified in the guinea pig. Previous reports included both adrenocortical adenoma and adrenal carcinoma (Manning, 1976). Two adrenocortical neoplasms, previously mentioned, resulted in a syndrome of hypercortisolism (Gaschen et al., 1998; Zeugsweter et al., 2007). A syndrome of multiple cortical nodules, presumably hyperplastic, was reported in association with neonatal castration.

In the absence of true thyroid disease, there have been several reports of thyroid neoplasia in this species; however, early reports did not clearly specify the tumor type (Manning, 1976). However, there is at least one well-documented case of thyroid papillary adenoma in the literature (LaRegina and Wightman, 1979). This well-encapsulated neoplasm was composed of papillary projections of cuboidal epithelial cells with well-developed incipient and mature follicles. A medullary carcinoma with osseous foci has also been reported (Zarrin, 1974). In short, thyroid neoplasia should be considered rare in this species.

REPRODUCTIVE SYSTEM

Unique Features of the Guinea Pig Reproductive System

There are a number of peculiarities of the reproductive tract and physiology of this species. In males, the curled seminal vesicles are extremely large, filling a large part of the caudal abdomen. Unlike most rodents,

the female has two conical inguinal nipples. Sows whelp one to two large precocious pup, whereas other rodent species generally have larger litters. In addition, the vagina of the female is generally closed by a waxy, hardened mucus plug, and is patent only during times of breeding and parturition.

Posthitis in Boars

Posthitis is an accumulation of bedding material and detritus within the preputial folds. This may occur in one of two ways: either following coitus, as the bedding adheres to the moist penis, or as a result of poor husbandry. As guinea pigs are indiscriminate and voluminous eliminators, it is not uncommon to see them sitting in moist, dirty litter. Accumulation of bedding, feces, hair, and other debris within the preputial orifice may further impair elimination, worsening the situation and perhaps preventing elimination altogether. Moreover, bacterial infections of the prepuce may result in adhesions and decreased fertility (Lee et al., 1978). Gentle removal of the debris by soaking in a dilute chlorhexidine solution and administration of systemic antibiotics has been recommended for treatment (O'Rourke, 2004). Other cases of preputial dermatitis in the guinea pig have been precipitated by trauma, possibly bite wounds.

Seminal Vesiculitis/Urethritis in Boars

Urethritis is an unrelated problem to posthitis in the boar, but also results in urethral plugging and post-renal obstruction (Wagner, 1976). In most cases, the plug is due to an inspissated plug of proteins from the seminal vesicles and sperm. In some cases, the plug can be visualized extending from the urethral orifice. Clinical signs include frantic activity followed by dullness and abdominal tension. Attempts to express the bladder are often unsuccessful. Microscopically, there is minimal reaction within the occluded region of the urethra, and hemorrhages within the wall of the urinary bladder. There may be suppurative inflammation within the seminal vesicles. Coliform bacteria, including *Pseudomonas aeruginosa*, have been cultured from the seminal vesicles of affected individuals.

Ovarian Cysts

Ovarian cysts are a very common age-related finding in sows. In one report, cysts were observed in 76% of sows between 18 and 60 months of age (Keller et al., 1987). As these cysts enlarge, they compress adjacent ovarian tissue, resulting in atrophy and eventual effacement of the ovary. The major result of these growing cysts is infertility of the older sow. However, they have been associated with other syndromes such as alopecia,

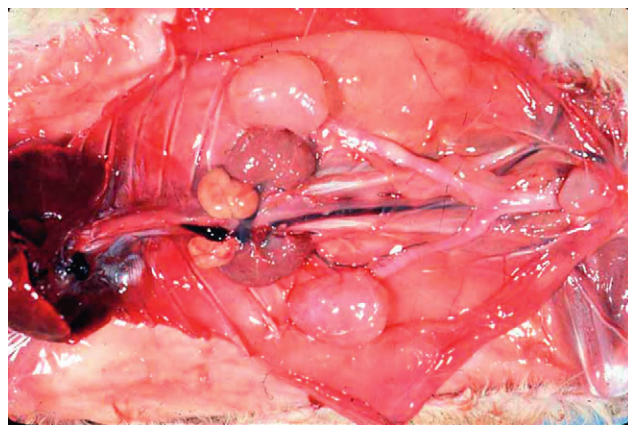


FIGURE 24.4 Older sow with bilateral multilocular cystic ovaries due to expansion of the ovarian rete. (Photo courtesy of Dr. John King.)

mucometra, cystic endometrial hyperplasia, endometritis, and various reproductive neoplasms, such as uterine fibroleiomyomas (Keller et al., 1987). The reproductive status of the individual sow appears to have no bearing on their development or eventual size (Nielsen et al., 2003).

Clinical signs of ovarian cysts include alopecia, abdominal distention, anorexia, and apathy. The cysts are readily identified by ultrasound examination, and ovariohysterectomy is the recommended treatment (O'Rourke, 2004).

Serial sections of ovaries from affected sows reveal that the cysts apparently arise from the epithelial-lined ovarian rete, and communicate with follicles and the outer mesothelial lining of the ovary (Percy and Barthold, 2007). Ovarian cysts range from a millimeter up to 7 cm in diameter (Figure 24.4). Small cysts are most commonly found near the ovarian hilus. The cysts may be uni- or multilocular, are filled with clear fluid, and are lined by flattened cuboidal ciliated epithelium. The condition is usually bilateral. Testosterone administration is reported to be an experimental cause of cyst formation within the ovarian rete in the guinea pig, as well as subsequent formation of epithelial ovarian neoplasms (Silva et al., 1997).

Dystocia

Dystocia is also a common problem in guinea pigs and the most common cause of perinatal mortality. Guinea pigs give birth to one to two large precocious pups per litter; in fact, female guinea pigs may gain up to 60% of their body weight during gestation.

The birth canal of the sow is narrow at the level of the os coxae. The release of relaxin by the pituitary gland and endometrium the week before parturition results in dissolution of the fibrocartilage of the pubic

symphysis and allows stretching and passage of the large offspring. However, if the sow is not bred prior to fusion of these bones at approximately 6 months of age, this separation of the pelvic bones cannot occur at parturition, and dystocia is far more likely to occur. Obesity in older sows may also result in dystocia.

Ectopic pregnancy has been reported with implantation in the peritoneal cavities of two multiparous strain 13/N guinea pigs (Hong and Armstrong, 1978).

Pregnancy Toxemia

This poorly named syndrome is another major cause of perinatal mortality in guinea pigs. Contrary to its name, the condition, while most commonly seen in pregnant or lactating guinea pigs, can also be seen in non-gravid females and even boars.

This metabolic syndrome of ketoacidosis shares some similarities with that seen in other species; however, studies in the guinea pig have identified two distinct conditions, the nutritional (or fasting) form, and the circulatory form (Percy and Barthold, 2007). Both forms are characterized by occurring during the last 2 weeks of gestation or the first week after parturition, as well as marked acidosis (even lowering the normal pH of urine from approximately 9.0 to 5.0–6.0), ketosis, ketonuria, proteinuria, and the clinical signs of lethargy, adipisia, hypersalivation, and incoordination (Brower, 2006).

The nutritional form is, in essence, the end result of reduced carbohydrate intake and marked systemic mobilization of fat stores for energy. While the name *pregnancy toxemia* indicates that it is primarily a disease of gestation, a similar condition occurs in guinea pigs undergoing carbohydrate reduction for a number of reasons – poor dietary management, changes in feeding times, or any other stressful condition that may put an animal off feed. Obese animals, presumably those with the largest fat stores, appear more at risk than lean ones. The disease has been experimentally reproduced in guinea pigs fed initially a high-carbohydrate diet, followed by sudden feed withdrawal (Lachmann et al., 1989). Gravid or lactating animals would develop the full manifestations of the syndrome, whereas non-gravid animals develop a non-clinical ketosis.

In the nutritional form, affected animals exhibit hypoglycemia (<60 mg/dl) and hyperlipidemia in addition to the aforementioned ketoacidosis, ketonuria, and proteinuria. Clinical depression occurs rapidly, with the animal becoming prostrate and dyspneic within 1 day (O'Rourke, 2004). The condition progresses to coma within 5–6 days. In this form, the primary gross finding is marked fat accumulation within hepatocytes, with yellow-to-white discoloration of the liver. The liver is moderately enlarged, with rounded edges. Microscopically, fat is present in large quantities within hepatocytes, renal

tubular epithelium, and cells of the adrenal cortex, and may be seen within vessels in glomerular and alveolar capillaries (Percy and Barthold, 2007).

A second form of pregnancy toxemia is the circulatory form. Thought to be a possible model for pre-eclampsia in humans, this form has other morphologic features of note, in addition to the metabolic derangements. In pregnant guinea pigs with pregnancy toxemia, one study demonstrated a 22% compression of the aorta in the area of the renal arteries with a resulting 30% reduction in blood pressure (Seidl et al., 1979). This reduction in arterial flow may result in vascular insufficiency to a number of abdominal organs including the liver, kidneys, adrenal glands, and the gravid uterus itself. Microscopically, there is hemorrhage and necrosis in these organs, including the placenta. Infiltration of white blood cells in areas of necrosis heralds a more long-standing insult. Diminished flow in these organs may also result in thrombosis, and eventually, consumptive coagulopathy. Experimentally, the condition has also been reproduced in gravid female guinea pigs by occlusion of the uterine vessels (Golden et al., 1980).

In both types of pregnancy toxemia, the prognosis is poor. Hypoglycemia may be treated with intravenous glucose, and shock may be treated with corticosteroids, calcium gluconate, and fluid replacement. Keys to prevention are keeping guinea pigs lean, especially breeding sows; eliminating potential stressors in a colony situation; and providing a varied, adequate well-balanced diet and water ad libitum. It is important to remember that pregnant sows in the last stages of gestation may triple their food intake.

Neoplasia of the Reproductive System

Tumors of the reproductive tract account for about 25% of spontaneous tumors in this species (Percy and Barthold, 2007). Tumors of the uterus are primarily benign and of smooth muscle origin (leiomyoma); however, leiomyosarcomas have been reported (Field et al., 1989). Other less common neoplasms of the uterus include fibromas and myxoid fibrosarcomas (myxosarcomas) (Blumenthal and Rogers, 1960).

In reports of spontaneous ovarian tumors, teratomas are well-represented. These neoplasms have well-differentiated tissues of ectodermal, mesodermal, and endodermal derivation, including skin, bone, muscle, and nervous tissue, among others (Frisk et al., 1978; Manning, 1976; Vink, 1970). Granulosa cell tumors and ovarian cystadenomas have also been reported in the guinea pig (Burns et al., 2001). Malignant epithelial neoplasms have been experimentally induced in the guinea pig ovary via the use of carcinogens in order to create an animal model for human ovarian carcinoma (Silva et al., 1997). Reports of spontaneous malignancies in the

guinea pig are rare, two papillary cystadenocarcinomas have been reported (Hong, 1980), as well as a single case of ovarian stromal sarcoma (Olson and Anver, 1980).

Interestingly, only one testicular tumor, an embryonal carcinoma, has been reported in the guinea pig (Manning, 1976). This may partially reflect the scarcity of intact males within colony settings and the pet populations.

MUSCULOSKELETAL SYSTEM

Hypovitaminosis C

Ascorbic acid is an important antioxidant and reducing agent. It is required for the hydroxylation of proline and lysine, a process that is essential in the formation of collagen. A number of animal species, including most fish, insects, fruit bats, guinea pigs, non-human primates, and man lack the enzyme gulonolactone oxidase, which catalyzes the last step of the synthesis of vitamin C, and require dietary intake. There is no storage of vitamin C within the body.

The lack of vitamin C (known traditionally as "scurvy") results in a host of systemic defects within susceptible individuals, but the most obvious are the result of blood vessel fragility. The lack of prolyl and hydroxyl hydroxylase results in formation of collagen fibrils that are not cross-linked and lack tensile strength, leading to blood vessel fragility and poor wound healing (Mahmoodian and Peterkofsky, 1999). Lesions associated with scurvy, such as subperiosteal, subcutaneous, intramuscular, and gingival hemorrhage, reflect this defect. Joint bleeding (Figure 24.5), one of the most obvious signs of scurvy in the guinea pig, is due to damage to synovial vessels and microfracture of bone. There may also be non-hemorrhagic joint effusions. In young growing individuals, significant changes occur in the growth plate due to defective and insufficient osteoid production. Clarke et al. (1980) reported changes in subclinical cases of scurvy to include attenuated and irregular epiphyses, and acute enteritis with numerous hemosiderin-laden macrophages in the intestinal lamina propria.

Hair loss and poor haircoat are reported; vitamin C is important in the disulfide bonding of hair. Tooth mobility may be the result of improper collagen formation in the dental socket and may lead to malocclusion. Conjunctival and intraocular bleeding is also common, and there is disruption of corneal epithelial and stromal organization with stromal vascularization in later stages. Animals with hypovitaminosis C also have poor wound healing. Vitamin C is known to promote wound healing through stimulation of collagen synthesis and deposition, increased fibroblast density, and tissue vascularity.



FIGURE 24.5 Bilateral femorotibial joint hemorrhage in a guinea pig with hypovitaminosis C. (Photo courtesy of the Armed Forces Institute of Pathology.)

Ascorbic acid is also an important antioxidant and reducing agent. It functions as a scavenger of free radicals and maintains the Fe^{++} and Cu^{+} cofactors of a variety of oxygenases in a reduced state. In this regard, ascorbic acid functions similarly to vitamin E in prevention of membrane peroxidation. Ascorbic acid also functions in the hydroxylation of dopamine to norepinephrine and serves to prevent oxidation of tetrahydrofolate, thereby maintaining folic acid stores. Furthermore, vitamin C increases the absorption of non-heme iron by catalyzing the reduction of ferric iron in the stomach, as well as increasing the amount of iron stored as ferritin (Padayatty and Levine, 2001).

Clinically, the earliest signs of vitamin C deficiency are generally non-specific, such as weakness, anorexia, diarrhea, and weight loss. Affected animals are reluctant to move and may vocalize and grind their teeth due to arthralgia and myalgia, as well as a result of hemorrhage into joints (especially the stifle joints) and muscles. Anorexia and weight loss are noticed within 2 weeks on a deficient diet. After 3 weeks, serum 25-OH vitamin D_3 , calcium, and albumin levels are significantly reduced, bone mineral density and bone content are significantly lower than normal, and bone volume is reduced in long bones (Fain, 2005).

Laboratory findings are non-specific. Anemia is seen as a result of folate deficiency more than the result of hemorrhage. Foods high in vitamin C are also sources of folate so folic acid deficiency is marked. Leukopenia and hypoalbuminemia, markers of malnutrition, are also common.

Gross skeletal lesions of scurvy include bowing of long bones, outward projection of the ends of the ribs ("scurvitic rosary," Figure 24.6), subperiosteal hematomas, loosening of teeth, and epiphyseal fractures. Cortices are thin due to decreased appositional growth and break easily.

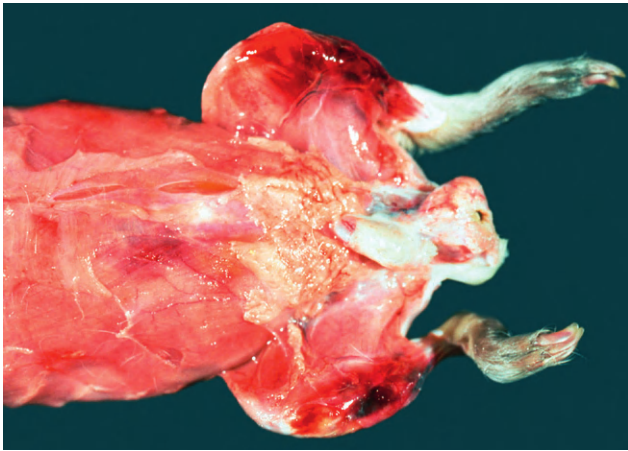


FIGURE 24.6 Flaring at the costochondral junction and hemorrhage in a guinea pig with hypovitaminosis C. (Photo courtesy of the Armed Forces Institute of Pathology.)

Microscopic skeletal features of scurvy are most profound within the growth plate, but may be seen in the bones of adults as well. As vitamin C is required for their differentiation from progenitor cells, osteoblasts are rare, and osteoid deposition on trabeculae of cartilage is scant or absent. In immature animals with open physes, a lattice of vascularized, calcified cartilage is formed in the metaphysis and is not replaced by bone as it increases in thickness. This “scorbutic lattice” is inherently weak and predisposed to microfracture and hemorrhage (Thompson, 2007). Scorbutic animals also have increased dilation of metaphyseal vessels which predispose to hemorrhage. The deep metaphysis often contains loosely arranged mesenchymal cells in areas normally occupied by hematopoietic cells (Percy and Barthold, 2007). Proliferation of spindle cells between the fibrous periosteum and the cortical bone surface thickens the periosteum of long bones, resulting in widening at the metaphyseal–epiphyseal junction (“metaphyseal flaring”). In mature bone, osteoid deposition on previously formed trabecular bone and endosteal and periosteal surfaces is also scant or absent.

Diagnosis is usually based on characteristic history, clinical signs, and radiographic findings, although serum ascorbic acid levels may be used in academic situations for diagnosis or to monitor treatment. Response to therapeutic trial of 50mg vitamin C per day may assist in presumptive diagnosis of the condition. Response to treatment is rapid, with most clinical signs reversed within a week of onset of adequate intake. In severely scorbutic guinea pigs, complete restoration of normal trabecular bone structure takes about 20–25 days.

Dietary recommendations for guinea pigs include 15–25mg/kg body weight of vitamin C per day, and up to 40mg/kg for pregnant sows. Most commercial chows are adequate but must be stored at or below 50°F

and fed within 90 days of milling (O’Rourke, 2004). Leafy green vegetables, such as kale or cabbage, should be part of the normal diet of guinea pigs as 50g of either can supply the recommended amount of vitamin C. Guinea pigs should not be fed rabbit chow, as it does not supply the recommended amount of vitamin C for guinea pigs.

Osteoarthritis

Osteoarthritis in Hartley guinea pigs resembles human osteoarthritis both morphologically and epidemiologically. Lesions first appear at about 3 months of age and progress with age (Jimenez et al., 1997). Obesity is a predisposing factor.

A 2003 study compared stifle joints of captive and wild-caught guinea pigs. No evidence of osteoarthritis was seen in wild-caught guinea pigs, suggesting osteoarthritis to be a problem of captivity, or of the “artificial environment” (Rothschild, 2003). The issue of genetic differences between “wild” and Hartley guinea pigs was not addressed in this study.

Radiographic changes in chronic osteoarthritis include marginal osteophytes of the tibia and femur, sclerosis of the subchondral bone of the tibial plateau, cystic changes of the femoral condyle, and calcification of the collateral ligaments (Jimenez et al., 1997). Histologically, unilateral focal degeneration of the hyaline cartilage of the joint surface, characterized by proteoglycan loss within the superficial zones, can be observed on the medial tibial plateau as early as 3 months. Over the next 3 months, these lesions worsen, and there is marked loss of the proteoglycan matrix of the superficial and middle zones, chondrocyte death, and fibrillation of the hypocellular matrix with proliferation of chondrocyte clones in deeper layers. Severe cases have degenerative changes within the medial femoral condyle, menisci, and eventually osteophytes will appear on the medial femoral and tibial condyles. Synovial changes include fibrosis, hyperplasia of synoviocytes and occasional papillary projections into the joint itself. Inflammatory cell infiltration is usually not marked. As a result of biomechanics of the stifle joint, lesions are seen primarily on the medial tibial plateau with sparing of the lateral side (Bendele and Hulman, 1988).

A recent study in guinea pigs demonstrated that high levels of vitamin C may actually worsen osteoarthritis in the guinea pig as a result of activating latent transforming growth factor-beta within osteophytes in damaged knees (Kraus et al., 2004).

Fibrous Osteodystrophy

Fibrous osteodystrophy has been reported in two guinea pigs with clinical signs of lethargy and apathy

(Schwarz et al., 2001). Radiographically, both animals had skeletal deformities, pathologic fractures, malocclusion, and marked osteoarthritis. Diagnosis of osteopenia was confirmed by use of dual-energy X-ray absorptiometry. The diagnosis of fibrous osteodystrophy was confirmed at necropsy and in the absence of renal disease, dietary calcium/phosphorus imbalance was considered to be the cause of this condition.

Nutritional Myopathy

A multisystemic condition with marked degenerative changes of skeletal muscles, especially of the hindlimbs, has been documented in guinea pigs deficient in vitamin E and selenium (Howell and Buxton, 1975). Clinical signs included hindlimb weakness, conjunctivitis, depression, decreased reproductive performance, and in severe cases, death. Laboratory values included elevated serum creatine phosphokinase. The disease has been reproduced experimentally in animals fed diets deficient in vitamin E as well as both vitamin E and selenium, with more severe lesions seen in animals deficient in both substances (Hill et al., 2001).

At necropsy, both skeletal and cardiac muscle may be affected; grossly, affected muscles are pale. Microscopically, myofiber changes include coagulative necrosis, hyalinization, fragmentation, and occasionally regeneration as evidenced by nuclear rowing. Mineralization of fibers is not a feature of this disease (Ward et al., 1977).

Testicular degeneration may be seen in boars, and encephalomalacia in fetuses in vitamin-E-deficient dams (Howell and Buxton, 1975). In one report, a group of affected animals showed a positive clinical response to injectable sodium selenite and alpha tocopherol (Howell and Buxton, 1975).

Traumatic Injury

Traumatic injury may result from falls, fighting within colonies, and improper flooring. Guinea pigs catching their legs in improperly slotted flooring may result in traumatic soft tissue injury, torn nails, and broken toes. Falls from cages to a hard floor have been reported to result in posterior paralysis from broken backs and spinal cord damage (Wagner, 1976). Guinea pigs have a very thin diaphragm. Traumatic injuries, including falls, may result in tears and extrusion of the abdominal viscera through a tear in the diaphragm near the esophageal hiatus into the thorax (Wagner, 1976).

Neoplasia of the Musculoskeletal System

Reports of neoplasia of the musculoskeletal system are rare. Osteosarcoma has been reported three times. In one report, the neoplasm arose in the lumbar vertebra in two animals, metastasizing widely to multiple organs in one (Hong and Liu, 1981). In a second report, the neoplasm originated within the femur and metastasized to the lung (Leader, 1937). In all cases, the neoplasm consists of large numbers of spindle cells which produce abundant osteoid. A single report of osteosarcoma arising in the proximal epiphysis of the right humerus and metastasizing to multiple organs is interesting in that the neoplasm was transplantable and was maintained through 11 generational transplants within both random-bred and inbred individuals (LaPorte and Sillard, 1963). Single reports of other neoplasms arising in the skeletal system include a fibrosarcoma of the maxilla, a chondrosarcoma of the ilium, and a rib enchondroma (Manning, 1976). Reports of neoplasms of the muscles are even rarer with a single report of an infiltrative lipoma (lipomyxofibroma) within the psoas muscle (Manning, 1976).

INTEGUMENTARY SYSTEM

Alopecia

Alopecia in the guinea pig is a common non-specific sign that can be the end result of a number of endocrine and non-endocrine diseases. Alopecia is present in almost all pregnant sows during late gestation and through lactation. This finding appears to be more severe in animals bred intensively, older sows, and those carrying a large litter. While a number of factors such as genetics and nutrition may play a part, the major reason is thought to be decreased anabolism of the maternal skin associated with fetal growth (Wagner, 1976). In affected dams, hair loss is most severe over the back, flanks, and rump, and will grow back in most, if not all, cases. Alopecia may be seen in pups at the time of weaning, but appears to be the result of a hair coat change as the more coarse "guard hairs" of the adults begin to appear.

Alopecia may be seen in any guinea pig as a result of stress or intercurrent disease – as the so-called "telogen effluvium." It is a documented clinical sign of hyperadrenocorticism in the guinea pig, and has been associated with cystic rete ovarii (O'Rourke, 2004). In one breeding colony study, alopecia was seen in animals receiving less than 200g of hay as part of their daily diet; addition of hay to the ration significantly improved both hair quality and quantity (Gerold et al., 1997).

Barbering

Barbering is a vice in individuals and evidence of a dominance hierarchy in group housing situations. In group situations, juvenile animals are usually barbered by adults. Individual animals may also barber, but hair loss is restricted only to places on the body that they can reach, so the head, neck, and shoulders are spared. Patterns of hair loss, occasional broken skin, and evidence of fighting should suggest the possibility of barbering in a group situation. Often, the dominant animal in the group will be the one without hair loss. In a group setting, separation of animals or removal of the dominant animal from the group may be the best option. Self-barbering is a more difficult issue for which provision of hay or supplemental leafy green vegetables has been recommended.

Ear chewing is a variant of this condition, and may result in notching, deformity, and in some cases, traumatic amputation (Wagner, 1976). Ear chewing is often a sign of aggression within an enclosure, and is usually attributable to males (Figure 24.7). Ear tags may often be the cause of ear chewing and may be rendered unreadable or pulled out.

Fight Wounds

Bite wounds, scratches, dermatitis, and alopecia are evidence of aggressiveness and fighting within an enclosure and usually can be attributable to a single individual. The composure of a particular group may have great impact on the predilection for fighting (Paterson, 1972). Intact boars should not be housed together – fighting is common with severe injuries and even death as a possible outcome. In general, older animals placed together have a greater tendency to fight than juveniles or adults that have lived together since they were juveniles.

Pododermatitis

Pododermatitis is a common problem in guinea pigs, especially pet ones. The condition is generally bacterial, with several predisposing. Heavy or obese animals kept in poor sanitation are predisposed. Wire or abrasive flooring and moist litter aggravate this condition, as do other debilitating diseases that inhibit movement. Clinical signs of pododermatitis include sedentary behavior, inactivity, vocalizing when moving, and lameness. One or multiple feet may be swollen, and both front and hind feet may be affected.

Clinically, foot pads and toes are erythematous, swollen, and may be eroded or ulcerated. Guinea pigs may vocalize when they are touched. The most common bacterial agent isolated from infected feet is *Staphylococcus aureus*, which usually enters through wounds on the



FIGURE 24.7 Trauma on the ear margin of a guinea pig. Ears are a common site of trauma between fighting individuals.

bottom of the feet. Rarely, traumatized tissues may be seeded from an infected site elsewhere in the body. Pododermatitis does not involve the formation of a discrete abscess which can be drained; the infection usually results in cellulitis, with infection of the skin and subcutaneous tissue, and a proliferation of granulation tissue. Osteomyelitis of the foot may accompany severe infections, and long-standing infections may result in systemic sequelae, such as amyloidosis.

Treatment of pododermatitis is prolonged, difficult, and often unrewarding (Brown, 2008). The vascularity of the granulation bed accompanying many such wounds predisposes to excessive, occasionally life-threatening bleeding when surgical options are pursued. Successful regimens include cleaning, dressing, administration of appropriate systemic antibiotics, and elimination of predisposing factors. Wound cleansing should focus on removal of detritus and necrotic tissue; use of wound cleansers which impair wound healing and leukocyte activity, such as povidone-iodine, chlorhexidine, and hydrogen peroxide should be avoided if possible. Hydrogel or hydrocolloid wound gels may be applied to the wound underneath a clean dressing. Dressings should allow mobility of the leg and avoid constriction and loss of circulation.

Systemic antibiotic therapy is usually required over a period of months. Ciprofloxacin or enrofloxacin are broad-spectrum antibiotics which do not result in dysbiosis. Analgesia may be required in some cases; buprenorphine at 0.03–0.05 mg/kg may be used up to three times daily (Brown, 2008). Treatment for pododermatitis generally requires several months, and periodic assessment is required. Elimination of any unsanitary practices, provision of dry, clean litter, and soft flooring is required for successful resolution.

Tumors of the Integument and Mammary Glands

The skin and subcutaneous tissues, after the respiratory system, are the second most common site for neoplasia in the guinea pig. In all reports, trichofolliculoma, a benign neoplasm arising from the epithelium of the pilosebaceous unit, is the most common (Tuch and Tuch, 1979; Zwart et al., 1981). These expansile neoplasms often arise in the lumbosacral area, and grossly are firm nodules which may have a central pore. Microscopically, the neoplasms are composed of a central cystic follicle with smaller variably well-differentiated follicular structures radiating from it in an arborizing pattern. Traumatized neoplasms may evoke a profound inflammatory response if keratin debris is extruded into the surrounding dermis. Surgical excision is curative.

Other cutaneous neoplasms are reported to be common, including fibrosarcoma (Steele, 2001), lipoma, sebaceous gland adenoma, and hemangioma (Hammer et al., 2005).

Several mammary gland neoplasms have been reported in guinea pigs, with benign fibroadenomas being the most numerous. These neoplasms are composed primarily of proliferating glandular tissue and large amounts of fibrous connective tissue. Approximately 30% of reported mammary neoplasms in guinea pigs are classified as mammary adenocarcinomas; most of these neoplasms were interpreted to be of duct origin (Greenacre, 2004). An unusually high incidence of adenocarcinoma in male guinea pigs has been reported (Andrews, 1976). As mammary adenocarcinomas have been reported to metastasize to the lung, preliminary workup should include a thoracic X-ray (Greenacre, 2004).

SPECIAL SENSES

Cataracts

Cataracts have been reported in guinea pigs of all ages on well-balanced rations. In strain 13/N guinea pigs, a mutation in the gene for lenticular zeta-crystallin results in juvenile cataracts as early as 13 weeks of age (Bettelheim et al., 1997). Diabetes mellitus and decreased levels of dietary tryptophan (Wagner, 1976) have been theorized to cause cataracts in this species. The guinea pig has been often used as an animal model for cataract research induced by UVB radiation (Hockwin et al., 1999) and galactosamine (Kosegarten and Maher, 1978).

Ocular Dermoid

Ocular dermoids have been reported in two unrelated outbred guinea pigs (Wapplier et al., 2002). The

dermoids consisted of dermal and epidermal elements, including stratified squamous epithelium, hair follicles, smooth muscle, and fatty mesenchymal elements. Both were removed by superficial keratectomy and corneal debridement.

Osseous Metaplasia of the Eye

Heterotopic bone, similar to that seen in the lungs, may appear in other tissues as well, including the eye. Well-differentiated bone has been reported in the ciliary body of a guinea pig (Donnelly and Brown, 2002).

Conjunctival Eversion (“Pea Eye”)

This condition, colloquially referred to as “pea eye,” is a focal eversion of the conjunctiva. This condition appears grossly as small white-to-pink extrusions of conjunctiva, usually on the lower lid. In the absence of corneal abrasion or obvious infection, treatment is not usually required. Large eversions of conjunctiva may be removed by laser therapy.

Ototoxicity

A number of drugs have been reported to cause ototoxicity in the guinea pig; most of these compounds are known for causing ototoxicity in other species. Topical gentamicin (Conlon et al., 1998), otic suspensions of neomycin and polymyxin B (Perry and Smith, 1996), and cisplatin (Saito et al., 1997) have all been reported to cause ototoxicity in experimental applications.

HEMATOLYMPHATIC SYSTEM

Foa-Kurloff Cells

One of the unusual features of the immune system of the guinea pig is the presence of a unique line of white blood cells, known as Foa-Kurloff (or simply Kurloff) cells. These lymphocytes possess a single large, granular mucopolysaccharide cytoplasmic inclusion which displaces the nucleus, and can be visualized in large numbers in PAS-stained sections or impression smears of the spleen. While the true nature of the Kurloff cell is unknown, these cells have been shown to possess anti-neoplastic capability both in vitro and in vivo against leukemia cells, suggesting they function like null killer cells in other species. Some authors surmise that the presence of these cells may be responsible for the relatively low incidence of neoplasms documented in this species.

Kurloff cells are seen in greater numbers in adult female guinea pigs, and their numbers peak during

pregnancy or following estrogen stimulation in sows. They occur in high numbers within the placenta, and their secretions, toxic for macrophages, are thought to help modulate local immunity and prevent rejection of the fetal placenta.

In non-pregnant animals, Kurloff cells are seen in highest numbers in the splenic sinusoids and lining the stroma of the bone marrow and thymus, but may make up 4% of the circulating leukocyte population (Pilny, 2008). These cells are rarely seen before 3 months of age, and numbers are approximately the same in boars and non-gravid females.

Cavian Leukemia

Cavian leukemia is a spontaneous hematopoietic neoplasm that can occur in inbred strains of guinea pigs (2/N and 13/N) as well as non-inbred lines. This disease tends to arise in young adults under 3 years of age and has been proposed as a model for acute lymphoblastic leukemia in humans (Kaplow and Nadel, 1979). In one colony, over a 3-year period, the reported incidence was 0.02%. Type C virus particles have been identified in neoplastic lymphoid cells by electron microscopy, suggesting the possibility of a retroviral cause; however, similar particles have been noted in lymph node germinal centers from normal guinea pigs. A guinea pig herpes-like virus has also been isolated from lymphoid cells derived from leukemic guinea pigs in vitro (Nayak, 1971). Its role, if any, is also unknown.

Experimentally,avian leukemia is 100% transplantable in inbred strains usually by intraperitoneal or subcutaneous inoculation of fresh or frozen blood or tissue. However, in non-inbred strains, transmissibility decreases quickly with age (Kaplow and Nadel, 1979).

Affected animals are usually young adults and may present with either a generalized lymphadenomegaly and/or a leukocytosis (up to 18000mm^3) with a significant number of circulating lymphoblastic cells. At necropsy, in addition to enlarged peripheral lymph nodes, there is marked splenomegaly and hepatomegaly. Microscopically, there is diffuse infiltration of lymphoblastic cells in the liver, lung, spleen, bone marrow, thymus, gastrointestinal-associated lymphoid tissue (GALT), heart, eyes, and adrenal gland. The prognosis for this condition is grave and animals generally die within 4 weeks (Pilny, 2008). Reports on the responsiveness of this condition to traditional chemotherapy vary widely.

Spontaneous T-Cell Lymphoma

Spontaneous T-cell lymphoma has been reported in a guinea pig with marked ocular involvement as well as peripheral lymphadenopathy (Steinberg, 2000).

Neoplastic cells were immunopositive for CD-3, a marker for T-cells. No specific cause was identified.

MULTISYSTEMIC CONDITIONS

Metastatic Calcification

Metastatic calcification occurs occasionally in guinea pigs older than 1 year of age. While the true cause of this condition has not been elucidated, a number of reports suggest an imbalance between dietary magnesium, calcium, and phosphorus to be responsible, most commonly diets high in phosphorus and low in magnesium (Galloway et al., 1964). A 1968 study of 140 guinea pigs necropsied between 20 and 24 months of age in a closed breeding colony found that 75% of animals had calcification, most commonly in the kidney. Other common sites of calcification include the periarticular soft tissues, stomach, aorta, lung, trachea, liver, uterus, and heart (Sparschu and Christie, 1968). Clinical signs in affected animals are non-specific and include alopecia, poor thrift, and muscle stiffness.

Amyloidosis

Amyloidosis occurs occasionally in guinea pigs, usually in association with chronic inflammatory conditions such as bacterial pododermatitis (Taylor et al., 1971). Amyloid deposits are of the AA-amyloid type, which is deposited in the tissues in chronic inflammatory conditions and are most commonly seen in the kidney, spleen, and liver, but may occur in any organ. Microscopically, the deposits appear as a waxy, homogeneous, pink material primarily within the walls of blood vessels, but occasionally extending into the surrounding interstitium. Renal failure resulting from amyloid deposition has not been reported in the guinea pig.

Alpha-Mannosidosis

Naturally occurring cases of alpha-mannosidosis have been reported by Crawley et al. (1999). Two guinea pigs with stunted growth, progressive mental dullness, and abnormal behavior, posture, and gait were examined at necropsy. Widespread neuronal vacuolation was observed throughout the CNS, as well as vacuolation in the pancreas, thyroid, epididymis, and ganglia. Extracts of whole liver, leukocytes, fibroblasts, and plasma showed a deficiency of lysosomal (acidic) alpha-mannosidase activity. Urine and tissue extracts contained stored and excreted oligosaccharides normally seen in human patients. The authors suggested that this line of guinea pig might prove to be a valuable model for human alpha-mannosidosis.

Lethal White Syndrome in Guinea Pigs

Approximately 25% of the offspring of the mating of color-diluted roan or dalmation guinea pigs are homozygous for the spotted roan or "lethal factor." Homozygosity for this allele is linked to a constellation of developmental defects that is known as the "lethal white syndrome" among fancy guinea pig breeders. There is no relationship to a similar condition in white overo foals, in which the main defect is aganglionosis of the large intestine.

The lethal white syndrome in the guinea pig consists of various combinations of microphthalmia, anophthalmia, partial or complete blindness, partial or complete deafness, missing or deformed incisors and cheek teeth with elongated roots, intestinal malabsorption, and decreased immune responses.

Lethal white guinea pigs have a decreased lifespan, often dying during weaning as a result of infectious diseases. Anecdotal reports suggest that affected guinea pigs can live for 2–3 years with excellent care.

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Guinea Pigs as Experimental Models

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INTRODUCTION

The guinea pig, like many animals used in biomedical research today, first became popular to enthusiasts and fanciers. So popular were guinea pigs in England that even Queen Elizabeth I kept one as a pet (Endersby, 2007). This popularity fostered familiarity with its behavior, physiology, and suitable husbandry conditions, and this knowledge about care and husbandry subsequently allowed successful transition into laboratory housing, making it a popular research model. Over time, “guinea pig” became synonymous with experimentation.

The guinea pig first served as a useful animal model of disease in the late 1700s when Antoine Lavoisier, a French biologist and chemist, used the species in his study of respiratory physiology. In the 1800s, guinea pigs were used in studies of infectious disease, and seminal work on the role of certain vital amines (aka vitamins) in maintaining health was done in the early 1900s. Through time it was learned that, beyond the virtues of its relatively small size and docile nature, the guinea pig possesses anatomical and physiological features that make it uniquely suited to certain studies (Wright and Churg, 2002; Wright et al., 2008). For example, guinea pigs are useful in reproductive studies

because they and humans share similarities in placental structure, their gestation period of 68 days can be divided effectively into trimesters akin to the human condition, and they can develop pregnancy toxemia (Golden et al., 1980; Seidl et al., 1979).

For the 2007 fiscal year, the USDA reports that 207,257 guinea pigs were used in research (APHIS, 2007), a slight increase of approximately 1% over the previous year, but this represents an overall steady decline in use from the all-time high of 599,000 used in 1985 (Harkness et al., 2002). The most commonly used variety is the Hartley albino stock, but the inbred strains 2 and 13, and euthymic hairless stock are also used with some frequency. A principal reason for the overall decline in use is, not surprisingly, the increased use of mice as a favored animal model. The paucity of inbred guinea pig strains and the limited number of specific reagents for use with the species are two factors that generally limit the use of this species in research in the foreseeable future.

MODELS OF DISEASE

Hearing

The National Institutes of Health estimate that 17 million Americans suffer from some degree of hearing loss. Cochlear hair cell loss from any number of causes such as toxins, acoustic trauma, or infection is the most common cause of permanent sensorineural hearing loss in humans (Raphael, 2002). Subsequent to the loss of hair cells, pathologic changes in the associated type I and type II spiral ganglion nerve cells also occur through retrograde degeneration (Dodson, 1997). The experimental method most commonly used in guinea pigs to model this condition is chemical deafening. Chemical deafening can be achieved through the administration of aminoglycoside compounds such as kanamycin, gentamicin, and neomycin. These compounds have been shown to cause complete inner and outer hair cell death within hours after administration and variable degrees of ganglion neuron death over subsequent days and hence this method quite accurately reflects sensorineural hearing loss in humans (Dodson, 1997; Dodson et al., 1994; Dupont et al., 1993). The partial death of ganglion cells is important because the survival of some neurons is necessary in order to test cochlear implants as a mode of therapy.

The two routes of aminoglycoside administration used most commonly are subcutaneous injection or direct infusion into the perilymph through the cochlear round window requiring a surgical approach (Shibata et al., 2009; Su et al., 2008). Systemic chemical deafening has the advantage of technical simplicity and will

deafen bilaterally, but there is some risk of nephrotoxicity, and the deafening is often not complete. In contrast, direct application of aminoglycoside within the cochlea is technically demanding, but systemic toxicity is not a concern, and the lesion in the affected ear is more robust, i.e. deafening is complete.

In addition to the induction of total deafness through chemical means, it is possible to induce partial hearing loss through the mechanical creation of lesions in the organ of Corti (Dong et al., 2009). A surgical approach is required for this technique, in this case so that a micropipette can be inserted into the organ of Corti and puncture Reissner's membrane. This technique is useful to study conditions like high-frequency hearing loss which is only a partial loss of function.

Subsequent to inducing hearing loss, the animals can be used to study therapeutic modalities such as cochlear implants and gene-mediated therapies. Both of these commonly studied therapies involved surgical approaches that come with inherent potential for negative clinical sequelae. In particular, animals used in studies of gene therapy can experience transient loss of equilibrium manifested as a head tilt associated with the injection of the fluid carrying the viral vector into the cochlear space (Husseman and Raphael, 2009). There is also the potential for inflammatory reactions to the viral vector, particularly adenoviruses. This issue is addressed by using vectors "stripped" of the ability to produce many endogenous antigens so that they incite less reaction (Husseman and Raphael, 2009; Thomas et al., 2003). It is also important to bear in mind that animals exposed to the vector require special consideration by the Institutional Animal Care and Use Committee (IACUC) in conjunction with the institution's biosafety committee.

There are several ways in which hearing physiology can be assessed in studies of therapy. These include structural analysis of hair cells and neurons using electron and light microscopy and functional measures. A method commonly used to study function is the auditory brainstem-evoked response (ABR), which assesses brainstem electrical potentials elicited by sound (Husseman and Raphael, 2009; Shibata et al., 2009). To measure ABR, electrodes are placed subcutaneously, often in the neck region, and the animal is subsequently subjected to clicks of varying duration and intensity (Grindal et al., 2009). A higher ABR threshold value indicates poor hearing function. A simple and effective way to determine the presence of hearing function in a guinea pig is to evaluate for the Preyer reflex, which is the "pricking" of the ears in response to noise. Although fairly insensitive, testing for the presence of a reflex is a good screening tool to determine if animals are suitable for study (Orita et al., 2009). Finally, behavioral response

such as lever pressing has been described as another means of assessing hearing function (Su et al., 2008).

Toxicology

Guinea pigs are often used in developmental and reproductive toxicology (DART) studies and studies of contact hypersensitivity or dermatitis (Rocca and Wehner, 2009). The former takes advantage of the prenatal brain development and the substantial gestation length of 68 days in the guinea pig (Cudd, 2005), which can be effectively divided into trimesters akin to humans, and the robust type IV hypersensitivity exhibited by guinea pigs makes them useful in studies of the latter. Guinea pigs are also one of the species, in addition to mice, used in the General Safety Test for toxic contaminants in biological products as defined by the Food and Drug Administration (FDA) in the Code of Federal Regulations, Title 21 (FDA, 2009).

In DART studies, the impact of alcohol on the developing fetus is a fairly robust area for study because trimesters of gestation can be targeted and brain development is rapid throughout fetal growth (Cudd, 2005; Gauthier et al., 2009), which is in contrast to other rodents such as rats and mice. The model is useful in studying the array of alcohol's effects on the developing fetus, termed fetal alcohol spectrum disorders (Olmstead et al., 2009). The basis of the model is oral intake of ethanol by the pregnant dam at varying gestational time points and for varying durations dictated by the objective of the study. Oral exposure can be accomplished by provision of a 4% ethanol solution in the drinking water, or by oral gavage at a dosage of 4g per kg of bodyweight (Gauthier et al., 2005; Kimura et al., 1996). Control animals are typically given a sucrose solution that is isocaloric to the ethanol administered to ensure that food intake and bodyweight gain are comparable among cohorts in the study. In the authors' experience, if ethanol is administered in the drinking water, it is imperative to monitor fluid intake at the onset of the study to ensure adequate hydration as some animals do not readily drink the ethanol solution.

In studies of a test compound's potential to cause contact dermatitis, there are several protocols that have been developed over the past 50 years. These include the Draize, the open epidermal, and the split adjuvant tests, all of which are used infrequently at this time. The two tests that are used most commonly with guinea pigs are the Buehler test and guinea pig maximization (MAX) test, also called the Magnusson-Kligman test, which were first described in the 1960s (Buehler, 1965; Magnusson and Kligman, 1969; Maurer, 2007; Weaver et al., 2003).

The Buehler test, the regimen most commonly used in the United States and the MAX test, often used in

Europe, are similar in many respects. It is recommended that animals to be used in the test are between 300–450 grams in bodyweight at the beginning of the study (Maurer, 2007). Both regimens include an induction phase where test subjects are exposed to the test compound repeatedly, typically in the scapular region following depilation of the area. Additionally, a bandage or occlusive material is used to enhance the absorption of topically applied agents. The principal difference between the two models is that the Buehler test uses topical administration typically repeated three times, while the MAX test includes intradermal injections of the compound combined with Freund's complete adjuvant (FCA) in an effort to reduce the number of false-negative reactions thereby increasing the sensitivity of the test (Maurer, 2007). Following the induction protocol, animals are exposed or challenged with a single, topical application of the compound in question. The degree of reaction is then scored on parameters such as erythema and swelling and can be compared to control compounds such as a negative control carrier vehicle and a positive control such as dinitrochlorobenzene (Tang et al., 2008).

In the General Safety Test, healthy guinea pigs weighing less than 400 grams must be used. Mice are also used. This test is required for all biological products administered to humans with some exceptions as put forth in CFR 21. Briefly the test is conducted by injecting at least two animals intraperitoneally with the test compound. Those animals are then observed for any untoward effects such as weight loss or death for 7 days, at which time the tested product is reported to have passed or failed the test. For additional details, the reader is directed to the statute (FDA, 2009).

The use of guinea pigs to study the toxic effects of other compounds are numerous and varied, and a complete discussion of those studies is beyond the scope of this chapter. Similar to their use in DART studies of alcohol, the effects of various agents such as cadmium (Trottier et al., 2002) or dioxin (Kransler et al., 2007) on the fetus are reported in the literature as well as the toxicity of xylene (Gagnaire et al., 2007) and dental resin (Seiss et al., 2009) on adult animals, to name a few. Looking forward, guinea pigs could become popular models for cutaneous toxicokinetic studies as it was recently reported that they exhibit dermal permeability properties nearly identical to those of humans (Barbero and Frasch, 2009).

Allergic Diseases

Guinea pigs were one of the first species to be used in studies of allergic conjunctivitis and remain popular for such studies. Allergic conjunctivitis, particularly the seasonal (more appropriately termed "intermittent")

variety, a type I hypersensitivity reaction is a very common condition affecting the human population (Sanchis-Merino et al., 2008). Beyond its designation as either seasonal or persistent, allergic conjunctivitis is further classified as either IgE-mediated or non-IgE-mediated, and guinea pigs are suitable models for both (Groneberg et al., 2003).

In the study of IgE-mediated disease, an active immunization protocol is typically followed. Animals are often sensitized to an antigen such as ovalbumin (OVA) using intraperitoneal injections (Groneberg et al., 2003; Meijer et al., 1996), although there are also studies using ragweed and Japanese cedar extracts (Merayo-Llves et al., 1995; Takada et al., 2000), which represent the allergens to which humans are routinely exposed in the environment. Subsequent to sensitization, the same antigen is instilled into the eye eliciting a robust inflammatory response in the tissues of the eye that can be evaluated as early as 30 minutes post-challenge. Endpoints examined can include clinical manifestations such as eyelid edema and ocular discharge, the nature and degree of cellular infiltration with eosinophils, and the level of inflammatory mediators such as substance P that are present. Once the model is induced, the effectiveness of therapies such as topical antihistamines can be investigated (Sanchis-Merino et al., 2008). An extension of these studies would include the examination of therapeutic effectiveness. Within this experimental framework it should be noted that highly immune-responsive (IMM/S) and a less immune-responsive (IMM/R) inbred strains have been developed and characterized (Lundberg et al., 1987). Studies of non-IgE-mediated disease are performed in much the same way except that a hapten is used to sensitize instead of a protein antigen (Saiga et al., 1992).

The guinea pig has recently been shown to be a valid model of allergic rhinitis, closely mimicking the clinical symptoms and pathology seen in the human condition. The methods employed are quite similar to those used in studies of allergic conjunctivitis and asthma and involve sensitization to an antigen such as OVA (Bahekar et al., 2008; Brozmanova et al., 2008), with subsequent exposure to the antigen. The principal differences are that the challenge exposure is via nasal instillation and that the endpoints studied such as degree of rhinorrhea reflect the symptoms of the disease.

Other allergic conditions for which guinea pigs have been used include food allergy, with most studies addressing cow's milk protein allergy (Helm and Burks, 2002; Kitagawa et al., 1995; Piacentini et al., 1994). Their use in such studies is limited however by their IgG response profile (Fritsche, 2003), which does not very accurately reflect the IgE-mediated disease in humans, making other species such as the mouse, dog, and pig more suitable (Helm and Burks, 2002).

Non-Infectious Respiratory Disease: Asthma and COPD

The guinea pig has been used extensively as a model of human asthma, airway hypersensitivity (Zosky and Sly, 2007), and other diseases that affect the airway. Airway anatomic features that make the guinea pig a good model of respiratory disease include abundant lung tissue, three major lobes in the right hemithorax and two in the left hemithorax, well-defined terminal bronchioles, alveoli that are nearly fully developed at birth, and a well-innervated trachea (Brewer and Cruise, 1997; Wright and Churg, 2002). Functionally, the guinea pig airway is quite reactive to certain inhaled compounds, and they exhibit a cough reflex similar to humans, attributes that have been useful in elucidating the mechanisms controlling cough (Canning and Chou, 2008; Oh et al., 2006).

Asthma is defined as a chronic inflammatory condition of the airway that compromises air flow resulting in clinical manifestations such as wheezing and coughing. The disease affects approximately 17 million Americans (Braun et al., 2008) and 300 million people worldwide (Bahadori et al., 2009). Models developed to study asthma hinge on a common theme, sensitization of the airway with compounds that produce a robust response. One method used to sensitize the airway is by exposing a guinea pig to OVA (Angeli et al., 2008; de Vries et al., 2006; Tohda et al., 2000). In this model, the animals receive repeated injections of OVA combined with an adjuvant such as aluminum hydroxide intraperitoneally (Muraki et al., 2008). The schedule of injections can vary, but a typical schedule is an initial injection repeated at days 2 and 21 (Muraki et al., 2008) or some variation on that schedule (Mokry et al., 2008). After undergoing this regimen, the animal is considered hypersensitized. These hypersensitized animals are then exposed to or "challenged" by OVA administered via nebulization, which induces a robust airway type I allergic and inflammatory response in the airways similar to what is observed in humans experiencing symptoms associated with asthma. As part of these studies, it is common to evaluate cellular infiltrates and tissue reactions using bronchoalveolar lavage and histopathology. The mechanical properties of affected airway smooth muscle such as contractility are also of interest and are evaluated following post-mortem tissue harvest (Angeli et al., 2008; Chitano et al., 2008). The ultimate goal of most studies is to determine therapeutic effectiveness of test compounds, with much attention paid to regulating mediators of the disease process such as leukotrienes, prostaglandins, and nitric oxide (Angeli et al., 2008; Canning and Chou, 2008).

Guinea pigs are also used to study chronic obstructive pulmonary disease (COPD), an inflammatory

condition that affects the airway much like asthma and is estimated to affect up to 10% of the human population (Wright et al., 2008). Like asthmas, several inflammatory mediators promote COPD, but in contrast COPD is a progressive disease leading to death in 30–70% of those diagnosed as a result of diminishing lung capacity (Seamark et al., 2007). The four anatomic lesions characteristic of COPD include emphysema, small airway remodeling, pulmonary hypertension, and excessive large airway mucus secretion (Churg and Wright, 2009). The most significant cause of COPD in the human population is smoking cigarettes. Exposure to cigarette smoke is one way to induce a COPD-like syndrome in guinea pigs that exhibits lung pathology mimicking in some ways that seen in humans, particularly small airway remodeling (Canning and Chou, 2008; Churg and Wright, 2009). Various inhalation chamber configurations are used to expose the animals, and protocols can range from acute exposure as short as 10 minutes to chronic exposure that lasts for months (Wright and Churg, 2002). In humans, chronic or latent respiratory viral infection is thought to exacerbate or cause COPD. Guinea pigs have been latently infected with adenovirus 5 prior to cigarette smoke exposure to model this condition, and they exhibit cellular and molecular changes similar to those seen in humans under similar conditions (Hogg, 2001; Vitalis et al., 1998).

Reproduction and Pregnancy-Related Diseases

Guinea pigs share many reproductive traits with humans and hence are used extensively in studies of reproductive physiology, anatomy, and endocrinology. Like humans, guinea pigs have a hemomonochorial placenta, trophoblast invasion patterns, and uterine vasculature akin to humans (Lee and DeMayo, 2004; Mess et al., 2007; Valdes et al., 2008). There is substantial evidence suggesting that guinea pigs can serve as suitable models of placental antibody transfer (Pentsuk and van der Laan, 2009), and genes encoding the proteins such as vascular endothelial growth factor (VEGF) and insulin-like growth factor (IGF) show expression patterns that are similar to humans (Grant et al., 2005; Valdes et al., 2008). Notable structural differences include the presence of a subplacenta in the guinea pig, a structure which seems to have no human counterpart (Carter, 2007; Oliveira et al., 2006) and a labyrinthine instead of the villous structure observed in humans (Mess, 2007).

Because of their gestational length and rapid brain development in utero, guinea pigs are the best rodent species for use in studies of chronic maternal stress and the effects of glucocorticoid administration on the dam, development of the neonate, and conditions such as chorioamnionitis that might impact brain development in utero (Emack et al., 2008; Kapoor and Matthews,

2008; Kapoor et al., 2009; Kemme et al., 2008; Owen and Matthews, 2007; Patrick et al., 2004; Setiawan et al., 2007; Sohlstrom et al., 1998). The long gestation period allows chronic, repeated or acute and precisely timed exposure (e.g. during maximal brain development at 50 days gestation) to various stressors such as food restriction, noise and light, and unfamiliar conspecifics with subsequent measurement of growth patterns and hormonal responses. These studies have shown that maternal glucocorticoid administration, commonly used in humans to promote lung development in at-risk pregnancies, can have profound effects on the hypothalamic–pituitary axis of the offspring. Guinea pigs are also considered by many to be the best non-primate model of hormonal control of parturition, in particular because their progesterone levels closely mirror those of humans during pregnancy (Mitchell and Taggart, 2009; Rodriguez et al., 2008; Welsh et al., 2005). Lastly, guinea pigs have been used somewhat infrequently to model intrauterine growth restriction (IUGR). To study IUGR as a result of compromised uterine blood flow, the uterine arteries or branches are surgically ligated with subsequent monitoring of fetal growth using imaging such as ultrasound (Turner and Trudinger, 2009).

Osteoarthritis

Osteoarthritis (OA) is the most common joint disease in humans, with 68% of adults showing some degree of the disease (Elders, 2000). The disease is progressive, involving all joint structures and is associated with distinctive histological changes in joint tissues, most notably cartilage loss (Bowyer et al., 2009). Guinea pigs have served as valid models, both spontaneous and induced, of the disease. Dunkin-Hartley guinea pigs, particularly males, develop notable, consistent degenerative lesions primarily in the medial tibial aspect of the stifle joint beginning around 3 months of age, and these lesions progress in severity with aging, becoming substantial by 9 months of age (Anderson-MacKenzie et al., 2005; Bendele et al., 1989; McDougall et al., 2009; Quasnichka et al., 2005). The lesions resemble those seen in humans with excellent fidelity with the exception of osteochondral and synovial blood vessel formation, which is less severe in the guinea pig compared to humans (Jimenez et al., 1997; Mapp et al., 2008). It has also been shown that Strain 13 and Bristol strain-2 can serve as suitable control strains as OA lesions are insignificant or absent respectively, and they express lower levels of pro-inflammatory biomarkers in synovial fluid and serum (Anderson-MacKenzie et al., 2005; Huebner and Kraus, 2006; Huebner et al., 2002; Quasnichka et al., 2005).

To induce disease, the most commonly performed procedure is a partial medial meniscectomy (Bendele, 2001; Pastoureaux et al., 2003). This procedure is easily

reproducible and the resultant OA lesions are consistent and develop by 1 month post-surgery with lesions becoming quite severe within 3 months (Bendele, 2001; Bolbos et al., 2008). As a non-surgical model, intracapsular injections of sodium mono-iodoacetate have also been used to induce chondrocyte death. A single injection, or two injections separated by 24 hours, is sufficient to induce lesions (Bendele, 2001; Vermeirsch et al., 2007). The clear advantages of both induced models over the spontaneous are the rapidity with which lesions form, and the fact that the contralateral joint can be used for comparison.

Atherosclerosis

Atherosclerosis is a condition that affects approximately 57% of the population between 40 and 59 years of age with an increased incidence associated with age (Kiechl and Willeit, 1999). It is considered a type of arteriosclerosis affecting large arteries characterized principally by endothelial damage with subsequent inflammation and lipid plaque (atheroma) formation (Badimon et al., 2009). Several risk factors such as elevated cholesterol and low-density lipoproteins, have been identified. While they are not used as commonly as some other species to study atherosclerosis, guinea pigs have unique attributes that make them well-suited to studies of this disease. Guinea pigs have triacylglycerol, lipoprotein, and cholesterol profiles that are influenced by gender, diet, and exercise in the same ways that are observed in humans (Ensign et al., 2002; Roy et al., 2000). The most unique feature of guinea pigs is that the majority of their cholesterol is present as low-density lipoprotein, which is not seen in most other animal species (Fernandez, 2001; Fernandez and Volek, 2006). Atherosclerosis is being recognized as a chronic inflammatory condition in humans, and guinea pigs exhibit a pro-atherogenic inflammatory profile in arteries when fed a high-cholesterol diet (Fernandez and Volek, 2006).

Infectious Disease

Introduction

Much of this chapter focuses on the common contemporary uses of guinea pigs in infectious disease research. Guinea pigs have provided significant contributions to research for some diseases for which they are no longer used, and these diseases are not covered in this chapter. For example, the guinea pig was a classic model for the study of *Treponema pallidum*, the causative agent for syphilis. This model was popular in the 1980s and 1990s, but no studies have been published using this model since 2001. Guinea pigs have also been used sporadically for other infectious diseases that are

beyond the scope of this chapter. Readers are referred to Padilla-Carlin et al. (2008) for a review of bacterial infections that have been studied utilizing guinea pigs, including syphilis. Guinea pigs have also been used for investigations of *Coxiella burnetii* (Aboudharam et al., 2000; Elliott et al., 1998; Heggers et al., 1975; Moos and Hackstadt, 1987; Russell-Lodrigue et al., 2006; Wilhelmsen and Waag, 2000), fungal infections with *Cryptococcus* (Carroll et al., 2007; Kirkpatrick et al., 2007) and parasite infections such as toxocariasis which are not included in this chapter (Faccioli et al., 1996, 1997, 1998; Fenoy et al., 2001; Sa-Nunes et al., 2003).

Genital Herpesvirus Infections

Genital herpes can be caused by herpes simplex virus (HSV)-1 or HSV-2, but is most commonly caused by HSV-2. Genital herpes caused by infection with either virus infects approximately 20–30% of adolescents and adults in the United States (Quenelle et al., 2008b; Rose et al., 2008). Most infected people develop subclinical or mild signs that they do not recognize as genital herpes lesions, but HSV infection can result in blisters that become ulcers around the genitals and rectum. The disease recurs within weeks to months between outbreaks, and the number of outbreaks tends to decrease over time. Infection can be severe in immunosuppressed people and neonates. There is currently no vaccine or cure, but antivirals such as acyclovir can decrease outbreaks and reduce transmission. Research utilizing the guinea pig model of HSV infections has primarily focused on developing more effective treatment and vaccine strategies (Bernstein, 2001; Bernstein et al., 2000; Rose et al., 2008).

The majority of research on genital herpesvirus infections in guinea pigs has focused on HSV-2. Guinea pigs are highly susceptible to HSV-2 and the pathogenesis associated with this virus has been well characterized. Guinea pigs develop genital lesions similar to those in humans, and a review of the pathology of this virus in guinea pigs is available (Hsiung et al., 1984). Females are usually inoculated by introducing the virus directly into the vagina, but may also be infected by placing viral solutions externally near the genital skin (Bernstein and Stanberry, 1986). Following intravaginal inoculation, viral shedding peaks prior to the development of genital lesions, with lesions detectable 3–4 days after infection (Hsiung et al., 1984; Lukas et al., 1975; Stanberry et al., 1982). The lesions progress to pustules, ulcers, and crusts. Lesions typically heal by days 10–15 post-infection, but resolution may require up to 18–20 days (Bernstein, 2001; Fotouhi et al., 2008b; Hsiung et al., 1984; Krinke et al., 1975; Manservigi et al., 2005; Stanberry et al., 1982). The virus initially replicates in the vagina and then spreads via the peripheral nerves to the external genital area, resulting in the typical ulcerative

lesions. The virus also spreads to the dorsal root ganglia and spinal cord which can lead to a severe ascending myelitis (Bernstein, 2001; Hsiung et al., 1984; Krinke et al., 1975; Lukas et al., 1975; Stanberry et al., 1982). Male guinea pigs are less frequently used, but can be inoculated on the penis or thigh with lesions and clinical sequelae similar to those seen in females (Kobrinskii et al., 1991; Stephanopoulos et al., 1989). Infection in neonates has rarely been used and is associated with higher mortality (Mani et al., 1996). Attempts at transplacental transmission have not been successful (Hsiung et al., 1984).

The HSV virus becomes latent in the dorsal root ganglia and spontaneously reactivates to cause recurring genital lesions (Hsiung et al., 1984; Landry and Bull, 1992; Rose et al., 2008; Scriba, 1976, 1981; Simms et al., 2000; Stanberry et al., 1982, 1985). Animal studies may last for 35–120 days in order to evaluate for recurrent disease. Recrudescence can be induced by UV radiation (Bratcher et al., 1993; Harrison et al., 1991; Stanberry, 1991). As in humans, virus can be isolated from the genital tract in between outbreaks. Inbred strain 2 and Hartley guinea pigs require the same dose to become infected, but strain 2 guinea pigs are less likely to develop initial and recurrent disease than Hartley guinea pigs (Fowler et al., 1992; Hsiung et al., 1984). Even within a guinea pig strain, infection and the incidence of lesion recurrence can be variable in animals from different sources or shipments (Hsiung et al., 1984). Parameters evaluated in these studies include genital lesion scores, incidence of recurrent lesions, the incidence of clinical sequelae and recovery of the virus from the dorsal root ganglia and genital swabs (Bernstein and Stanberry, 1986; Bernstein et al., 2000; Harrison et al., 2001; Heineman et al., 2004; Quenelle et al., 2006; Stanberry et al., 1982, 1989).

The clinical sequelae associated with HSV infections in guinea pigs can be severe. The neurologic side effects can include urinary retention, hindlimb paralysis, and colonic stasis between 5 and 10 days after infection. These neurologic effects often resolve 5–10 days later if animals recover from the infection (Stanberry et al., 1982; Thornton et al., 1984). Other clinical sequelae that can occur with the initial disease include anorexia, weight loss, mild fever, secondary bacterial and yeast infections, encephalitis and death (Hsiung et al., 1984; Lukas et al., 1975; Manservigi et al., 2005; Stanberry et al., 1982; Thornton et al., 1984). Primary disease is more severe than recurrent disease, and supportive care should be considered even if death is not used as an endpoint (Thornton et al., 1984). Urinary retention should be assessed and relieved by pressing on the abdomen to empty the bladder. Although virulence varies with viral strain, the severity of lesions is primarily affected by the dose of virus used and most

strains that are commonly used can be associated with high mortality (Heineman et al., 2004; Hsiung et al., 1984; Landry et al., 1982; Prichard et al., 2005; Pronovost et al., 1982; Stanberry et al., 1982).

Guinea pigs have been inoculated with HSV-2 and HSV-1 to evaluate host responses and factors that influence pathogenesis of genital herpesvirus infections. This model has been used to evaluate the host immune response to infection including local and systemic cell-mediated immunity (Stanberry, 1991). The immune response and disease progression have been evaluated with HSV-2 genital reinfection, following previous genital infections with HSV-2 or HSV-1 or oral infections with HSV-1 (Bernstein et al., 1989; Hoshino et al., 2009; Landry and Zibello, 1988; Stanberry et al., 1986). These models reflect situations that commonly occur in humans, and reinfection is usually associated with decreased lesion severity relative to infections in naïve animals. This model has also been used to look at the pathogenesis associated with acyclovir-resistant viral strains (Bernstein et al., 2000). The differences in pathogenesis between HSV-1 and HSV-2 have been studied because both can cause genital infection, but they differ in their efficiency of reactivation that causes recurrent disease. HSV-1 reactivates more readily from trigeminal ganglia, while HSV-2 reactivates more readily from dorsal root ganglia (Bertke et al., 2007). The genetic factors that affect latency and reactivation have been studied, including creating mutant viruses or HSV-1 and HSV-2 chimeras (Bertke et al., 2007; Blakeney et al., 2005; Bourne et al., 1994; Burke et al., 1991; Krause et al., 1995; Lekstrom-Himes et al., 1998; Wang et al., 1997; Yoshikawa et al., 1996).

Guinea pigs have been used extensively in evaluating treatments for genital HSV infections. Acyclovir is a nucleoside analog that is commonly used for the management of HSV infections. It has been tested in both adult and neonatal guinea pigs by a variety of routes, including oral, topical, intramuscular, and intraperitoneal (Bravo et al., 1996; Ellis and Barry, 1985; Hsiung et al., 1984; Kern, 1982; Landry et al., 1982; Pronovost et al., 1982; Stanberry et al., 1990). Although these studies primarily focus on the treatment of HSV-2, acyclovir treatment for genital HSV-1 infections has also been described (Myerson and Hsiung, 1983). Because some viral strains have developed resistance to acyclovir, research has also focused on other treatments including acyclovir derivatives, other nucleoside analogs, other antivirals, and non-steroidal anti-inflammatories (Bratcher et al., 1993; Bravo et al., 1993; Jennings et al., 1999; Kern et al., 2000; Kleymann et al., 2002). This has included evaluating topical microbicides in their ability to prevent or reduce disease transmission (Bernstein et al., 2003; Bourne et al., 1999a, 1999b; Chen et al., 2004). In addition to resistance, antivirals also have

limitations in that recurrence occurs at previous levels once the medications are discontinued (Bernstein, 2001). These limitations have led to the development of immunotherapy strategies with vaccines or immunomodulators that could prolong the therapeutic effects of standard treatments (Bernstein, 2001; Bernstein et al., 1993, 2001; Bourne et al., 2002; Burke, 1991; Fujihara et al., 1989; Harrison et al., 1991, 2001; Hoshino et al., 2005; Manservigi et al., 2005; Miller et al., 1999; Pyles et al., 2002; Quenelle et al., 2008b; Rose et al., 2008; Wachsmann et al., 2001).

Guinea pigs have also been used extensively for studying vaccines to protect against genital HSV-2 challenge. Because treatment options are limited, vaccine development has been a large segment of the research related to HSV. Parameters measured often include the presence of neutralizing antibodies, cellular immunity, infection rates, virus replication in the genital tract, lesion severity, lesion recurrence, and virus isolation from the dorsal lumbosacral ganglia. Vaccinations with HSV glycoproteins have been the focus of the research, and are administered by various strategies including protein subunits, recombinant glycoproteins, and in plasmid expression vectors (Berman et al., 1985; Bourne et al., 1996, 2003, 2005; Bowen et al., 1992; Burke, 1991; Byars et al., 1994; Erturk et al., 1991; Fotouhi et al., 2008a, 2008b; Hoshino et al., 2005, 2009; McClements et al., 1996, 1997; Mester et al., 1999; O'Hagan et al., 1999; Quenelle et al., 2006, 2008a; Simms et al., 2000; Stanberry, 1991; Stanberry et al., 1987, 1990). Recombinant viral vectors expressing HSV glycoproteins have also been used as a vaccine, including vesicular stomatitis virus, varicella-zoster virus, and vaccinia virus (Bernstein et al., 2000; Heineman et al., 1995, 2004; Natuk et al., 2006). Another common strategy is to utilize live virus vaccines attenuated by genetic manipulation (Boursnell et al., 1997; Da Costa et al., 1997, 2001; McLean et al., 1994; Morrison et al., 1998; Prichard et al., 2005; Quenelle et al., 2008b; Spector et al., 1998; Wachsmann et al., 2001). Although most vaccines are administered by the subcutaneous or intramuscular route, subunit vaccines have also been evaluated by alternative routes including vaginal and nasal administration. These alternative routes have had variable responses relative to the traditional routes for vaccination (Bernstein 2000; Bowen et al., 1992; Hoshino et al., 2005; Morrison et al., 1998; O'Hagan et al., 1999). Optimizing the adjuvants included in the vaccine preparation is also crucial for efficacy, and a variety of adjuvants have been used (Berman et al., 1985; Bourne et al., 2003; Bui et al., 1994; Ho et al., 1992). Many of these vaccines have had efficacy in decreasing primary disease and recurrence, but have variable results in the ability to reduce viral shedding and incidence of infection. The degree of protection for some vaccines against primary disease correlates with the

humoral immune response, but this has not correlated with protection in human vaccine trials (Prichard et al., 2005; Simms et al., 2000; Stanberry 1991). Vaccines have also been evaluated in animals that had been exposed to HSV-1 prior to HSV-2 challenge because a glycoprotein vaccine was partially efficacious in HSV-1-seronegative but not -seropositive women (Hoshino et al., 2009).

Cytomegalovirus

Human cytomegalovirus (CMV) is a Betaherpesvirus that is distributed worldwide. It is the most common congenital infection and occurs in 0.5–2% of all births (CDC, 2006; Kern, 2006; Schleiss, 2006). In the United States, the majority of adults are infected by the age of 40 because people do not clear the infection (CDC, 2006). Although most infections are asymptomatic, the virus can cause both temporary disease and permanent disabilities in newborns including decreased birth weight, purpura, seizures, and liver damage (CDC, 2006; Levinson, 2008). Permanent disabilities often involve the nervous system and sensorineural hearing loss is a common lesion with congenital infection, but it can also result in mental retardation, vision loss, and death (CDC, 2006; Schleiss, 2006). CMV can also cause serious complications in immunocompromised adults, such as HIV-infected patients and transplant recipients. These infections can become systemic leading to one or multiple conditions, including pneumonia, CNS disease, hepatitis, enteritis, and retinitis (Booss and Kim, 1989; Levinson, 2008). In healthy adults, CMV rarely causes a temporary mononucleosis that is accompanied by fever and lethargy (Levinson, 2008).

The guinea pig model relies on a host-specific CMV because human CMV will not infect other species of models of this infection, including the guinea pig (Barry et al., 2006; Bia et al., 1982, 1983; Reddehase et al., 2008; Schleiss, 2005; Yue and Barry, 2008). Naturally occurring guinea pig cytomegalovirus (GPCMV) has historically been called salivary gland virus because it is primarily found in the salivary gland, but inclusions may be found in other organs as well (Van Hoosier, 1976). Natural infection of guinea pigs is usually subclinical, but reports of clinical disease do exist (Pappenheimer and Slanetz, 1942; Van Hoosier et al., 1985). Experimental studies in guinea pigs typically use a strain isolated from the salivary glands of guinea pigs infected by Hartley in 1957 (Schleiss, 2006). GPCMV was first described in 1920, and early studies characterized the nature of intranuclear and intracytoplasmic inclusions, pathogenicity, and transmission of the virus (Bia et al., 1983). Research efforts were later focused on using this model for the development of prevention and treatment options in humans, which remain the primary goals of current research. Although CMV vaccines have been tested in humans, at the time of this writing there is no

effective vaccine for preventing congenital infections. In addition, the treatments that are currently used for CMV infections have significant side effects, may require long-term therapy, and are not adequate for drug-resistant strains. These drawbacks could be particularly dangerous for pregnant women and immunocompromised patients, therefore more options are needed (Kern, 2006). Guinea pigs are preferable to other rodent models for congenital infection because the virus crosses the placenta in guinea pigs, similar to its pathogenesis in humans. Guinea pig models of congenital disease have primarily been used to test vaccines, but are also used to test treatments. Guinea pigs may also be used as models for disseminated infection to simulate disease in immunocompromised patients. More recently, the complete viral genome of GPCMV has been sequenced, and recent studies have focused on genetic manipulations of GPCMV (Schleiss, 2002; Schleiss et al., 2008b; Schraff et al., 2007; Yamada et al., 2009). These genetic studies could contribute to understanding the similarities between guinea pig and human CMV, pathogenesis, vaccine development, and treatment.

The guinea pig model of congenital CMV infection usually involves inoculation of the dam with GPCMV during pregnancy. Early studies characterized the distribution of infection and outcomes in congenitally infected pups and established that the disease is similar to that seen in human newborns. The outcomes of congenital infection in the pups can be variable, and include labyrinthitis and auditory defects, CNS damage, disseminated disease, and death (Griffith et al., 1982, 1986; Katano et al., 2007; Kern 2006; Schleiss 2006; Woolf et al., 1989).

A common protocol for current vaccine studies is vaccination of a young, Hartley guinea pig prior to mating, followed by CMV inoculation in the late second or third trimester. This timeframe for inoculation is chosen to increase the chance that infection will result in newborn mortality instead of fetal resorption (Kern, 2006). Parameters that are evaluated for experimental outcomes include maternal viremia, maternal immune response, maternal weights, maternal mortality, pup viremia, pup mortality, congenital transmission rates and infection distribution in the pups (Crumpler et al., 2009; Schleiss et al., 2004). A review of vaccine developments in this model is available (Schleiss, 2008). This model has been used to test many vaccine strategies including live attenuated, subunit vaccines and DNA vaccines (Bia et al., 1980, 1982; Crumpler et al., 2009; Schleiss et al., 2004).

Some studies of congenital infections of GPCMV rely on modes of infection other than inoculation of the mother during pregnancy. Treatment options are difficult to evaluate in congenitally infected newborns because of the high mortality rates. Newborn guinea

pigs have been inoculated IP within 48 hours of birth to test antivirals, although mortality is still considerable at 50% (Bravo et al., 2003; Kern 2006). Viral labyrinthitis can be replicated by intracochlear or intrathecal injections of newborns (Woolf et al., 1985). The model of intracochlear injections has also been used in weanling guinea pigs (200–350 g) to study the pathogenesis and potential treatments of CMV-associated hearing loss (White et al., 2006).

The severity of disease and mortality associated with GPCMV depends on multiple factors, including animal strain, pregnancy status, and viral dose. Pregnant animals are more susceptible to disease than non-pregnant, and mortality can also vary depending on when during pregnancy the animal is infected (Griffith et al., 1982). Hartley guinea pigs are the most commonly used guinea pigs for GPCMV studies, but they are less susceptible to infection than the inbred strain 2 guinea pigs that can have high mortality even as adults. Mortality will also vary depending on the dose of the virus used, but mortality is lower in Hartley guinea pigs than strain 2 even at the same dose (Bia et al., 1980; Griffith et al., 1986). For example, one study demonstrated that a dose of virus that caused 50% mortality in strain 2 guinea pigs resulted in only 15% mortality in Hartley guinea pigs (Bia et al., 1980; Woolf et al., 1989).

Because of their decreased susceptibility to disease, non-pregnant Hartley guinea pigs have been used as a model of mild disease that is associated with mononucleosis, as can occur in immunocompetent adult people. Subcutaneous inoculation in 1–2-month-old Hartley guinea pigs results in hematopoietic and lymphoid changes similar to human CMV mononucleosis (Griffith et al., 1981). This model of mononucleosis has also been used to evaluate treatment (Lucia et al., 1984).

The increased susceptibility of strain 2 guinea pigs provides a good model of the disseminated CMV infection that can occur in immunosuppressed people. Strain 2 guinea pigs can develop generalized infection, including common features of the disease in humans such as pneumonia (Bia et al., 1982). Strain 2 guinea pigs develop generalized infection within 5–17 days of inoculation, with 44–100% mortality 8–13 days after inoculation (Bia et al., 1982; Fong et al., 1983). The low end of the mortality range does not include animals that are euthanized due to a moribund condition (Bia et al., 1982).

In addition to strain 2 guinea pigs, other guinea pig models have been utilized for diseases associated with disseminated CMV infection. Disseminated disease can be reproduced in Hartley guinea pigs with the administration of immunosuppressive agents. Cyclophosphamide can increase mortality associated with infection to 87% by 19 days after infection (Schleiss et al., 2008a). Young Hartley guinea pigs (8–18 days old)

are more susceptible than adults, and may also be used as a model of generalized CMV (Connor and Johnson, 1976). Others have developed a model of CMV encephalitis, a complication associated with AIDS, using intracerebral inoculation (Bia et al., 1983; Booss and Kim 1989; Booss et al., 1990). Although not frequently used, intratracheal administration of the virus can produce a pneumonia, which is a common clinical feature of disseminated disease in humans (Bia et al., 1983).

Hemorrhagic Fevers

BACKGROUND

Viral hemorrhagic fever viruses are a group of RNA viruses belonging to the families Arenaviridae, Bunyaviridae, Filoviridae, and Flaviviridae. Of the arenaviruses that cause hemorrhagic fevers, guinea pigs have primarily been used as models for Lassa fever and Argentine hemorrhagic fever (Junin virus). They are less commonly used to study Venezuelan hemorrhagic fever (Guanarito virus) and Bolivian hemorrhagic fever (Machupo virus) (Hall et al., 1996; Khmelev et al., 2009; Tesh et al., 1994). Guinea pigs have also been used as models for the filoviruses, Marburg and Ebola. A few studies also utilized guinea pigs to research the bunyavirus that causes Crimean-Congo hemorrhagic fever, primarily focusing on transmission by the tick vector (Dohm et al., 1996; Gordon et al., 1993). The hemorrhagic fever viruses can cause severe disease and mortality in humans. Early clinical signs include flu-like symptoms that can progress to polysystemic signs, hemorrhage, and death. Filoviruses cause sporadic outbreaks, but infections can result in mortality of up to 90% depending on the viral strain (Bausch et al., 2008). The South American arenaviruses are less pathogenic than filoviruses but can be associated with significant mortality. For example, Junin and Guarnito viruses have 15–30% mortality if untreated (Doyle et al., 1998; Tesh, 2002). Lassa fever has a lower mortality rate than many other hemorrhagic fevers, but this virus is more common and results in approximately 100 000–300 000 infections and 5000 deaths per year in West Africa (CDC, 2004).

Guinea pigs have been used primarily to study the pathogenesis and host interactions with these viruses, but have also been used for developing vaccines and treatments. Although non-human primates are the gold standard as models for these diseases, the high virulence and BSL-4 conditions associated with these infections make rodents valuable models for practical and ethical reasons. Most of these viruses have animal reservoirs, but the viruses do not cause significant disease in the reservoir species and hence do not serve as good models (Gowen and Holbrook, 2008). It is important that the rodent models have a similar pathogenesis as in

primates; the virus infects macrophages and dendritic cells and then spreads to cause a systemic infection (Gowen and Holbrook, 2008). Rodents are not always reliable predictors for treatment or vaccine efficacy in humans, but are useful as models for screening preliminary products and continue to be used for understanding virulence and pathogenesis (Bente et al., 2009).

LASSA FEVER

Guinea pigs can develop severe disease with Lassa fever virus infection and this model is used primarily for vaccine development. Mortality in strain 13 guinea pigs can be 80–100% within 14–16 weeks of inoculation (Auperin et al., 1988; Carrion et al., 2007; Clegg and Lloyd, 1987; Jahrling et al., 1982). Hartley guinea pigs are less susceptible to lethal disease with infection (Jahrling et al., 1982). After inoculation, the virus is found in many organs including spleen, lymph nodes, and liver, but the brain is minimally involved. Interstitial pneumonia is a consistent finding in animals with fatal infection. Clinical signs in guinea pigs include fever, weakness, labored breathing, hindlimb paralysis, anorexia, and death (Carrion et al., 2007; Clegg and Lloyd 1987). Infected animals can also have elevated liver enzymes. Neutralizing antibodies are only found in outbred guinea pigs that survived infection (Jahrling et al., 1982). Aerosol transmission of the virus has been documented and appears to have the same tissue tropism as subcutaneous inoculation (Stephenson et al., 1984). Virulence for some viral strains has correlated well between guinea pigs and humans, particularly those strains isolated from pregnant women and infants (Jahrling et al., 1985). The guinea pig has been used to test live-attenuated vaccines that are clones of the Lassa fever virus (Carrion et al., 2007; Lukashevich et al., 2005) and recombinant viral vectors (Auperin, et al., 1988; Bredenbeek et al., 2006; Clegg and Lloyd 1987; Morrison et al., 1989). This model has also been used to test treatments such as antivirals and neutralizing antibodies (Huggins 1989; Jahrling 1983; Jahrling et al., 1985).

Pichinde virus is a favorable surrogate for Lassa fever because it does not cause disease in humans and can be handled as a BSL-2 agent. The classic model involves Pichinde virus infection in inbred strain 13 guinea pigs. Pichinde virus is more virulent in strain 13 than Hartley guinea pigs, and the pathogenesis in strain 13 guinea pigs more closely models the pathogenesis of Lassa fever virus infections in people that develop severe disease (Gowen and Holbrook, 2008; Jahrling et al., 1981). Pichinde virus must be passaged in guinea pigs to become lethal in this species (Aronson et al., 1994, 1995; Lan et al., 2009). Inoculation with as little as three plaque-forming units (PFU) of adapted Pichinde virus is fatal in strain 13 guinea pigs with death in

12–19 days after post-inoculation (Aronson et al., 1994, 1995; Jahrling et al., 1981). Mortality can be as high as 100% with adapted strains, and as low as 15% with less adapted strains (Aronson et al., 1994, 1995). Mortality is usually preceded by fever, followed by hypothermia, severe weight loss, and decreased water consumption (Aronson et al., 1994, 1995; Guo and Liu, 1995; Jahrling et al., 1981). Virus is present in many tissues, and pathologic changes are similar to those seen in non-human primates and humans, including hepatocellular necrosis and fatty changes, splenic necrosis, adrenal necrosis, and interstitial pneumonia. Strain 13 guinea pigs also develop coagulation disorders including abnormal platelet function and thrombocytopenia (Cosgriff et al., 1987).

Although Hartley guinea pigs are not as susceptible to disease from Pichinde virus infections as strain 13, Hartley stock are becoming more common in these studies. In addition to the greater availability of Hartley guinea pigs, some researchers believe that the variability in susceptibility more closely reflects the disease condition in humans (Scott and Aronson, 2008). The reason for this variability is unknown, and could provide a model for evaluating disease resistance factors (Gowen and Holbrook, 2008). Resistant animals have been shown to have immune responses different to susceptible animals (Scott and Aronson, 2008). In addition, Pichinde virus strains that can be highly lethal in Hartley guinea pigs have been developed (Lan et al., 2008). For example, a low passage (P2) strain only causes mild clinical signs and no mortality, while a higher passage (P18) strain causes 88–100% mortality (Fennewald et al., 2007; Lan et al., 2009). The disease progression of the P18 strain in Hartley guinea pigs is similar to the classic lethal Pichinde virus infections seen in strain 13 guinea pigs. The typical progression includes a pronounced fever during the first week of infection followed by severe weight loss and death by 12–21 days. Death coincides with hypothermia, metabolic acidosis, microvascular leakage, and myocardial suppression (Scott and Aronson, 2008).

Pichinde virus in both inbred strain 13 and Hartley guinea pigs is used to study the pathogenesis and host responses to viral infections. Pichinde virus has a tropism for macrophages and monocytes. After intraperitoneal injection, the virus infects the peritoneal, liver, and alveolar macrophages and then spreads to the spleen and other reticuloendothelial organs (Aronson et al., 1994; Fennewald et al., 2002). The viral loads in the liver are higher than in serum. Liver pathology includes fatty changes, minor necrosis, elevated liver enzymes, and decreased coagulation factors (Chen et al., 1993; Lucia et al., 1990). Animals can also develop respiratory failure due to obstruction of the small airways (Guo and Liu 1991; Schaeffer et al., 1993). Other pathologic changes include intestinal villous blunting and

infiltration of macrophages, leaky gut capillaries, and splenic necrosis (Aronson et al., 1994; Katz and Starr, 1990). Infected animals can also develop cardiovascular dysfunction (Qian et al., 1994).

The guinea pig's immune response to Pichinde virus infection has been well-characterized. The virus suppresses the normal macrophage response to infection (Fennewald et al., 2007). Studies have compared the immune response to infection with avirulent to virulent strains to determine how the viruses differ in modulation of the host immune response (Aronson et al., 1994, 1995; Bowick et al., 2009; Fennewald et al., 2002). The genetic sequences of both virulent and avirulent strains have been reported, and have led to the development of molecular clones that mimic the pathogenesis of natural viruses (Lan et al., 2008, 2009; Liang et al., 2009). This will facilitate analysis of the genetic differences that contribute to variability in mortality. Potential treatment options have also been evaluated in Pichinde-virus-infected guinea pigs (Soares et al., 2008).

FILOVIRUSES

The guinea pig model for Ebola virus uses the Zaire viral subtype that has been passaged in guinea pigs. The wild-type Zaire virus causes only transient febrile illness and is not lethal in guinea pigs (Bente et al., 2009; Chepurinov et al., 2001). Adapted viral strains produce a disease that is similar to that seen in humans and non-human primates (Connolly et al., 1999). The virus targets macrophages throughout the infection and viremia increases in the first 7 days. The accumulation of viral proteins in different cells has been evaluated (Steele et al., 2001a). Guinea pigs develop clinical signs within 4–7 days post-inoculation, including fever, anorexia, weight loss, and dehydration (Bente et al., 2009; Connolly et al., 1999). Gross evidence of hemorrhage is not typically a clinical feature of the disease in guinea pigs. The disease quickly progresses to death by days 8–11 post-infection. Virus is detected in multiple tissues, with viral loads highest in the spleen and liver. Animals often have elevated liver enzymes, a neutrophilic leukocytosis, coagulopathy, and a thrombocytopenia, but are not typically anemic (Connolly et al., 1999). Findings at necropsy can include enlarged, reddened lymph nodes, pale friable livers, petechiae, gastric erosions, and hemorrhage in the stomach and colon (Bray et al., 2001). Histologic lesions include splenic necrosis, lymph node necrosis, hepatic necrosis, and inflammation and diffuse marked necrosis of the lamina propria of the colon (Bray et al., 2001; Connolly et al., 1999). The inflammation in the liver can be severely granulomatous (Ryabchikova et al., 1996a). Rodent adapted viral strains are lethal in both strain 2 and strain 13 guinea pigs (Bray et al., 2001). The host immune response has been evaluated using both adapted and non-adapted

strains to determine differences that are associated with viral virulence (Chepurinov et al., 2001, 2003).

Guinea pigs have been used to evaluate prophylactic and treatment options for Ebola virus. Guinea pigs are commonly used to study vaccines prior to trials in non-human primates (Burton and Parren, 2000). Vaccines that have been tested include viral vectors or plasmids expressing glycoproteins and nucleoproteins (Bukreyev et al., 2009; Feldmann et al., 2007; Yang et al., 2008), live attenuated Ebola virus (Halfmann et al., 2009), recombinant proteins (Mellquist-Riemenschneider et al., 2003), and DNA vaccines (Steele et al., 2001b). There have also been efforts to develop a general vaccine for filoviruses, and a single vaccine with virus-like particles has protected guinea pigs from challenge with both Ebola and Marburg viruses (Swenson et al., 2005). Neutralizing antibodies have had some efficacy in treatment in guinea pigs with Ebola (Jahrling et al., 1999; Kudoyarova-Zubavichene et al., 1999; Takada et al., 2007). Small interfering RNAs that disrupt viral RNA have also had some efficacy in treating guinea pigs (Mellquist-Riemenschneider et al., 2003).

Guinea pigs have been used less frequently for the study of Marburg virus. Similar to Ebola, experimental infections with wild-type virus only causes a mild febrile disease (Bente et al., 2009). Lake Victoria Marburg virus has been adapted through serial passage in guinea pigs to make the virus lethal in this species (Bente et al., 2009; Gowen and Holbrook 2008). Some viral variants are non-lethal, and the genetic differences between variants with different virulence have been analyzed (Lofts et al., 2007). The adapted virus is lethal 7–17 days after infection (Bente et al., 2009). The pathogenesis of viral migration from the liver to the vascular system has been studied (Kolesnikova et al., 2007; Ryabchikova et al., 1996b). Infected animals show weight loss, fever, edema, and impaired clotting times. The pathogenesis after respiratory challenge has also been characterized (Ryabchikova et al., 1996b). The immune response to infection has been studied in efforts to develop novel treatments for the virus (Ignatyev et al., 2000). Vaccines of glycoprotein subunits and viral vectors have offered some protection against infection (Hevey et al., 1998). Vaccines using virus-like particles or viral vectors have offered protection from multiple isolates of Marburg virus (Swenson et al., 2005, 2008; Wang et al., 2006; Warfield et al., 2004). Treatment with monoclonal antibodies was also partially protective in infected animals (Hevey et al., 1998).

SOUTH AMERICAN ARENAVIRAL HEMORRHAGIC FEVERS

Guinea pigs have been useful for understanding the pathogenesis of Junin virus, the etiologic agent of Argentine hemorrhagic fever. Guinea pigs infected with

virulent strains produce the main features of Argentine hemorrhagic fever including fever, weight loss, hemorrhage, leukopenia, thrombocytopenia, and lymphoid necrosis (Carballal et al., 1981; Enria et al., 2008; Yun et al., 2008). The virus has tropism for lymphoid tissues and has severe cytopathic effects in these tissues, depresses the humoral and cellular immune response, and animals can die without detectable antibody to the virus. Evidence from electron microscopy of peripheral blood and bone marrow has suggested that viral activity on megakaryocytes is responsible for the acute thrombocytopenia (Carballal et al., 1977). Guinea pigs develop some but not all of the coagulation and complement abnormalities of humans (Molinas et al., 1989). Inbred and outbred guinea pigs can both be highly susceptible to the disease (Yun et al., 2008). Immunosuppression can increase the pathology associated with avirulent strains but does not affect the course of disease with virulent strains (Kenyon et al., 1985). Interestingly, cyclophosphamide actually delayed time to death for infected animals, but this was more likely to do with the antiviral characteristics of the drug than immunosuppression (Ponzinibbio et al., 1989).

The pathogenesis of Junin virus is highly dependent on viral strain and history of in vivo or in vitro viral passage (Kenyon et al., 1988). Viscerotropic viruses replicate primarily in lymph nodes, spleen, and bone marrow, with progression to necrosis of those organs and hemorrhage. Viremia increases from day 7 post-infection until death at approximately days 13–15 (Enria et al., 2008; Kenyon et al., 1988). Neurotropic strains have only transient replication in the spleen and lymph nodes, followed by distribution to the brain. These viruses cause poliomyelitis and animals frequently exhibit rear leg paralysis followed by death 28–34 days after inoculation (Kenyon et al., 1988; Medeot et al., 1992). The pathogenesis associated with attenuated strains has also been characterized and is more similar to the neurotropic viruses (Boxaca et al., 1984a; Laguens et al., 1983). Attenuated viruses are more likely to be found in the brain and cause a meningoencephalitis with lower mortality than virulent strains. Increased passage of the virus in guinea pigs can enhance the neurotropism of the virus (Boxaca et al., 1984a). More recent studies have indicated that the XJ viral strain, which was once highly virulent in guinea pigs, has become avirulent through in vitro passage of the virus (Yun et al., 2008). Transplacental transmission has been documented using both attenuated and virulent strains (Boxaca et al., 1984b; Gomez and Boxaca, 1985; Sangiorgio and Weissenbacher, 1983). This model of Junin virus in guinea pigs has been used to evaluate host immune responses and potential treatments (Dejean et al., 1988; Gonzalez et al., 1987). Immune serum treatment decreases mortality, but

surviving animals can develop a hindlimb paralysis, encephalitis, and meningitis (Enria et al., 2008). This is similar to humans in that neurologic complications can develop after treatment with serum. Ribavirin has been used with variable efficacy in this model (Huggins, 1989; Kenyon et al., 1986).

Influenza

Influenza viruses are enveloped RNA viruses that belong to the family Orthomyxoviridae. Influenza viruses are divided into three types (A, B, and C) and influenza A viruses are further divided into subtypes based on the two surface proteins hemagglutinin (H) and neuraminidase (N). Influenza B viruses are not classified by subtype but do have different strains. Influenza viruses are responsible for both the annual seasonal flu and periodic pandemics. Each year in the United States, approximately 21000–41000 deaths are associated with the seasonal flu (CDC, 2009; Lowen et al., 2006). The CDC recommends a yearly vaccine for the seasonal flu, but additional vaccine strategies are needed because some populations that are particularly susceptible to complications from the infection, such as the elderly and immunocompromised, may not respond well to the vaccine (Fiore, 2008; Glezen, 2006; Kumar and Burns, 2008). Guinea pigs have been used primarily for studies on the pathogenesis and transmission of the influenza A viruses, but have also occasionally been used for studies on vaccines, treatments, and diagnostics (Havlickova et al., 2006; Robuccio et al., 1995; Van Hoeven et al., 2009).

The pathogenesis and host response to influenza infection in the guinea pig have been characterized. Animals are usually inoculated intranasally, but aerosol infection has also been used. Intragastic inoculation does not produce infection (Kwon et al., 2009). Animals are typically evaluated using clinical observations, weights, body temperature, replicating virus in nasal washes and tissues, and histology. Early studies evaluated cellular and humoral immune responses after intranasal infection with influenza A viruses (Kauffman et al., 1978; Phair et al., 1979). Guinea pigs are susceptible to infection without prior host adaptation of the virus, but do not develop obvious clinical illness or fevers with most influenza A viruses (Kauffman et al., 1978; Lowen et al., 2006; Mubareka et al., 2009; Phair et al., 1979; Van Hoeven et al., 2009). Animals in one study developed severe bronchopneumonia and weight loss with some influenza A viruses, but the authors intentionally produced a severe infection by using a dose higher than is considered standard (Tang and Chong, 2009). Guinea pigs have also been used to evaluate the virulence of high pathogenicity influenza viruses, including the avian influenza (H5N1) and the H1N1 virus that caused the 1918 pandemic (Kwon et al.,

2009; Van Hoeven et al., 2009). In contrast to mice and ferrets that have severe disease with these highly pathogenic strains, guinea pigs only develop modest lethargy and weight loss followed by recovery from the infection.

The guinea pig has been suggested as a good model for non-lethal influenza virus infection in humans because of their decreased susceptibility to disease relative to mice and ferrets (Azoulay-Dupuis et al., 1984; Lowen et al., 2006; Van Hoeven et al., 2009). Infection in guinea pigs is characterized by lesions appearing approximately 24 hours after infection, followed by a focal interstitial pneumonia at 96 hours and widespread inflammation by 7 days. However, the virus is cleared from the nose and lungs by day 9, and the pathology is reversible with complete repair within 5 weeks. Even with highly pathogenic viral strains, guinea pigs only develop a mild to moderate interstitial pneumonia (Kwon et al., 2009; Van Hoeven et al., 2009).

Guinea pigs have been suggested as an alternative to ferrets as a model for influenza transmission. Mice are the most commonly used species for influenza studies, but mice fail to transmit the virus to other mice housed in close proximity. Ferrets are suitable for transmission studies, but guinea pigs are easier to obtain and house and are less likely to have been previously exposed to the virus (Lowen et al., 2006). For transmission studies, guinea pigs are infected by intranasal or aerosol inoculation and then placed in transmission cages. These cages have wire tops and perforations on the side, and can be placed next to each other or at a distance to simulate short- or long-range aerosol transmission (Mubareka et al., 2009). Guinea pigs can transmit both seasonal influenza A, highly pathogenic viruses, and 1918 influenza pandemic virus by direct contact, short- and long-range transmission (Lowen et al., 2006). Guinea pigs have been used to demonstrate that influenza A viruses vary in their efficiency of transmission by aerosol or fomites (Mubareka et al., 2009). This model has also been used to evaluate the effect of environmental temperature and humidity on viral transmission, vaccine efficacy and how drug resistance affects transmission (Bouvier et al., 2008; Lowen et al., 2008, 2009).

Influenza virus infections can be severe in neonates, and one study evaluated the pathology after the intranasal infection of pregnant guinea pigs because of the similarities in human and guinea pig placental structure. Guinea pigs have primarily transplacental transmission of antibodies as do humans, which was the impetus for another study that evaluated the susceptibility of infant guinea pigs to influenza based on the immune status of the mother (Ali et al., 1989). Additional uses of this model have included the pathogenesis of influenza A in otitis media and after intraocular inoculation (Ohashi et al., 1991, 1993; Rozina et al., 1987).

Respiratory Syncytial Virus

Respiratory syncytial virus (RSV) is a member of the family Paramyxoviridae that was initially isolated from a chimpanzee, and since then has been recognized as a common pathogen in humans (Blount et al., 1956; Byrd and Prince, 1997). RSV causes mild disease in healthy adults, but can be severe in infants, children, and the elderly. This virus is the leading cause of bronchiolitis and pneumonia in children less than 1 year old in the US (CDC, 2008b). Infants that recover from lower respiratory disease may go on to develop asthma (Sly and Hibbert, 1989; Sutton et al., 2007). The immune response in humans provides only short-term protection, and there is currently no vaccine available (Byrd and Prince 1997; CDC, 2008b). Previous attempts at developing a vaccine with formalin-inactivated virus resulted in widespread infection with up to 80% of children hospitalized and two deaths (Byrd and Prince, 1997; Kim et al., 1969). Many species have been used as models of RSV infection, including mice, cotton rats, ferrets, cows, guinea pigs, hamsters, chimpanzees, and other non-human primates.

Guinea pigs develop bronchiolitis and airway hyper-responsiveness after RSV infection. Guinea pigs, particularly juveniles, are useful for this model because of the extensive knowledge available about airway physiology and reactive disease in this species, even though they are not as easily infected as some other models (Byrd and Prince, 1997). One study evaluated immunosuppressed animals with cyclophosphamide to more closely approximate the disease usually observed in humans (Tan et al., 2008). Airway hyper-responsiveness is usually determined by the response to an acetylcholine challenge, although a histamine challenge has also been used (Bramley et al., 1999b; Riedel et al., 1997). The peak viral replication occurs at 3 days and maximal bronchial hyper-responsiveness and inflammation is usually observed 6–7 days after inoculation (Dakhama et al., 1997; Robinson et al., 1996; Streckert et al., 1996). Histology also indicates a reactive component with an abundance of eosinophils. Inoculation with 4×10^3 PFU results in replicating virus in 90% of animals by day 6, but bronchiolitis and replicating virus decreases by 14 days post-infection (Hegele et al., 1993). RSV-specific antibodies are present as early as 7 days after infection (Dakhama et al., 1997). After the acute disease resolves, guinea pigs develop persistent lung infection (Bramley et al., 1999a; Dakhama et al., 1997; Sutton et al., 2007).

The reactive component of the disease and persistent infection in guinea pigs is a common area for research because asthma can be a long-term effect of RSV infections in humans. Outbred guinea pigs are routinely used because they are more susceptible to allergic sensitization than strain 2 or strain 13 guinea pigs, but

strain 2 have been used as allergy-resistant models and compared to outbred guinea pigs or strain 13 (Bramley et al., 2003; Sutton et al., 2007). Early experiments indicated that viral RNA and viral proteins continued to be present at least 60 days after inoculation despite negative viral cultures, and a later study confirmed that this correlated with replicating virus (Dakhama et al., 1997; Hegele et al., 1994). Guinea pigs have been evaluated 100 days after inoculation and still exhibited increased airway hyper-responsiveness and increased eosinophils (Bramley et al., 1999a). Although inflammation does decrease after acute infection, it does persist with the virus (Hegele et al., 1994; Riedel et al., 1997). Recent studies have evaluated specific immune components that may contribute to reactive disease (Sutton et al., 2007; Tan et al., 2008). Some researchers have also proposed that a predisposition to allergies may correlate with a predisposition to severe consequences of RSV infection. This was studied in guinea pigs by comparing infections in the allergy-resistant strain 2 guinea pigs with the allergy-susceptible strain 13 guinea pigs, or by inducing allergic sensitization by inhalation exposure to OVA in outbred guinea pigs (Bramley et al., 2003; Robinson et al., 1997). While guinea pigs are primarily used to study pathogenesis and immune response in these infections, they have sporadically been used for other studies including vaccine, treatments, and diagnostics (Dakhama et al., 1998; Tayyari et al., 2005).

Mycobacterium tuberculosis

Tuberculosis (TB) continues to be a major focus of medical research because of its widespread distribution and devastating consequences. *M. tuberculosis* infects approximately one-third of the world's population, resulting in disease in 9 million people and almost 2 million deaths annually. The bacteria are spread primarily by airborne transmission, and active disease usually involves the lungs but other organ systems can be affected. The majority of infections are latent and these people do not develop symptoms and are not contagious, but 5–10% of this population will develop active disease if not treated. Reactivation is a particular concern in immunosuppressed individuals, and TB is a leading cause of mortality in people with HIV (LoBue, 2009). One factor contributing to the failure of disease control is the lack of an effective vaccine. The Bacille Calmette-Guerin (BCG) vaccine is the only available vaccine but has variable efficacy. Another concern is the emergence of drug-resistant TB which is associated with increased treatment cost and treatment failure. Multi-drug-resistant (MDR) TB is resistant to two first-line antibiotics used, isoniazid and rifampin, and extensively drug-resistant (XDR) TB is also resistant to the next most commonly effective drugs. Both of these

strains have been found in the US and with increasing frequency worldwide. National and international organizations have indicated that animal models are necessary for TB research priorities such as vaccine development and treatment, particularly of drug-resistant strains. In addition, animal models are still needed to understand basic bacterial biology, pathogenesis, and host–pathogen interactions, including understanding reactivation of latent disease (LoBue, 2009).

The guinea pig is a well-characterized model that has both historical and current relevance for tuberculosis research. Robert Koch used guinea pigs for his research on tuberculosis, which led to the identification and naming of the etiologic agent as *Mycobacterium tuberculosis* in 1882 and a Nobel Prize in 1905 (Kaufmann and Schaible, 2005). Koch fulfilled the third of his postulates for the definition of microbial pathogens by inducing disease in guinea pigs inoculated by the intraperitoneal route with bacteria derived from the lung of an infected person. Many reviews are available that cover tuberculosis in animal models, including guinea pigs (Basaraba 2008; Orme and Gonzalez-Juarrero 2007; Padilla-Carlin, et al., 2008; Smith and Wiegeshaus 1989). Guinea pigs are a valuable model because of their high susceptibility to the disease which facilitates evaluation of treatment and vaccines (Gupta and Katoch, 2009). Unlike the mouse model, guinea pigs develop a caseating necrosis with TB lesions that is a hallmark of the disease in humans (Helke et al., 2006; Nuermberger, 2008). The current guinea pig model usually involves aerosol infection of young, Hartley guinea pigs (200–350 grams). Some studies use slightly older animals of up to 500 grams, but fully grown animals are rarely used (Baldwin et al., 1998; Basaraba et al., 2006; Garcia-Contreras et al., 2006; Henao-Tamayo et al., 2009; Ordway et al., 2007). Guinea pigs can be infected with as few as 3–5 infectious bacilli, but 30 or more are often used (Ahmad et al., 2009; Ly et al., 2008; Orme and Gonzalez-Juarrero, 2007). A specific protocol using the Madison Chamber developed at the University of Wisconsin for aerosol exposure has been described (Orme and Gonzalez-Juarrero, 2007). These procedures are carried out under animal biosafety level (ABSL) 3 conditions.

Once infected with tuberculosis, guinea pigs develop a progressive and eventually fatal disease (Helke et al., 2006). They develop classic granulomas with Langerhans multinucleated giant cells in the lungs and pulmonary necrosis followed by dissemination to and necrosis of extra-pulmonary tissues including lymph nodes and spleens (Palanisamy et al., 2009; Rao et al., 2009). The enlargement and necrotizing lymphadenitis of the hilar lymph nodes in infected guinea pigs can be severe, which is similar to the disease in children (Basaraba et al., 2006). Extra-pulmonary lesions

can occur in the eyes, brain, small intestine, pancreas, adrenal gland, and heart (Palanisamy et al., 2008; Rao, et al., 2009). Guinea pigs do have several drawbacks as a tuberculosis model. They are more expensive and have fewer reagents available for immunologic and molecular techniques than mice, although more are being developed (Flynn, 2006; Gupta and Katoch, 2009; Yamada et al., 2005). The pathology is different from humans in that most humans do not develop active disease and guinea pigs do not develop liquefaction and cavitary lesions (Gupta and Katoch, 2009; Orme 2006; Orme and Gonzalez-Juarrero, 2007).

Although TB infection is invariably fatal in guinea pigs even with a low number of bacilli inoculated, the disease usually takes months to a year to be fatal. Weight loss and mortality typically occur 100 days after inoculation (Flynn, 2006; Orme and Gonzalez-Juarrero, 2007). Study endpoints range from death or moribund status to predetermined time points (Ahmad et al., 2009; Baldwin et al., 1998; Orme and Gonzalez-Juarrero 2007). If study endpoints necessitate that an animal is allowed to develop clinical disease, monitoring for weight loss and euthanasia prior to death is preferred from both an animal welfare perspective and in allowing the collection of viable samples (Orme and Gonzalez-Juarrero, 2007). Weight loss of 15–20% has been used as a benchmark, although some studies that use weight loss as an endpoint do not indicate what percentage was used (Cardona et al., 2009; Palanisamy et al., 2008; Weldingh and Andersen, 2008; Williams et al., 2005). Some studies have used endpoints based on other parameters such as anorexia, lethargy, and a modified Karnofsky score (Weldingh and Andersen, 2008; Xing et al., 2009). The Karnofsky Performance Status Scale score was initially developed in 1948 for the assessment of status in human oncology patients. This scoring system has been modified for many conditions and uses physical parameters such as weight loss, appearance, and respiratory rate. The time course of disease often results in studies that last for months. After euthanasia, most studies evaluate bacterial loads and lesions in the lungs and other relevant tissues to assess virulence, treatment, or vaccine efficacy.

Guinea pigs have been used to study both the pathogenesis and host–pathogen interactions associated with tuberculosis. Many naturally occurring strains of *M. tuberculosis* vary in phenotype, and guinea pigs have been used to characterize the difference in pathology and virulence associated with different clinical strains (Palanisamy et al., 2008, 2009). Guinea pigs have also been used to evaluate the host immune response to infection after aerosol or intravenous infection, particularly cell-mediated immunity which is a significant component of the human host reaction (Lasco et al., 2005; Ly and McMurray, 2009; Ly et al., 2008; Ordway

et al., 2007). Specifically, the host immune response may play an important role in the establishment of secondary lesions in the lung. In the guinea pig, TB causes a primary lung lesion at the site of entry, and is then transported to lymph nodes, spleen, and then lung. Secondary lesions in the lung occur through hematogenous spread back to the lung instead of spread from the primary lesion (Ly et al., 2008; McMurray, 2001; Ordway et al., 2007; Turner et al., 2003). The immune responses associated with these primary and secondary lesions differ, which could have implications for humans because the secondary lesions are the most likely cause of reactivated TB disease (Balasubramanian et al., 1994; Ly et al., 2008; McMurray, 2001; Turner et al., 2003). Guinea pigs do not easily replicate the process of reactivation from latency because of their sensitivity to the initial exposure, but the use of mutant strains of tuberculosis can be helpful in evaluating this process (Drumm et al., 2009; Kashino et al., 2008). The host immune response has also been studied in the context of a guinea pig model of pleurisy. Bacteria were injected into the pleural space bilaterally, and animals were euthanized to collect pleural fluid and focal granulomatous tissue for bacterial counts and cell analysis (Allen and McMurray, 2003; Ly et al., 2009; Phalen and McMurray, 1993).

Guinea pigs have been widely used to assess treatments and treatment failures of TB. Basaraba (2008) and Nuermberger (2008) offer two independent reviews on this topic. Because of the similarity in lung lesions between guinea pigs and humans, they have been used to evaluate the interaction between lesion morphology and treatment efficacy. In contrast to other rodents, guinea pigs may be a better model for studying treatment efficacy if the caseous necrosis alters the bacteria's ability to persist or makes treatment more difficult (Basaraba, 2008; Nuermberger, 2008). Guinea pigs have been used widely to test antibiotic efficacy and relapse after treatment (Basaraba, 2008; Nuermberger 2008). For example, isoniazid treatment is immediately effective in improving clinical outcomes but this drug can select for tolerant strains which leads to decreased drug efficacy later in disease (Ahmad et al., 2009). Guinea pigs have also been used to test aerosolized versions of drugs, which could improve efficacy by reducing treatment frequency, decreasing systemic side effects, and increasing concentrations in tissues where it is most needed. For example, rifampicin-loaded polymeric microspheres have been compared to nebulized rifampicin suspension (Garcia-Contreras et al., 2006; Suarez et al., 2001). Nanotechnology has been proposed as an improved method of drug delivery that could increase bioavailability and deliver controlled-release drug with either oral or aerosol administration. Guinea pigs have been used to determine pharmacokinetics and tissue

concentrations after delivery of intratracheal administration of rifampicin encapsulated in nanoparticles (Pandey and Khuller, 2006; Sung et al., 2009). Guinea pigs have also been used to specifically study potential treatments for multi-drug-resistant strains of TB (Stover et al., 2000).

Research related to the development of tuberculosis vaccines has been a large component of recent efforts utilizing guinea pigs, and multiple reviews are available (Gupta and Katoch, 2009; Gupta et al., 2007; Orme, 2006). Mice are usually the first model used to evaluate a vaccine because of lower cost and availability of reagents associated with the former species, but guinea pigs are considered a better model and are often used in subsequent studies of vaccines that demonstrated promising results in mice. The guinea pig is a good model for vaccine candidates because the disease is consistently fatal without protection (Flynn, 2006; Gupta and Katoch, 2009). In addition, the BCG vaccine is more effective in the guinea pig than other rodent models, which allows more sensitive comparisons of efficacy between BCG and new vaccines (Gupta and Katoch, 2009). The BCG vaccine itself has been studied extensively in the guinea pig (Basaraba, 2008; Basaraba et al., 2006; Cho and McMurray, 2007; Fok et al., 1976; Harding and Smith, 1977; Jeevan et al., 2006; Lasco et al., 2005; Ly et al., 2008; Ordway et al., 2008; Roche et al., 1995; Smith and Wiegshauss, 1989; Smith et al., 1970; Wiegshauss et al., 1970; Yamamoto et al., 2007). After vaccine administration, challenge with bacteria is done at least 1 month later or at longer intervals. Even though the BCG vaccine has limitations in its use in humans, the vaccine's benefits such as safety and long-term immunity have led some researchers to investigate a recombinant BCG vaccine that may have increased immunogenicity in guinea pigs (Gupta et al., 2007; Horwitz and Harth, 2003; Horwitz et al., 2000; Orme 2006; Sun et al., 2009). DNA vaccines have been tested in guinea pigs with variable results (Baldwin et al., 1998; Gupta et al., 2007; Khera et al., 2005; Orme, 2006). In addition, guinea pigs have been used to study subunit (Gupta et al., 2007; Olsen et al., 2004; Reed and Lobet, 2005) and attenuated live vaccines (Cardona et al., 2009; Sampson et al., 2004). Another vaccine strategy is using a novel vaccine as a booster to the BCG vaccine, which has also been evaluated in guinea pigs (Orme, 2006; Williams et al., 2005). Recombinant viral vectors such as adenovirus and poxvirus have also been shown to have some efficacy in the guinea pig model (Williams et al., 2005; Xing et al., 2009). Studies have also looked at the potential benefits of post-exposure vaccination (Henao-Tamayo et al., 2009).

Guinea pigs have been used less frequently for other aspects of TB research. For example, a few studies have looked at diagnostic tests for TB in guinea pigs,

including characterizing lesions on MRI (Geisbrecht et al., 2006; Weldingh and Andersen, 2008). In addition, several classic studies have described the use of guinea pigs to detect airborne transmission of tuberculosis in hospital wards and this process has been used more recently as a potential method to assess mechanisms to decrease airborne transmission of the bacteria (Escombe et al., 2007; Koenig, 2008; Riley et al., 1995). Basically, the animals are exposed to air from tuberculosis wards and tested routinely by the tuberculin skin test. Guinea pigs that test positive on the skin test are submitted for necropsy to confirm infection. Guinea pigs have also been used to study the relationship between malnutrition and infection or vaccine efficacy (Cegielski and McMurray, 2004; Roche et al., 1995).

Chlamydia

Chlamydia trachomatis is an obligate intracellular bacterium that is a leading cause of sexually transmitted disease and preventable blindness (Wilson et al., 2009). Most people with reproductive tract infections do not develop symptoms, but untreated infections in women can lead to pelvic inflammatory disease, infertility, and ectopic pregnancy. This bacterium is also the etiologic agent of trachoma, which is a chronic follicular conjunctivitis that can lead to corneal ulceration, opacities, and blindness secondary to inversion of the eyelashes. The host's immune response is an important factor in the pathology of the disease for both reproductive and ocular infections (Wilson et al., 2009).

The guinea pig model of reproductive chlamydial infection utilizes the guinea pig pathogen *Chlamydomphila caviae*. This bacterium is the etiologic agent of guinea pig inclusion conjunctivitis, and was formerly the guinea-pig-associated strain of *Chlamydia psittaci*. Natural conjunctival infections in guinea pigs can range from asymptomatic to severe with conjunctivitis and uveitis, and the disease can progress to the lower respiratory tract (Lutz-Wohlgröth et al., 2006). *Chlamydomphila caviae* is species-specific for guinea pigs, but it has been associated with clinical disease in other species including horses, rabbits, and dogs (Pantchev et al., 2009). Reviews of both the male and female guinea pig model are available (Padilla-Carlin et al., 2008; Rank and Whittum-Hudson 1994).

Guinea pigs are a useful model because of the similarities with humans in the pathology and immune response to chlamydial infection. The guinea pig female reproductive tract is more similar than mice to humans, and chlamydial infections in female guinea pigs have a propensity for ascending infection, which has been suggested as a model for pelvic inflammatory disease (Rank et al., 1993). Both human and guinea pig offspring can acquire chlamydial conjunctivitis from the mother during parturition (Padilla-Carlin et al., 2008).

Chlamydial infection in male guinea pigs also models the condition in humans in that they are usually asymptomatic, with no urethral discharge (Padilla-Carlin et al., 2008). Another key similarity that guinea pigs share with humans is they are the only animal model where sexual transmission of the bacteria has been documented. Guinea pigs differ from non-human primates and humans in that they develop acute, short infections that steadily diminish to a negative culture state, whereas humans and non-human primates can have intermittent positive cultures (Golden et al., 2000).

Investigations of chlamydial infections in guinea pigs usually use young but sexually mature animals (usually about 500 g bodyweight). Females are infected by intravaginal inoculation or sexual transmission. For intravaginal infection, the vaginal membrane must be broken prior to inoculation. Males can be infected with bacterial suspension placed on the extruded penis or directly into the urethra. After inoculation, infection is usually assessed by the percentage of inclusion bearing cells or isolation of the bacteria from urethral or vaginal scrapings. For sexual transmission, male guinea pigs are infected by administration of the bacteria into the urethra and then mated with females. The model of sexual transmission has been used to determine an inoculum for artificial infection that most closely resembles natural infection (Rank et al., 1993). The course of artificial infection with 10^2 inclusion-forming units (IFU) most closely resembled natural infection, and infected 70% of the animals, which was similar to the infection rate resulting from mating. Infection with 10^4 IFU was required for a 100% infection rate. The authors recommended 10^2 – 10^3 FU based on these results, but many prior studies had used higher doses (Darville et al., 1995, 2000; Rand and Whittum-Hudson, 1994).

The pathology and disease course after infection with *C. caviae* have been well-characterized. The histology associated with genital infections in female guinea pigs has been described (Barron et al., 1979; Rank, 1994). Bacterial numbers and inflammation are most pronounced in the cervix, with peak inclusion scores usually at 6–9 days post-inoculation (Barron et al., 1979; Rank, 1994). The bacteria are present in approximately 80% of uterine horns and oviducts within 7 days of inoculation, although the incidence of salpingitis was lower (Rank, 1994; Rank and Sanders, 1992). Inflammation resolves and inclusion scores decrease considerably by approximately 20 days (Barron et al., 1979; Rank, 1994). Early studies used cyclophosphamide for immunosuppression to assess ascending infection, but studies have indicated that increasing the sample size and refining timepoints allows for the evaluation of ascending infection without immunosuppression (Rank and Sanders, 1992; White et al., 1979). Infection can also result in long-term oviductal damage that is

seen at 75–80 days (Rank and Sanders, 1992; Rank and Whittum-Hudson, 1994). The course of infection is not altered by the stage of the estrous cycle during inoculation, but timing can affect the incidence of pathologic findings (Rank et al., 1993). The pathology of disease in the females can be altered by hormones. This has been evaluated because many women take oral contraceptives. Contraceptive hormones, particularly estradiol, prolong infection, delay the humoral immune response and increase the incidence of salpingitis, endometritis, and cystitis (Barron et al., 1988; Pasley et al., 1985; Rank and Barron, 1987; Rank et al., 1982). In the male, intraurethral infection can last for 2 weeks (Howard et al., 1976). Cyclophosphamide increases the number of successfully infected males and prolongs the time the bacteria can be found in the bladder from 18 days to 50 days (Patterson and Rank, 1996; Rank et al., 1981).

Both male and female guinea pigs have at least partial immunity to reinfection of *C. caviae* in the genital tract after primary infection, but reinfection in females can increase the chance of tubal damage (Rank et al., 1995). Females that are reinfected have a shorter disease course with decreased shedding following intravaginal inoculation or sexual transmission of the bacteria (Golden et al., 2000; Lamont et al., 1978; Rank and Barron, 1983). Animals are most resistant to reinfection 30 days after the initial infection (Rank et al., 1988). Although males are resistant to reinfection of the genitals, they are still susceptible to ocular infections after primary genital infection (Howard et al., 1976).

The host immune response is a factor both in the pathology and resolution of the diseases associated with chlamydial infections, and both the humoral and cellular responses have been studied in the guinea pig (Darville et al., 1995, 2000; Howard et al., 1976; Rank, 1994; Rank et al., 2000). The immune responses of guinea pigs and humans share more similarities compared with mice and humans (Rank et al., 2000). Studies have evaluated the antibody response and both serum and local antibodies have a role in resolution and protection (Batteiger et al., 1993). Resistance to reinfection is dependent on the humoral immune response, but cellular immunity also plays a role (Igietsme and Rank, 1991; Rank and Barron, 1983; Rank and Batteiger, 1989). Because of the partial protection conveyed by infection and serum-derived antibody from infected animals, a few studies have used this knowledge of the immune response to look at protection conferred by vaccines (Rank and Batteiger, 1989). The vaccines studied included both inactivated bacteria and subunit vaccines, but they have failed to provide full protection (Batteiger et al., 1993; Patterson and Rank, 1996; Rank et al., 1990).

As a model of human conjunctivitis, guinea pigs have demonstrated value. The model was first described in the 1960s, and simply involves inoculating the

conjunctiva with the organism (Murray, 1964). This model differs from humans and non-human primates in that the guinea pig model is more acute and has a low incidence of crossover if only one eye is infected (Rank and Whittum-Hudson, 1994). Lesions are graded based on the presence and extent of erythema, edema, and exudates. Animals are evaluated by the lesion scores and the presence of organisms in conjunctival scrapings. The pathology lasts for a mean of 7 days and is the most severe at 1.5 days post-inoculation (Wilson et al., 2009). The lesions can progress to pannus with multiple challenges of the bacteria (Rank and Whittum-Hudson, 1994). Inbred and outbred strains are both susceptible to ocular infection (Rank and Whittum-Hudson, 1994). This model has primarily been used to study pathology of the infection (Rank et al., 2008, 2009). Infection of the eye can result in immunity to reinfection of both the eye and genitals in male guinea pigs (Howard et al., 1976). It is interesting to note that estradiol does not alter the course of ocular infection as it does for reproductive infection (Rank and Barron, 1987).

Legionella pneumophila

Legionella pneumophila is a Gram-negative, facultative intracellular bacterium that is the etiologic agent of Legionnaires' disease. This disease was originally described in an outbreak at a 1976 convention of the American Legion in Philadelphia. The primary lesion is pneumonia, and clinical signs can include high fever, chills, cough, muscle aches, and headaches. Symptoms usually begin 2–14 days after exposure. Many people that are infected have no or mild symptoms and are not diagnosed. However, it can progress to severe pneumonia and approximately 8000–18000 people are hospitalized annually in the US and fatalities can occur in 5–30% of these cases (CDC, 2008a). Most healthy people respond to treatment with antibiotics, but the disease is more severe and difficult to treat in the elderly, immunocompromised, and people with underlying respiratory disease. The bacteria are not readily spread from person to person, but are found naturally in the environment. Outbreaks are usually caused by exposure to a common source of contaminated water vapor, such as whirlpool spas.

Guinea pigs develop severe pneumonia with *L. pneumophila* and have been a classic model for Legionnaires' disease in humans. Reviews of the guinea pig as a model of this disease are available (Armstrong and Haas, 2007; Padilla-Carlin et al., 2008). Other rodents are relatively resistant to infection and have immune systems that share fewer similarities with humans than guinea pigs (Armstrong and Haas, 2007; Davis et al., 1982; Winn et al., 1982). Guinea pigs are highly susceptible to infection even without passage of the bacteria through this species, but some studies do

passage strains before experimental infection (Berendt et al., 1980; Davis et al., 1983a, 1983b). The guinea pig pneumonia model usually relies on aerosol infection, but intratracheal and intranasal instillation of the bacteria have also been used. Aerosol is a more efficient mechanism for infection than intranasal inoculation (Fitzgeorge et al., 1983). Organisms are less virulent when administered intraperitoneally, but the bacteria can cause a peritonitis and mortality at high doses (Berendt et al., 1980; Dournon and Rajagopalan, 1988; Fitzgeorge et al., 1983). Aerosol infection usually involves a mechanism to deliver droplets less than or equal to 5 μm . The numbers of retained bacteria are estimated based on an assumed 50% retention rate and/or by evaluating the dose retained in the lung at necropsy. Aerosol infection produces a severe pneumonia and clinical signs include fever, ruffled fur, weight loss, lethargy, and dyspnea, often associated with high mortality (Baskerville et al., 1981; Berendt et al., 1980; Davis et al., 1982, 1983a, 1983b; Fitzgeorge et al., 1983). The bacterial strains vary in virulence in guinea pigs, and the same strain can lose virulence over time by many passages in culture (Jepras et al., 1985). For example, inoculation with the Philadelphia-1 strain originally resulted in 100% mortality (Berendt et al., 1980), while later studies indicated no mortality (Jepras et al., 1985). Virulence can also be affected by growth temperatures for the bacteria prior to infection (Edelstein et al., 1987).

The pathology and host defenses associated with *L. pneumophila* infection have been extensively studied in the guinea pig model. Animals develop pneumonia by 1–3 days after infection characterized by diffuse foci of mixed inflammation and consolidation (Baskerville et al., 1981; Davis et al., 1983a; Fitzgeorge et al., 1983). Bronchoalveolar lavage done at different time points during infection indicates that an increase in bacteria coincides with an influx of inflammatory cells that begins at 16 hours after infection, peaks at 3 days and subsides by 11 days if the animals are going to recover (Davis et al., 1983a, 1983b). Most viable bacteria are associated with alveolar macrophages (Davis et al., 1983a; Hui et al., 2009). Both humoral and cell-mediated immune responses have been studied, and cellular immunity is the primary factor in preventing lethal infection in surviving animals (Blander and Horwitz, 1991, 1993; Klein et al., 1984; Weeratna et al., 1994). Activated monocytes and alveolar macrophages inhibit intracellular multiplication of the bacteria and are a large component of host defenses against this disease (Blander and Horwitz, 1991, 1993). In addition, studies have evaluated lesions induced by particular proteases of the bacteria to determine their role in the pathology of the lesions (Baskerville et al., 1986; Conlan et al., 1986). Studies have also evaluated the genes that may be involved in pathogenesis by infecting guinea

pigs with mutant strains and analyzing bacteria–macrophage interactions (Edelstein et al., 1999). Similar to humans, transmission of the bacteria from one animal to another does not readily occur (Katz et al., 1982).

Guinea pigs have been used to assist with diagnosis of *L. pneumophila*, including the virulence of clinically isolated strains. Historically, guinea pigs were exposed to test water and clinical samples for detection of the bacteria because it is slow-growing and difficult to isolate (Edelstein et al., 1982; McDade et al., 1977; Meenhorst et al., 1983). Since 1979, it has been recognized that *Legionella* antigens can be detected in the urine of infected patients and that is now a common method for diagnosis. Guinea pigs have been suggested for use as a model of refining these urinary antigen tests but are used infrequently (Kim et al., 2003; Williams and Lever, 1995). They have also been used to test the reliability of PCR tests for diagnosis and have been compared to protozoan models and found to be a reliable predictor of virulence (Fields et al., 1986; Maiwald et al., 1995). Guinea pigs continue to be a good model for human risk extrapolation because of their high susceptibility to the disease as well as similar disease course and macrophage–bacterial interactions (Armstrong and Haas, 2007).

Guinea pigs are also useful in testing vaccine and therapeutic options for *L. pneumophila* infections. The animals in these studies are often challenged with lethal doses of the bacteria in order to maximize the difference between control and experimental groups. Killed bacteria elicit high antibody responses but do not elicit a cellular immune response and are not protective against subsequent aerosol challenge (Blander and Horwitz 1991, 1993; Breiman and Horwitz 1987). In contrast, vaccines composed of *L. pneumophila* membranes or proteins induce strong cellular immunity and protection (Blander and Horwitz, 1991, 1993; Breiman and Horwitz, 1987). Edelstein (1995) offers a review of research related to antimicrobials for this disease, including the use of the guinea pig model. Generally, efficacy in humans is consistent with efficacy demonstrated in the guinea pigs (Edelstein, 1995). Novel fluoroquinolones and macrolides have been studied, and are usually compared to erythromycin for efficacy (Dournon and Rajagopalan, 1988; Dournon et al., 1986; Edelstein et al., 1999, 2001a, 2001b; Garcia-de-Lomas et al., 1998).

Staphylococcus aureus

Guinea pigs are used for a variety of investigations of *S. aureus* infections because of their size, but are most commonly used to study wound infections. Some of these studies also include infection with other bacteria including other *Staphylococcus* species, *Streptococcus*, *Bacteroides*, and *Pasteurella*. *S. aureus* is a common cause

of surgery-related infections and can be difficult to manage, particularly with the emergence of drug-resistant strains such as methicillin-resistant *Staphylococcus aureus* (MRSA). A common guinea pig model uses superficial dorsal lacerations to the level of the fascia, followed by bacterial contamination. One study used liquid nitrogen to break down the skin integrity before application of the bacteria (Kratzer et al., 2007). For the superficial wound infection model, at least 10^4 bacteria are needed to cause reliable infection (Stratford et al., 2002). Animals are subsequently evaluated by quantitative bacterial counts of the tissue and/or clinical scores of the lesions. This model has been used to evaluate the efficacy of different antibacterial irrigating solutions, including iodine, normal saline, and cefazolin (Howell et al., 1993a, 1993b; Lammers et al., 2001). Additionally, antibiotic creams for treating MRSA, the anti-inflammatory effects of ketoconazole, the effects of local anesthetics on infection, and the use of silver products to control infection in skin grafts have been examined in guinea pigs (Kawai et al., 2001; Kratzer et al., 2007; Mazurak et al., 2007; Stratford et al., 2002; Van Cutsem et al., 1991).

A superficial wound model has also been used to study the association of various types of suture and tissue adhesives with bacterial infections (Edlich et al., 1968; Howell et al., 1995; Ming et al., 2007, 2008; Quinn et al., 1997; Storch et al., 2004). This guinea pig model has been used to evaluate the use of topical antibiotics and antibiotic-impregnated beads for surgical wounds infected with both drug-susceptible and MRSA bacterial strains (Boon et al., 1985; Galandiuk et al., 1997). Also, because hypothermia is commonly associated with anesthesia, researchers have studied the relationship between hypothermia and infection utilizing guinea pigs (Sheffield et al., 1994).

S. aureus infection associated with surgical implants is a significant clinical problem. This infection is commonly simulated in guinea pigs with the use of a tissue cage implanted subcutaneously. The tissue cage is a perforated Teflon tube with or without additional foreign bodies inside the tubes. The perforations allow for the influx of inflammatory cells and fluid. After the animal has healed from the surgery for the cage implantation, a fluid sample is taken percutaneously from the tissue cage to confirm sterility. Bacterial suspensions are then inoculated into the tissue cage for subsequent tests of pathogenesis and treatments of foreign body infections. Fluid samples can also be analyzed for toxins and RNA expression of various genes to determine factors involved in the pathogenesis of infection (Goerke et al., 2001; Senn et al., 2005). In order to study the factors influencing adherence of *S. aureus* to materials, plastic or glass foreign bodies are placed inside the cage and tested for viable bacteria at the study's end (Falcieri et al., 1987; Fluckiger et al., 2005; Francois et al., 2003;

Wolz et al., 2002). This model is also useful in evaluating antibiotic therapies. The fluid samples can be analyzed for bacterial counts and drug concentrations to determine efficacy and compared to in vitro models for reliability (Blaser et al., 1995). The same model has been used to evaluate antibiotics such as fluoroquinolones, vancomycin, and rifampicin for the prophylaxis and treatment of both drug-susceptible strains and MRSA (Baldoni et al., 2009; Bouchenaki et al., 1990; Cagni et al., 1995; Schaad et al., 1994; Vaudaux et al., 2002). The treatment studies also have included the use of foreign bodies inside the cages, and culture results from fluid samples have correlated well with those taken directly from the foreign body itself (Cagni et al., 1995).

The tissue cage model is not the only model of foreign-body-related infection in guinea pigs. Metallic implants have been fixed onto the iliac bones or implanted subcutaneously to mimic infections associated with orthopedic devices (Fischer et al., 1996). A model utilizing dextran microbeads as a foreign body has been developed (Kaiser et al., 1992). The microbeads are mixed with a bacterial suspension and administered by intramuscular injection over the back, between the panniculus carnosus and superficial trunk musculature. Punch biopsies are taken for bacterial cultures at necropsy 3–4 days later. This model has been used to test the prophylactic and treatment efficacy of drug-susceptible and -resistant strains with treatments such as of ampicillin, cefazolin, vancomycin, fluoroquinolones, immunomodulatory agents, and drugs that inhibit bacterial entry into the cell (Kaiser et al., 1992; Kernodle and Kaiser, 1993a, 1993b, 1994; Kernodle et al., 1998; Menzies et al., 2002). Early experiments that used this model had high numbers of deaths from colitis secondary to the antibiotic therapy, and were able to decrease mortality from 40% to less than 10% by using gentamicin and polymyxin B in the water (Kaiser et al., 1992). These antibiotics are poorly absorbed from the gastrointestinal tract, so they did not affect the experimental infections.

Because of its ubiquitous nature, *S. aureus* can be associated with many other types of infections. *S. aureus* is a common cause of Gram-positive sepsis, and guinea pigs have been inoculated intravenously or by implantation of an intraperitoneal osmotic minipump to simulate disseminated disease. These studies have focused on pathogenesis including the development of disseminated intravascular coagulation (DIC), vascular leakage, and even the role of diet (Imamura et al., 2005; Kessler et al., 1991, 1997; Nelson et al., 1996). This model can be associated with high mortality. Guinea pigs have also been used to compare the virulence of drug-resistant and -susceptible strains (Cutler, 1979; Mizobuchi et al., 1994). A burn injury model was developed where injuries are created under anesthesia and then experimentally infected with *S. aureus* to evaluate the host immune

response and treatments (Bjornson et al., 1984; Kaufman et al., 1990; Orenstein et al., 1997). Other conditions that have been infrequently studied include *S. aureus* infective endocarditis (Maurin et al., 1997), mastitis (Vandeputte-Van Messom et al., 1995), dermonecrotic reactions (Miedzobrodzki and Tadeusiewicz, 1987), and middle ear infections (Sutbeyaz et al., 1996).

Listeria monocytogenes

Listeria monocytogenes is a Gram-positive foodborne pathogen that primarily affects immunocompromised, pregnant, or elderly people. It has been a reportable infection since the year 2000 with 2500 cases and 500 deaths in the United States annually (CDC, 2008d; Nieves, 2009). Listeriosis can vary from a mild, flu-like illness to gastroenteritis, sepsis, and severe CNS infections including meningitis and encephalitis. Infections in pregnant women can lead to stillbirth, spontaneous abortions, premature delivery, and disease in the neonate. The bacteria enter through the small intestine and then spread to target organs, including liver, spleen, and the fetus (Cabanes et al., 2008). Many laboratory animal species have been used as models. Early studies determined the LD50 of *L. monocytogenes* in the guinea pig after intraperitoneal and intravenous administration (Dustoor et al., 1977; Kautter et al., 1963). The advantage of the guinea pig model is that the bacteria cross the intestinal epithelium and cause gastroenteritis similar to the disease in humans (Cabanes et al., 2008). *Listeria* has a surface protein, internalin A that interacts with an intestinal epithelium receptor, E-cadherin (Lecuit et al., 1999, 2001). The E-cadherin of mice and rats differs in amino acid sequence from humans, which inhibits interaction with internalin A. In contrast, the E-cadherin homolog in guinea pigs permits entry of *Listeria* in the intestinal epithelium (Lecuit et al., 1999, 2001). Although subsequent spread to the brain and placenta does not occur as frequently in guinea pigs as it does in humans, placental infections do occur in guinea pigs and pregnant animals are used as models of fetoplacental transmission (Cabanes et al., 2008). The low incidence of spread to the CNS has been argued as one of the model's shortcomings (Gray and Killinger, 1966; Lecuit et al., 2007). Another potential limitation is that the guinea pig may lack one of the specific receptors for bacterial surface proteins thereby affecting tissue tropism (Khelef et al., 2006; Lecuit et al., 2007). Despite these potential limitations, the model continues to be popular. Readers are referred to Lecuit et al. (2007) for a review of the disease and animal models.

The guinea pig models often rely on administration of a product inoculated with *Listeria monocytogenes* into the gastrointestinal tract. Young Hartley guinea pigs are frequently used for these studies. Because it is a BSL2 pathogen, experimental procedures must involve the

appropriate precautions. Although specific protocols have been published that describe gavage and intragastric administration (Cabanes et al., 2008; Dussurget et al., 2002; Garner et al., 2006; Pang et al., 2007), many studies rely on consumed palatable products (Irvin et al., 2008; Jensen et al., 2008; Roldgaard et al., 2009; Williams et al., 2007). Some of these studies fasted animals for 12–48 hours, but most do not indicate a fasting period. Whipping cream is often used as the vehicle because the high-fat content mimics many of the natural modes of infection through high-fat foods such as cheese. During the study, fecal pellets are collected for bacterial counts. Animals are then euthanized at determined time points depending on the goals of the study, and bacterial counts determined in target organs such as jejunum, spleen, and liver and less commonly lymph nodes. Sublethal infective doses are typically used, because severe clinical signs and mortality can result from high doses (Bakardjiev et al., 2005; Cabanes et al., 2008; Lecuit et al., 2001).

Strains of *Listeria monocytogenes* vary greatly in virulence, and guinea pigs have been used to assess virulence and the potential risk the bacteria could pose to consumers. Bacterial strains that have been found in food-processing plants have been compared to clinical strains using the guinea pig model (Jensen et al., 2008a). Jensen et al. (2008) demonstrated a difference in fecal shedding between bacterial strains after oral administration that was consistent with predictions from other models of bacterial virulence, which helps validate the guinea pig as a method for distinguishing virulence. Anderson et al. (1980) showed that environmental conditions in which the bacteria grow prior to infection can affect virulence, which has implications for human safety if virulence varies for the same strain. Bacteria grown under oxygen-restricted conditions resulted in significantly higher bacterial counts in feces and tissues after oral administration. This study also attempted to evaluate a novel method of *L. monocytogenes* infection that would reduce the animal numbers needed to complete experiments. Multiple bacterial strains or monocultures were administered to the animals, and fluorescent labels were used to differentiate the different strains. Based on these results, the authors suggest that mixed infections do not decrease the ability of the model to discriminate between strain virulence and can utilize fewer animals than administering the bacteria as monocultures. These results were supported by another study that used these techniques to compare virulence between three strains (Roldgaard et al., 2009). Results were similar for each strain regardless of whether they were administered as monoculture or mixed infection.

Pregnant guinea pigs have been used to compare strain virulence and evaluate factors that can contribute to placental infections with *L. monocytogenes*. As with

other infections, the guinea pig model can be a useful model because both guinea pigs and humans have a hemochorial placenta (Bakardjiev et al., 2006). Pregnant guinea pigs are usually administered the bacteria via the gastrointestinal system or intravenously and euthanized prior to delivery (Bakardjiev et al., 2004, 2005, 2006; Irvin et al., 2008a; Jensen et al., 2008b; Williams et al., 2007). Parameters that are evaluated include maternal weight loss and clinical signs, fecal shedding of the bacteria, premature delivery or abortions, fetal viability, and bacterial counts in tissues from both the fetus and the sow. Fetal viability is determined based on physical characteristics indicating if the fetus had reached an expected stage of development and the general appearance including cyanosis, the placental blood and cord, and skin color. Williams et al. (2007) helped define this model by evaluating different infective doses administered orally to determine the appropriate dose for inducing stillbirths and infections of desired tissues. That study determined that 10^7 CFU was sufficient for this strain to adversely affect 50% of the pregnancies and result in disease with pathology similar to humans. Jensen et al. (2008b) evaluated orally administered strains found at a food-processing plant and determined that the strain isolated from the plant was capable of crossing the placenta. Oral inoculation in pregnant guinea pigs has also been used to evaluate the immune response and placental apoptosis as potential factors for negative consequences for the fetus during infection (Irvin et al., 2008a, 2008b). Several studies have utilized intravenous infection via the pedal or ear veins in pregnant guinea pigs to model vertical transmission. These studies characterize infection of the placenta and how it can serve as a nidus of infection to reinfect the sow (Bakardjiev et al., 2004, 2005, 2006). This model also results in bacteria crossing the placenta and inducing abortion with doses of at least 10^6 CFU of bacteria, which can be achieved without clinical illness in the mother.

L. monocytogenes infection has been used sporadically in other guinea pig studies. Pang and co-authors (2007) used older guinea pigs (approximated 2 years old) to determine the effects of immunomodulation with the immunostimulant vitamin E or the immunosuppressant cyclosporin A on the outcome of infection. There have also been reports of attempts to inoculate by other routes such as intratesticular (Sanui et al., 1982, 1983) and CNS (Nairn et al., 1995; Sanui et al., 1982). Bacteria have also been genetically altered to determine the role of certain proteins in pathogenesis after administration by both the gastrointestinal and intravenous routes (Dussurget et al., 2002; Garner et al., 2006).

Streptococcus pneumoniae

Streptococcus pneumoniae can cause a variety of clinical diseases in humans including pneumonia, bacteremia,

otitis media, meningitis, sinusitis, peritonitis, and arthritis. Sequelae can include tympanosclerosis from otitis media and sensorineural hearing loss from pneumococcal meningitis (Ozcan et al., 2008; Skinner et al., 2004). These diseases can be fatal, and high-risk groups are particularly susceptible to serious disease including the elderly, children under 2 years old and those with underlying medical conditions. The use of vaccines has decreased rates of infection since 2001, and the CDC currently recommends vaccination of infants, young children, and adults (CDC, 2008c). As with other bacteria, resistant bacteria are an emerging problem and beta-lactam resistance is common. *S. pneumoniae* is a pathogen for many species, and has caused outbreaks in guinea pig laboratory colonies (Saito et al., 1983). This bacterium can also be found in the respiratory tract of normal animals and disease may not occur unless precipitated by a stressor. Guinea pigs are used as models of this disease primarily for research related to bacterial pathogenesis and the development of treatments. Although this generally involves the CNS or inner ear, guinea pigs have also been used to test antibiotics after experimentally induced fatal pneumonia from intratracheal instillation of *S. pneumoniae* (Ponte et al., 1996).

A guinea pig model of intrathecal administration has been used to evaluate pathogenesis and antibiotic effectiveness for meningitis. This model can involve intrathecal injection of bacteria into the cistern magna (Blank et al., 1994; Force et al., 2008, 2009). Others have injected bacteria into the subarachnoid space after drilling a hole over the parietal skull bone (Winter et al., 1996, 1997). Infection can lead to labyrinthitis, meningitis, and death. After infection, cerebrospinal fluid (CSF) is taken at various time points to assess for inflammation, protein, and bacterial counts. Blood may be evaluated to assess for bacteremia. Other parameters measured may include auditory integrity and evaluation of the organ of Corti by electron microscopy or histology (Blank et al., 1994; Winter et al., 1996, 1997). This model has been used to test antibiotics such as meropenam, rifampin, ceftriaxone, and vancomycin (Force et al., 2008, 2009; Nairn et al., 1995). Force et al. (2008) compared the guinea pig to the traditional rabbit model of meningitis. The disadvantage of the guinea pig is that their small size only allows for CSF collection at a maximum of two time points, whereas rabbits allow collection of a larger volume. However, the authors suggest that guinea pigs are a more reliable model for testing efficacy of certain drugs. For example, rabbits easily hydrolyze the drug meropenam and metabolize it more quickly than guinea pigs and humans.

Guinea pigs have also been used to study the ototoxicity of *S. pneumoniae* by direct administration into the ear as a model of otitis media. Bacteria or pneumococcal

proteins are inoculated through the tympanic cavity and directly into the middle ear or cochlea. Parameters often measured include otomicroscopic exams and auditory nerve function, followed by euthanasia for histology and bacterial cultures (Cook et al., 1999; Hori et al., 2000; Ozcan et al., 2008; Skinner et al., 2004; Winter et al., 1998). This model has also been used to evaluate treatments such as topical doxycycline (Hori et al., 2000; Ozcan et al., 2008; Winter et al., 1998).

Helicobacter pylori

Helicobacter pylori causes active chronic gastritis and is a factor in the development of peptic ulcer disease and gastric cancer in humans (Keenan et al., 2003; Lee, 2000). The Mongolian gerbil is the model with the most severe pathology, but many animal models including non-human primates, cats, guinea pigs, ferrets, pigs, and mice have been used (Lee, 2000). Unlike other rodents, guinea pigs have several characteristics in common with humans that may facilitate the significant gastritis that occurs with *H. pylori* infection (Lee, 2000; Shomer et al., 1998). Guinea pigs have a homolog of interleukin-8, and *H. pylori* induces IL-8 secretion which attracts neutrophils and this cytokine may contribute to the gastric pathology (Keenan et al., 2003; Lee, 2000; Shomer et al., 1998). The anatomy of the guinea pig stomach is also similar to humans because it lacks a non-glandular portion that is present in other rodents (Shomer et al., 1998). In addition, guinea pigs have dietary requirements for vitamin C and levels of vitamin C in gastric juices comparable to humans. This is important because humans with *H. pylori* infections have low levels of vitamin C in gastric juices, which may contribute to the progression of disease (Nedrud, 1999; Shomer et al., 1998; Sjunnesson et al., 2003). Guinea pigs may also be good models because they have no natural *Helicobacter* species as pathogens that could complicate the model (Sjunnesson et al., 2003).

The pathology associated with *H. pylori* in guinea pigs has been well established. Most studies use the rodent-adapted Sydney strain or human clinical isolates that have been passaged in guinea pigs, but some do use clinical isolates directly from human biopsies. The adapted strains are associated with higher recovery of bacteria from the tissue after infection and better colonization of tissue than non-adapted strains (Rijpkema et al., 2001; Sturegard et al., 1998). *H. pylori* is usually administered via the oral cavity, but intra-gastric administration in several doses is also used, in both cases followed by euthanasia to evaluate pathology utilizing techniques such as culture, PCR, and histology (Shomer et al., 1998; Sturegard et al., 1998). *H. pylori* produces reliable infection in guinea pigs with severe antral gastritis and the formation of lymphoid follicles, and the lesion distribution is similar to that seen

in humans (Shomer et al., 1998; Sjunnesson et al., 2003; Sturegard et al., 1998). Infection with the Sydney strain of *H. pylori* can persist for at least 5 months (Sjunnesson et al., 2003). Recovery of bacteria and gastritis scores can be lowered by supplementing guinea pig diets with antioxidants, including vitamin C (Sjunnesson et al., 2001). Investigations have also evaluated the role of bacterial outer-membrane proteins and gastric proteins in the pathogenesis and ability to colonize the stomach (de Jonge et al., 2004; Lueth et al., 2005). Studies have also evaluated immune responses, and animals reliably seroconvert after infection (Rijpkema et al., 2001; Shomer et al., 1998; Sjunnesson et al., 2001; Sturegard et al., 1998).

In addition to pathogenesis, guinea pigs have been used to study treatments and prevention of *H. pylori* infections. Gonzalez-Segovia et al. (2008) looked at the effectiveness of the flavanoid quercetin on the inflammation and lipid peroxidation induced by *H. pylori*. Studies on the use of vaccines have indicated that the vaccine can induce an immune response and may reduce infection, but the humoral immune response does not correlate with protection (Durrani and Rijpkema, 2003; Keenan et al., 2003).

Superficial Fungal Infections

Guinea pigs are used as a model for superficial fungal infections because of their susceptibility to dermatophytosis. In humans, onychomycosis and dermatophytosis are most commonly caused by the organisms of the genera *Trichophyton*, *Microsporum*, and *Epidermophyton* (Ghann, 2009). Dermatophytosis is described more specifically as infections of the scalp (tinea capitis), feet (tinea pedis), and body (tinea corporis). Children in particular are prone to tinea capitis (Saunte et al., 2007). *Trichophyton* is the most common causative agent of tinea pedis (Treiber et al., 2001). *Microsporum canis* is primarily a pathogen of dogs and cats, but is contagious to other species including humans and is a frequent cause of tinea capitis (Saunte et al., 2007; Vermout et al., 2008). Animal models are necessary, particularly for the development of new treatment options, because of the side effects that can be associated with systemic antifungals and the possibility of treatment failures or relapse after standard treatments are discontinued (Ghannoum et al., 2009; Nagino et al., 2000; Saunte et al., 2008).

The guinea pig model of infection with both *T. mentagrophytes* and *M. canis* has been characterized. Since early studies indicated that guinea pigs could be reliably infected with multiple dermatophytes, including *T. mentagrophytes*, *M. canis*, and *Epidermophyton floccosum*, studies have attempted to optimize these protocols (Chittasobhon and Smith, 1979; Greenberg et al., 1976). Some researchers perform these studies under ABSL-3 conditions because of the high risk of transmission to other animals, but dermatophytes do not meet

the typical characteristics of ABSL-3 agents from the perspective of human health (Saunte et al., 2007, 2008). Guinea pigs are usually shaved with electric clippers, and then the area is abraded prior to inoculation. Items used for abrasion have included pumice stone, sandpaper, hand razor, roughened glass pestle, razor blades, or even repeated application of tape (Chittasobhon and Smith, 1979; Ghannoum et al., 2004; Nagino et al., 2000; Petranyi et al., 1987; Saunte et al., 2007; Treiber et al., 2001). However, one study that compared removing hair with a razor or tape found reliable infection with both *T. mentagrophytes* and *M. canis* without additional abrasion, and the hand razor was the preferred method only for practical reasons (Saunte et al., 2008). Studies have indicated that *T. mentagrophytes* is most infectious 10–12 days after inoculation in culture, so that is the best time to use the fungus for animal inoculation (Treiber et al., 2001). Although some studies use occlusive bandages during infection, most studies do not and results indicate that it can have variable effects (Kerbs and Allen, 1978; Saunte et al., 2008; Valiant and Frost, 1984). Studies have also evaluated the optimal concentrations of *T. mentagrophytes* and *M. canis* for infection and found the severity of infection was associated with the inoculum concentration for *T. mentagrophytes* but not *M. canis* (Ghannoum et al., 2004; Saunte et al., 2008). After infection, animals are assessed based on clinical scores of the lesion that consider erythema, alopecia, crusting, and duration of lesions and mycology by microscopy, culture, histopathology, and the hair root invasion test. The hair root invasion test involves incubating hairs on a Petri dish for 2 days followed by counting the number of hairs showing fungal growth at the hair root (Saunte et al., 2008; Treiber et al., 2001). Majima and co-authors (2005) created a novel method for evaluating treatment efficacy with a mycological assessment that focuses on the size of colonies grown from skin specimens in guinea pigs, while the conventional mycological assessment relies on positive culture but is limited in ability to quantify recovery of the fungus. A model of tinea pedis has also been developed that results in consistent infection with the appropriate dose of inocula with *T. mentagrophytes* without the need for abrasion (Arika et al., 1990; Fujita and Matsuyama, 1987; Majima et al., 2005; Ohmi et al., 1991).

The guinea pig model has primarily been used to assess topical and systemic treatments for superficial fungal infections. The efficacy of drugs against both *T. mentagrophytes* and *M. canis* has been compared with medications such as allyamines (e.g. terbinafine) and azoles (e.g. ketoconazole, econazole, fluconazole) and mitotic inhibitors (griseofulvin) and others (Arika et al., 1990; Borgers et al., 1993; Ghannoum et al., 2004; Meerpoel et al., 2005; Nagino et al., 2000; Ohmi et al., 1991; Petranyi et al., 1987; Saunte et al., 2008; Uchida

and Yamaguchi, 1984; Yamaguchi and Uchida, 1984). Ghannoum and colleagues (2009) evaluated terbinafine solutions with nail penetration enhancers that would allow treatment of deep nail infections, although this study still evaluated the treatments using the standard skin models instead of nails (Ghannoum et al., 2009; Uchida and Yamaguchi, 1984). In addition to standard mycological and clinical assessment, drug studies also may measure drug concentrations in the blood and in skin biopsies (Saunte et al., 2007). This model has also been used to assess alternative treatments that may be supplements to traditional treatments, such as compounds isolated from Japanese cypress oil (Lee et al., 2007).

The guinea pig model has been used to study other aspects of dermatophytosis, including pathogenesis, diagnostics, and vaccines. Vermout et al. (2008) studied proteins involved in pathology of dermatophytosis by testing the proteins' ability to induce skin reactions and by detecting expression of these proteins in experimental infections. PCR has been found to be more sensitive for diagnosis of superficial fungal infection in guinea pigs than the standard diagnostics of microscopy and culture (Saunte et al., 2007, 2008). Guinea pigs have also been used to produce spores of fungi for analysis from clinical samples when only mycelial forms are available (Moriello et al., 2004). Subunit and DNA vaccines have been evaluated and can be partially effective, but efficacy does not correlate with antibody response (Descamps et al., 2003; Mignon et al., 1999; Vermout et al., 2004).

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Taxonomy and History

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TAXONOMY

Hamsters are classified within Rodentia, the largest mammalian order. Rodents are classically divided into three suborders: Sciuromorpha, Myomorpha, and Hystricomorpha. Muroidea, a superfamily within the suborder Myomorpha, includes the closely related families Muridae and Cricetidae, murine rodents and hamsters, respectively. Taxonomists believe that Cricetidae were the older radiation once common in North Asia, Europe, and Africa that then extended to North and South America. Muridae evolved from an early cricetine rodent and spread to Southeast Asia, Australia, Europe, North Asia, and Africa. The family Cricetidae includes 681 species distributed world-wide except Australia. The subfamily Cricetinae includes seven genera: *Allocricetulus*, *Cansumys*, *Cricetulus*, *Cricetus*, *Mesocricetus*, *Phodopus*, and *Tscherskia*. Cricetinae are all found in Europe, Asia, and the Middle East (Wilson and Reeder, 2005). The Syrian hamster, *Mesocricetus auratus*, was first described and originally named *Cricetus auratus* by Waterhouse in 1839. The original description was based on study of the skin and skull of a female. Nehring (1902) was the second known person to study the Syrian hamster. He used a preserved female at the

Beirut Museum, and in 1898 established the genus *Mesocricetus* for certain hamsters from southeastern Europe and Asia Minor. In 1902, he renamed the Syrian hamster *Mesocricetus auratus*. However, for several decades, the Syrian hamster was still considered to be a subgenus of *Cricetus* and was termed *Cricetus (Mesocricetus) auratus*. In 1940, Ellerman pointed out that *Mesocricetus* was a well-characterized and distinct genus (Granados, 1951).

GEOGRAPHICAL DISTRIBUTION

The natural habitat of Syrian hamsters is a small area in northwest Syria in the vicinity of Aleppo. Syrian hamsters construct deep, chambered burrows in which they store grain and other feedstuffs foraged from the fields (Billingham and Silvers, 1963). Continued presence of Syrian hamsters in the wild was most recently confirmed by expeditions to northern Syria and southern Turkey in 1997 and 1999 (Gatterman et al., 2001). Their natural habitat is dry, rocky steppes or brush slopes. Hamsters are primarily granivorous, but also eat green plant parts and shoots, roots, insects, and fruit. Adults generally live one to a burrow and will readily

fight one another (Anderson and Jones, 1984; Nowak and Paradiso, 1983).

ORIGIN AND DOMESTICATION

Laboratory hamsters are believed to have originated from three or four littermates captured near Aleppo in Syria in 1930. Thus, they not only have a very narrow genetic base, but they have been domesticated for a relatively short time in comparison with mice, rats, and guinea pigs. More recently, additional wild hamsters have been trapped and studied (Murphy, 1971) and a comprehensive history of the capture and domestication of the Syrian hamster has been published (Murphy, 1985).

In the late 1920s, Dr. Saul Adler of the Hebrew University, Jerusalem, was studying leishmaniasis in Chinese hamsters, the only suitable experimental animals for these studies at the time. Chinese hamsters did not reproduce well in captivity and it was necessary to import them from the Far East; thus, he was interested in finding an indigenous species for experimental use, preferably *Cricetulus phaeus*, a close relative of the Chinese hamster (Yerganian, 1972). In 1930 Professor I. Aharoni, a zoologist from Hebrew University agreed to collect and provide specimens of *C. phaeus* for Dr. Adler's work. Professor and Mrs. Aharoni went on a zoological expedition to Syria where they collected *C. phaeus*, but also recovered a litter of *Mesocricetus auratus*. A local Sheikh hired a team to excavate a hamster burrow on a farm located 30 km from Aleppo, Syria (latitude, 40° 10'; longitude 44° 30'). A nest 30 cm in diameter containing a female with 11 young was found 2.5 meters underground. Professor and Mrs. Aharoni successfully reared nine hamsters from this litter and presented them to Mr. H. Ben-Benachen, head of Hebrew University Animal Facilities. Unfortunately, the animal caretakers underestimated the hamster's ability to escape and only one or two females survived to produce young. (There is discrepancy regarding the sex and number of the remaining hamsters. In Adler's account, one male and two females remained (Adler, 1948) but Aharoni stated in his memoirs that only one female and three males remained (Yerganian, 1972).) The first known litters born in captivity were produced from these animals. Thereafter, a laboratory-reared colony of Syrian hamsters was quickly established.

Dr. Adler quickly realized that Syrian hamsters were useful laboratory animals. He decided to distribute the animals as widely as possible to avoid the risk of maintaining a valuable species in one laboratory. In 1931, he carried a few animals to Professor Edward Hindle of the Wellcome Bureau of Scientific Research in England (Bruce and Hindle, 1934). He also distributed them



FIGURE 26.1 Gulf Hamstery ad from *Mechanix Illustrated*, circa 1949.

to the Medical Research Council and the College of France. In 1938, animals were sent from Palestine to the U.S. Public Health Service Hospital, Carville, Louisiana and Western Reserve University in Ohio (Bond, 1945). A third colony of Syrian hamsters was established at the Rockefeller Foundation in 1938 (Poiley, 1950). Therefore, it appears that most of the Syrian hamsters used as laboratory animals or pets are descendants of the original three or four surviving littermates.

After 1950, research demands for laboratory hamsters were met by two major commercial suppliers in the United States: Engle Laboratory Animals in Farmersburg, Indiana and Lakeview Hamster Colony, Newfield, New Jersey.

Engle Laboratory Animals was founded in 1949 by a 14-year-old boy, Everett Engle, when he responded to a classified ad in *Mechanix Illustrated* placed by Albert F. Marsh, Gulf Hamstery, Mobile, Alabama (Figure 26.1). With money loaned from his father, young Everett purchased a trio of two females and one male hamster for \$15; included with his purchase was *The Hamster Manual*, a complete manual for breeding and raising of hamsters, authored and published by Mr. Marsh. His father's coal shed was furnished for raising hamsters and within 2 years Everett was filling orders for Gulf Hamstery and shipping hamsters by rail express out of Farmersburg to customers throughout the Midwest (Figure 26.2). Customers began calling Everett's high school to place orders until the principal found out; Everett's customer service department was thereafter limited to his home phone. Upon graduation from high school, Everett married his high-school sweetheart; her father had reluctantly consented to giving his daughter's hand in marriage to the young hamster entrepreneur. Everett worked for the A & P Food Plant until his mid-20s when he decided to devote all of his efforts to his hamster business. The A & P manager was skeptical and would not permit him to resign but instead gave him a leave of absence. Everett never returned to his job at the A & P. His early customers were Midwestern pharmaceutical firms (Pfizer, Mead Johnson, Dow and Lilly), but many more customers came on board after he began advertising in *Laboratory Animal Care*, the bimonthly journal



FIGURE 26.2 Hamster production, Everett Engle, 1951.

Pregnant Hamsters

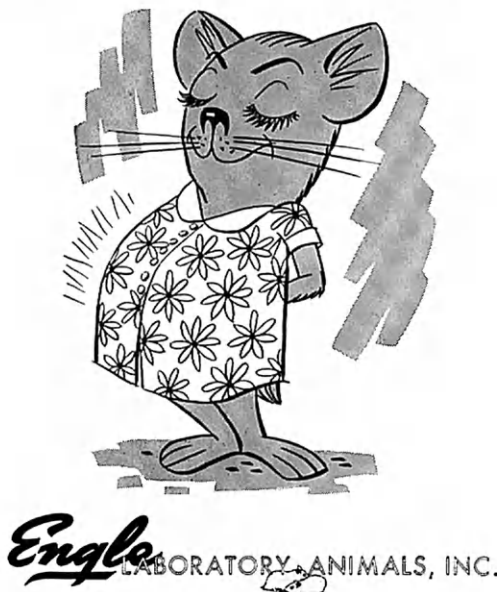


FIGURE 26.3 Advertisement for Engle Hamsters.

published by the American Association for Laboratory Animal Science (Figure 26.3). In 1961, the Engle family's graciousness was acknowledged by the White House when they received two hamsters to replace Caroline Kennedy's escaped pet. By 1970, Engle Laboratory Animals was producing 14000 hamsters per week for research laboratories throughout the world. Engle attributed his success in raising hamsters to minimizing stress during weaning and shipping. "Wet tail" was not a problem if shipping boxes were not overcrowded and carefully packed by the Engle family. In 1984, Engle Laboratory Animals and the hamster colony were sold to Harlan Laboratories; Everett has retired and his three sons Mike, Mark, and Tony have continued their careers in laboratory animal production with Harlan (Engle, 2009).

LAKEVIEW HAMSTERS

PROFITABLE — ENTERTAINING — EASY TO RAISE

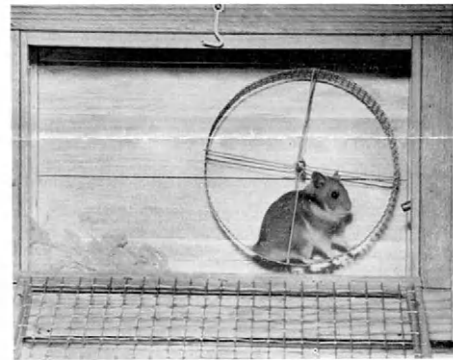


FIGURE 26.4 Lakeview Hamster Colony brochure.

The field of laboratory animal science is indebted to a glass company, Owens-Illinois, for asking one of their ceramic engineers, Mr. Steven Slater, to transfer from their New Jersey location to a job in Ohio. Mr. Slater turned down the job move because he wanted to keep his family in New Jersey and founded his own company, Lakeview Hamster Colony in 1949 (Figure 26.4). His vision and business plan were to establish and market the Syrian hamster to the research community on the eastern seaboard. Lakeview hamsters were established with breeding stock from Gulf Hamstery (Mobile, Alabama); Hudson Hamstery, (Newfoundland, New Jersey); and the South Jersey Hamster Colony (Newfield, New Jersey). The Hudson Hamstery was also established with breeding stock from Gulf Hamstery. In 1951, the Lakeview Hamster Colony was merged with a hamster colony that was purchased from Mr. Angelo from Leesburg, New Jersey; Mr. Angelo's colony was established from stock obtained from Albert Hayner of Normal, Illinois. After 1951, Lakeview Hamster Colony was closed to the introduction of new breeding stock. Mr. Slater diligently marketed the Lake View hamster to research laboratories in the Philadelphia/New York area. Much of the growth in the use of hamsters for infectious disease research was attributed to research publications from poliovirus pioneers Salk and Sabin (Slater, 2009).

Endemic wet-tail was initially a challenge for the Lake View organization; the incidence was greatly reduced by selecting replacement breeders from litters derived from parents that produced progeny without wet-tail. In spite of consulting with a leader in the field, Dr. Lisabeth Kraft, Mr. Slater was unable to develop the germ-free hamster by foster suckling or hand-feeding young derived by caesarian section. Slater developed the defined-flora hamster by first using diagnostic assays to select a large cohort of viral antibody-free hamsters. These hamsters were treated with antibiotics

and introduced through a Wescodyne® dunk tank into a germ-free isolator where they were reassociated with a defined flora cocktail. In 1969, Lake View was sold to Charles River Laboratories and Gil Slater, Steven's son, continued to serve in the business of laboratory animal production.

Both major U.S. suppliers of research hamsters, Lake View and Engle, obtained their breeding stock from the Gulf Hamstery colony in Mobile, Alabama. The owner of this business, Albert F. Marsh (Figure 26.5), told a reporter from the Mobile Press-Register that he had won a Syrian hamster as payment of a \$1 gambling debt (Helms, 2008). Most likely this hamster originated from the colony held at Carville, Louisiana. Mr. Marsh marketed the hamster through several common periodicals and published 80000 copies of the *The Hamster Manual* during the period 1948 to 1951 (Marsh, 1951).

Since the Aharoni expedition in 1930, an additional 12 Syrian hamsters were captured by Syrian farmers and imported to the United States. The hamsters were sent to the Massachusetts Institute of Technology, for observations on their natural history and comparisons with domesticated stock (Murphy, 1971). Descendants of these animals are maintained at the National Institutes of Health, Bethesda, Maryland (Murphy, 1985). New breeding stock were also transported to Germany from expeditions to Syria and Turkey in 1997 and 1999 (Gatterman, 2001).

Syrian hamsters were introduced to the scientific community by Adler and Theodor (1931), who reported on their use in Kala-azar studies (visceral leishmaniasis). They were quickly accepted as research animals and were used in a variety of studies (Anonymous, 1969; Granado, 1951). Some examples of early studies involving hamsters included breeding and growth (Bruce and Hindle, 1934); susceptibility to *Brucella abortus* (Tchernomoretz and Ellenbogen, 1937); susceptibility to human leprosy (Adler, 1937); reproductive physiology (Klein, 1938); reproductive cycle (Deansely, 1938); susceptibility to tubercle bacilli (Griffith and Pagel, 1939); host for St. Louis encephalitis (Broun et al., 1941); influenza virus infection (Wheeler and Nungester, 1942); effect of hormones on reproduction (Peczenik, 1942); experimental rickets (Jones, 1945); experimental cholesterol arteriosclerosis (Altschul, 1946); tumor transplantation (Crabb, 1946); inoculation with Newcastle disease virus (Reagan et al., 1947); effect of X-radiation (Kivy, 1947); biological exchange of trace elements (Smith, 1947); and toxic effect of beryllium (Sprague et al., 1948).

Before 1954, over half of the publications regarding use of Syrian hamsters involved microbiology, parasitology, or dental disease (Magalhaes, 1954). By 1963, the relative use of the Syrian hamster in these fields of study had declined, but hamsters were being actively used in cancer research and reproductive physiology



FIGURE 26.5 Albert F. Marsh, founder and owner of Gulf Hamstery, Mobile, Alabama.

(Magalhaes, 1968). Use of hamsters in research reported to the United States Department of Agriculture has gradually declined in absolute number and percentage animal use since 1972. In the federal Fiscal Year 2006–2007, registered research facilities in the United States reported use of 172498 hamsters or 19% of total animal use reported (United States Department of Agriculture, 2008).

GENETICS

In the recommended rules for a nomenclatural system, the symbol for *M. auratus* is (SYR) (Institute of Laboratory Animal Resources, 1970). Information regarding inbred strains, genetically defined stocks, and mutants for coat color and other coat characteristics has been provided (Nixon, 1979; Nixon et al., 1979; Slater, 1979; Yoon and Peterson, 1979). Variants include white, cream, piebald, albino, hairless, and longhair (teddy bear) (Fukuta, 1993). Studies of strain-specific responses have been published for the following strains: CAN, CN, APA, APG, CBN, GN, and ACNT (Frank, 2004); LPN (Férezou et al., 2000); Bio F1B (Cheema and Cornish, 2007); BIO 1.5 and Bio 87.20 (Vitaterna, 1993); KVL (Salem et al., 1993); MHA, PD4, LSH, CB, LVG, and LHC (Genovesi, 1988).

Acknowledgments

This chapter is a revision of "Historical Perspectives and Taxonomy" by J. Derrell Clark from Laboratory Hamsters, G.L. Van Hoosier, Jr., and C. W. McPherson, eds. Academic Press, 1987. Much of the original text and references was unchanged because Dr. Clark's astute perspective should be maintained.

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Anatomy, Physiology, and Behavior

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INTRODUCTION

The anatomical, physiological, and behavioral characteristics of the laboratory golden Syrian hamster (*Mesocricetus auratus*) are summarized in this chapter.

EXTERNAL FEATURES

An adult Syrian hamster is approximately 14–19 cm in length and weighs approximately 114–140 grams.

Hamsters have thick compact bodies with short legs, a short tail, large cheek pouches, and excess loose skin. Dorsal flank glands, which are more prominent in males, are another noticeable external feature. Flank glands are sebaceous glands that are responsive to androgens and used to mark territory. A reddish gold dorsum with a grayish white ventral surface is the most common coloration pattern of the short and smooth haircoat found in hamsters purpose-bred for biomedical research. The number of pattern mutant and coat color genes observed in hamster colonies has increased

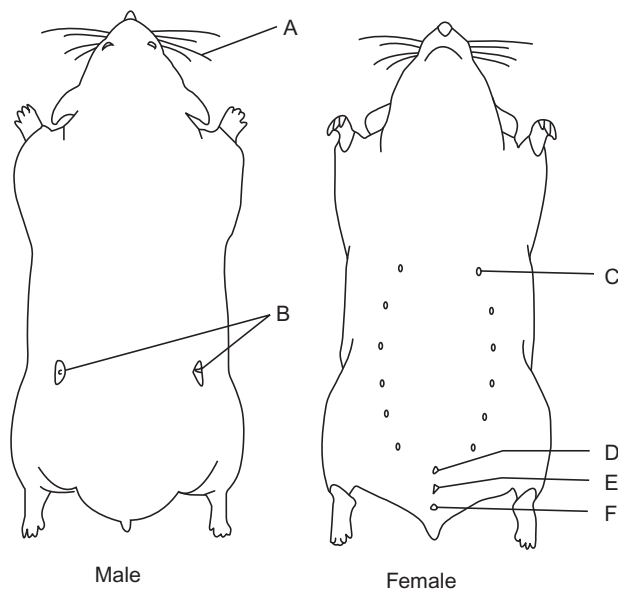


FIGURE 27.1 Dorsal view of a male hamster (left) and ventral view of a female hamster (right). A, Vibrissae; B, flank glands; C, mammary glands; D, urethral opening; E, vaginal opening; F, anus.

during the past six decades (Robinson, 1990). A variety of colors and hair lengths, such as the popular long-haired or “teddy bear” have been bred and maintained in the pet industry. Dorsal and ventral views of an adult hamster are outlined in Figure 27.1. The sexually mature male is characterized by an enlarged, rounded posterior when the testes are in the scrotal sacs, a long anogenital distance, and a penis that is retracted. The mature female has a pointed posterior end, a short anogenital distance, a vaginal opening, and a separate urethral opening. As with other rodents, newborns can be sexed by comparing the distance between the anus and the genital papilla. The distance is greater in the male than in the female and the male genital papilla is larger than that of the female.

MUSCULOSKELETAL SYSTEM

The hamster vertebral formula is seven cervical, 13 thoracic, six lumbar, four sacral, and 13–14 caudal (Salih and Kent, 1966). There are four digits on the front paws and five digits on the hind paws. There is little information in the literature regarding the musculature system of the hamster. Salih and Kent (1964) describe the muscle groups including origins and insertions of the epaxial musculature. Several inbred lines of Syrian hamsters, BIO 1.50 and BIO 12.14, have been reported to develop muscular dystrophy and are described by Homberger (1972).

DIGESTIVE SYSTEM

Oral Cavity

The free margins of the lips form a three-cornered flap which seals the mouth opening when closed and aids in filling the cheek pouches. The oral opening is approximately 12 mm × 17 mm (Schwarze and Michel, 1960). The hard palate, 15.0–16.5 mm in length, begins at the caudal edge of the incisors and extends to the end of the last molar where the soft palate, 4–6 mm in length and smooth, begins. The hard palate mucous membranes form two groups of seven ridges located on either side of the midline. The ridges slope labially near the molars and in a pharyngeal direction when not near the molars. The nasopalatine duct enters the oral cavity on the labial surface of the first pair of ridges.

Dentition

The dental formula is: 2/2 incisors 1/1, canines 0/0, premolars 0/0 and molars 3/3. Incisors are open-rooted and continually erupt throughout the hamster’s life. Dentition is monophyodont, bunodont, and brachyodont. Incisors erupt shortly after birth with molars eruption beginning between days 7–10 (M1), days 12–20 (M2) and days 30–35 (M3) (Gaunt, 1960; Hinrichsen, 1955; Keyes and Dale, 1944; Schwarze and Michael, 1960). Age, sex, diet, and season may affect rate of incisor growth. The short (2.5 ± 0.5 mm) maxillary incisors can replace themselves in 1 week while the longer (10.0 ± 1.8 mm) mandibular incisors can require 2.5–3 weeks for replacement. Both incisor sets extend lingually and end in a notch at the gingival margin. The maxillary diastema which separates the incisors from the molars is larger (9–11 mm) than the mandibular diastema (4–6 mm). Mandibular incisors may be located on either the labial or lingual side of the maxillary incisors. The mandibular symphysis may not fuse throughout life (Schwarze and Michel, 1960).

The molar teeth form a convex arch with the maxillary molars pointing outward and mandibular molars pointing inward (Schwarze and Michel, 1960). Molar cusps are conical, pointed, and in rostrocaudal alignment. The cusps are separated by broad buccolingual sulci with deep occlusal fossae and grooves (Keyes and Dale, 1944). Newly erupted molar crowns are completely covered by enamel, and roots are covered by a thin layer of cell-free cementum. Attrition causes exposed primary dentin to become metamorphosed with secondary dentin deposited on the underlying pulpal wall. Secondary dentin is deposited at the root apices. Occlusion is by cuspal interdigitation. Attrition is initiated on the mesial slopes of mandibular cusps and distal slopes of maxillary cusps, and spreads over the occlusal surface (Keyes and

Dale, 1944). Morphologically, the crowns allow retention of fine food particles, making the hamster susceptible to caries and a model for human dental caries (Arnold, 1942; Dale et al., 1941; Lovelace et al., 1958).

Salivary Glands

The hamster salivary glands consist of submaxillary, retrolingual, parotid, and sublingual (Boerner-Patzelt, 1956) and are compound tubuloacinar structures. Acinar elements complete their differentiation early in postnatal development. Convolved granule tubules have a more gradual and prolonged postnatal development, becoming completely differentiated upon sexual maturity. Submaxillary gland acini are morphologically and histochemically intermediate between mucous and serous acini (Shackelford, 1961). The sialic acid content of the submaxillary gland is influenced by the presence of female sex hormones, therefore the content is greater in females than males (Shackelford and Klapper, 1962a). The organization of the parenchyma of the hamster submaxillary gland is similar to that of the rat and mouse (Flon and Gerstner, 1968). Narrow intercalated ducts connect the acini to the branches of the granular convoluted tubules. These tubules are continuous with the striated duct segments of the excretory system. The major part of the submaxillary gland consists of acinar elements, closely resembling the rat's gland (Devi and Jacoby, 1966). The tubule/acinus ratio, size, and appearance of the cells of the convoluted tubules are similar for both male and female.

The parotid gland, located below the ear, is structurally similar to other mammals (Shackelford, 1961). Four salivary ducts (two dorsal, one central, and one ventral) emerge from each parotid salivary gland. The gland is covered by the cheek pouch and ends near the maxillary edge of the entrance to the cheek pouch, 3mm labially from the first maxillary molar (Schwarze and Michel, 1960). In the hamster, most proteolytic enzyme activity is confined to the parotid gland (Shackelford, 1961), whereas the rat and mouse have the most proteolytic enzyme activity in the submaxillary gland (Shackelford and Klapper, 1962b). Proteolytic enzyme activity is lowest in the hamster submaxillary gland.

The sublingual gland is composed of mucous acini, serous demilunes, and numerous myoepithelial cells (Alm et al., 1973; Shackelford, 1961).

Cheek Pouches

The hamster has large bilateral buccal cheek pouches that are located along the side the head. These distensible, well-vascularized structures are thin-walled, lined with stratified squamous epithelium, devoid of lymphatic tissue and rich in mast cells (deArruda and

Montenegro, 1995). The cutaneous mucosa has fine folds covered with tiny protrusions that are more numerous near the entrance of the pouch (Schwarze and Michel, 1960). The pouch wall is composed of four layers: stratified squamous epithelium on the surface, dense fibrous connective tissue, longitudinal striated muscle fibers, and loose areolar or submuscular connective tissue where the pouch joins the underlying structures. Blood vessels and nerves are found in the connective tissue and muscle layers (Billingham et al., 1960; Fulton et al., 1946; Lindenmann and Strauli, 1968).

The pouches are approximately 35–40mm long and 4–8mm wide when empty and up to 20mm wide when full. They are relatively easy to evert. Feed and bedding may be stored in cheek pouches. Female hamsters with newborn pups may place the young in cheek pouches if stressed. The pouches are emptied by a massaging action with the front feet and action of the tongue. The pouches have been termed, "immunologically privileged" due to the lack of an intact lymphatic drainage system (Bergstresser et al., 1980). The unique anatomical and physiological characteristics of the cheek pouches have contributed to their extensive use for microvascular studies of tumor growth, carcinogenesis, and inflammation (Gimenez-Conti and Slaga, 1993; Hedqvist et al., 1990; Svensjo, 1990).

Stomach

The hamster stomach is approximately 3.5cm long and 2.0cm wide and is divided into two distinct areas: glandular stomach and non-glandular forestomach. The forestomach and glandular stomach are separated from each other by the incisurae of the greater and lesser curvatures (Magalhaes, 1968). The esophagus enters into the forestomach, which is lined with keratinized epithelium, has a higher pH than the glandular region, and contains microorganisms that enable fermentation which may aid in the digestive process. The pyloric region of the glandular stomach empties into the small intestine.

Small Intestine

The duodenum begins at the pylorus of the stomach and runs caudally as the descending duodenal loop. This loop is located ventral to the transverse colon and the beginning of the jejunum. The duodenum then reverses and returns to the left as the ascending duodenal loop. At the point where the ascending loop crosses the descending duodenal loop it becomes the jejunum. The jejunum, which is formed into multiple small loops, is approximately 2.5 times the length of the duodenum. Duodenal villi first appear at 13.5 days of gestation. By birth the villi have changed from a square

to a columnar shape. After birth, absorbed colostrum appears as distinct round lipid droplets in the intestinal epithelium. The droplets can be found for up to 6 days (Koruhmaru et al., 1979). The ileum is short, less than 2 cm in length, and connects the jejunum to the cecum (Malgalhaes, 1968).

Cecum

The hamster cecum is slightly sacculated and divided into apical and basal portions (Krueger and Rieschel, 1950). A groove in the external cecal wall corresponds to the location of a semilunar valve delineating the division between the two portions (Yonce and Krueger, 1952). There are four valves in the hamster ileocecal–colic junctions: the ileocecal valve, the apicobasal valve, the basal semilunar valve, and the chevron valve. The ileocecal valve has a semilunar lip projecting into the lumen of the ileum. The apicobasal semilunar valve, located on the apical side of the ileocecal junction, functions to shunt the contents of the small intestine directly into the colon. The basal semilunar valve is located 5 mm toward the colon from the apicobasal semilunar valve, while the chevron valve is located at the cecal–colonic junction and is formed from the ends of the muscular chevrons located in the colon (Krueger and Rieschel, 1950).

Large Intestine

The ascending colon runs cranially from the cecal–colonic junction, under the jejunum, before turning to form a horseshoe-shape that becomes the transverse colon. The transverse colon runs from right to left, just caudal to the liver and stomach prior to turning caudally to become the descending colon which empties into the rectum and anus.

Pancreas

The hamster pancreas consists of three lobes: gastric, splenic, and duodenal, and averages 0.46 g of weight or approximately 0.4–0.5% of total body weight. The gastric lobe of the pancreas, which comprises approximately 25% of the weight of the pancreas, is attached to the stomach and pylorus by the mesogastric membrane formed from the greater omentum. The splenic lobe, which comprises approximately 40% of the pancreatic weight, is located caudal to the spleen along the greater curvature of the glandular stomach. The splenic lobe has a connective tissue attachment to the cranial descending colon. The smallest lobe, the duodenal lobe, comprises approximately 12% of the pancreatic weight and is enclosed in mesentery between the duodenum and cranial ascending colon (Takahashi et al., 1977).

The common bile duct passes through the head of the pancreas and merges with the three major pancreatic ducts prior to emptying into the duodenum, similar to mice and rats, but different than other mammals including man.

Organogenesis of the pancreas begins at 10.5 days of gestation (Boyer, 1968). The islets of Langerhans develop during the first 2 weeks of life and double in size by 4 weeks of age but do not reach full size until about 1 year of age. The acini of the hamster pancreas reach full size at 4 weeks of age (Orgrowsky et al., 1980).

Liver and Gall Bladder

The hamster liver consists of four lobes: right dorso-caudal, left dorsocaudal, dorsal median, and ventral median. The dorsal median lobe is divided into a large right dorsal part and a smaller left dorsal part. The ventral median lobe is divided by the umbilical fissure into a right cranioventral part and a lateral left cranioventral part (Nettleblad, 1954). The gall bladder is located in the right cranioventral section of the ventral median lobe of the liver. The gallbladder wall consists of three major layers: mucosa, muscularis, and serosa. Goblet cells are completely absent from the epithelium, while cask-like and crayon-like cells are prominent in the bottom folds (Yamada, 1959, 1969). Hamster bile acids have a similar composition to human bile (Reyes and Kern, 1979).

Liver weight significantly increases during pregnancy and at a rate that is proportionately greater than the increase in body weight. Concurrent with this increase in liver weight is a decrease in bile flow such that the mean rate of bile flow in an 8-day pregnant and a 14-day pregnant hamster is reduced by 37% and 64%, respectively, when compared to a non-pregnant female hamster (Reyes and Kern, 1979). In addition, a reduced secretion of total bile acids due to decreased cholic acid secretion, a decrease in sodium, potassium, ATPase activity and a decrease in the acid-dependent bile flow is observed (Reyes and Kern, 1979).

CIRCULATORY SYSTEM

Heart and Vascular System

The four-chambered heart is located at the level of the third to fifth thoracic ribs. The pulmonary and aortic valves are tri-cuspid. Both the left and right atrioventricular valves have two major and two minor accessory leaflets. Figures 27.2 and 27.3 are schematics of the major blood vessels. Walls of the pulmonary vein have muscle fibers similar to myocardium, like mice and rats. The media of the coronary arteries in the hamster

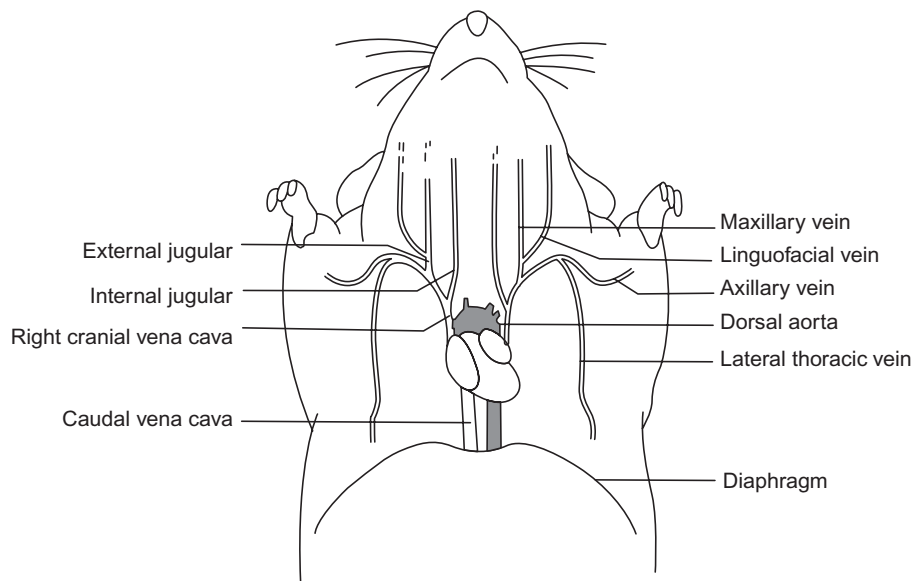


FIGURE 27.2 Schematic of the major veins of the golden hamster.

consists of circular muscle, which is different than the rat, where the media is composed of longitudinal muscle fibers (Kovalevskii, 1966). Physiologic characteristics of the cardiovascular system are described in Table 27.1.

Orbital Venous Sinus

The hamster has an orbital venous sinus similar to the mouse. This sinus is an elongated venous channel formed by the confluence of the ophthalmic veins in a groove of bone formed in the presphenoid and maxillary bones. Pre-rinsing blood collection equipment with an anticoagulant such as 1:1000 heparin is recommended to facilitate blood collection from the hamster due to a relatively rapid coagulation time (Edwards, 1967; Pansky et al., 1961; Timm, 1979).

Lymphatic System

The lymphatic system in the hamster is depicted in Figure 27.4. The 16 groups of lymph nodes are located in similar areas in the hamster as those locations described for other rodents (Belisle and Saint-Marie, 1981; Kawashima, 1972).

PULMONARY SYSTEM

The respiratory system of the hamster consists of the nasal cavity, nasal sinuses, pharynx, larynx, trachea, and lungs. The hamster nasal cavity consists of vestibular, olfactory, and non-olfactory portions. The larynx is composed of the epiglottic, thyroid, cricoid, and arytenoid cartilage. The trachea bifurcates at the level of the fourth rib. The lung lobes, illustrated in Figure 27.5,

consist of a single lobe on the left and four lobes on the right (Malgalhaes, 1968). Limited glandular structures are found in the conductive airways of the hamster (Hayes et al., 1977). Various respiratory physiologic characteristics are listed in Table 27.2.

GENITOURINARY SYSTEM

Kidneys and Ureters

The kidneys are reddish brown, bean-shaped organs located on either side of the median plane between the second and fourth lumbar vertebrae. The right kidney is located more cranial than the left kidney and generally weighs less as well. The kidney has rounded poles, a flat dorsal surface, and a protruding ventral surface.

The hamster kidney is further characterized as having a type II renal pelvis which enables high urine concentration (Pfeiffer, 1968). It has a single, long papilla that extends into the ureter, allowing for in vivo collection of urine from a single collecting tubule (Jarausch and Ullrich, 1957).

Hamster kidneys are very responsive to estrogen because the same embryonic germinal ridge develops into the urogenital and reproductive tracts. Renal tumors induced by estrogen administration in male hamsters have been used as a model for human renal cancer (Li et al., 1993).

Bladder and Urethra

The gross anatomy of the hamster's bladder and urethra is similar to that described for the dog (Schwarze and Michel, 1960). The dog and the hamster are two

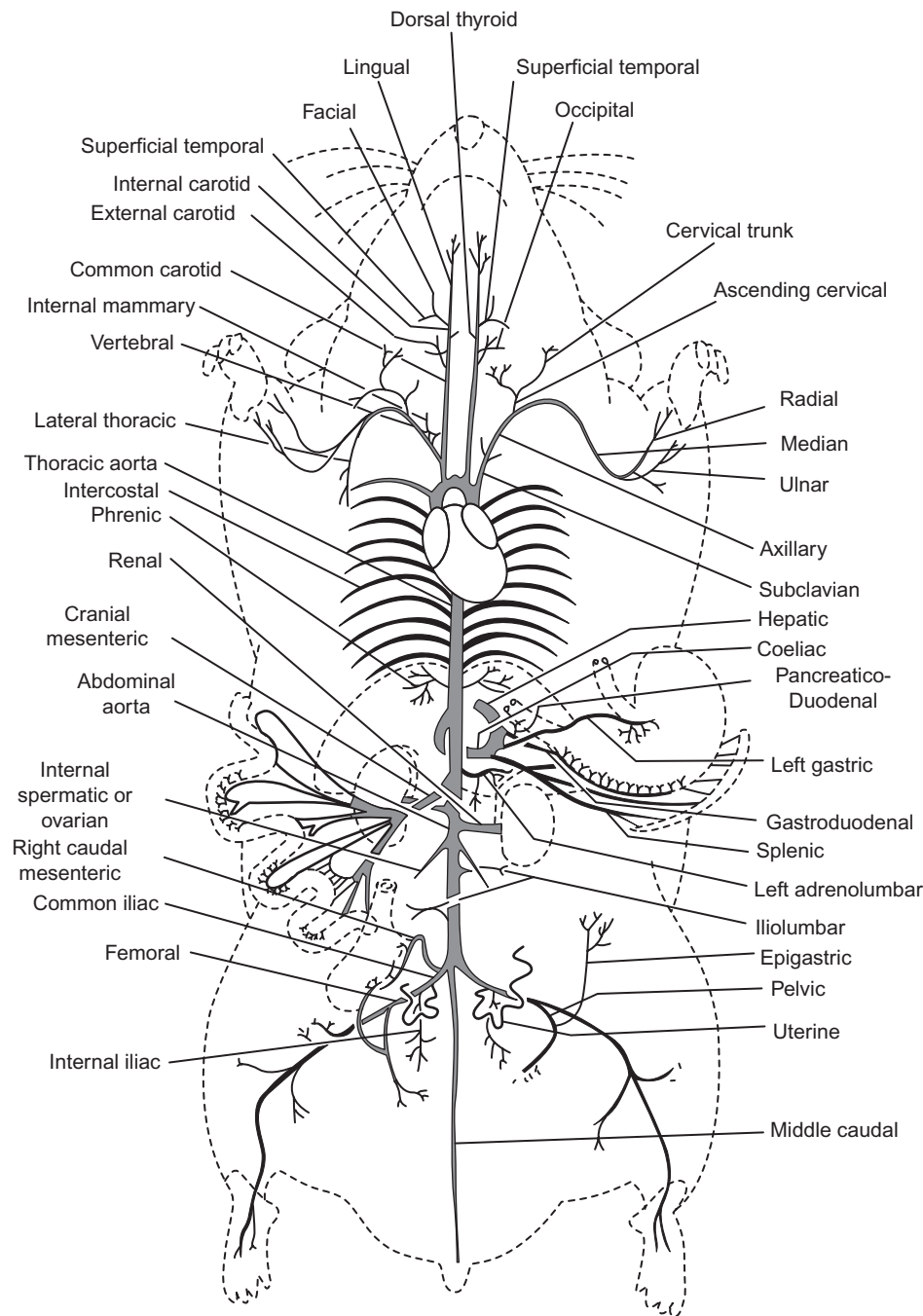


FIGURE 27.3 Schematic of the major arteries of the golden hamster.

of the most reliable models for studying the effect of chemical carcinogens on the urinary bladder (Van Hoosier and Ladiges, 1984).

Male Reproductive System

The urogenital system of the mature male hamster is depicted in Figure 27.6. The gonads and accessory reproductive organs grow and continue to develop during the first 2 weeks after birth. By 26 days of age,

TABLE 27.1 Cardiovascular Function of the Hamster^a

Parameter	Range
Heart rate (beats/minute)	350–500
Mean systolic pressure (mmHg)	90–111
Blood volume (percent of body weight)	7.4 ± 3.1

^aFrom Robinson, P. (1968). General aspects of physiology. In "The Golden Hamster – Its Biology and Use in Medical Research" (R.A. Hoffman, P.F. Robinson, and H. Magalhaes, Eds.). pp. 111–118. Iowa State University Press, Ames.

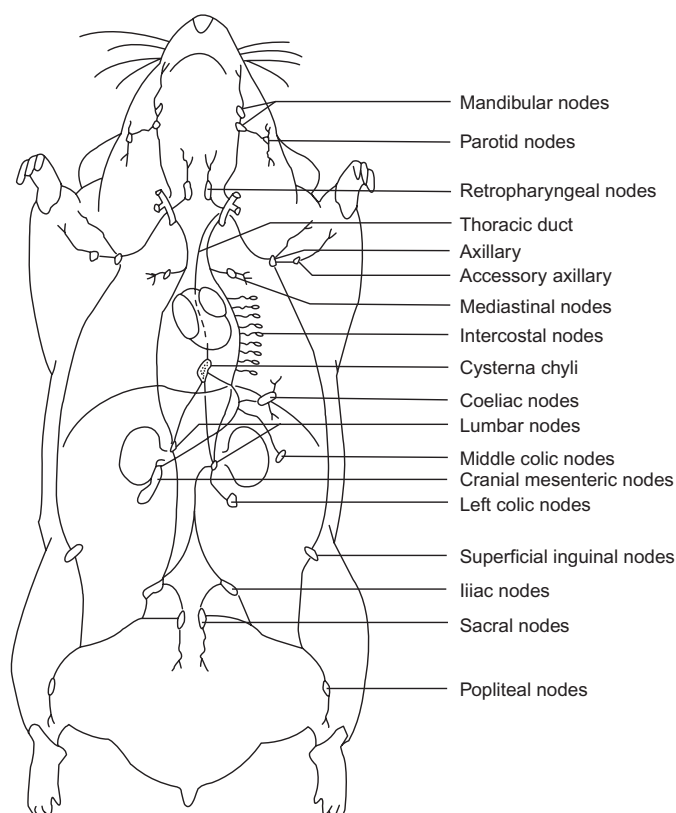


FIGURE 27.4 Schematic of the lymphatic system of the golden hamster.

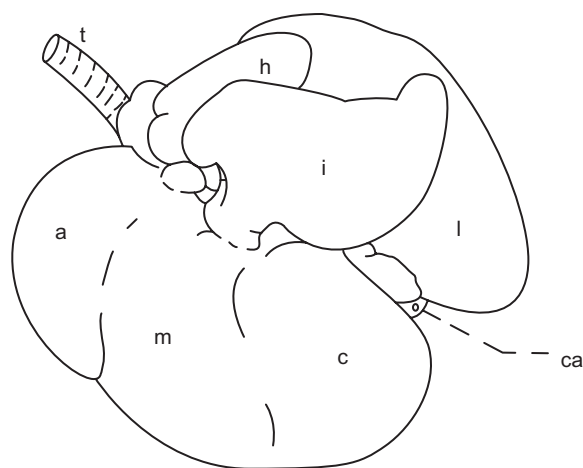


FIGURE 27.5 Lobes of the hamster lung, (a) cranial lobe; (m) middle lobe; (c) caudal lobe; (i) intermediate lobe; (ca) caudal accessory lobe; (l) left lobe; (h) heart; (t) trachea.

all male accessory glands have fully developed and the testes have descended (Ortiz, 1948). Hamster spermatazoa have sickle-shaped and flattened heads, similar to other rodents (Yanagimachi and Noda, 1970). The male has an os penis with a dorsal prong and two distal lateral prongs (Callery, 1951). Male accessory glands include interior and exterior ampulla glands, seminal

TABLE 27.2 Respiratory Function of the Hamster^a

Parameter	Range
Respiratory rate (breaths/minute)	33–127
Tidal volume (ml)	0.42–1.4
Mean minute volume (ml)	30–42

^aFrom Robinson, P. (1968). *General aspects of physiology*. In "The Golden Hamster – Its Biology and Use in Medical Research" (R.A. Hoffman, P.F. Robinson, and H. Magalhaes, Eds.). pp. 111–118. Iowa State University Press, Ames.

vesicles, coagulating glands, a three-lobed prostate, and bulbourethral glands (Schwarze and Michel, 1960). Like mice and rats, adult male hamsters have a urethral plug which is a white, rubber-like cast of the urethra (Campean et al., 1980; Kunstner et al., 1982; Rosenbauer et al., 1980).

Sexual maturity in the male hamster occurs at approximately 12 weeks of age and 90 grams of body weight (Van Hoosier and Ladiges, 1984). Exposure to at least 12.5 hours of light per day is required for normal levels of testosterone and breeding activity in male hamsters (Elliot, 1976; Gaston and Menaker, 1967; Morin and Zucker, 1978). The major source of testosterone is the interstitial cells of Leydig which contain abundant endoplasmic reticulum in the reproductively active hamster. Compared to other mammals which have an agranular tubular form of endoplasmic reticulum, the hamster has a flattened cisternae form. Serum cholesterol levels are inversely related to the amount of agranular tubular reticulum, thus the hamster has high levels of serum cholesterol (Wing and Lin, 1977).

Hamsters, like mice and rats, have superficial rete testis which lies immediately beneath the tunica albuginea. The extratesticular rete is particularly well developed in the hamster (Martin, 1976). As the hamster ages, there is little change in the reproductive hormone profile. Serum follicle-stimulating hormone (FSH) levels increase between 4- and 11-month-old hamsters but then remain at that level. There is no significant difference between serum testosterone and luteinizing hormone (LH) level in hamsters ranging in age from 4 month to 31 months of age. There are also very few differences observed with age in the weight of the testes and accessory sex organs as hamsters age, regardless of breeding activity. This is different to both rats and mice which show age-related declines in both serum hormones (testosterone, LH, and FSH), as well as weights of testes and accessory sex glands (Swanson et al., 1982).

Female Reproductive System

The urogenital system of a mature female hamster is depicted in Figure 27.7. Unlike most rodents where

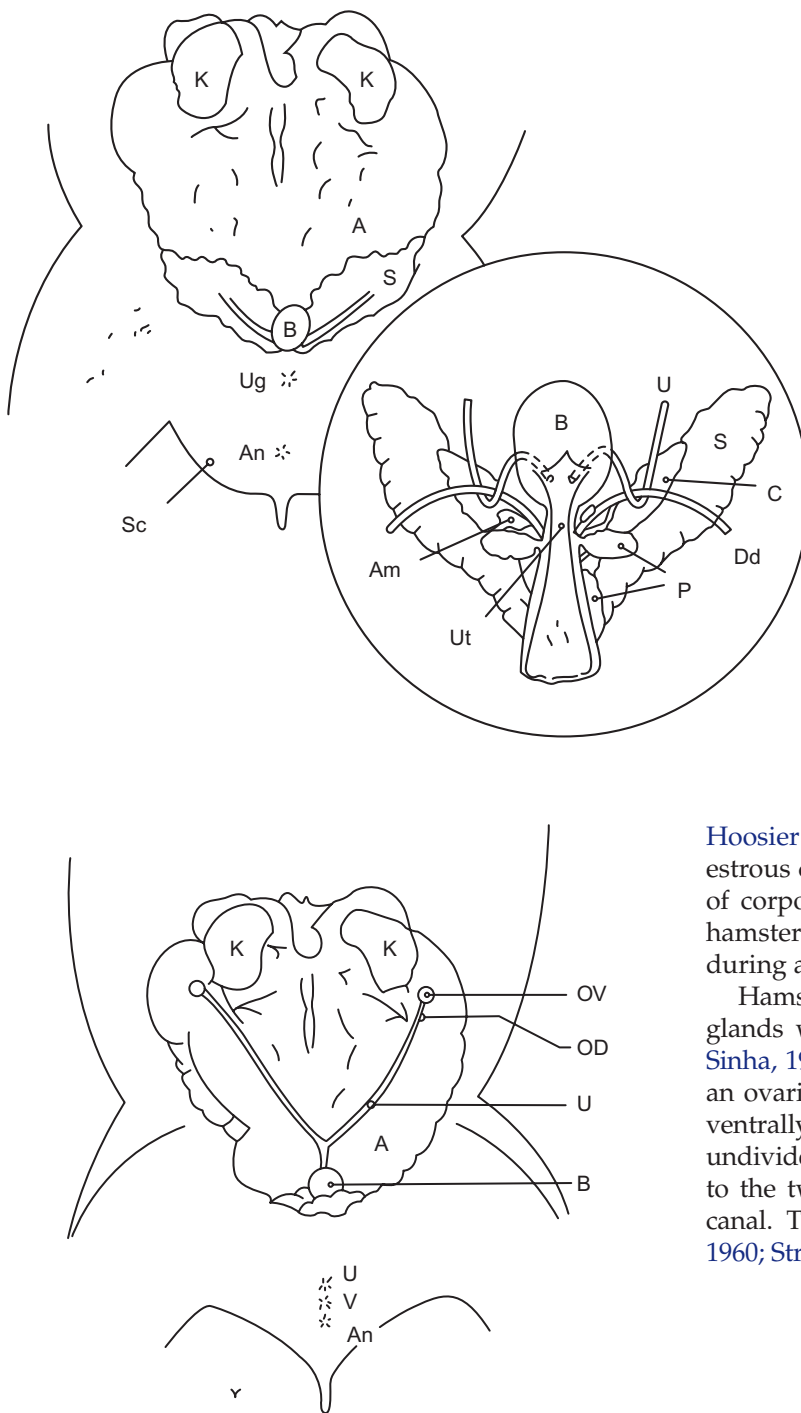


FIGURE 27.6 Ventrodorsal view of the male urogenital system of the hamster illustrating the kidney (K), bladder (B), seminal vesicle (S), urogenital opening (Ug), anus (An), scrotum (Sc), and adipose tissue (A). The insert is a ventral view of the accessory sex organs. The bladder and urethra have been opened to show entrances of ducts. Shown in the insert are the seminal vesicle (S), ureter (U), ductus deferens (Dd), urethra (Ut), coagulating gland (C), prostate (P), and ampullary gland (Am). A bulbourethral gland enters the urethra further caudally and is not illustrated.

the vagina opens at approximately sexual maturity, the hamster vagina opens when the animal is approximately 10 days old (Ortiz, 1948, 1955). Sexual maturity in the female hamster occurs at approximately 12 weeks of age and at a body weight of about 90–100 grams (Van

Hoosier and Ladiges, 1984). Hamsters have a 4-day estrous cycle. Unlike mice and rats, where multiple sets of corpora lutea are present from previous cycles, the hamster corpus luteum rapidly develops and regresses during a single cycle (Nakano, 1960).

Hamsters have an average of seven pairs of mammary glands with a range of six to 11 pairs (Anderson and Sinha, 1972). Paired, oval-shaped ovaries surrounded by an ovarian bursa and covered by fat, are located dorso-ventrally to the kidneys. The uterus is duplex with an undivided part and two horns. Dual cervical canals lead to the two uterine horns. The caudal cervix is a single canal. The oviducts are long and tightly coiled (Oeri, 1960; Strauss, 1956).

Hamsters have an average of seven pairs of mammary glands with a range of six to 11 pairs (Anderson and Sinha, 1972). Paired, oval-shaped ovaries surrounded by an ovarian bursa and covered by fat, are located dorso-ventrally to the kidneys. The uterus is duplex with an undivided part and two horns. Dual cervical canals lead to the two uterine horns. The caudal cervix is a single canal. The oviducts are long and tightly coiled (Oeri, 1960; Strauss, 1956).

NERVOUS SYSTEM

The average total weight of the hamster brain is 1.4 grams. The cerebral hemispheres consist of large frontal, lateral, and occipital lobes. The sulcus parame-dius is low and flat, while the caudal uvula is lengthened considerably. The paraflocculus and flocculus have only one layer. The paleocortex is very broad and the caudal area is wider than the rostral area. The pons and medulla oblongata are longer than wide. The corpus callosum is short and narrow. The medial region of the cerebellum, near the sigmodon, is differentiated into ten layers (Pilleri, 1960).

The hamster has eight cervical, 13 thoracic, six lumbar, three sacral, and 2–3 caudal pairs of nerves. Nerve plexuses are located in the cervical, brachial, and lumbosacral areas (Michel, 1963).

SPECIAL SENSES

Vision

The newborn hamster is an excellent model for experimental manipulation of the developing visual pathway as it is born with a poorly developed visual system (Tiao and Blakemore, 1976). The hamster has a panoramic field of vision, long streak-shaped specialization areas in the retina, an obvious area centralis or fovea and a large binocular visual field. The hamster has a rather uniform retinal organization. The center of the area centralis does not occupy corresponding points in the two eyes (Greiner and Weidman, 1978; Tiao and Blakemore, 1976).

Visual function in the hamster appears to be divided between the visual cortex and the superior colliculus. This hypothesis of two visual systems within a single brain provides mechanisms for identifying novel stimuli, commanding the orienting response in the colliculus and for pattern recognition in the visual cortex (Tiao, 1976). In addition, there is little physiological evidence to support the theory that the visual cortex is more involved with the identification of visual stimuli than the colliculus (Chalupa, 1981).

The divergent area centralis points into the middle of the hamster's view of its own vibrissae. In the superior colliculus there is good correspondence between the visual and somatic maps, consistent with the fact that the presumed region of maximum resolution in the retina is the vibrissae which the hamster relies on for somatic sensory input (Tiao, 1976; Tiao and Blakemore, 1976a).

Marked sexual dimorphism of the Harderian gland has been described in Syrian hamsters but not other species of hamsters. The dimorphism is androgen-dependent, with female hamsters secreting 100–1000 times more porphyrin than males and containing a different lipid profile (Buzzell, 1996).

Olfaction

The hamster, like other mammals, has keen olfactory capabilities. The hamster has four nasal endoturbinates and three ectoturbinates which project into the lumen of the nasal cavity. Localized olfactory neurons are found within the nasal turbinate mucosa (Constanzo and O'Connell, 1980). The hamster has several nasal serous glands which open into the internal ostium of the external nares, some nasal glands with mucus components and the vomeronasal gland (Adams and McFarland, 1972).

The vomeronasal organ is located bilaterally in the rostromedial nasal septum. It is completely enclosed in a bony capsule and consists of a 7 mm long tubular structure which is embryologically derived from the olfactory placode. The cranial end communicates with the nasal cavity through a rostral orifice. The lateral wall is lined with non-sensory respiratory epithelium, devoid of goblet cells, and the medial wall is lined with sensory olfactory epithelium with surface cilia and microvilli. PAS-positive mucous and serous acini are located on the dorsolateral and ventromedial sides of the lumen (Taniguchi and Mochizuki, 1982; Taniguchi et al., 1982). The male hamster has vomeronasal chemoreceptors that are important in sexual behavior (Meredith et al., 1980; Powers and Winans, 1975; Winans and Powers, 1977).

Somatosensation

Vibrissae are important for spatial orientation and communication in many species. The hamster is unique in that it moves its cranial vibrissae in various complex patterns keyed to particular exploratory situations. This movement repertoire suggests that a precisely operating motor system controls the vibrissae. The facial musculature, vibrissal vasculature, and vibrissal connective tissue have been proposed as important components of the vibrissal motor system (Wineski, 1985).

Taste

Four different types of papillae are located on the hamster tongue: filiform, fungiform, foliate, and vallate. Filiform papillae are abundant, conical in shape with the vertex pointed caudally, and are located on the rostral, dorsal, and lateral surfaces of the tongue. The dorsal surfaces of fungiform papillae have a single taste bud. Fungiform papillae are found on the dorsal rostral surface of the tongue. A single vallate papilla is located on the dorsal midline of the tongue. Multiple taste buds are found in the epithelial depressions on either side of the vallate papilla. Foliate papillae are found on the lateral caudal surface with taste buds arranged in longitudinal rows in the epithelium of the interpapillary clefts (Miller and Chaudhry, 1976).

Morphologically, all taste buds are spindle-shaped and 100–125 µm long by 50–80 µm wide. They communicate with the oral cavity through a taste pore and the taste buds extend through the stratified squamous epithelium of the tongue to the basement membrane (Miller and Chaudhry, 1976). Ultrastructurally, hamster taste buds are similar to those of other mammals (Farbonann, 1965; Murray and Murray, 1967; Uga, 1969).

Each taste bud responds to one or two basic tastes. If two, the combinations are either acid-salt or sweet-salt. Sweet and acid responses are negatively correlated.

The ordering for stimuli for sweet receptors is sweet > bitter > salt > acid, while the ordering for acid receptors is acid > salt > bitter > sweet (Frank, 1973).

BEHAVIOR

Hamsters are nocturnal animals that live singly or as a mother with litter in the wild. Hamsters spend a significant amount of their time asleep (41%) and the amount of time spent sleeping increases with age. When unexpectedly startled or awakened, hamsters may exhibit aggressive behavior towards handlers as well as cage-mates. Hamsters are prone to escape if the cage top is not secure. Gnawing and hoarding are common hamster behaviors and may be stereotypic (Arnold and Estep, 1994).

In the laboratory setting, both male and female group-housed animals may fight. If animals are group housed when young, stable groups may be formed. Use of enrichment devices such as PVC pipe and glass jars may reduce cage-mate aggression (Arnold and Westbrook, 1998).

Both male and female hamsters build nests, with nest-building activity greatly increased during pregnancy and lactation (Richards, 1966). The hormonal basis of nest building in female hamsters is dependent on the presence of both estrogen and progesterone, unlike female mice where progesterone alone was sufficient to increase nest-building activity (Richards, 1969).

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Management, Husbandry, and Colony Health

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INTRODUCTION

This chapter provides a review of the management, husbandry, and colony health requirements of the Syrian or golden hamster, *Mesocricetus auratus*, utilized in biomedical research. Hamsters are rodents belonging within the family Cretidae. Members of the Cretidae family are characterized by large cheek pouches, relatively thick bodies, and a short tail. Additional publications that provide comprehensive descriptions of hamster husbandry and care are [Hankenson and Van Hoosier \(2002\)](#) and [Hoffman et al \(1968\)](#).

HOUSING

Caging Systems

Hamsters are generally housed in cages that are appropriate for housing other laboratory rodents. Contemporary cages are generally made of commercially manufactured rigid plastic materials (i.e. polycarbonate, polysulfone, and polypropylene) or stainless steel. Hamsters are agile and have considerable ability to escape by means of chewing or gnawing on the cage material and by dislodging the cage lid. To reduce the

TABLE 28.1 Cage Space Requirements for Hamsters^a

Body Weight (g)	Minimum Floor Area/Animal in ² (cm ²) ^b		Height ^c in (cm)
<60	10	(64.5)	6 (15.2)
60–80	13	(83.8)	6 (15.2)
80–100	16	(103.2)	6 (15.2)
>100	19	(122.6)	6 (15.2)

^aSummarized from CFR (rev. 2008) and ILAR (2011).^bA nursing female hamster with litter must be housed in a cage without other adult hamsters and that provides at least 121 in² (780.5 cm²).^cMeasured from cage floor to cage top.

opportunity for escape by chewing, caging should not be manufactured with wood or soft metals such as aluminum. Hamsters may also chew plastics, around the hole in the cage for the water bottle, and escape. Caging must also be equipped with secure, tight and close-fitting doors and lids to prevent escape of animals. Plastic shoebox-style cages with tight-fitting wire bar lids are commonly employed in research institutions and commercial breeding operations. Hamsters may be housed successfully in solid-bottom cages with contact bedding. Excluding breeding colonies, hamsters may be housed in suspended cages with raised floors, i.e. caging with stainless steel wire-mesh, slatted, or perforated suspended floors over catch pans. Suspended wire floor caging is not recommended for breeding colonies as smaller animals may fall through or become entrapped in the wire mesh flooring. Preference testing has indicated that hamsters generally prefer solid floor caging with contact bedding over stainless steel suspended floor caging, but the preference for solid floor caging decreased with increased duration of exposure to suspended wire flooring (Arnold and Estep, 1994).

Requirements for both floor space and cage height for hamsters are defined in the Animal Welfare Regulations (CFR, rev. 2008). The Institute of Laboratory Animal Resources (ILAR, 2011) provides similar non-regulatory recommendations for cage size and height for hamsters (see Table 28.1). Nursing female hamsters, together with a litter, must be housed without other hamsters and provided a minimum of 121 sq. in. (780.6 sq. cm.) of floor space (CFR rev., 2008). Cage size has been shown to affect core body temperature and febrile response in hamsters (Kuhnen, 1999). Housing hamsters in small cages was associated with increased baseline rectal temperature when compared with hamsters housed in large cages. Febrile response to lipopolysaccharide injection was greatest in hamsters housed in large cages and smallest in animals housed in small cages.

The cage sanitation practices applied to the common laboratory rodents also apply to hamster caging. Soiled bedding material should be removed and replaced with

clean material, or animals transferred to a clean bedded cage, as often as necessary to keep the cage occupants clean and dry. Unlike most common laboratory rodents, hamsters tend to defecate and urinate in one corner of the cage and, because they are desert-adapted animals, hamsters produce relatively scant amounts of urine. U.S. Animal Welfare Regulations (CFR rev. 2008) prescribe that, at a minimum, hamster cages must be sanitized at least once every 2 weeks (14 days).

Bedding

A variety of contact bedding materials can be used with hamsters, including processed wood shavings and chips, corn cob, pelleted wood, as well as virgin and recycled paper products. Preference testing of bedding materials with hamsters is limited. Lantaigne and Reeb (2006) showed that without nesting material, hamsters have a preference for pine shavings over aspen shavings, corn cob over wood pellets, pine shavings over corn cob, and aspen shavings over wood. The preferences were eliminated, however, when animals were provided a piece of paper towel as nesting material. Provision of long-term access to running wheels can result in development of foot lesions in hamsters, but the development of lesions was not affected by use of pine shavings or hard wood chip bedding (Beaulieu and Reeb, 2009). Hamsters build nests, and, for breeding animals, the addition of nesting material (i.e. tissue paper, cotton wool) is recommended. It is not recommended to use cedar and untreated (non-kiln-dried) soft wood bedding, such as pine, as it contains aromatic hydrocarbons capable of inducing hepatic microsomal enzymes and cytotoxicity in rodent species, including hamsters (Harkness, 1994; Vesell, 1967).

ENVIRONMENTAL CONDITIONS

Ventilation

Specific ventilation rates for the primary enclosure (cage) and the secondary enclosure (room) housing hamsters have not been described and ventilation rate recommendations for housing other research rodents are acceptable. Animal Welfare Regulations (CFR rev. 2008) require that facilities housing hamsters be adequately ventilated to provide for the health and comfort of the animals and to minimize the development of drafts, odors, and moisture condensation. In contrast, ILAR (2011) provides a general recommendation of 10–15 air changes per hour (ACH) for the secondary enclosure housing research animals, including hamsters. The use of individually ventilated caging systems, which provide intra-cage ventilation rates in excess of 10–15 ACH, may

allow decreased air exchange rates in the rooms housing the caging system, while preserving an acceptable cage environment for the animal (Reeb et al., 1998).

Illumination

Recommendations for acceptable illumination levels within vivaria are generally based on providing levels sufficient to allow safe working conditions for personnel, good housekeeping, adequate observation of animals, and to preserve circadian cycles (ILAR, 2011). The level of illumination required for optimal maintenance and health of hamsters has not been described and light intensity levels of approximately 325 lux measured approximately 1 m (3.3 ft) from the floor are generally acceptable to allow adequate illumination for husbandry purposes and observation of animals (ILAR, 2011).

Hamsters are strictly nocturnal in captivity, whereas females in the wild are primarily diurnal (Gattermann et al., 2008). Hamsters are seasonal breeders that in a natural setting will modulate reproduction in association with photoperiod, the duration of light and dark contributing to a 24-hour cycle. In a natural setting, hamsters restrict reproduction to the long days of spring, summer, and fall. In captivity, hamsters can be maintained under a 12:12 light cycle (12h light:12h dark) for general research purposes. For reproductively active animals, such as breeding colonies, a long 14-hour light cycle is recommended and thus breeding colonies are maintained under 14:10 light:dark cycle (Brainard et al., 1986). Alterations of photoperiod in hamsters have been shown to affect circulating levels of luteinizing hormone (LH) and follicle stimulating hormone (FSH) and to produce altered testicular development and spermatogenesis (Ellis and Follett, 1983). Alteration in the photoperiod during pregnancy of hamsters has been shown to affect the timing of parturition (Viswanathan and Davis, 1992).

Temperature

The Syrian hamster originates from an arid, desert environment and their thermoneutral zone, the temperature range associated with minimal metabolic expenditure, is approximately 82.4–86°F (28–30°C) (Gordon, 1993). Unless required for scientific purposes, hamsters are commonly housed and maintained in research institutions at temperatures appropriate to other common laboratory rodents. The Animal Welfare Regulations specify that hamsters maintained within an indoor housing facility must be provided ambient temperatures that neither fall below 60°F nor rise above 85°F (CFR rev. 2008). ILAR (2011) recommends that hamsters be maintained in a range of 68–79°F (20–26°C). Hamsters are adaptable to cooler temperatures, and

short-term hibernation can be induced in hamsters by exposure to cold temperatures, approximately 3–5°C, and a shortened light cycle (Hoffman, 1968). A study by Gumma et al. (1967) showed that pre-hibernation hamsters may show a preference for 8°C whereas post-hibernation hamsters show a preference for higher temperatures (24°C).

Humidity

As with previous recommendations for ambient temperature, hamsters are commonly housed and maintained at relative humidity levels generally acceptable for other common research rodents. Recommendations are that relative humidity be maintained between 30–70% for most research species, including hamsters (ILAR, 2011).

Environmental Enrichment

A primary goal of environmental enrichment is to provide animals with the opportunity to perform species-specific behavioral patterns (Hutchinson et al., 2005). Numerous enrichment devices have been described for use with hamsters including tubes, pipes, shelters, nest material, and deep bedding. In the wild, hamsters are solitary, living either singly or as mothers with nursing pups in burrow systems. Burrow systems include a nest chamber, a chamber for urination, and a chamber for food storage (Gattermann et al., 2001; Sorensen et al., 2005). Because hamsters spend considerable time underground in the wild, it is generally recommended that some form of burrow, pipe, tube, and shelter be provided to hamsters (Arnold and Westbrook, 1998; Baumans, 2005). McClure and Thompson (1992) showed that aggression in hamsters housed in suspended wire cages was diminished after the addition of a polyvinyl chloride (PVC) pipe. Provision of nesting material, or bedding that provides the ability for nest building, is generally recommended as both wild and captive-bred hamsters will create nests (Gattermann et al., 2001; Lanteigne and Reeb, 2006); and female nest-building behavior has been shown to increase during pregnancy (Richards, 1969). Hamsters that were provided deep bedding (i.e. 40 and 80 cm deep) showed significantly less gnawing of cage wire bars, a possible indicator of sub-optimal housing conditions, and increased the construction of burrows used for sleeping than did animals provided shallow amounts (10 cm) of bedding (Hauzenberger et al., 2006). Hamsters readily utilize exercise wheels (i.e. running wheels), and the use of exercise wheels has been proposed as a method of environmental enrichment as well as a means to measure activity in chronobiology research (Beaulieu and Reeb, 2009; Gattermann et al., 2004). Hamsters naturally forage and hoard food and they

should be provided with food pellets inside the cage, preferably on the cage floor (Baumans, 2005; Sorensen et al., 2005).

NUTRITION

Feed Type

Hamsters are generally provided commercially produced pelleted rodent diet intended for mice and rats, and animals raised on such diets show normal growth and reproduction (Hankenson and Van Hoosier, 2002; Slater, 1972). Hamsters can also be raised and maintained successfully on purified and semi-purified diets. Colocolic intussusception has been described in hamsters following a change in diet from a standard pelleted rodent chow to a semi-purified diet with sucrose as the primary carbohydrate (Cunnane and Bloom, 1990). Although once common, when fed a nutritionally adequate diet formulated for mice and rats, dietary supplementation with grains, fruits, and vegetables is generally considered unnecessary (Slater, 1972). Additionally, dietary supplementation with grains, fruits, and vegetables may increase the risk of exposure to unwanted microbiologic agents and contaminants (Coates, 1991).

The method of feed presentation to hamsters differs from that for mice and rats, in that hamsters are generally provided food on the cage floor. Harkness et al. (1977) observed that when a hamster colony originally provided pelleted rodent food from feed hoppers with 7/16-inch slots were fed from feed hoppers with narrower 5/16-inch slots morbidity and mortality of animals in the colony increased significantly. Adult animals showed cachexia, delayed weight gain, broken incisors, and an inability to obtain food from the feed hopper. Nursing hamsters demonstrated agalactia resulting in pup mortality and decrease in conception rate. Upon feeding hamsters from the cage floor, animals regained a normal pattern of weight gain. It was postulated that because hamsters have a relatively broad muzzle compared with similarly sized rodents, they have difficulty accessing adequate food from narrow-slotted feed hoppers. Consequently, providing food solely from feed hoppers is not recommended and hamsters are commonly provided food on the cage floor. In addition, young hamsters will begin to eat solid food at 7–10 days of age and nursing hamsters with litters may become preoccupied with hopper-bound food and neglect their young (Harkness and Wagner, 1995).

Nutritional Requirements

Hamsters maintained on commercially produced rodent diet show normal growth and reproduction.

General nutrient levels associated with acceptable rates of growth and reproduction have been reported with diets consisting of 15–25% protein, 35–40% carbohydrate, 4–5% fat, and 5% crude fiber (Arrington et al., 1966, 1979; NRC, 1995; Newberne and Fox, 1980; Newberne and McConnell, 1979). Feed consumption in hamsters is similar in males and females, with published ranges between 5.0–8.9 g per day for growing and adult animals (Arrington et al., 1979; Banta et al., 1975; Newberne and McConnell, 1979).

Although hamsters are commonly maintained on laboratory diets formulated for mice and rats, relatively little has been published on the specific nutritional requirements of hamsters, and the nutritional requirements of hamsters are not identical to those of other rodents (NRC, 1995; Newberne and McConnell, 1979). Hamsters possess a distinctly compartmentalized stomach consisting of a forestomach and a glandular stomach. The forestomach shares physical characteristics with a rumen, contains microorganisms similar to those found in a rumen, is a source of volatile fatty acid production, and has been postulated to allow fermentation, prior to passage of ingesta into the gastric stomach (Hoover et al., 1969; Warner and Ehle, 1976). It has been proposed that the apparent rumen-like functions of the hamster forestomach may allow the animal to digest and utilize lower-quality protein in comparison to other rodents such as the rat (Newberne and McConnell, 1979). The forestomach, however, is not required for maintenance of proper nutrition, survival, or growth of the hamster fed a nutritionally adequate diet, as surgical removal of the forestomach was shown not to affect growth and feed utilization in the animal (Ehle and Warner, 1978).

Published requirements for amount of protein in the hamster diet are variable. For hamsters fed semipurified diets utilizing casein as the sole source of protein, satisfactory growth rates have been reported for diets containing 12–24% casein; satisfactory weight gain has been associated with natural-product diets containing 5–25% protein (Newberne and McConnell, 1979). Soybean meal as a protein source has been shown to promote increased weight gain and improved protein utilization (i.e. increased protein efficiency ratio) compared with diets containing wheat gluten and fish protein concentrate (Banta et al., 1975). Nutritional studies have also shown increased survival of hamsters fed a diet containing 20 g lactalbumin/100 g diet (Birt et al., 1982). Cornstarch has been shown to be a good carbohydrate source for hamsters, promoting satisfactory growth, reproduction, and longevity and it is generally provided in the range of 30–40% as a source of dietary energy (Newberne and McConnell, 1979). Dietary carbohydrates can affect circulating glucose

and lipid metabolism in hamsters. Hamsters fed a diet containing 60% fructose showed increased food consumption and development of obesity, hyperinsulinemia, and hypertriglyceridemia (Kasim-Karakas et al., 1996). The dietary fat requirement for hamsters has been described for growing hamsters as 5%; commercially prepared rodent diets with 4% fat also appear adequate for growth, reproduction, and maintenance (Knapka and Judge, 1974; Newberne and McConnell, 1979). Requirements for fiber have not been described for the hamster and 4–5% fiber found in commercial rodent diets appears appropriate for normal growth, reproduction, and maintenance. Diets containing high levels of refined sugars and no fiber have been associated with high mortality, and the substitution of corn-starch for glucose or sucrose or the addition of 12–20% alfalfa to the diet increased survival (Ershoff, 1956). There is a paucity of information regarding mineral and vitamin requirements of hamsters. It has been recommended that levels for copper, magnesium, potassium, and zinc should be increased in comparison to dietary requirements of the rat; recommended levels in semipurified diets are 12ppm copper, 0.12% magnesium, 20ppm potassium, and 0.6% zinc (Newberne and Fox, 1980; Newberne and McConnell, 1979). Studies have described minimal levels of dietary vitamin A for the hamster as 1.1mg/kg retinol and 2mg/kg retinyl palmitate/kg in semi-purified diet (Newberne and McConnell, 1979; Rogers et al., 1974). The hamster does not require a dietary source of vitamin D when provided a calcium–phosphorus ratio of 2:1 (NRC, 1995; Newberne and McConnell, 1979). Hamsters are coprophagic and it is presumed that a considerable amount of vitamin K is provided by ingestion of feces (NRC, 1995). Minimal dietary requirement for vitamins B₆, B₁₂, E, biotin, choline, folic acid, niacin, pantothenic acid, riboflavin, and thiamin have not been described for the hamster and sufficient dietary levels for growth and reproduction are provided in diets formulated for the rat. Satisfactory dietary levels for vitamins and minerals for the hamster are provided in Table 28.2.

Hydration

Hamsters should be provided access to clean, potable water at all times. Water can be provided via water bottles with stainless steel sipper tubes or automatic water systems with stainless steel lixits. Hamsters have been reported to consume, on average, 8.5ml water per 100g body weight. Consumption of water can vary significantly; male consumption ranges from 4.5–5ml/100g body weight and female consumption ranges from 13.6–14ml/100g body weight (NRC, 1995).

TABLE 28.2 Satisfactory Vitamin and Mineral Dietary Levels for the Hamster

Vitamin/Mineral	Natural Product Diet (mg/kg)	Semi-purified Diets (mg/kg)	
	Banta et al. (1975)	Arrington et al. (1966)	Rogers et al. (1974)
A	15.3	90.0	2.0
C	–	900.0	–
D ₂	–	5.0	62.1
E	1100.	100.0	600.0
K	5.2	45.0	4.0
Choline	2000.0	150.0	2000.0
p-Aminobenzoic acid	100.0	100.0	6.0
Inositol	100.0	100.0	200.0
B12 (µg/kg)	32.0	28.0	50.0
Niacin	93.0	90.0	100.0
Pantothenate	54.0	60.0	40.0
Riboflavin	12.0	20.0	15.0
Thiamin	14.0	20.0	25.0
Pyridoxine	10.1	20.0	6.0
Folic acid	3.1	1.8	4.0
Biotin	0.9	0.4	0.6
Calcium, %	0.54	0.59	0.41
Phosphorus, %	0.58	0.30	0.39
Magnesium, %	0.13	0.09	0.06
Potassium, %	0.79	0.82	0.61
Sodium, %	0.19	0.15	0.21
Iron	180.0	140.0	154.0
Manganese	15.9	3.65	9.0
Copper	12.6	1.6	7.0
Zinc	9.4	–	9.2
Iodine	0.02	1.6	1.7
Cobalt	–	0.2	–
Fluoride	–	–	0.2

Nutrition-Related Disease

Nutrition-related diseases are uncommon in hamsters fed commercially produced rodent diets. Numerous nutritional deficiencies have been induced in the hamster by feeding diets formulated to be deficient in specific nutrients, minerals, and vitamins. For a comprehensive review see NRC (1995). Hamsters fed a diet deficient in vitamin E, starting at weaning,

develop testicular degeneration, muscular dystrophy, and experience decreased growth, which is reversible by treatment with dietary vitamin E (Granados, 1968). Supplementation of vitamin E has been shown to reduce aortic fatty streak accumulation in hypercholesterolemic hamsters (Xu et al., 1998). Deficiency of vitamin E, possibly combined with oxidative stress, may be important in development of clinical disease in cardiomyopathic hamsters (Sakanashi et al., 1991). Spontaneous hemorrhagic necrosis (SHN) of the central nervous system of fetal hamsters has been described when pregnant dams are fed vitamin-E-deficient diets, and the syndrome is alleviated by intraperitoneal administration of vitamin E (Keeler and Young, 1979). Vitamin A deficiency, similar to other rodent species, can lead to abnormal development, alopecia, a coarse hair coat, xerophthalmia, and altered morphology of tracheal epithelium (Chopra et al., 1990; Keeler and Young, 1979).

BREEDING

Reproductive Biology

Sexual maturity in the hamster generally occurs at approximately 6 weeks (42 days) of age, although copulatory activity can begin as early as 4 weeks of age. Sexual maturity can be verified in the male hamster by performing the penile smear technique to identify the presence of spermatozoa on the glans penis (Vandenbergh, 1971). Female hamsters generally begin estrus between 6–8 weeks of age. It is recommended that breeding animals be allowed to mature to 90–100 g body weight before breeding to achieve maximum production. Females are generally 8–10 weeks of age and males 10–12 weeks of age prior to initial breeding. The use of slightly older and larger males appears beneficial when placing males with more aggressive females (Slater, 1972). Reproductive senescence occurs at approximately 14 months of age in both sexes.

Female hamsters have a regular 4-day estrous cycle. The end of ovulation, typically day 2 of the 4-day estrous cycle, is identifiable by the presence of a readily observable postovulatory vaginal discharge. The vagina, and often the vaginal orifice, is filled with an opaque, white, viscous discharge. The postovulatory discharge is indicative of the animal having reached estrus the day before and it is predictive of the next estrus 3 days after the appearance of the discharge, followed by estrus on days 7, 11, and so forth. Female hamsters can be successfully mated in the evening of the third day following the postovulatory discharge. In commercial breeding operations, large groups of hamsters can be selected based on the postovulatory discharge and mated with a resulting 90% breeding success rate (Balk and Slater, 1987). Females

may show pseudopregnancy lasting 9–10 days following non-fertile copulation, with return of a postovulatory vaginal discharge on postcopulatory days 10–11 (Richards, 1966). Pregnant animals are identifiable by rapid weight gain and abdominal distension at 10 days post mating. Implantation occurs on day 6 of pregnancy. Postpartum estrus in hamsters is generally anovulatory (Harkness and Wagner, 1995).

The female hamster is tolerant of the presence of a male only during estrus, and females will readily attack males if paired together outside of estrus. When in estrus and receptive to males, the female will display lordosis upon approach of a male and maintain the posture while the male remains in the vicinity. When copulation occurs, males will mount the female multiple times. Females in estrus will accept males beginning approximately 1 hour before start of the dark cycle (i.e. lights out) and end receptivity to the male approximately 15 hours later (Ciaccio and Lisk, 1971).

As mentioned earlier, hamsters are seasonal polyestrous breeders that will modulate reproduction in association with photoperiod. For reproductively active animals, such as those in breeding colonies, a long 14-hour light cycle is recommended and thus breeding colonies are maintained under 14:10 light:dark cycle (Brainard et al., 1986). Alterations of photoperiod in hamsters have been shown to affect circulating levels of luteinizing hormone (LH) and follicle stimulating hormone (FSH) and to produce altered testicular development and spermatogenesis (Ellis and Follett, 1983). Alteration in the photoperiod during pregnancy of hamsters has been shown to affect the timing of parturition (Viswanathan and Davis, 1992).

The gestational period for the hamster is approximately 15.5 days. Parturition is preceded by the female displaying an increase in activity and restlessness with alternating bouts of eating, grooming, and nest building. Immediately prior to parturition, an increase in respiration rate and a bloody vaginal discharge are observed. Litter sizes range from 4–16 pups, with average litter sizes reported as 5–9 (Harkness and Wagner, 1995) and 11 (Slater, 1972). The first litter from a primiparous female is generally smaller than subsequent litters.

Common Breeding Systems

Multiple breeding schemes have been described for hamsters including hand-mating, pair mating, and polygamous mating (Harkness and Wagner, 1995). A common method for breeding hamsters utilizes hand-mating of the animals. On the third day following a post-ovulatory discharge, the female hamster is placed into a male hamster's cage 1–2 hours prior to the start of the dark cycle. Animals are then observed for fighting, and the female removed if she shows aggression towards the male. If the female is receptive, she will

show a posture of lordosis with tail erect and hindlimbs spread apart. Animals will often mate within 5–10 minutes of being placed together, at which time they may be separated; or animals may be left paired overnight and separated the following morning. Hamsters may also be bred by polygamously pairing one male with 1–2 females for 7–14 days, followed by separation of the animals and housing of the females individually for parturition and until pups are weaned. Monogamous pairing of one male with one female continuously has also been described. This breeding scheme requires placing a prepubital male and female together, and allowing them to remain together throughout breeding and parturition. Sequential monogamy mating of hamsters involves rotating seven females through a male's cage in series, with each female paired with the male for 7 days. Using this breeding system, after a female weans a litter at 3 weeks of age, the female is rotated back to the male's cage every 7th week. Because female hamsters can be aggressive, breeding pairs of hamsters must be checked daily for fight wounds and animals separated if needed.

Care of Young

Approximately 2 days prior to parturition, females should be transferred to a clean cage and provided with nesting material. Sufficient food should be added to the cage to allow the female to remain undisturbed with a newborn litter for 7–10 days following parturition. Disturbing a dam, especially a primiparous dam, with a newborn litter can lead to litter abandonment and cannibalism by the dam. During the first 7–10 days following parturition, food and water may be added to a cage if needed, but bedding should not be changed. If it is necessary to disturb a dam with a young litter, the dam should be provided with fresh food with which she may fill her cheek pouches reducing the likelihood of cannibalism of the new pups ([Hankenson and Van Hoosier, 2002](#)). When moving neonatal hamsters between cages, care should be taken to transfer the neonates within their nest to minimize the disturbance as much as possible.

Hamsters are born hairless, with teeth, and with eyes and ears closed. Ears open at 4–5 days of age, eyes open at 14–16 days of age, and hamsters begin to eat solid food at 7–10 days of age. Consequently, water should be available by 10 days of age when the young hamster begins to ingest solid food. Care should be taken to ensure that if animals are provided water via a bottle that the sipper tube is appropriately sized to provide accessibility to the neonates from the floor of the cage. Hamsters can be weaned as early as 15 days of age, but it is suggested that young be left with the mother until at least 19 days of age ([Slater, 1972](#)), and it is common to

wean hamsters at 21–28 days of age. At weaning, animals are generally separated and housed in groups by age and sex.

COLONY HEALTH

Preventive Medicine

A preventive medicine program for hamsters is contingent upon selection of a high-quality source of hamsters. A quality source of hamsters should have a well-defined health-monitoring system in place to allow periodic assessment of the breeding colony for common infectious agents of hamsters. As with other rodents, infection by adventitious microbial agents can adversely affect animal health, alter the physiology of animals, introduce experimental variables that can alter experimental outcomes (i.e. data) generated from the use of animals, and pose a zoonotic risk to the people working with the animals. For a summary of natural pathogens of hamsters and the effects of those organisms on research, see [Baker \(2003\)](#). If hamsters intended for research originate from a non-commercial source without adequate health monitoring information for the colony, animals should be placed into quarantine and microbiologic testing performed to identify infectious agents that may compromise the health of the animals or their usefulness in research. A rigorous biosecurity program, defined as all measures taken to prevent the introduction, contain, and when necessary eradicate adventitious infections, is also required to protect the microbiological integrity of hamsters maintained and used in research and breeding facilities ([Shek and Gaertner, 2002](#)). Maintenance of healthy hamsters within a research facility is also contingent upon providing adequate husbandry and care, including provision of a nutritionally complete diet formulated for laboratory rodents, continuous access to clean water, appropriately designed and maintained caging, proper sanitation of cages, equipment, and facilities supporting the hamsters, and appropriate environmental conditions as outlined earlier in this chapter.

Colony Health Monitoring

Infection of rodents, including hamsters, by adventitious agents can negatively affect animal health and interfere with research outcomes derived from the animals ([Baker, 2003](#)). Health monitoring of research rodent colonies involves subjecting animals to a battery of assessments, including macro- and microscopic pathology, bacteriology, parasitology, and serum and microbiologic diagnostic tests with a goal of identifying whether the test animal, a surrogate for the source colony, is infected with specific microbiological agents considered important for

the particular rodent species under study. Although this process is often referred to as health monitoring, microbiologic monitoring may be the more appropriate term (Hansen, 2003) and is used as a synonymous term. Shek and Gaertner (2002) describe the components to be considered when designing and implementing a microbiologic monitoring program for rodents, including detailed discussions on diagnostic methodologies, design and implementation of a surveillance program, and interpretation of results. Important parameters that must be defined when developing a microbiologic monitoring program are the number of animals to test, the frequency of testing, the agents for which to screen, and the diagnostic methodology that is most appropriate for each agent. Selection of the appropriate testing methodology for hamster infectious agents is beyond the scope of this chapter and the reader is referred to the relevant literature (Livingston and Riley, 2003; Shek and Gaertner, 2002; Waggle et al., 1994). Frequency of testing is dependent upon many variables, including historical contamination rates, general health quality of the colony, i.e. gnotobiotic health status versus specific pathogen-free status, financial resources, and sensitivity of the research to contamination by common hamster infectious agents. Many organizations develop schedules whereby samples are submitted for serologic testing relatively frequently, i.e. quarterly, in conjunction with submissions of live animals that combine serologic testing with more comprehensive testing including bacteriology, parasitology, and pathological exam less frequently, i.e. once to twice yearly.

Selection of agents to include in a microbiological testing program for hamsters should be based on selection of agents that pose a risk to human health (i.e. zoonotic agents), cause disease in hamsters (i.e. primary pathogens), and interfere with research outcomes. When possible, decisions regarding frequency of testing and the selection of specific agents to include in a monitoring program should also involve a risk-based approach that requires knowledge of the general prevalence of infectious agents found within the research population of interest (Pritchett-Corning et al., 2009; Shek and Gaertner, 2002). The prevalence of infectious agents in research mice and rats has been well-documented (Jacoby and Lindsey, 1997; Livingston and Riley, 2003; Pooley, 1970; Pritchett-Corning et al., 2009; Schoondermark-van de Ven et al., 2006), whereas prevalence rates of infectious agents in research hamsters is limited (Suzuki et al., 1982). In general, prevalent agents are more likely to contaminate a facility and such agents should be tested for more frequently than agents that are rarely found in research colonies. Table 28.3 provides a summary of positive test results for hamster samples, excluding samples from commercial rodent vendors, submitted from 2003–2008 to a commercial diagnostic laboratory (Charles River Research Animal

Diagnostics Service, Wilmington, MA), unpublished data (Cosentino, 2009). The Federation of European Laboratory Animal Science Associations (FELASA) has provided recommendations on microbiologic agents to include in a hamster health monitoring program (Nicklas et al., 2002).

The following agents are commonly included in hamster microbiologic monitoring programs. The primary zoonotic concern associated with research hamsters is infection with lymphocytic choriomeningitis virus (LCMV), a virus of the family *Arenaviridae*, genus *Arenavirus*. Human cases of LCMV have been reported after exposure to infected research and pet hamsters (Baum et al., 1966; Bigger et al., 1975; Lewis et al., 1965). Additional viral agents for which colonies are routinely screened are pneumonia virus of mice (PVM) and Sendai virus. PVM is a *Pneumovirus* in the family *Paramyxoviridae*. Infection of hamsters with PVM is clinically silent, and experimental infection has produced interstitial pneumonia in hamsters (Baker, 2003). Sendai virus (parainfluenza 1) is a *Paramyxovirus* within the family *Paramyxoviridae*. Sendai virus infection in hamsters can produce respiratory disease including tracheitis, bronchitis, and interstitial pneumonia and it may alter renal potassium flux (Genovesi and Peters, 1987). The prevalence of LCMV, PVM, and Sendai virus in research hamster colonies is considered to be low (Baker, 2003).

A limited number of bacterial agents are generally included in hamster microbiologic monitoring programs. Infection with *Clostridium piliforme* (i.e. Tyzzer's disease) can cause disease and mortality in hamsters (Motzel and Gibson, 1990), although reports of disease in research colonies are rare. Screening for *Salmonella* spp. is recommended by FELASA (Nicklas et al., 2002) and practiced by many commercial hamster breeders; reports of salmonellosis in research hamsters appear rare, although outbreaks have been reported (Innes et al., 1956). A number of bacteria, including *Pasteurellaceae* and *Streptococcus pneumoniae* that were associated with pneumonia in a survey of hamster diseases (Renshaw et al., 1975) and *Corynebacterium kutscheri*, which can colonize the upper respiratory tract of hamsters (Amao et al., 1991), are frequently also reported on hamster health monitoring reports. Their importance in producing clinical disease in hamsters is unclear. A number of *Helicobacter* spp. have been isolated from research hamsters, and infection by several species of *Helicobacter* has been associated with clinical disease (Whary and Fox, 2004). Hamsters are an established host for *H. cinaedi*, although the prevalence in research colonies has not been described, and hamsters have been proposed as a reservoir species for zoonotic helicobacteriosis (Gebhart et al., 1989; Orlicek et al., 1993).

Hamsters can also serve as hosts for a variety of parasitic agents. Acariasis is a common finding during

TABLE 28.3 Prevalence of Common Rodent Infectious Agents in Hamsters, 2003–2008^a

Agent	Assay Method ^b	No. of Samples Tested	No. Positive	Prevalence (%)
VIRUSES				
Lymphocytic choriomeningitis virus (LCMV)	ELISA	7445	2	0.027
Parvovirus genus (NS-1)	ELISA	5928	0	–
Pneumonia virus of mice (PVM)	ELISA	7437	1	0.013
Reo-3 virus	ELISA	7426	0	–
Sendai virus	ELISA	7446	3	0.040
BACTERIA				
<i>Bordetella bronchiseptica</i>	Culture	207	0	–
β -hemolytic <i>Streptococcus</i> spp.	Culture	207	1	0.48
<i>Campylobacter jejuni</i>	Culture	413	2	0.48
<i>Corynebacterium kutscheri</i>	Culture	207	0	–
<i>Klebsiella oxytoca</i>	Culture	207	1	0.48
<i>Klebsiella pneumoniae</i>	Culture	207	0	–
<i>Helicobacter</i> genus	PCR	84	55	65.5
<i>Helicobacter hepaticus</i>	PCR	84	1	1.19
<i>Mycoplasma pulmonis</i>	Culture	146	0	–
<i>Pasteurella multocida</i>	Culture	207	0	–
<i>Pasteurella pneumotropica</i>	Culture	207	55	2.42
<i>Pseudomonas aeruginosa</i>	Culture	415	8	1.93
<i>Staphylococcus aureus</i>	Culture	207	37	17.9
<i>Streptococcus pneumoniae</i>	Culture	207	0	–
ECTOPARASITES				
<i>Demodex</i> spp.	Direct	431	52	12.1
INTESTINAL PROTOZOA				
<i>Entamoeba</i> spp.	Wet mount	215	22	10.2
<i>Giardia</i> spp.	Wet mount	215	39	18.1
<i>Hexamastix</i> spp.	Wet mount	215	40	18.6
<i>Reortamonas</i> spp.	Wet mount	215	3	1.40
<i>Spirotrunculus</i> spp.	Wet mount	215	33	15.4
<i>Trichomonas</i> spp.	Wet mount	215	169	78.6
INTESTINAL NEMATODES				
<i>Aspicularis tetraptera</i>	Direct	215	3	1.40
<i>Syphacia</i> spp.	Direct	215	6	2.79

^aSamples originated from pharmaceutical, biotechnology, academic, and government institutions in North America and were submitted to Charles River Animal Diagnostic Services (Wilmington, MA) from 2003–2008, unpublished data (Cosentino, 2009).

^bELISA (enzyme-linked immunosorbent assay of serum), PCR (polymerase chain reaction of feces), Direct (examination of pelt under a stereomicroscope for ectoparasites; examination of macerated cecum and colon with stereomicroscope for nematodes), Wet mount (high-magnification phase-contrast microscopy of wet mount of duodenal and cecal mucosa scrapings for protozoal agents).

colony health screening of hamsters; the two common mites identified are *Demodex criceti* and *D. aurati*. Prevalence of *Demodex* infestation in research colonies is high, although clinical signs are uncommon (Baker, 2003). A number of protozoal organisms have been identified in the intestinal tracts of hamsters, but their association with enteric disease is unclear, as their presence is common in both healthy and ill animals (Hankenson and Van Hoosier, 2002). The primary nematodes found in the intestinal tract of hamsters are pinworms of the genus *Syphacia*; hamsters may be infected by *S. mesocriciti*, *S. criceti*, *S. obvelata*, and *S. muris* (Baker, 2003; Hankenson and Van Hoosier, 2007). The prevalence of pinworm infections in hamsters has not been described.

RECORD KEEPING

Use of Records

Record keeping is an important aspect of any scientific endeavor and fundamental to good science. Records show what data have been collected and under what conditions the data were collected. Accurate records provide accountability and are necessary for a laboratory to replicate earlier experimental procedures and research outcomes. In animal studies, the requirement for accurate and full record keeping extends to the care and husbandry provided to the animals. Minimal animal identification records for hamsters are often satisfied by a cage card with the following data: source of animal, strain or stock, name(s) of responsible individual (i.e. investigator), pertinent dates (i.e. purchase date, date of birth, dates of experimental procedures), and Institutional Animal Care and Use Committee protocol number, when applicable. If animals are bred for research, accurate records must be maintained to document items such as parentage of animals, litter size, and age of animals as these parameters are important for proper management of breeding colonies and may provide information on the health of the colony. Health records are necessary to accurately treat and evaluate the response of treatment of ill and injured animals and medical records provide valuable information on the general health of research animal colonies.

Breeding Records

The maintenance of accurate breeding records is essential for proper management of breeding and production colonies. For ongoing breeding programs, such as in a commercial breeding operation and for research efforts that rely on multi-generational breeding of

hamsters, a computer database is helpful for maintenance of records. For small-scale breeding programs, typical in research settings breeding small numbers of hamsters, breeding records may be maintained on paper such as in a laboratory notebook or by retention of animal cage cards. Minimal information that should be recorded and maintained in breeding records includes animal identification (i.e. unique animal identification numbers for males and females), the date a breeding pair or breeding group is established, the date of birth of the litter, total number of pups born, the number of pups by sex, the number of surviving animals at weaning, segregated by sex, and the date of weaning. If ongoing breeding of females is planned (i.e., multiple litters from the same female), it is recommended to routinely evaluate the litter size for each female. A decrease in litter size is commonly observed as an animal ages and this information is used as criterion to retire breeders. For a detailed discussion on management and design of breeding colonies, including development of foundation colonies and breeding colony structure, see White (2007).

Husbandry Records

Records documenting the environmental conditions within the vivarium as well as the activities of animal facility staff provide important information to animal facility management staff, veterinary care staff, and researchers. Common environmental records maintained within animal facilities include documentation of housing room temperature, humidity, and light cycle. These parameters can be recorded automatically via electronic building management systems or manually via paper records. Electronic building management systems are beneficial as alarm parameters can typically be programmed to detect when environmental parameters exceed predetermined limits (i.e., high and low temperature limits), and the building management system will automatically notify personnel that can then take action to rectify the problem before animal welfare is adversely impacted. If using a paper-based documentation system, animal husbandry staff can be tasked to record temperatures and humidity levels daily within each animal holding space. Under this system, it is recommended to utilize a thermometer that registers both the peak (high) and low temperature over a set period of time, typically daily, and record both temperatures. This allows assessment of temperatures within the space during periods when staff are not present. Relative humidity in an animal housing space can be measured using a hygrometer or sling psychrometer. If housing room light cycles are not controlled through a centralized system, individual room light timers

must be routinely assessed to insure that timers are working properly and that animals are not exposed to alterations in light cycle. Husbandry activities that are important to maintaining animal health and wellbeing should also be recorded. Such records include dates when animals are provided clean food and water, cages are cleaned, and when animal rooms are cleaned and sanitized. Sanitation records are also useful for tracking key aspects of the cage sanitation process. For example, facilities utilizing mechanical cage washers routinely maintain records indicating that rinse water temperatures reach prescribed minimums to achieve adequate sanitation. Recording this information provides animal care management, research personnel, and regulatory officials the ability to regularly review key aspects of animal care, husbandry, and housing environment provided to the animals to insure they meet acceptable standards.

Health Records

Health records, also referred to as clinical records, can be maintained as group records for large populations, such as commercial breeding colonies, or as individual animal records. Health records should include pertinent clinical observations and diagnostic information, history of surgical procedures, postoperative care, and information on experimental use as it pertains to clinical care of the animal. For rodents, this information may be relatively simple and maintained on the cage card accompanying an animal or extensive and maintained as a stand-alone record.

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Biomedical Research Techniques

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INTRODUCTION

The use of hamsters in biomedical research peaked at over 500 000 animals per year in the mid-1970s and then progressively declined to the current level of approximately 146 000 animals per year (United

States Department of Agriculture, 2010). Nevertheless, hamsters are still used for a multitude of biomedical research purposes including studies in oncology, dentistry, cardiology, and neuroscience. The purpose of this chapter is to provide the reader with descriptions of specific techniques, or references to techniques, that



FIGURE 29.1 Lifting a Syrian golden hamster. Note the large amount of loose skin in the dorsal cervical area.

have important applications in biomedical research. Some techniques, such as those that are more appropriate for use by exotic animal practitioners or are infrequently used in research, have not been included. Unless otherwise noted, the descriptions presented are for the Syrian golden hamster (*Mesocricetus auratus*).

GENERAL NON-SURGICAL PROCEDURES

Handling and Restraint

Most laboratory hamsters do not relish being handled and can inflict a painful bite if given the opportunity to do so. There is a large amount of loose skin by the dorsal cervical area that can be used to restrain and lift the animal, but be sure to include a generous amount of that skin when lifting a hamster, otherwise the animal can turn around and bite (Figure 29.1). Hamsters that are regularly handled by their owners tend to be calmer and less prone to biting. Hamsters also are very apt to bite when awakened from their sleep (they will often roll over on their back before biting); therefore, it is a good idea to ensure the animal is awake before handling. Interestingly, in the experience of some authors, gentle arousal of hamsters does not lead to biting (Quesenberry and Carpenter, 2003). Perhaps the latter finding is more typical of pet hamsters. Although Syrian golden hamsters will hibernate at low temperatures (e.g., 5°C), that is unlikely to occur in typical laboratory research environments and therefore it is doubtful that a sleeping hamster is hibernating. Nevertheless, this author has noticed that the European hamster (*Cricetus cricetus*) will enter

a torpor-like state when the outside daylight becomes shorter, even though the laboratory light cycle is maintained at 12h light:12h dark (Silverman and Chavannes, 1977).

Numerous other methods have been used to help restrain or move hamsters short distances, such as scooping the hamster up into one's cupped hands or scooping the animal into a wide-mouthed jar or similar container. Caution is advised when using these methods because hamsters are prone to jump out of such loose confinement and in the author's experience they can injure themselves severely if they fall all the way to the floor. If you are unfamiliar with handling hamsters, consider learning how to do so while working at floor level.

Radiology

Either permanent or portable radiograph machines can be used, but as recommended by Silverman and Tell (2005) the machine should be capable of 5.0–7.5 mA exposures (at least 300 mA at 0.017 seconds or faster), have a range of 40–100 kVp and be adjustable in 1–2 kVp increments. We commonly use 3M Ultra Detail Plus Veterinary X-ray Film (3M Animal Care Products, St. Paul, MN) with a 3M Veterinary SA2 Fine Detail, Green Light Emitting, Rare Earth intensifying screen (3M Animal Care Products, St. Paul, MN) using a tabletop technique of 102 cm focal-film distance for whole body imaging and 97 cm for extremities. For most applications the hamster must be sedated or anesthetized. An easily removed paper adhesive tape or radiolucent foam can be used to aid positioning the animal.

Another imaging option is digital radiography. This technology uses a digital detector consisting of a scintillator that converts X-rays into light. The light is captured by pixels (similar to those in a digital camera) which in turn are transmitted to a computer monitor. Because there is a large gray-scale bandwidth the image can be enhanced, filtered, softened, sharpened, etc. It can also be saved to a file or sent electronically to another computer. Although the initial outlay can be expensive, by using a high-quality detector in a small frame (i.e., a small footprint), digital radiology of hamsters and other small research animals can be more cost-effective than the use of larger digital units designed for clinical veterinary practice with species such as dogs. Specific machine settings are not provided in this chapter because a high-quality digital radiograph unit incorporates AEC (automatic exposure control) technology which will take a pre-exposure image and provide electronic feedback to the X-ray source. In turn, the machine is automatically calibrated to provide the best image possible. The entire process, from pre-exposure to seeing the image on a computer monitor, may take as few as 12–15 seconds.

Magnetic Resonance Imaging (MRI)

Eichhorn et al. (2008) performed contrast-enhanced MRI studies on hamsters. The MRI scans were performed on a 9.4-Tesla magnetic resonance microimaging system with a maximum gradient strength of 200mT/meter and a 1H birdcage resonator with a 38mm inner diameter transmit/receive coil. The isoflurane anesthetized and pre-positioned hamsters were placed in the resonator and electrocardiogram and breathing were continuously monitored with a MRI-compatible monitoring unit. Body temperature was maintained with a warm air heating system.

Using a special plastic restrainer, Parzy et al. (2007) used MRI to image the heart of unanesthetized cardiomyopathic hamsters. After 15–30 minutes the animals remained still enough to be imaged with a 4-Tesla horizontal bore 4/60 spectrometer/imager equipped with shielded gradients delivering 200mT/meter with 260 ms rise time.

Body Temperature Measurement

It is difficult and impractical to use a standard rigid glass rectal thermometer to obtain a hamster's body temperature. However, a variety of thin, flexible, and rigid temperature thermocouples and thermistors are available commercially from scientific supply houses for use with electronic thermometers. Rectal temperatures are displayed in a matter of seconds. Young hamsters 6–14 days old have a rectal temperature of 35.1°C, 1-month-old animals have a temperature of approximately 37°C, while 160-day-old animals were found to have a rectal temperature of 37.4°C (Desautels et al., 1985). Infrared thermometers, which do not directly contact the animal, have been used for hamsters (e.g., Ohta et al., 2006) but may have limited applications because infrared measurements are typically taken from the tail surface, a notably small feature in the hamster. Body temperature can also be monitored via an implantable temperature transponder.

Respiratory Function Assessment

Although hamsters are often used in respiratory disease research, very few studies have focused on techniques specifically designed for measuring their respiratory functions. Reviews of invasive and non-invasive methods for studying pulmonary functions in mice have been published (Glaab et al., 2007; Pichavant et al., 2007) and they can be adapted to hamsters. Raub et al. (1983) tracheotomized hamsters and measured lung function in a whole-body plethysmograph. They found an end-expiratory volume of approximately 1.4ml, vital capacity of 5.7ml, residual volume of 0.75ml, total lung capacity

of 6.5ml, and respiratory system compliance of 0.4ml/cm water.

Sato et al. (1989) used a whole-body plethysmograph and a hot-wire flow sensor that measured gas volume respired through a tracheal cannula. This allowed for the measurement of airway resistance in male hamsters that was free of artifacts caused by temperature and humidity. Those authors found airway resistance to be 0.44 ± 0.06 (SE) cm H₂O.ml⁻¹.second. This had a strong correlation with pulmonary resistance (0.42 cm H₂O.ml⁻¹.second, $r = 0.93$). Mean respiratory frequency was $77.2 \text{ min}^{-1} \pm 6.8$ (S.E.), tidal volume was $0.61 \text{ ml} \pm 0.01$, and functional residual capacity was $1.52 \text{ ml} \pm 0.06$.

Lung volume has been estimated in hamsters by traditional radiography (Canals et al., 2005) and in mice by magnetic resonance imaging (Martin et al., 2008). The former method used a standard small animal radiograph machine to help develop formulas for estimating lung volume in anesthetized hamsters weighing ~42g. Radiographically calculated lung volume was $2.44 \text{ ml} \pm 0.28$ (S.D.) while the measured lung volume from water displacement was $1.56 \pm 0.37 \text{ ml}$.

Dental Procedures

Tooth Trimming

As with mice, rats, and gerbils, hamsters have open-rooted (elodont) incisors that grow continuously. To prevent malocclusions the incisors must be worn down by their proper occlusion with each other and by their diet. In contrast, the molar teeth (there are no premolars) do not grow continuously (anelodont). Significantly overgrown incisors are fairly easy to see and they can be trimmed back by the use of a variety of different types of tooth clippers (e.g., toe nail clippers). Although this sounds easy, in reality it can be quite difficult to determine, without radiographs, the amount of tooth to trim. This is particularly true with the lower incisors, which are normally longer than the upper ones. One must also assure that the hamster's tongue is not cut during the clipping process. Tooth clipping can lead to a tooth fracture or disrupt the periodontal membrane; therefore, if possible the tooth should be ground down with a dental burr. Unfortunately, this is not often possible in a research setting. Overgrown teeth frequently must be trimmed every 2–3 weeks. The technician or veterinarian should become very familiar with the normal appearance of hamster teeth.

Dental Abscesses

Dental abscesses of the molars are occasionally seen in hamsters. Unless the laboratory animal practitioner has the prerequisite dental training to treat or remove these teeth, these therapeutic procedures should be left



FIGURE 29.2 Endotracheal intubation using a fiberoptic endoscope.

to a veterinarian with the appropriate experience and skills.

Endotracheal Intubation and Intratracheal Instillations

Endotracheal intubation in hamsters is complicated by the small size of the oral cavity and glottis. Before attempting intubation, first make sure that there is no food in the oral cavity and especially in the cheek pouches. The endotracheal tubes used for hamsters rarely completely occlude the tracheal lumen and it is important to minimize the potential for food particle inhalation. We prefer to use a technique similar to that described by [Hawkins and Graham \(2007\)](#). The anesthetized hamster can be hand held; however, we prefer to have the animal placed on a nearly vertical board (about 70° angle) suspended by its upper incisors. Use of a laryngoscope with a size 0 blade (used for premature infants) may help to visualize the glottis. [Costa et al. \(1986\)](#) described a custom-made fiberoptic laryngoscope for small rodents. A similar commercial product (BioLite, [Figure 29.2](#)) is now available as part of a rodent intubation system (Bioseb, Chaville, France). Otherwise, a narrow otoscope cone with most of one side cut out can be used. One drop of 2% lidocaine is placed on the larynx to help prevent laryngospasm. The animal's tongue is either pulled out of the way with a forceps or pushed to the side with a cotton-tipped applicator.

A variety of different tubes can be used for endotracheal intubation (e.g., thin rubber urinary catheters, intravenous catheters, plastic tubing). Tubes should be approximately 1.5 mm in diameter (slightly smaller than a 14-gauge needle). The length should be no less than 6.5 cm ([Costa et al., 1986](#)). The sides and oral opening of the endotracheal tube must be smooth. Small

endotracheal tubes are often very flexible and difficult to insert, therefore, if necessary, a blunt stylet can be passed into the trachea prior to passing the tube over the stylet (the stylet is then removed). It is important that the oral end of the endotracheal tube is secured to the hamster and is capable of being attached to a standard anesthesia tube fitting if the latter will be used. Similar to [Hawkins and Graham \(2007\)](#), we have also found that mucus plugs in the tube can be a problem.

For intratracheal instillations once an endotracheal tube is in place, approximately 0.15 ml of liquid or suspension can be administered per 100 g of body weight ([Brain and Valberg, 1979](#)).

Pulse Oximetry

Pulse oximetry measures hemoglobin oxygenation. The rapid heart rate of the hamster requires pulse oximetry equipment that can record at well over 400 heart beats/min. Fortunately, such equipment is now available (e.g., MouseOx[®], Starr Life Sciences, Oakmont, PA; PulseSenseTMVET, Nonin Medical, Inc., Plymouth, MN). The sensor can be placed on an ear, a foot, and in some instances on the hamster's small tail.

Electrocardiography (ECG)

Cardiomyopathic hamsters have a long history of use in biomedical research. The Syrian golden hamster's rapid heart rate (~400 beats/min) requires special recording equipment. The animal is anesthetized and alligator clips attached to a hypodermic needle, spring clip electrodes, or needle electrodes are placed in one of the standard ECG arrangements. To prevent excessive cooling of the animal minimal alcohol or gels should be used ([Hawkins and Graham, 2007](#)).

To record the ECG in conscious hamsters one can use a surgically implantable telemetry device (e.g., Mini-Mitter, Bend, OR; Data Sciences, St. Paul, MN) or an ECG recording platform. The latter is an array of gel-coated ECG electrodes embedded in a platform on which the animal stands. It has been described for recording the ECG in unanesthetized mice ([Chu, 2001](#)) and would likely be applicable for use by hamsters. A commercial version of the recording platform is the ECGenieTM (Mouse Specifics, Inc., Quincy, MA).

Blood Pressure Measurement

Direct blood pressure measurements are made with techniques similar to those used in other species. For example, catheters can be inserted into the carotid artery (see below). Indirect measurement of blood pressure is more difficult due to the hamster's relatively small size and lack of a long tail. [Kato and colleagues](#)

(2003) described the use of a modified tail-cuff placed around the left brachial artery of anesthetized hamsters which weighed ~123 g. Details of the modification were not provided, however, a statistical analysis indicated strong positive correlation with a simultaneous direct blood pressure measurement.

A Doppler ultrasonic probe can also be used to detect arterial flow when used with a pressure cuff to occlude arterial flow and a sphygmomanometer or oscillometric device to measure the blood pressure (Hawkins and Graham, 2007). Nevertheless, this can be difficult to use in the hamster. ECG methods for assessing general systolic function and other indices of left ventricular function have been reported for the Syrian golden hamster (Martino, 2008; Salemi et al., 2005).

Electroencephalography (EEG)

Hamsters are often used in neuroscience and behavioral research. A procedure for hamster electroencephalography has been described (Bali et al., 2008). After light isoflurane anesthesia seven EEG needle electrodes were placed subcutaneously over the left and right frontal, parietal, and central areas of the unshaved skull and recordings made as required for the study.

INJECTIONS AND INTUBATIONS

Gastric Intubation

The technique used for gastric intubation is similar to that used in other species. The length of the gavage needle should be a little shorter than the distance from the tip of the animal's nose to the end of its rib cage (~4 cm). A curved or straight, flexible or rigid, 18–20-gauge gavage needle (with a ball head) is inserted into the mouth of a manually restrained hamster. When using a straight needle, the needle is slid caudally along the roof of the animal's mouth while using the needle as a lever to assure the hamster's head is in a straight line with the remainder of its body. At times, slight dorsoflexion of the animal's head, using the needle, makes the intubation easier. With gentle pressure the needle is advanced down the esophagus and into the stomach. The animal is generally not anesthetized for this procedure and if anything more than mild resistance is felt the gavage needle should be removed and redirected. Even with a small ball head on the gavage needle the chance of entering the trachea is extremely low and is readily detected via the animal's gag reflex. A maximum volume of 3–5 ml/100 g body weight can be administered (Heatley and Harris, 2009).

For newborn hamsters a silicon catheter of 0.63-mm outer diameter can be passed directly into the stomach



FIGURE 29.3 Subcutaneous injection in the flank.

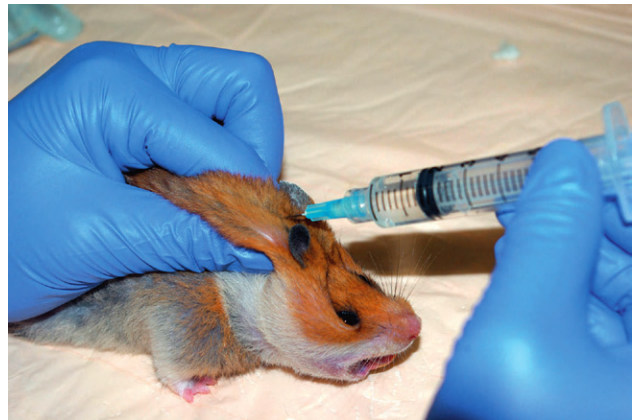


FIGURE 29.4 Subcutaneous injection between the scapulae.

(Silverman, 1987). The animal is gently restrained with the thumb and index finger of one hand. The catheter, gently held by a forceps, is directed into the animal's mouth and then into the stomach.

Subcutaneous (SC, SQ) Injection

The flank area of the hamster, as in other small mammals, can be used for SC injections (Figure 29.3). The abundant amount of loose skin along the back of the hamster also allows for easy SC injections, although caution must be used to properly restrain the animal while making the injection (Figure 29.4). It is suggested that if multiple SC injections must be used as part of a research project, the site of the injections should be rotated if that is compatible with the study. The hamster is lifted and restrained with one hand and the injection is made with the syringe and needle that is in the other hand. Once the needle is inserted under the skin it is moved slightly left and right to assure that it can be seen moving, thereby assuring it is not intraperitoneal. The plunger on the syringe is then depressed.

It is suggested that the novice practice on an inanimate object to assure that his or her fingers are positioned in a manner that allows the plunger of the syringe to easily be depressed while the needle is under the skin. A maximum of 3–5 ml can be injected (Heatley and Harris, 2009).

Intraperitoneal (IP) Injection

Intraperitoneal injections are typically made in the lower right or left quadrant of the hamster's abdomen, not on the midline. A relatively short needle (e.g., 0.5-inch or less) is suggested to help prevent puncture of the intestines or cecum. Once the animal is properly restrained (as described above for SC injections) the injection site is visualized, the needle is inserted at approximately a 60° angle to the body wall and the plunger on the syringe immediately depressed or, if desired, withdrawn slightly to assure the needle is not in an organ or hollow viscera. Injections that enter viscera, solid organs, or are SC rather than IP, will usually be absorbed but often slower than when the injection is truly IP. As with SC injections, it is suggested that an inexperienced person first practice on an inanimate object. A maximum of 3–4 ml can be injected (Heatley and Harris, 2009).

Some texts (e.g., Field and Sibold, 1998; Hrapkiewicz and Medina, 2006) have recommended tilting the animal's head downwards when performing IP injections, presumably to have the intestinal viscera slide forward and decrease the potential for injections into hollow viscera such as the cecum. A search of the biomedical literature did not discover any research that supports or refutes the rationale for this practice.

Intramuscular (IM) Injection

Hamsters have relatively little muscle mass in their hind limbs and IM injections must be made carefully. The only site we routinely use is the muscle mass anterior to the femur (the quadriceps group). A small needle (e.g., 0.25-inch, 25-gauge) is advanced into the muscle, somewhat parallel to the femur, and the injection is made. The author does not routinely use the muscles caudal to the femur due to the increased potential for injecting into or near the sciatic nerve. It is suggested that two people be used to perform IM injections (one to restrain the animal and the other to make the injection). A maximum of 0.1 ml/site is injected (Heatley and Harris, 2009).

Intrarectal (IR) Instillation

Although relatively easy to perform, IR instillations should be performed under light anesthesia to prevent struggling and possible colonic perforation. A standard

ball-tipped feeding needle can be used (e.g., 18 gauge, 4 cm). The animal is placed in dorsal recumbency and, if compatible with the research, a light coating of water-soluble lubricant is placed on the anus to ease the passage of the feeding needle. The needle is inserted to the desired length and the infusion is made slowly. The skin around the anus should be held somewhat taut to prevent its invagination into the anus (Silverman, 1987).

Intraosseous (IO) Injection

An IO catheter can be used for the rapid injection of non-toxic fluids, for maintaining open access to the vascular system over a period of days, and for therapeutic needs when intravenous catheterization is not possible, which is often the case with laboratory hamsters. The catheter itself is the needle that is used to penetrate the bone, and it should be ~30–50% of the length of the long bone into which it will be inserted (typically the tibia or femur) (Hawkins and Graham, 2007). It should occupy 30–60% of the marrow cavity at its thinnest point (Lennox, 2008). A 24–25-gauge needle is adequate for most adult hamsters. For a healthy hamster general anesthesia is required and post-procedural analgesia should be considered. If the tibia is to be used, the proximal area should be prepared for aseptically and the catheter is introduced at the tibial crest, approximately where the patellar ligament inserts (Lennox, 2008). A 4-0 monofilament stainless steel suture can be used as a stylet in a 25-gauge needle to help prevent blockage of the catheter's lumen (Hawkins and Graham, 2007). It is important to avoid penetration into the femorotibial joint. The catheter is advanced until it is stopped by the bony cortex. After insertion, the catheter is flushed with 1:10000 heparinized saline. An injection cap is then placed on the needle, the area of insertion is covered with neomycin-polymixin-bacitracin antibiotic ointment, and the catheter is secured with a bandage-tape butterfly and sutures (Hawkins and Graham, 2007). If possible, the entire area can be bandaged to help prevent gnawing through to the catheter.

Intraspinal (IS) Injection

An early description for IS injections was provided by Habel and Li (1951) who performed the procedure through a skin incision in anesthetized mice and hamsters at the "lumbar enlargement" of the spinal cord, using a 0.25-inch, 25-gauge needle on a 0.25-ml syringe. The needle, inserted between the slightly flexed vertebrae, was held at a 45° angle with the tip pointed cranial. It was inserted just to the right of midline and also pointed slightly toward the midline. They stated that when the needle entered the spinal canal a "giving" sensation was felt. Twenty µl could be injected. Kimberline

et al. (1987), using adult female hamsters, cut out a small window in the skin over the area of the 9th–11th thoracic vertebrae. Using a 30-gauge needle they injected 30 μ l over a period of 5–10 seconds so as to avoid damaging the spinal cord by excessive pressure.

Intravenous (IV) Injection

Ferm (1967) indicated that 2.0ml may be administered IV over a 1-min period while Gaertner et al. (2008) recommended a maximum of 0.3ml as a bolus and 25ml/kg body weight by infusion.

1. Cephalic vein. There are a limited number of readily accessible veins for IV injections. Although small, the cephalic vein can be accessed using a 27–30-gauge needle. The injection technique is similar to that used with cephalic IV injections in other species (the vein is initially constricted proximal to the injection site, the needle bevel is faced upwards and the needle is inserted nearly parallel to the vein).
2. Lateral saphenous vein. This vein can be entered where it crosses the tarsus, although this author has not had much success in using it. The technique used is the same as that for the cephalic vein, described above.
3. Retroorbital venous sinus. The retroorbital venous sinus, which is often used for blood collection (see below), can also be used for IV injections. (Popesko et al. (1990) illustrate an orbital venous plexus (*plexus orbitalis*). Other authors (House et al., 1961; Reznik et al. 1978; Tassoni 1955) all state that there is an orbital venous sinus in the Syrian golden hamster but none provide any documentation. In a newsletter article, Timm (1980) reported having prepared latex injected and corrosion casts of the venous structures of the head and diagrammed a venous sinus occupying the caudal one-half to two-thirds of the hamster orbit.) The hamster is lightly anesthetized and the eyelids are gently retracted manually, which will proptose the eye. A 25–27-gauge needle on a 1-ml syringe is inserted through the conjunctiva of the medial canthus of the orbit, at about a 45° angle and in a caudal–medial direction (Figure 29.5). Care must be taken not to pierce the thin bone of the skull. The needle is carefully advanced into the post-orbital venous sinus (a backflash of blood is occasionally seen in the syringe) and the injection is made. Do not inject more than 0.3ml. Because the injection is directly into the bloodstream, no clumped material should be used although Handler (1968) indicated that about 0.05ml of a cell suspension can be inoculated by this method. After removing the needle put direct pressure over the eye for about 30 seconds. Injections should not be made more often than every other day in the same eye.



FIGURE 29.5 Intravenous injection into the retroorbital venous sinus of the anesthetized hamster.

4. Jugular vein. The jugular vein can be used for IV injection by inserting an indwelling catheter using the general approach described below for jugular vein catheterization while the animal is under general anesthesia. In addition, a simple venous cutdown is often sufficient for a single injection using a 25–27-gauge, 0.5-inch needle. Additionally, the technique described later in this chapter for percutaneous collection of blood from the jugular vein can also be used for injections into the vein. Yohn et al. (1968) indicated that the left jugular vein of a newborn hamster could be used for IV injection of 0.05ml of virus inoculum or 0.1 ml of a cell suspension but details of the technique were not provided.
5. Lingual vein. This vein can be used for IV injections using the general technique for blood collection from that vein as described later in this chapter. A maximum of approximately 0.3ml can be injected.

Jugular Vein Catheterization

Catheterization of the jugular vein is typically performed when repeated blood collections or infusions are required. The jugular veins are located on either side of the neck, running in the jugular groove that parallels the trachea. In the anesthetized and surgically prepped hamster, a 1.5–2.0-cm incision is made over the caudal aspect of the right jugular vein while the hamster is in dorsal recumbency. The vein is located by blunt dissection and surrounding connective tissue and muscle is cleared away. Silk ligatures (3-0 or 4-0) are carefully passed under the cranial and caudal aspects of the cleared vein and the cranial ligature is tied tightly enough for the venous flow to be occluded. A small cut is made in the caudal part of the exposed vein using fine iris scissors. Using a fine forceps the sterile silicon or polyurethane catheter is threaded into the cut vein, pointing toward the heart. The

caudal suture is then tied to secure the catheter in place, being careful not to tie it so tight as to occlude the lumen of the catheter. A typical catheter size for an adult female hamster is 0.65mm inner diameter and 1.2mm outer diameter (Lisciotto and DeBold, 1991). The catheter can be advanced about 1.8–2.0cm into the vein to reach the right atrium of a 150-g hamster (Terpstra et al., 1999). The open end of the catheter can be exteriorized to the dorsal cervical area and secured to the skin in a standard manner (e.g., sutured to the skin directly or by using a catheter bead). The skin incision is closed routinely. Terpstra et al. (1999) have described an imbedded prosthesis to help secure the catheter, although commercially available catheter beads can be used. Once inserted, the catheter can be used for either withdrawing blood or administering intravenous products. The catheter should be capped and flushed daily with a 1:10000 heparin:saline mixture.

Carotid Artery Catheterization

Only a general outline is presented here. The reader should refer to the original publication (Elliott et al., 2005) for a comprehensive description. A 2-cm incision is made over the trachea. The left carotid artery is exposed by blunt dissection in the left paratracheal groove, and it is ligated cranially. A temporary second ligature is placed around the artery at the caudal end of the incision. A small arteriotomy is made in the artery and PE-10 polyethylene or another suitable tubing cannula is inserted into the arteriotomy incision. A third ligature is placed between the first and second one, within 1mm of the incision, and tightened around the cannula with a single half-hitch. The temporary ligature is then released. The cannula can then be attached to a 27-gauge needle for research procedures (e.g., blood transfusion). After the study is completed, the half-hitch ligature is further tightened to close off the artery and the surgical openings are closed with simple interrupted sutures.

Arterial Injection into the Pancreas

The induction of pancreatic cancer in hamsters by SC or IP chemicals such as N-nitrosobis(2-oxopropyl)amine or N-methyl-N-nitrosourea is an established and valuable research technique. Nevertheless, only one study has addressed vascular access to the arterial side of the pancreas (Sørensen and Seljelid, 2003), a procedure with importance for chemotherapy and immunotherapy research. The arterial blood supply of the pancreas is primarily derived from the celiac artery. Branches of this artery can be used for experimental therapeutic injections. A midventral incision is made just posterior to the xiphoid process and the stomach, spleen, duodenum, and transverse colon are exteriorized. The liver is retracted as needed. Using a surgical microscope, the

gastroduodenal artery is ligated as it branches off the celiac artery. The celiac artery itself is then ligated distal to where the splenic artery branches off. The celiac artery is punctured with a 30-gauge cannula close to the aorta and the cannula is advanced 5–6mm into the celiac artery. The cannula is then secured to the artery and exteriorized (if appropriate to the study). The exteriorized organs are replaced and the wound is closed routinely.

BIOLOGICAL SPECIMEN COLLECTIONS

Blood Collection

Blood collection is often required in studies with hamsters but because of the animal's very short tail, sampling from that location is impractical and techniques using other sites are described below. If collected blood is to be separated for plasma or serum, consider the use of commercially available micro-collection tubes such as the Microtainer® (Becton Dickinson, Franklin Lakes, NJ). In general, the use of heparin as an anticoagulant is preferred over ethylenediaminetetraacetic acid (EDTA) (McClure, 1999). For most research purposes a volume of blood equal to approximately $0.01 \text{ ml} \times \text{body weight}$ (in grams) can be safely sampled. Thus, for a healthy 100-g hamster one can safely remove about 1.0ml of blood. This can be repeated in approximately 3 weeks (Joint Working Group, 1993). Even with dwarf hamsters (e.g., *Phodopus sungorus*) modern equipment allows plasma chemistry analyses on as little as 150µl of whole blood (Jenkins, 2008).

1. Retroorbital. Retroorbital blood collection is not difficult but the operator must be skilled so as not to damage the bony orbit or the eye. The animal is lightly anesthetized and placed on its side. The ocular globe is easily proptosed by having the index finger and thumb of one hand gently pulling the eyelids apart while the same thumb is used to exert slight pressure over the area of the jugular vein. A small capillary tube (such as those used for hematocrit determinations) is advanced into the lateral canthus, perpendicular to the long axis of the body. The capillary tube is gently rotated until the conjunctiva is ruptured and the underlying orbital venous sinus is reached. After a sufficient amount of blood is collected in the tube, remove the tube and exert direct pressure over the ocular globe for about 30 seconds. Although a hypodermic needle (e.g., 22-gauge) can also be used to withdraw blood from the retroorbital sinus, one must be very careful not to perforate the bone of the orbit or injure the eye itself. MacDonald (1988) reported obtaining 1 ml of blood by retroorbital collection and suggested first flushing

the collecting tube with 1:10 000 heparin:saline, stating that hamster blood is "highly coagulable." Alternately, commercially available heparinized tubes can be used.

The medial canthus also can be used as a site for capillary tube insertion, but the insertion should be directed medially and posterior. Usually, more blood can be obtained using the lateral rather than the medial canthus. Another site, which is quite effective with hamsters, is along the superior border of the ocular globe, half way between the medial and lateral canthi (Silverman, 1987). A capillary tube is inserted through the conjunctiva and blood is collected as described above.

2. Small veins. Hawkins and Graham (2007) described methods for collecting blood from small vessels. A 0.3-ml insulin syringe with a swaged-on 27–29-gauge needle is used. The syringe plunger is removed, the needle is introduced into a small vessel (e.g., the saphenous vein) and blood is collected by introducing a heparinized microhematocrit tube into the syringe barrel. The syringe barrel acts as a stabilizing device. Blood also can be collected by inserting a small gauge needle into a vein and using a heparinized microhematocrit tube to collect blood as it exits through the rear of the needle, without the use of the insulin syringe.

A skilled person can obtain a small amount of blood from the cephalic vein or the caudal branch of the lateral saphenous vein as it crosses the tarsus. In both of these techniques the fur is wetted with alcohol and, if possible, a small tourniquet is placed proximal to the vein. A little silicon grease or petroleum jelly can be applied to prevent clotting. The vein is punctured (not cut) with a small needle (e.g., 23-gauge). Alternately, for the cephalic vein, it is possible to directly insert a 27-gauge needle attached to a tuberculin or insulin syringe and gently withdraw a small amount of blood. The technique requires one person to hold off the vein and another to withdraw the blood. Anesthesia is usually not required for the cephalic vein, but we have found that anesthesia or a tranquilizer is often needed for the lateral saphenous vein due to the movements of the hamster. A detailed description of saphenous venipuncture has been published (Hem et al., 1998). The hamster sublingual vein (lingual vein) is relatively easy to use for blood collection. The animal is anesthetized and held in a supine position by the scruff of the neck to slightly compress the jugular and lingual veins. A second person manually extends the animal's tongue and the caudal part of the sublingual vein is punctured with a 24-gauge, 1-inch hypodermic needle. The animal is turned ventrally and held horizontally over a blood collection tube. To

stop the bleeding, release the pressure on the neck. Approximately 750 μ l can be collected (Heimann et al., 2009).

3. Nail clip. Clipping a nail short can provide a drop of blood, but it is not a recommended procedure as it is likely to be painful, some animals have developed infections in that area, and subsequent serum chemistries can be compromised by tissue fluids. If this procedure is used the bleeding nail should be cauterized with silver nitrate or other means.
4. Cranial (anterior) vena cava. This procedure is used to collect relatively large volumes of blood. A detailed technique, using isoflurane anesthesia, has been described by Picazo et al. (2009). The hamster is maintained in dorsal recumbency. On the animal's left or right side, a 25-gauge needle on a 1-ml syringe is inserted 0.4 cm lateral to the manubrium of the sternum and cranial to the first rib. The needle is inserted at a 30° angle in the direction of the head of the opposite femur. Pulling on the syringe plunger to create slight negative pressure once the skin is pierced, the needle is inserted 0.3–1.0 cm until blood begins to flow. After the collection the needle is removed and direct pressure is applied for 30 seconds at the injection site. A slight modification of this technique is suggested by Hawkins and Graham (2007) who use a 27-gauge, 0.5-inch needle, inserted at a 45° angle.
5. Jugular vein. Percutaneous jugular vein blood collection can be performed using the general technique described by Kassel and Levitan (1953) or Hoff (2000). For a right-handed person the anesthetized hamster is held in the left hand with its head toward the operator's fingers. The area over the right jugular vein is either clipped or wetted with alcohol. The neck is hyperextended by placing a thread or piece of gauze sponge over the upper incisors, pulling it backwards and holding it with the left hand, along with the animal. The needle (23–25-gauge) is introduced in a caudal to cranial direction, over the sternum, puncturing the skin about 2 mm below the sternoclavicular junction. Remove the blood slowly. Jugular blood also can be collected via a catheter, as described earlier in this chapter.
6. Cardiac puncture. Cardiac puncture can be used for blood collection in an anesthetized animal. Approximately 3 ml can be collected from an adult hamster. The animal is placed in dorsal recumbency. Use a 1–3-ml syringe with a 22–23-gauge, 0.5-inch needle. The needle is inserted in a cranio-dorsal direction, at about a 30° angle to horizontal, and a little to the left of the xiphoid cartilage. If inserted correctly blood will enter the needle. Slowly withdraw the syringe plunger. It is suggested that cardiac blood collection be used as a

terminal procedure due to the potential for cardiac tamponade. Often, blood can be aspirated from the heart immediately after carbon dioxide euthanasia as the heart continues to beat for a short while.

7. Major blood vessel cut. Large amounts of blood can be collected as a terminal procedure by anesthetizing the animal and cutting any of the major blood vessels. Commonly, the axillary vessels, caudal vena cava, and abdominal aorta are used.

Urine Collection

Non-surgical catheterization is not practical in the male. The use of a standard metabolic cage is recommended if urine collection is required over a period of time. For a single urine collection, restraining the hamster often causes the animal to urinate one or two drops. If the animal urinates on a stainless steel table top or even in a plastic bag, the small amount of urine present can be used for a basic urinalysis using a commercially available reagent test strip.

Females can be catheterized if necessary. In the past polyethylene tubing has been used, but the typical PE-10 tubing (about 0.60mm outer diameter) does not have a smooth end and can injure the mucosa of the urethra. Waynforth and Flecknell (1992) suggest using the catheter part of an over the needle IV catheter as an alternative for rats. This can be tried for hamsters. Another option is to use a 2-French polyurethane catheter (0.64mm outer diameter). Although polyurethane catheters with a rounded tip are available in varying degrees of stiffness, the stiffness sold commercially for most IV applications is suitable. Polyurethane is available pre-sterilized by ethylene oxide (e.g., Solocath,TM Instech Solomon, Plymouth Meeting, PA). The actual procedure is performed on an anesthetized animal. For a right-handed operator the dorsally recumbent animal is placed in the left hand with its head toward the operator. The urethral papilla is stretched somewhat by using the left thumb to pull the caudal abdominal skin somewhat cranially. The catheter is now placed in the urethral orifice and inserted as far as it can go without excessive pressure. The catheter is then tilted caudally while the operator continues to push it gently cranially until it enters the bladder.

Pancreatic Duct Catheterization

The basic technique has been described by Permert et al. (2001). A dissecting microscope is used to aid the surgery. After a 12-h fast a midline laparotomy is performed and the pancreatic duct is identified and ligated at its entrance into the duodenum. The duct is opened with fine scissors just proximal to the ligature and a PE-10 polyethylene or other suitable cannula is inserted and secured. Although not noted in the published

surgical description, for survival surgery the tubing must be exteriorized and secured to the body wall. One way of doing this is to pass a large-bore needle through the abdominal wall (from the exterior to the interior), pass the open end of the tubing through the needle, remove the needle and then secure the tubing to the body wall (Waynforth and Flecknell, 1992).

Bile Collection

The hamster is an appropriate model to study human cholesterol metabolism (Spady and Dietschy, 1983). Like the mouse, but unlike the rat, the hamster has a gallbladder. The hamster gallbladder is attached to the middle lobe of the liver. After anesthesia and surgical preparation a midline abdominal incision is made up to the level of the caudal aspect of the xiphoid cartilage. A large-bore needle is passed through the aseptically prepared skin, pointed toward the hamster's back, and then into the abdominal cavity (Waynforth and Flecknell, 1992). The needle's bore will later be used for exteriorizing the catheter. The proximal part of the common bile duct (*ductus choledochus*) is located and dissected from the tissues of the hepatoduodenal ligament. The bile duct (*ductus cysticus*) is ligated with either a suture or a hemostatic clip. A small longitudinal cut in the common bile duct is made with fine scissors and the common bile duct is cannulated with a 33-cm, 1.0-mm inner diameter and 1.8-mm outer diameter silicon catheter (Fuchs et al., 1992) or PE-10 polyethylene tubing, directed toward the liver. The tubing is secured to the duct. The open end of the catheter is pushed through the bore of the large-bore needle and secured to the skin.

Cerebrospinal Fluid (CSF) Collection

After anesthesia the hamster is placed in a stereotaxic holder with its neck maximally ventroflexed. The dorsal cervical area and skull is prepped for surgery. A 5–7-mm midline incision is made from between the scapulae to the dorsum of the skull. A sterile 30-gauge needle (without the hub) is attached to approximately 25cm of 0.25-mm inner diameter microbore tubing. A 1.0-ml syringe is attached to the other end of the tubing via a needle on the end of the syringe. The 30-gauge needle is placed in the arm of the stereotaxic device and inserted vertically into the neck 4-mm caudal to the occipital crest. Maintaining mild suction with the syringe, the needle is slowly advanced until fluid is seen entering the tubing. Thirty to 70µl can be collected (modified from Morrey et al., 2007). The surgical wound is then closed. A slight modification of this method was used in rats by Nirogi et al. (2009) who did not make an initial incision in the skin.



FIGURE 29.6 Cerebrospinal fluid collection in the anesthetized hamster.

Another method of CSF collection is similar to that of [Nirogi et al. \(2009\)](#) in that a skin incision is not made but it is further modified by holding the anesthetized hamster in the operator's hand with the animal's head ventroflexed. After the needle insertion area is surgically prepped a 29-gauge, 0.5-inch needle on a 0.5-ml syringe is inserted on the midline just posterior to the most caudal aspect of the skull (the *squama occipitalis*). The needle is pointed rostrally at approximately a 45° angle ([Figure 29.6](#)). The needle is advanced into the cisterna magna and using slight negative pressure on the syringe plunger the CSF is collected.

Saliva Collection

Hamsters are injected IP with 3.0mg of pilocarpine hydrochloride. Over a period of 2–3h about 0.5ml can be collected from one hamster if the animal is placed in a restraint apparatus with its head over a beaker ([Haakenstad and Coe, 1971](#)). Gravity collection from a hand-held hamster can be used to recover smaller amounts of saliva.

Milk Collection

Milk can be collected from anesthetized or unanesthetized lactating hamsters by using a simple suction device as described for mice ([Haberman, 1974](#)), however, the collection pipette is made somewhat longer due to the longer teat of the hamster. Specifically, we drill two holes into a rubber stopper which is used to plug a 30–40-ml test tube. A smaller test tube (~3.5ml) is placed in the larger tube and the narrow end of a Pasteur pipette is passed through one of the drilled holes and into the smaller test tube. This Pasteur pipette (with or without some minor modifications) is the collecting pipette. A small piece of glass tubing is passed through the second hole in the stopper and eventually

connected to a simple suction pump via flexible tubing. The collecting pipette is placed over the nipple of the hamster and the milk flows into the smaller test tube.

With a lactating hamster it is usually not necessary to prime milk letdown with oxytocin, but if a greater quantity of milk is needed or if milk letdown is minimal, 0.5–1.0IU of oxytocin can be administered SC. This dosage is based on limited experience by the author. Others have suggested 0.1IU ([Haakenstad and Coe, 1971](#)) or 6.25IU/kg ([Nordenhäll et al., 1995](#)). Typically 0.1–0.3ml of milk can be collected from each hamster during the first 2 weeks postpartum with no milk being collected after the second week ([Nordenhäll et al., 1995](#)). The hamster can have milk expressed manually from the gland if a smaller quantity is sufficient.

Superovulation and Ova Collection

Sexually mature hamsters are superovulated by IP injection of 30IU of pregnant mare's serum gonadotrophin on day 1 of the estrous cycle and 30IU of human chorionic gonadotropin (hCG) is injected 48–56h later. The animals are euthanized 15–16h after the hCG administration. Ova with their cumulus oophorus masses are collected by flushing the oviducts with normal saline at 22–25°C ([Yuan and Shi, 2000](#)).

An alternate method of superovulation was described by [Venditti et al. \(2009\)](#). Ovulation is determined by placing a male hamster briefly into a female's cage during the dark phase of the light cycle and observing her behavior. The day in which the female shows lordosis is designated as estrous day 4, indicating ovulation will occur just prior to lights on. The hamsters are superovulated by intraperitoneal injection of 25IU pregnant mare serum gonadotropin dissolved in 0.1ml water 1h after the lights come on.

Sperm Collection

Electroejaculation has been described in many species; however, no literature references have been found for using this technique in hamsters. [Bedford and Yanagimachi \(1992\)](#) studied hamster sperm activation by allowing males to mate with females and then removing the sperm from the vagina of the female subsequent to euthanasia. Ejaculate without sperm can be collected by the IP injection of 400–600mg/kg body weight of potassium chloride ([Rockhold and Hiestand, 1956](#)).

OTHER SURGICAL PROCEDURES

Eye Enucleation

Hamster eyes normally appear somewhat more proptosed than other rodents and from time to time

the laboratory animal practitioner will be required to remove an eye damaged from fighting, other forms of trauma, or for research requirements. Given the small size of the eye, the technique is much easier than that required for a larger species such as a dog. After general anesthesia a small lateral canthotomy can be made if needed. The eye is either further proptosed or the sclera is grabbed with a hemostat or other suitable instrument and pulled away from the orbit. The periorbital and ocular tissues (including muscles, optic nerve, and blood vessels) are cut with iris scissors and the eye is removed. [Arce et al. \(1995\)](#) described the removal of a healthy eye for research needs. The technique is similar to that just described, however, they placed a ligature around the optic nerve and cut the nerve distal to the ligature. Direct pressure is used to suppress any bleeding (or a small amount of oxidized cellulose can be placed in the orbit) and the lids can be oversewn with absorbable suture material. Post-operative analgesia is provided.

Adrenalectomy and Adrenal Medulla Resection

The technique is similar to that used in other species ([Waynforth and Flecknell, 1992](#)). The anesthetized and surgically prepped hamster has a 1–2-cm skin incision made on the dorsal midline, over the general area of the kidneys. The skin incision is retracted to either side of the midline and scissors and forceps are used to bluntly (and sharply when needed) dissect the connective tissue. A small longitudinal incision is made behind the ribs and a few mm ventral to the spinal epaxial muscles (i.e., about a third of the distance down from the hamster's dorsum for the left adrenal and about a quarter way down for the right adrenal). The peritoneum is opened and using two pairs of curved hemostatic forceps (one to hold the spleen out of the way and the other to search for the adrenal gland), the adrenal gland is found in a mass of fat anterior to the kidney. It must be carefully teased out of the incision by pulling out the fat. It is important not to use the forceps to grasp the actual gland as a piece may break off and remain in the abdomen. The tissue and vessels are double-clamped at the base of the gland and the clamping forceps pulled apart to effect hemostasis. Muscle suturing is usually not needed although the skin incision must be routinely closed.

To remove the adrenal medulla, bilateral incisions are made as described for adrenalectomy. The adrenal glands are gently extirpated but not clamped. With the aid of a dissecting microscope a small incision is made on the adrenal cortex and the adrenal medulla is removed using minimal pressure to squeeze it out. The adrenal cortex is left intact ([Demas et al., 2004](#)).

Liver Perfusion

Many laboratories have developed their own procedures for liver perfusion, based on unique research needs. Two are presented here. One common *in situ* method is to make a ventral midline incision on the anesthetized animal from the xiphoid cartilage to the pubis and place a loose tie around the caudal vena cava near the liver. A 21-gauge butterfly needle is inserted into the portal vein and secured. As soon as the perfusion is started the loose loop around the caudal vena cava is tightened and the vein is cut caudal to the ligature to allow the perfusate to drain freely. The right atrium is cut with a scissor for further draining of the perfusate ([Silverman, 1987](#)).

A second perfusion method can be used in preparation for isolated liver perfusion. After a large midline incision is made on the anesthetized animal, the bile duct (*ductus cysticus*) is tied off. The common bile duct (*ductus choledochus*) is cannulated with PE-10 polyethylene tubing and a 21-gauge angiocatheter is placed into the portal vein. The liver is flushed with oxygenated Krebs Ringer bicarbonate and the caudal vena cava is cut. An angiocatheter is placed into the right cranial vena cava by entering it through the right atrium after a thoracotomy. The caudal vena cava is ligated cranial to the right kidney. The isolated liver is removed and placed in a perfusion chamber ([Cheng et al., 1996](#)).

Orchiectomy

Hamsters can be castrated using a closed or open technique. In a closed castration, which is preferred as a consequence of the open inguinal canal of hamsters, a single midline incision is made just anterior to the testicles. After the skin and subcutaneous tissue are cut, one testicle is exteriorized through the incision and a 4-0 ligature is passed around its spermatic cord and tunics. The ligature can be transfixed through the cremaster muscle for extra fixation. Once ligated, the spermatic cord is transected distal to the ligature and removed. The second testicle is then exteriorized through the same midline incision and the process is repeated. The tunics and subcutaneous tissue are sutured closed. The skin is then closed with appropriate sutures, skin staples, or surgical adhesive.

Our method for an open castration is to prepare the area for aseptic surgery and then incise the skin and subcutaneous tissue over the testicle. The incision is continued through the spermatic cord fascia and parietal vaginal tunic. The ligament of the tail of the epididymis is stretched or separated to exteriorize the testicle. The vascular cord and ductus deferens are individually ligated and the tunic closed with sutures. The testicle is removed distal to the ligature and the process is repeated for the second testicle.

Ovariectomy and Ovariohysterectomy

After anesthesia and surgical site preparation the hamster is placed in dorsal recumbency and a 1.0–1.5-cm midline incision is made between the umbilicus and pubis. The ovaries, which are found in a large fat pad just caudal to the kidneys, are easily exteriorized, ligated, and removed. If an ovariohysterectomy is required, one of the two fat-surrounded uterine horns is exteriorized and its blood vessels are ligated if required. The horn is then double-ligated and transected just cranial to the cervix, leaving the ligated stump in the abdomen. The same procedure is followed for the other horn, and the skin and subcutaneous tissues are then closed in a routine manner. The surgeon may also choose to transect both uterine horns at the same time by ligation of the uterus just cranial to the cervix, rather than ligating each horn separately. (The hamster has a separate cervix for each uterine horn but during ovariohysterectomy they can be ligated together as if there was a single cervix.)

Alternatively, for an ovariectomy, a dorsal approach can be used. The animal is placed in ventral recumbency and the dorsum is shaved and prepped. A 1–2-cm, midline dorsal skin incision is made approximately half way between the middle of the back and the base of the tail. The skin incision is retracted to one side, allowing for an incision to be made through the muscle belly posterior and parallel to the last rib and approximately half way down the side of the body (~3mm ventral to the spinal epaxial muscles). The ovary on that side can then be pulled out through the incision by grasping the periovarian fat. Care should be used not to grasp the ovary, as small pieces may detach and remain active in the peritoneal cavity. The junction of the Fallopian tube and the uterine horn is located and the distal end of the Fallopian tube and proximal end of the uterus are crushed with mosquito forceps. The ovarian vessels may be ligated, cauterized, or transected along with the Fallopian tube (just proximal to the crushed portion), allowing for complete removal of the ovary. If the muscle incision is small enough (e.g., ~0.5cm or less), no sutures are required, or, if necessary, a single suture can be used. The process is repeated on the other side and the skin incision closed.

Vomeronasal (VM) Organ Removal

Mating in naïve male Syrian golden hamsters requires sensory input from either the main olfactory or VM systems. To surgically remove all or most of the VM organs a midline incision is made through the anterior part of the hard palate of the anesthetized hamster. This exposes the bony VN capsules on either side of the midline. The natural openings in the palatine bones are

extended (i.e., the palatine fissures) anteriorly with a dental drill. A U-shaped groove is then made with the drill, forward and across the midline, to connect the two openings. A forceps is used to break the medial palatine process of the maxillary bones in order to disconnect the VN capsules at the posterior end. The VN capsules are separated by pressing the midline bony suture with a scalpel. The last connection with the palate is broken by drilling anterior to their rostral connection. Using a small forceps each capsule, with one VN organ, is removed separately. The incision is closed with sutures and sealed with tissue glue (Westberry and Meredith, 2003).

Olfactory Bulbectomy

In Syrian golden hamsters the olfactory bulbs (bulbus olfactorius) provide tonic inhibition of the hypothalamic–pituitary–gonadal (HPG) neurosecretory system (Pieper and Newman, 1999). This is in contrast to the Siberian hamster (*Phodopus sungorus*) where the bulbs provide little functional input to the HPG axis (Prendergast et al., 2009). Surgical removal of the bulbs is accomplished by immobilizing the anesthetized animal's head in a stereotaxic holder and making a 1-cm longitudinal midline skin incision in the frontal bone near the caudal extent of the nasal bones (approximately behind the position of the second molar tooth). The skin is retracted as needed and a hole is made in the anterior portion of the left and right frontal bones using a 0.4-mm diameter stainless steel burr. A 200- μ l pipette is inserted into each burr hold in an anterior–ventral direction. The pipette tip is connected to a vacuum pump with a water trap and the olfactory bulb is aspirated from each hole, being careful not to disturb the superior sagittal sinus. The resulting bone defect can be filled with oxidized cellulose if needed to control bleeding. The skin incision is then closed. The bulbectomy procedure removes the main olfactory bulb, the accessory olfactory bulb, and the *nervus terminalis* (Pieper et al., 1994).

Pinealectomy

Syrian hamsters are seasonal breeders and the effects of the exposure to a short photoperiod on their reproductive organs can be prevented by pinealectomy (Juszczak et al., 1997). Anesthetized hamsters can be handheld but it is recommended to use a stereotaxic holder to stabilize the animal's head. The method of Gündüz et al. (2008) is suggested. After anesthesia and surgical preparation a longitudinal midline scalp incision is made between the ears and eyes. The periosteum and any overlying muscle insertions are scraped away. Using a dental burr drill, an incomplete-depth circular cut (~5mm diameter) is made where the readily visible transverse and superior sagittal

sinuses meet. Fine scissors are used to carefully remove somewhat more than one-half of the posterior part of the circle. The piece of cranium covering the pineal gland is then lifted and folded anteriorly. If still adherent, the dura mater is removed with fine scissors. Using fine-tipped forceps, the pineal gland is grasped and removed. The bone flap is then replaced, using a small amount of absorbable gelatin sponge to help control bleeding. The scalp incision is closed routinely. A modification of this technique involves double ligating the superior sagittal sinus using a curved atraumatic needle, well anterior (rostral) to the confluence of the superior sagittal and transverse sinuses. This helps control bleeding and allows for easier removal of the gland at the confluence of the sinuses (Senoglu et al., 2009). Analgesia should be used postoperatively.

Hypophysectomy

Hypophysectomy requires strict attention to technique as the operator is working close to the jugular veins and numerous nerves. The parapharyngeal technique is the method most often used in hamsters and the surgical procedure noted herein is that previously described by Silverman (1987). The anesthetized animal is placed in dorsal recumbency with the animal's tail toward the operator. An endotracheal tube is not usually used. The animal's body, and particularly its head, must be well secured so that it does not move during surgery. A 1.5-cm incision begins in the mid-cervical area and extends anteriorly almost to the mandibular symphysis. The skin and mandibular salivary glands are retracted, exposing the trachea, sternohyoid, and omohyoid muscles. Using two 4.5-inch fine forceps, the muscles are separated and then retracted. The trachea is carefully retracted, but only when needed to obtain better visualization of the underlying cranium. A dental scraper is used to remove about a 1-cm circle of periosteum from the ventral cranium immediately dorsal to the trachea. This will reveal a faint blue suture line between the basisphenoid and occipital bones, which will be the area for subsequent trephining. The bone can now be removed by first gently scoring with a small trephine and then using a second trephine to remove the bone or by using a dental drill with a burr. The pink-colored gland is now visible and it can be sucked out using a 16–18-gauge needle (with a blunted end) and 10lb of vacuum pressure. Any bleeding is controlled with cotton-tipped or oxidized cellulose swabs. The pars distalis of the hamster pituitary extends forward and laterally making it somewhat difficult to fully remove and the gland often separates into numerous pieces during removal (Knigge, 1954). Postsurgical analgesics are routinely provided and a 10% glucose solution, given orally, will help prevent hypoglycemia.



FIGURE 29.7 Manual eversion of the hamster cheek pouch.

A detailed description of parapharyngeal hypophysectomy in the mouse is available (Hoff et al., 2006).

Cheek Pouch Preparations

The everted hamster cheek pouch can be used to study microcirculation and human tissue grafts. The thin pouch is very vascular and easy to transilluminate. The pouch has been called an immunologically privileged site, based on a deficiency of an afferent pathway to drain antigenic material to a lymphoid area which would recognize it and initiate an immune response (Hochman et al., 2004). The hamster is anesthetized and any food or bedding in the cheek pouch is washed out. The pouch can be everted manually by pushing from the outside of the pouch (Figure 29.7) or by using Adson-Brown tissue forceps to grasp the tissue deep in the pouch. The forceps should be kept closed while being inserted. When the forceps reaches the end of the pouch the pouch is grasped and slowly everted (Handler and Shepro, 1968).

To implant tissue, a small incision is made with fine scissors in an avascular area of the pouch, cutting through the epithelium. The cut is initially made near the buccal end of the pouch, carefully avoiding the retractor muscle fibers. (The retractor muscle originates from the lumbodorsal fascia and the spinous processes of the last three thoracic vertebrae. It then passes ventrocranially to insert on the cheek pouch. It lies just below the panniculus carnosus and platysma muscles and serves to prevent accidental eversion of the cheek pouch (Priddy and Brodie, 1948) and increases pouching efficiency for food items subsequent to the first item (Buckley et al., 2007).) Beginning at the cut end a tunnel is undermined in the pouch, in the aboral direction, taking care to avoid blood

vessels. The tissue fragment is placed in the aboral end of the tunnel and the epithelial borders of the incision are united by simple digital compression. The everted pouch is invaginated back into the oral cavity (Hochman et al., 2004).

To observe cheek pouch microcirculation, a number of techniques are available as acute preparations. The most basic is to evert the cheek pouch as described above and then pin the pouch over an acrylic or similar transparent support. A longitudinal cut is then made parallel to the saccular arteries and a transverse cut is made along the aboral border. This allows the pouch to be viewed as a single-layer preparation (Duling, 1973). The evaginated pouch is kept moist (e.g., using Ringer's solution). A non-acute (i.e., survival) preparation can be made using the same technique, but the cheek pouch epithelium is not cut (a so-called double-layered membrane preparation). This has the disadvantage of poor optical resolution when used for certain purposes and the operator should consider using analgesia after the procedure.

Although the above methods are satisfactory for many purposes, they put stress on the retractor muscle, which in turn may affect the circulation of the pouch. To avoid this problem, other researchers have developed methods to cut through the skin of the hamster's cheek and neck, ligate and cut the retractor muscle, and withdraw the pouch through the skin incision (Yamaki and Kritchman, 1981). Modifications of the skin incision method, using glass support and viewing chambers have been described (Davis and Gore, 1985; Greenblatt et al., 1969).

Yet another option for the chronic observation of vascular patterns of implanted tumors in cheek pouches is the use of a tissue chamber. The inner part of an acrylic disk is placed as far inside the pouch as possible. The skin, subcutaneous tissue, and muscle are removed and the two parts are joined by nylon rivets which pass through the pouch. Tumor tissue is inoculated at the time of insertion of the chamber and the operator uses microscopy to examine the growth of tumor and vasculature (Handler and Shepro, 1968).

Cheek pouch "painting" is the topical application of a carcinogen (or other material) to the epithelial surface of the everted cheek pouch. The entire procedure (including eversion of the pouch) can be performed without anesthetizing the animal, as described by Haisley (1980).

Dorsal Skin Fold Preparation

The dorsal skin fold preparation is another way of viewing the hamster's microcirculation and is conceptually similar to the tissue chamber described above. The major advantage is that microcirculatory studies can be performed repeatedly in unanesthetized animals

over a period of 3–4 weeks (Menger et al., 2002). The advantage of using the hamster over rats or mice is that in hamsters the retractor muscle is only loosely attached to the underlying panniculus carnosus muscle. This allows easy removal of the retractor muscle and aids later microscopic analysis. To prepare a dorsal skin fold, the skin of the back, just caudal to the neck of the anesthetized hamster, is lifted upwards (tenting) and maintained in that position with stay sutures. Using a stereomicroscope a 15-mm circular area of skin, subcutis (with the panniculus carnosus), and retractor muscle are completely removed. This is essentially a full-skin-thickness removal. Next, remove the retractor muscle from the inner surface of the opposite side of the tented skin. This leaves the subcutis and panniculus carnosus of the opposite side available for study of the microcirculation. The tented skin is then sandwiched between two titanium plates (which have a hole or "window" to observe the microcirculation) and the plates are joined with bolts. A removable cover glass is positioned in the titanium window to provide access to the tissue under investigation. Details of the procedure have been published (Menger et al., 2002).

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Bacterial and Fungal Diseases

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INTRODUCTION

The incidence of bacterial diseases in laboratory hamsters has declined in recent decades. This decrease is primarily due to improved husbandry activities including more frequent and effective sanitation practices, and the use of microisolation and barrier technologies. A reduction in occurrences of bacterial diseases is also associated with the reduced use of hamsters as a laboratory animal species. However, bacterial diseases of hamsters remain significant because many of these diseases have the potential for high morbidity, high mortality, zoonosis, and economic loss. Conversely, mycotic infections are extremely rare and are poorly documented in the scientific literature.

BACTERIAL DISEASES

Proliferative Ileitis

Background

Proliferative ileitis is a serious infectious disease of hamsters and usually results in high morbidity and mortality. The disease is characterized clinically by diarrhea in weanling hamsters and pathologically by segmental proliferative lesions of the epithelium of the terminal ileum. The disease, proliferative ileitis, has also been referred to as regional enteritis, terminal ileitis, enzootic intestinal adenocarcinoma, atypical ileal hyperplasia, hamster enteritis, and "wet-tail". All of the above synonyms describe the same disease syndrome, which will be referred to as proliferative ileitis in this chapter. The term "wet-tail" has been used, especially in the pet industry, to denote this disease. However, this descriptive clinical appearance can be caused by several conditions that may cause diarrhea; and, thus, the term's use can result in confusion. The literature on proliferative ileitis has been reviewed by several authors (Cooper and Gebhart, 1998; Frisk, 1987; Frisk

and Wagner, 1977b; Jacoby and Johnson, 1981; Lawson and Gebhart, 2000).

The first major outbreak of proliferative ileitis recorded in the literature was in 1958 (Friedman, 1965). The naturally occurring disease in hamsters has only been reported from North America (Jasni et al., 1994b). The search for the etiologic agent of proliferative ileitis of hamsters has included many diagnostic and experimental studies. In the past, speculation as to the cause of the characteristic hyperplastic lesions included bacteria (Amend et al., 1976; Booth and Cheville, 1967; Friedman, 1965; Frisk et al., 1981; Jacoby and Johnson, 1981; Jacoby et al., 1975; La Regina and Lonigro, 1982; Lentsch et al., 1982; Wagner et al., 1973), viruses (Tomita and Jonas, 1968), and nutrition (Goldman et al., 1972). A review of the early studies regarding the quest for the causative agent was done by Frisk (1987). Since the 1980s, research regarding proliferative ileitis was focused almost exclusively on identification of the intracytoplasmic organism observed within the affected ileal epithelial cells. Identification of this organism was made very difficult because of its inability to be propagated on artificial media. This organism was ultimately characterized and named *Lawsonia intracellularis* (McOrist et al., 1995).

Several intestinal organisms have been shown to act as opportunists in hamsters with proliferative ileitis including *Campylobacter jejuni* (Lentsch et al., 1982), enteropathogenic *Escherichia coli* (Frisk et al., 1978), *Chlamydia trachomatis* (Fox et al., 1993), *Cryptosporidium* sp. (Davis and Jenkins, 1986), and protozoan parasites (Booth and Cheville, 1967; Jackson and Wagner, 1970; Sheffield and Beveridge, 1962). The above-listed bacterial and parasitic agents are not consistently present in all cases of proliferative ileitis, and may be observed or isolated from hamsters without proliferative lesions. Also, typical lesions of proliferative ileitis can be experimentally produced without the presence of these agents (Amend et al., 1976; Johnson and Jacoby, 1978; Stills, 1991).

Etiology

In 1995, *Lawsonia* was established as a new genus through 16S ribosomal genetic sequence analysis (McOrist et al., 1995). It was shown to be genetically similar to but distinct from *Desulfovibrio* (Fox et al., 1994; Smith and Lawson, 2001). The bacterium was classified in the Proteobacteria subdivision and was named *L. intracellularis* (McOrist et al., 1995). Isolates from proliferative lesions show close 16S ribosomal genetic sequence similarities between pigs and several different species including hamsters (Cooper et al., 1997a, 1997b; Peace et al., 1994).

L. intracellularis is a Gram-negative, non-spore-forming, slightly curved rod (1.5 by 0.35 μm). Organisms within ileal epithelial cells are usually not membrane bound, being observed free in the cytoplasm. *L. intracellularis* are observed in the apical cytoplasm just beneath the terminal web. These bacteria can not be grown on artificial media or in embryonated eggs. *L. intracellularis* has been isolated and propagated in cell culture on rat enterocyte and human fetal Intestine 407 cell lines in a microaerophilic environment (Smith and Lawson, 2001; Stills, 1991).

L. intracellularis is an obligate intracellular bacterium which is the causative agent of the proliferative enteropathies that occur in pigs, hamsters, ferrets, horses, deer, ostriches, and rabbits (Cooper et al., 1997a, 1997b; Fox et al., 1994; Lawson and Gebhart, 2000). The organisms isolated from many of these species have been compared using 16S ribosomal gene sequencing. Pure cultures of *L. intracellularis* isolated from hamsters have fulfilled Koch's postulates having been shown to cause proliferative ileitis in hamsters (Stills, 1991). Oral inoculation of pure cultures of *L. intracellularis* isolated from proliferative lesions of pigs can cause proliferative ileitis in weanling hamsters (Jasni et al., 1994a). This is suggestive that the organisms from these disparate species are the same or at least very closely related.

Epizootiology

Proliferative ileitis is a contagious disease, which is caused by *L. intracellularis*. The disease is transmitted by the fecal-oral route. Cannibalism of affected hamsters by cage mates may contribute to spreading the disease (Jacoby and Johnson, 1981).

Although the incidence of proliferative ileitis has decreased (Cooper and Gebhart, 1998), high morbidity and mortality in colony outbreaks makes proliferative ileitis one of the most feared diseases of commercial hamster breeders. It can complicate research projects by causing clinical signs and death in hamsters on experimentation. Increased severity and development of the disease have been associated with overcrowding, transport, surgery, limited diets, purified diets (Decker and Henderson, 1959), transplantation of neoplasms

(Lussier and Pavilanis, 1969), and experimental visceral leishmaniasis (Frenkel, 1972).

A proliferative ileitis outbreak is typically characterized by variable morbidity (20–60%) and high mortality (up to 90%) (Boothe and Cheville, 1967; Friedman, 1965; Goldman et al., 1972; Jonas et al., 1965). In affected hamster colonies, proliferative ileitis is first manifested as an epizootic disease (Friedman, 1965; Jackson and Wagner, 1970). Usually only a few hamsters in a disease outbreak recover; and, in some instances, no survivors have been reported (Jonas et al., 1965). Some animals may initially recover, but later succumb to ileal obstruction associated with chronic scarring of the ileum (Boothe and Cheville, 1967; Jacoby et al., 1975).

Hamsters affected with proliferative ileitis are usually between 3 and 8 weeks of age, but cases in adults have been reported (Jonas et al., 1965; Trum and Routledge, 1967). In experimental transmission studies, hamsters were susceptible to infection by oral inoculation of ileal homogenates up to 9 weeks of age (Frisk, 1987). Jacoby and Johnson (1981) showed that susceptibility to experimentally induced disease is age-related. Hamsters were less susceptible beginning at 6 weeks of age and resistant at 10 weeks.

Clinical Manifestations

Hamsters usually die 24–48 hours after clinical signs of proliferative ileitis are noted, and deaths may occur in animals without premonitory signs. Early signs include lethargy, anorexia, irritability, ruffled hair coat, and rapid weight loss. As the disease progresses, there is the appearance of a fetid, watery diarrhea which causes moist matted fur on the perineum, tail, and ventral abdomen (Figure 30.1). Signs associated with the diarrhea include dehydration, inactivity, and a hunched back which has been proposed to be indicative of

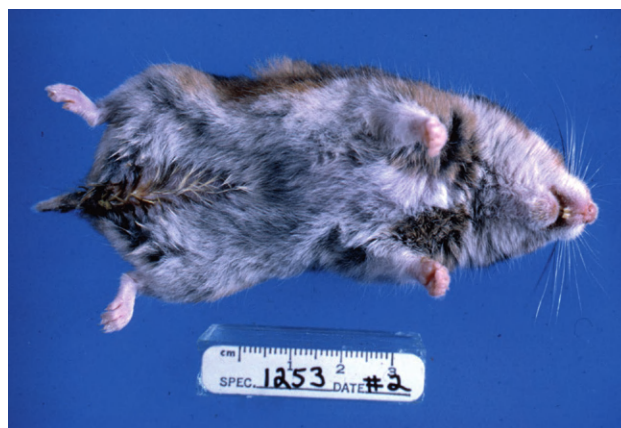


FIGURE 30.1 Weanling hamster with clinical signs of proliferative ileitis ("wet-tail") including matting of fur with feces in the perianal and posterior ventral abdominal areas.



FIGURE 30.2 The dissected gastrointestinal system (esophagus to rectum) of a hamster with proliferative ileitis demonstrating significant enlargement of the ileum.

abdominal pain (Friedman, 1965). A severe drop in body temperature, abdominal distention, and convulsions can occur just prior to death. Prolapse of the rectum or more commonly, intussusceptions with extension through the anus can be observed. Fresh blood around the anal region usually indicates an internal intussusception, the prognosis being grave.

Jacoby et al. (1975) closely observed hamsters after experimental transmission of proliferative ileitis and divided clinical signs into acute, subacute, and chronic. Acute signs occurred in 10% of hamsters 7–10 days after inoculation, the primary sign being hemorrhagic diarrhea. Subacute signs of retarded growth and diarrhea appeared 21–30 days after transmission. The chronic disease did not produce clinical signs, these animals showing normal growth rates.

Pathology

GROSS LESIONS

Gross lesions described with proliferative ileitis vary from acute enteritis to chronic inflammation with fibrosis. Acute enteritis lesions include hyperemia of the ileum which contains a fetid, yellow-gray fluid, blood, and/or gas (Boothe and Cheville, 1967; Sheffield and Beveridge, 1962). Sheffield and Beveridge (1962) reported that the mucosal surface of the cecum was sometimes ulcerated.

Segmental proliferative ileal lesions are observed in outbreaks of the disease. Typically, the posterior 4–8 cm of small intestine is progressively thickened, which abruptly terminates at the ileocecal junction (Figure 30.2). Occasionally, the proximal colon or cecum can be involved (McOrist et al., 1989). Affected ileal walls are 4–6 times thicker than normal, turgid, edematous, and friable (Figure 30.3). White patches of necrotic mucosa are commonly observed. Ileal serosa appears hyperemic,



FIGURE 30.3 Abdominal viscera of a male hamster with proliferative ileitis showing a markedly enlarged ileum.

rough, or granular. Subserosal abscesses are occasionally present, which can rupture causing focal or generalized peritonitis (Boothe and Cheville, 1967; Sheffield and Beveridge, 1962). There may be focal fibrinous lesions between affected intestinal segments and adjacent intestines, mesentery, or peritoneum. Intussusceptions of the ileum are observed, some of which extend into the cecum, colon, and at times through the anus. Intussusceptions of the colon and prolapses of the rectum have also been described. Chronic ileal lesions are characterized by a circumferential fibrotic scar which may cause partial or complete obstruction.

Peyer's patches and mesenteric lymph nodes of hamsters with proliferative ileitis are typically enlarged two to three times, hyperemic, and edematous.

MICROSCOPIC LESIONS

Microscopic lesions have been classified into the categories of acute enteritis, proliferative ileitis, proliferative ileitis with epithelium in muscle layers, and chronic ileitis (Frisk and Wagner, 1977b). Lesions of acute enteritis range from mild catarrhal enteritis to severe diffuse hemorrhagic necrosis of ileal mucosa. Proliferation of absorptive epithelial cells is not observed in cases with acute lesions (Frisk and Wagner, 1977b).

Proliferative lesions, which are characteristic of the disease, typically show ileal villi that are wider and longer than normal which may eventually fuse (Figure 30.4). There is usually a distinct demarcation between the affected ileum and a normal-appearing cecum. In studies involving the experimental production of the ileal lesions, epithelial cell proliferation is first observed in crypt epithelium (Frisk and Wagner, 1977a; Jacoby, 1978). "Focal proliferation" (Jackson and Wagner, 1970) is characterized by individual crypts which show proliferative changes among normal-appearing crypts.

Hyperplasia first occurs with the movement of crypt epithelium onto the villus within 10 days after

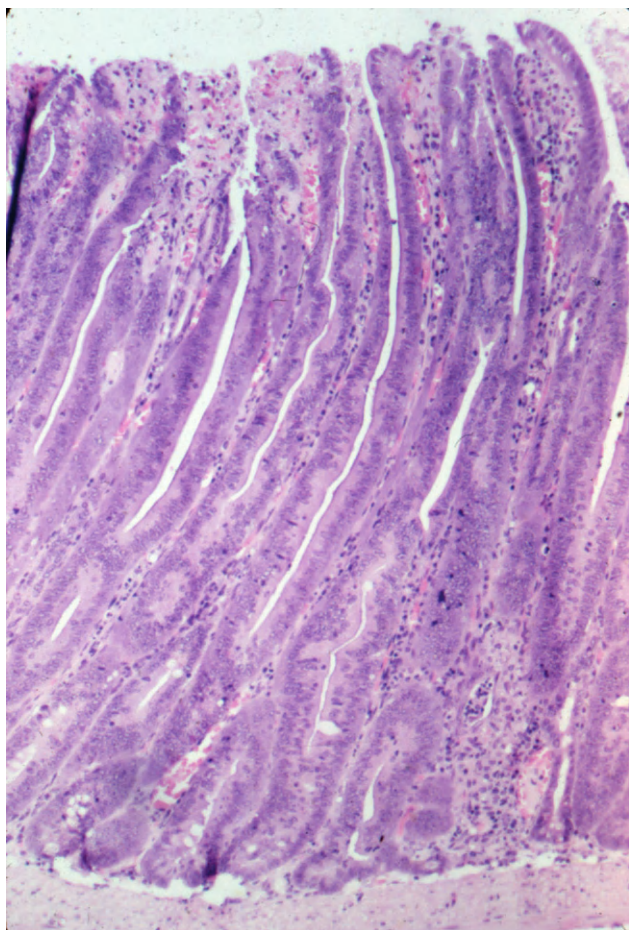


FIGURE 30.4 Microscopic section of ileum from a hamster with proliferative ileitis showing hyperplastic epithelium covering elongated villi. Hematoxylin-eosin stain. Magnification: 3130.

experimental infection (Jacoby, 1978). These hyperplastic epithelial cells are enlarged two to four times and contain large hyperchromatic nuclei with prominent nucleoli (Jonas et al., 1965). Their cytoplasm appears more basophilic than unaffected ileal epithelial cells. Absorptive epithelium is markedly thickened and pseudostratified beginning near the villar base. Immature epithelium ascends to eventually replace normal mature villar epithelium (Jacoby, 1978). Increased numbers of mitoses are observed within crypts, and mitotic figures are also observed on affected villi. The number of epithelial cells from the crypt to the villar tip is increased two to three times 3–4 weeks after infection (Jacoby, 1978). There is total absence of goblet cells.

Silver stains (Warthin-Starry) reveal myriads of curved bacteria in the apical portion of hyperplastic ileal epithelial cells (Jasni et al., 1994b; Stills, 1991) (Figure 30.5). Intracytoplasmic inclusions within epithelial cells have been described (Frisk and Wagner, 1977a; Jacoby, 1978; Jonas et al., 1965); however, this finding is not consistent.

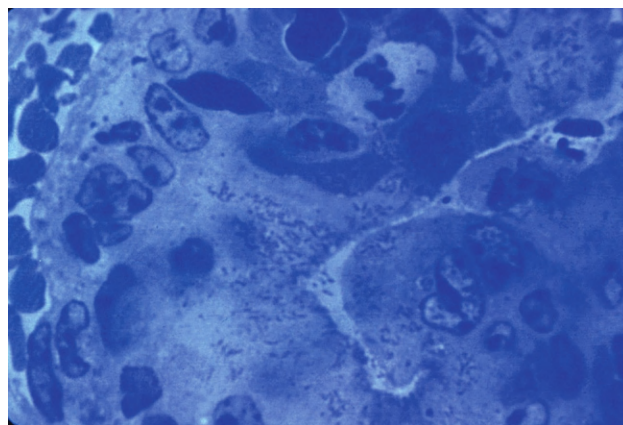


FIGURE 30.5 Hyperplastic ileal epithelial cells from a hamster with proliferative ileitis showing *Lawsonia intracellularis* organisms in the apical portion of epithelial cells. Toluidene Blue stain. Magnification: 31000.

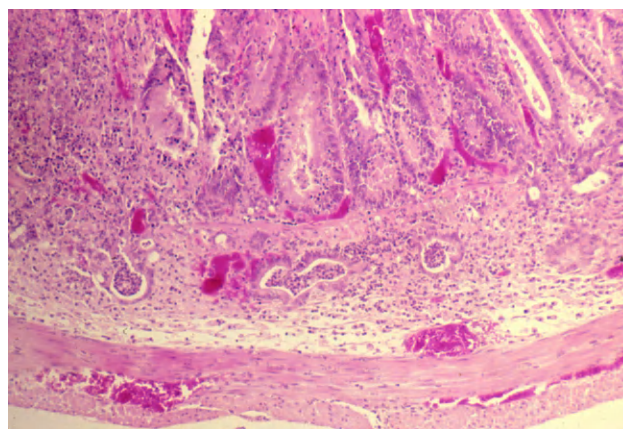


FIGURE 30.6 Light micrograph of an ileum from a hamster with proliferative ileitis demonstrating inflammation within the lamina propria and extension of crypt epithelium through the muscularis mucosa. Hematoxylin-eosin stain. Magnification: 3100.

Necrosis of villi and infiltration of the lamina propria by neutrophils, lymphocytes, and macrophages are common features. This inflammation becomes pyogranulomatous as the lesions progress. Subserosal abscesses may develop, which may communicate with the lumen via crypts. Hyperplastic epithelial cells may penetrate the muscularis mucosa and muscle layers and extend into inflamed tissue to produce pseudodiverticula (Figure 30.6). This type of lesion led Jonas et al. (1965) to classify the disease as neoplastic rather than inflammatory describing this change as “carcinoma in situ.” Jacoby and Johnson (1981) critically interpreted this “intrusive behavior”, and felt it was due to mechanical pressures from proliferation and was not invasion of a neoplastic epithelium.

Chronic lesions are characterized by marked thickening and hypertrophy of muscle layers, which may be

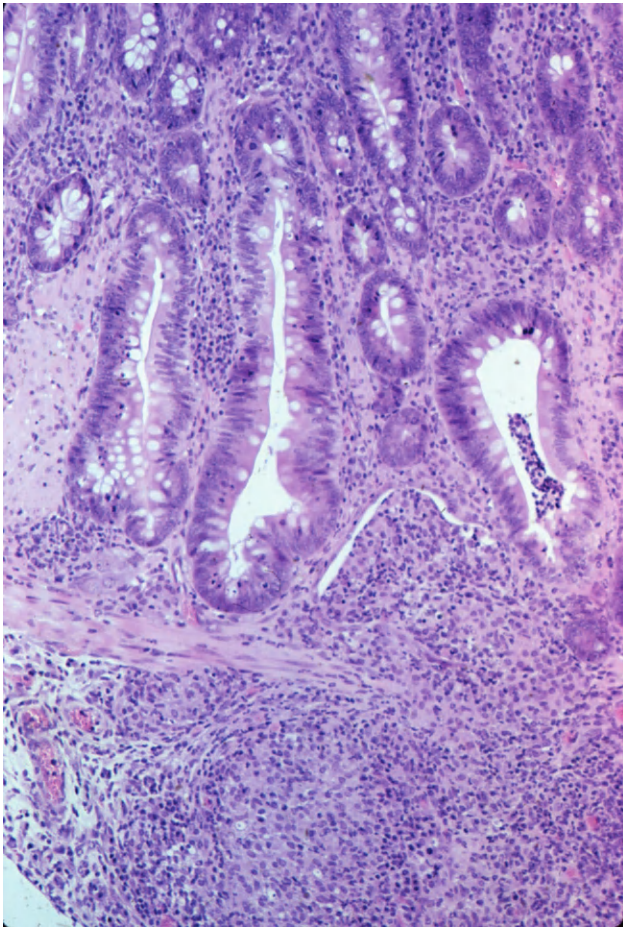


FIGURE 30.7 Microscopic section of ileum from a hamster with lesions of proliferative ileitis that demonstrate chronicity including increased numbers of goblet cells in crypts and granulomatous inflammation extending from the lamina propria into smooth muscle layers. Hematoxylin-eosin stain. Magnification: 3130.

secondary to partial luminal obstruction (Boothe and Cheville, 1967). Fibrosis of the ileocecal valve may lead to stenosis which may cause fatal obstruction (Jacoby et al., 1975). Scattered granulomas and epithelial-lined diverticula are present in the submucosa and subserosa. The ileal epithelium consists of large numbers of goblet cells (Figure 30.7).

Mesenteric lymph nodes can show reticuloendothelial hyperplasia, edema, granulomatous inflammation, and focal necrosis. Epithelial cells have not been observed within lymph nodes. Focal lesions of hepatitis are occasionally observed (Frisk and Wagner, 1977b).

TRANSMISSION ELECTRON MICROSCOPY

L. intracellularis have been described within the cytoplasm of hyperplastic ileal epithelial cells in natural and experimental cases of proliferative ileitis (Frisk and Wagner, 1977a; Jasni et al., 1994b; Johnson and Jacoby, 1978; Wagner et al., 1973). Cells containing organisms



FIGURE 30.8 Electron micrograph from a hamster with proliferative ileitis showing many profiles of *Lawsonia intracellularis* in the apical portion of the cytoplasm of ileal epithelial cells.

have been observed as early as 5 days after experimental inoculation of hamsters (Frisk and Wagner, 1977a; Johnson and Jacoby, 1978). Intracellular organisms are slightly curved, rod-shaped, measure $0.3\text{--}0.4 \times 1\text{--}2\mu\text{m}$ and divide by binary fission (Frisk and Wagner, 1977a; McOrist et al., 1989). Organisms are most prominent in the apical portion of hyperplastic cells (Figure 30.8) and are more numerous in epithelial cells that are further out on the villus (Johnson and Jacoby, 1978). *L. intracellularis* are usually free within the cytoplasm, however, occasionally they are observed bounded by lysosomal membranes (Frisk and Wagner, 1977a; Jasni et al., 1994b). Clusters of organisms observed by electron microscopy within the cytoplasm of hyperplastic epithelial cells could account for the occasional observation by light microscopy of intracytoplasmic inclusion bodies. Degenerative or toxic changes of the invaded cell are not observed. *L. intracellularis*, in fact, appear to have a cell-stimulating effect on infected cells, being reported primarily within hyperplastic cells and within cells in normal stages of mitosis (Frisk and Wagner, 1977a; Johnson and Jacoby, 1978).

Hyperplasia of ileal epithelial cells is observed by 10 days after experimental inoculation (Johnson and Jacoby, 1978). Hyperplastic cells resemble undifferentiated crypt cells with microvilli that are much shorter, less regularly arranged, and less densely packed than

mature villus epithelial cells (Kim and Jourden, 1977; Smith and Lawson, 2001). There may be focal necrosis of crypt epithelial cells and penetration of the muscularis mucosa as evidenced by bacteria between myofibers. Macrophages and leukocytes infiltrate crypts, lamina propria, submucosa, and adjacent muscle layers.

In chronic cases, goblet cells appear which do not contain *L. intracellularis*. Absorptive epithelial cells contain increased numbers of lysosomes and large membrane-bound vacuoles containing degenerative organisms (Frisk and Wagner, 1977a).

SCANNING ELECTRON MICROSCOPY

By scanning electron microscopy, Johnson and Jacoby (1978) noted that villi from hamsters with proliferative ileitis were elongated, broader, and flatter than normal. The crypt-villus junction was elevated above the basal lamina. In fractured blocks examined with the scanning electron microscope, crypts were observed to be four to five times normal length with only rudimentary villi at the surface. Villi sometimes appeared leaf-like, were fused into plateaus and the surface obscured by bacteria and cellular debris. Microvilli were sparse on villi covered by hyperplastic cells.

Pathogenesis

Although the causative agent of proliferative ileitis has been determined to be *L. intracellularis*, the detailed pathogenesis of the disease processes has not been completely elucidated. The literature regarding the pathogenesis of proliferative enteropathies in several species including hamsters associated with *L. intracellularis* has been reviewed (Smith and Lawson, 2001).

The pathogenesis of proliferative ileitis in hamsters has been studied by experimentally reproducing the disease by orally inoculating weanling hamsters with either pure cultures of *L. intracellularis* grown in cell culture (Jasni et al., 1994a, 1994b; Stills, 1991; Yeh et al., 2006) or with ground ileal lesions (Amend et al., 1976; Frisk and Wagner, 1977a; Jacoby, 1978; Jacoby et al., 1975; Johnson and Jacoby, 1978; Stills and Hook, 1989).

Proliferation, which is the hallmark of this disease, has been shown to be associated with the intracytoplasmic organisms identified as *L. intracellularis* (Frisk and Wagner, 1977a; Jasni et al., 1994; Johnson and Jacoby, 1978). Jacoby et al. (1975) showed by indirect immunofluorescence that experimentally infected hamsters produced antibodies to this intracytoplasmic organism. Antibody was detected by 10 days after infection (Jacoby, 1978). Attempts at protecting hamsters by passive immunization with hyperimmune serum failed (Jacoby and Johnson, 1981).

L. intracellularis enter the enterocytes of the ileum from the lumen. The mechanism of entry into the cell has been described (Jasni et al., 1994b). Organisms enter ileal

epithelial cells via membrane-bound endocytic vacuoles at the brush border (Jasni et al., 1994b). Once organisms are within the cell, vacuoles immediately breakdown, which leaves *Lawsonia* free within the cytoplasm.

Once established within the cell, *L. intracellularis* do not appear to have a degenerative effect on infected cells (Frisk and Wagner, 1977a; Jasni et al., 1994b; Johnson and Jacoby, 1978; Wagner et al., 1994). The onset of hyperplasia is closely associated with the intracellular presence of *L. intracellularis*. Organisms can be observed within normal-appearing mitotic cells (Frisk and Wagner, 1977a; Johnson and Jacoby, 1978). The physiological interaction between *L. intracellularis* and the enterocyte to cause hyperplastic lesions remains to be discovered. A synergism between normal intestinal microflora and *L. intracellularis* may be necessary to produce epithelial hyperplasia (Cooper and Gebhart, 1998).

Proliferative ileitis of hamsters has similarities to porcine proliferative enteropathy. Cross species transmission has been demonstrated with hamsters being susceptible to the pig agent (McOrist and Lawson, 1987). *L. intracellularis* has been identified as the causal agent of both diseases. The clinical and epizootiological aspects of swine with proliferative enteritis are very similar to hamsters with proliferative ileitis. The gross, microscopic, and electron microscopic changes observed in swine with proliferative enteritis are identical to those in hamsters with proliferative ileitis (Lomas and Glock, 1982; McOrist and Lawson, 1987; Rowland and Lawson, 1974, 1975). Because the same etiological agent can cause proliferative lesions, the hamster and swine diseases most likely have a very similar pathogenesis, and thus data and information learned about the disease in one species may be helpful in understanding the disease in the other.

L. intracellularis is not believed to be infectious for humans. The experimental production of proliferative ileitis of hamsters could be exploited as a model for studying the regulatory mechanism of mucosal proliferation (Jacoby and Johnson, 1981). Crypt hyperplasia occurs in man as a response to mucosal injury, such as cytolytic agents, radiation injury, and tropical and celiac sprue. The mechanism by which crypt hyperplasia is produced has been proposed to be through a feedback regulatory system, and this particular hamster lesion may give investigators valuable insights into this system (Jacoby and Johnson, 1981).

Diagnosis

Proliferative ileitis should always be suspected with the observance of clinical signs of diarrhea in young hamsters. Diarrhea may occur with other diseases such as salmonellosis, hymenolepiasis, Tyzzer's disease, or colonic hyperplasia. Their exclusion is primarily based

on gross and microscopic lesions. The proliferative changes involving the ileum, so typically observed, are pathognomonic for the disease. With Warthin-Starry silver stains, diagnosis can be confirmed by observing intracytoplasmic organisms within ileal epithelium. PCR assays for *L. intracellularis* are available commercially. Palpable, ropelike intra-abdominal masses can be detected 2 weeks post exposure, prior to the observation of clinical signs at 3 weeks (Jacoby et al., 1975).

Prevention and Therapy

Antibiotics administered orally have been the most commonly recommended treatment for proliferative ileitis but are only moderately successful. Tetracycline hydrochloride (400 mg/liter drinking water) was found to be most effective in reducing the numbers of hamsters contracting proliferative ileitis after experimental inoculation (La Regina et al., 1980). Dimetridazole (500 mg/liter drinking water) was much less effective than tetracycline, and neomycin (125 mg/liter drinking water or 10 mg/hamster) was considered ineffective when compared to untreated controls. Jacoby and Johnson (1981) reported that metronidazole (2 mg/ml drinking water) was nearly as effective as tetracycline, and neomycin was ineffective at a dose rate of 2000 mg/liter of drinking water.

Strict quarantine of affected hamsters and good sanitation practices are useful in controlling proliferative ileitis. Amend et al. (1976) stated that filter covers were effective in preventing cage-to-cage transmission of the disease. Colony depopulation, thorough sanitation of the facility, and repopulation with hamsters from a source without a history of the disease are the most effective steps to eliminate proliferative ileitis.

Colibacillosis

Background

Enteropathogenic *Escherichia coli* have been isolated from hamsters associated with some outbreaks of proliferative ileitis. Prior to *L. intracellularis* being identified as the etiological agent of proliferative ileitis, the possible role of *E. coli* in the pathogenesis of the disease was investigated (Frisk and Wagner, 1977a; Frisk et al., 1978, 1981; Wagner et al., 1973). Although *E. coli* isolated from hamsters with proliferative ileitis may cause secondary intestinal inflammatory lesions, it does not contribute to the production of ileal hyperplasia. *E. coli* isolated from hamsters have been demonstrated to be enteropathogenic and can cause acute enteritis (Amend et al., 1976; Boothe and Cheville, 1967; Frisk and Wagner, 1977a; Frisk et al., 1981).

E. coli was isolated from the intestines of hamsters with enteritis without proliferative lesions by Sheffield

and Beveridge (1962). They surmised that the *E. coli* was the cause of the fatal diarrhea and compared the condition to infantile diarrhea of man; and colibacillosis in piglets, chickens, and calves. Thomlinson (1975) isolated *E. coli* from a colony of hamsters with a fatal form of acute enteritis. The isolated serotype was identified in eight of ten affected and one of six unaffected hamsters. The organism was cultured from stomach, intestine, and spleen. This particular isolated serotype has also been reported as an intestinal pathogen of calves. Thomlinson (1975) believed that stress was a part of the enteritis disease similar to colibacillosis of calves and pigs.

Etiology, Epizootiology, and Clinical Manifestations

The *E. coli* isolated from hamsters with signs of diarrhea, including those with lesions of proliferative ileitis, have been shown in some cases to be enteropathogenic. The *E. coli* have been shown with some isolates to invade ileal epithelial cells and are either non-lactose or slow lactose fermenters.

Within the first 9 days after inoculation with ground proliferated ilea, Frisk and Wagner (1977a) showed by isolation, electron microscopy and indirect fluorescent antibody techniques, that a non-lactose-fermenting *E. coli* was present within ileal epithelial cells. These intracellular *E. coli*, with serologic and pathogenic characteristics similar to shigella, were found in experimentally infected hamsters (Frisk and Wagner, 1977a). Lesions of acute enteritis were present concurrent with these organisms. The finding of an intracellular *E. coli* after inoculation with ground proliferative ileal lesions is not consistent, Johnson and Jacoby (1978) did not observe *E. coli* by electron microscopy.

E. coli organisms were observed within ileal epithelial cells by Frisk and Wagner (1977a). These organisms were identified as *E. coli* by indirect fluorescent antibody techniques and measured $0.5\text{--}1 \times 2.2\text{ }\mu\text{m}$ by electron microscopy. Organisms were present within ileal epithelial cells in hamsters 1–9 days after inoculation. Detection of *E. coli* was associated with microscopic lesions of acute enteritis.

Pathology and Pathogenesis

Frisk et al. (1981) inoculated a non-lactose-fermenting *E. coli* that was isolated from ilea with proliferative lesions into weanling hamsters. The organism was able to produce clinical signs of diarrhea and lesions of acute enteritis. By electron microscopy and indirect fluorescent antibody techniques, *E. coli* were observed within intestinal epithelial cells. This organism was able to divide within cells and produce a disease which was comparable to shigellosis. Amend et al. (1976) isolated a slow-lactose-fermenting *E. coli* from hamsters with

proliferative ileitis. Oral inoculations of this *E. coli* into weanling hamsters resulted in lesions of acute enteritis. [Boothe and Cheville \(1967\)](#) isolated an *E. coli* from hamsters with typical lesions of proliferative ileitis. When weanling hamsters were orally inoculated with pure cultures of the isolated *E. coli*, diarrhea and generalized enteritis were observed.

Production of acute enteritis with pure cultures of *E. coli* inoculated into weanling hamsters is not a consistent finding. [Jackson and Wagner \(1970\)](#) isolated a hemolytic *E. coli* from hamsters with proliferative ileitis, but they also reported similar cultures from hamsters without proliferative lesions. From a separate outbreak, [Wagner et al. \(1973\)](#) isolated a non-lactose-fermenting *E. coli* from proliferated ileal lesions. Pure cultures inoculated orally into weanling hamsters did not produce disease. [Jacoby et al. \(1975\)](#) isolated a slow-lactose-fermenting *E. coli* from hamsters with proliferative ileitis lesions. Pure cultures of *E. coli* could not consistently produce disease.

[Frisk et al. \(1978\)](#) investigated the enteropathogenicity of several *E. coli* isolates from hamsters with proliferative ileitis. In an intestinal loop technique developed in weanling hamsters, three *E. coli* isolated from hamsters with proliferative ileitis were shown to be enteropathogenic, when compared to a non-enteropathogenic *E. coli* that was isolated from a normal hamster.

Campylobacteriosis

Background, Etiology, and Epizootiology

Campylobacter jejuni has been recognized as a major cause of enteritis in humans and has been isolated from a wide range of domestic and wild animals ([Fox, 1982](#)). There appears to be a high incidence of campylobacter infection in hamsters from the United States, up to 100% of hamsters tested in some colonies. In a survey of hamsters obtained from pet stores, 80% were carriers of *C. jejuni* ([Fox et al., 1983](#)).

C. jejuni is a motile, slender, comma-shaped, Gram-negative rod that requires a microaerophilic environment for growth. Route of transmission from animal to animal is by the fecal-oral route. *C. jejuni* has been recovered from ileal, cecal, jejunal, duodenal, and colonic contents ([Fox et al., 1986](#)).

Clinical Manifestations

Most hamsters naturally infected with *C. jejuni* are asymptomatic. Signs of diarrhea have been observed in a small percentage of animals ([Fox et al., 1983](#)). In hamsters with proliferative ileitis, there appears to be a high incidence of *Campylobacter*-positive animals ([La Regina and Lonigro, 1982](#); [Lentsch et al., 1982](#); [Stills et al., 1989](#)).

Pathogenesis

In the past, studies investigated the role of *Campylobacter* spp. in the pathogenesis of proliferative ileitis. [Lentsch et al. \(1982\)](#) isolated *C. jejuni* from fecal swabs and proliferated ilea of hamsters that were from a natural outbreak of the disease. The organism was isolated from all animals showing gross lesions of ileal proliferation. [La Regina and Lonigro \(1982\)](#) isolated *Campylobacter* sp. from hamsters with experimentally induced proliferative ileitis. Inoculations of *C. jejuni* isolated from hamsters with proliferative ileitis were attempted ([La Regina and Lonigro, 1982](#); [Lentsch et al., 1982](#)). Colonization of the intestinal tract was established in all inoculated hamsters, mild enteritis being noted in a few. However, typical lesions of proliferative ileitis were not produced ([La Regina and Lonigro, 1982](#); [Lentsch et al., 1982](#); [Stills et al., 1989](#)).

In a survey of three commercial hamster colonies, *Campylobacter* sp. was isolated from a colony that had previously experienced proliferative ileitis ([La Regina and Lonigro, 1982](#)). Hamster breeders with no history of proliferative ileitis were negative for *Campylobacter* sp. ([La Regina and Lonigro, 1982](#)).

L. intracellularis has since been identified as the causal agent of proliferative ileitis, and the isolated *C. jejuni* from affected hamsters is considered an opportunist. Although *Campylobacter* sp. has the ability to colonize the intestine and stomach, hamsters inoculated with pure cultures typically do not develop significant disease ([Fox et al., 1982](#)).

Diagnosis

Detection of *C. jejuni* is made by sampling feces or intestinal contents. Culture may be attempted on selective media incubated under microaerophilic conditions. Commercially available PCR assays are used to detect *Campylobacter* spp. In humans, PCR was found to be a more sensitive detection method than culture ([Sinha et al., 2004](#)).

Prevention

Because of the relatively high incidence of *Campylobacter* carriers, hamsters should be considered as a potential reservoir for zoonosis for pet owners and laboratory personnel ([Fox et al., 1983, 1986](#)).

Helicobacter

Background

Hamster intestines are naturally colonized with several *Helicobacter* spp. including *H. cinaedi*, *H. mesocricetorum*, *H. cholecystus*, *H. aurati*, and unnamed *Helicobacter* spp. ([Fox et al., 2009](#); [Nambiar et al., 2006](#); [Whary and Fox, 2004](#)). Clinical disease is usually not associated with these carrier animals.

Etiology

Helicobacter spp. are Gram-negative bacteria, motile, and are curved to spiral to fusiform in shape. Isolation of this organism requires microaerophilic conditions. Speciation is accomplished with biochemical testing and PCR analysis. This genera of bacteria shares many characteristics with *Campylobacter* spp. A number of helicobacters have been isolated from hamsters from research and commercial colonies.

Epizootiology and Pathology

HELICOBACTER MESOCRICETORUM

H. mesocricetorum was isolated from the feces of normal hamsters (Simmons et al., 2000). Gross and microscopic lesions were not observed. The organism was thought to be a non-pathogenic commensal of the intestine.

HELICOBACTER CINAEDI

H. cinaedi was isolated from the intestinal tract of clinically normal hamsters (Gebhart et al., 1989). Pathologic lesions in hamsters were not associated with the organism. *H. cinaedi* is a significant pathogen for immunocompromised humans. This organism may cause enteritis, proctocolitis, and rectal infection in man (Gebhart et al., 1989; Whary and Fox, 2004). Septicemia and meningitis occurred in a 5-day-old infant whose mother cared for pet hamsters during the first two trimesters of her pregnancy (Orlicek et al., 1993). *H. cinaedi* was isolated from blood and cerebrospinal fluid of the newborn. The organism could not be isolated from the mother. Hamsters should be considered a reservoir host for *H. cinaedi* and may be a source of infection for humans.

HELICOBACTER CHOLECYSTUS

H. cholecystus can cause cholangiofibrosis and centrilobular pancreatitis in hamsters (Franklin et al., 1996). *H. cholecystus* was isolated from the gallbladders of 3–8-week-old hamsters. Histologically, hepatic lesions were characterized by portal lymphoplasmacytic infiltration, some forming lymphoid follicles. In severe cases, bile duct hyperplasia was extensive and periportal fibrosis bridged to adjacent portal triads. Centrilobular pancreatitis consisted of extensive periductular inflammation and extension of inflammatory cells into ductal epithelium. Pathogenesis of this disease was suggestive of an ascending *H. cholecystus* infection of the common bile and pancreatic ducts, which resulted in the hepatic and pancreatic lesions.

HELICOBACTER AURATI

In aging (18–24 months) hamsters infected with *Helicobacter* spp. (including *H. aurati*), chronic progressive typhlocolitis and associated dysplastic lesions

were noted in the large intestine (Nambiar et al., 2006). Clinical signs were either absent or included chronic weight loss and poor body condition. Gross lesions included a thickened ileoceocolic junction with serosal cysts. Histopathologically, inflammation of the intestine was characterized by expansion of lamina propria by mixed inflammatory cells and multiple large cystic crypts. Inflammation extended into the submucosal and muscular layers and multiple crypt abscesses were evident. Dysplastic lesions were noted, which were similar to lesions of humans with irritable bowel disease. Dysplastic lesions include atypical, tortuous, hyperplastic crypts with areas of inflammation. Areas of epithelial hyperplasia showed loss of goblet cells. Although two tumors (round cell sarcoma and malignant fibrous histiocytoma) were noted, their association with the chronic inflammatory process was suggestive but not established. Although three *Helicobacter* spp. were isolated from all affected hamsters, the organisms could only be implicated as causal. Naturally occurring progressive proliferative and dysplastic typhlocolitis in aging hamsters may be a valuable model for studying irritable bowel disease of humans (Whary and Fox, 2004). Because *Helicobacter*-free hamsters have not been established, experimental inoculations of hamsters with pure cultures are not possible.

H. aurati was isolated from the inflamed stomach and cecum of adult hamsters (Patterson et al., 2000a). Chronic gastritis and intestinal metaplasia without cancer was observed in 7–12-month-old hamsters naturally infected with *H. aurati* and two other microaerobic species (Petterson et al., 2000b). The most severe gastric lesions were found in the distal antrum near the pyloric-duodenal junction. Gastric inflammation extended from the mucosa to the serosa. Increased numbers of goblet cells were indicative of intestinal metaplasia. In a follow-up study, a 2-year-old hamster positive for *Helicobacter* spp. had a gastritis-associated adenocarcinoma which was invasive but not metastatic (Nambiar et al., 2005). *Helicobacter*-infected aging hamsters may be a potential model of the development of intestinal metaplasia and gastric adenocarcinomas in chronic *H. pylori*-infected humans (Whary and Fox, 2004).

HELICOBACTER SP.

A novel *Helicobacter* sp. was identified by *Helicobacter*-specific PCR from livers of aged hamsters (Fox et al., 2009). The livers from these hamsters had lesions of chronic hepatitis, hepatic dysplasia, and biliary hyperplasia. Hepatic inflammation was also a prominent feature including cellular infiltration by lymphocytes, neutrophils, and macrophages. This novel *Helicobacter* sp. is closely related to *H. bilis* and may have a role in hepatobiliary disease.

Tyzzler's Disease

Background

Prior to reports of spontaneous outbreaks of Tyzzler's disease in hamsters, it was shown that hamsters could be infected experimentally (Craigie, 1966; Takagaki et al., 1966). During an outbreak of Tyzzler's disease in a colony of gerbils, unconfirmed cases were reported in hamsters (White and Waldron, 1969). Naturally occurring confirmed cases of Tyzzler's disease have been sporadic (Nakayama et al., 1975; Takagaki et al., 1974; Timmons and Dickey, 1981; Zook et al., 1977). *Clostridium piliforme* is able to infect and cause an enterohepatic disease in many different species. In addition to hamsters, Tyzzler's disease has been reported in the mouse, rat, rabbit, gerbil, cat, rhesus monkey, horse, guinea pig, and dog.

Etiology

The causative agent of Tyzzler's disease is the obligate intracellular bacterium, *C. piliforme*. The organism is a Gram-negative, motile, pleomorphic bacillus measuring $0.5 \times 8\text{--}10\mu\text{m}$, some being up to $40\mu\text{m}$ in length. *C. piliforme* is spore-forming, showing monilial subterminal swellings. Spores play an important role in establishing infection by the oral route. Contaminated materials can be infective up to one year at room temperature (Craigie, 1966). *C. piliforme* has not been grown on cell-free artificial media. The organism can be grown in the yolk sac of embryonated chicken eggs and in cell culture (Waggie et al., 1987; Zook et al., 1977).

The antigenicity of *C. piliforme* isolated from hamsters has been compared to isolates from other species by complement fixation, immunofluorescence, and double-diffusion techniques (Fujiwara et al., 1974). Hamster organisms and the organism isolated from a kitten during a hamster outbreak showed strong cross reaction. There was weak or no cross reaction with Tyzzler organisms isolated from rats or mice. Nakayama et al. (1975) showed common antigens by complement fixation when organisms from hamsters with enteric lesions were compared with organisms from hamsters with hepatic lesions. It appeared that the antigenic specificity of *C. piliforme* was dependent upon the host species from which it was derived. Franklin et al. (1994) demonstrated that the *C. piliforme* isolated from a natural case of Tyzzler's disease in a hamster was specific for that host and, when inoculated experimentally, did not infect mice or rats. This host specificity may be due to bacterial and host factors most likely involving the organism's ability to attach to and enter host enterocytes and hepatocytes. A *C. piliforme* isolated from a hamster outbreak was shown to produce a cytotoxin which may result in a more severe disease than isolates that are non-toxicogenic (Franklin et al., 1994; Riley et al., 1992).

In tissue sections, organisms are intracytoplasmic and are most often arranged in parallel bundles or starburst arrays. Tyzzler organism has a predilection for multiplication in the hepatocyte and epithelial cell of the intestine. Organisms are poorly visible in tissues stained with hematoxylin-eosin and Gram stains. The organisms are most easily visible, as light brown to black filamentous rods, with the silver stains of Warthin-Starry or Levaditi (Ganaway et al., 1971). Other effective stains are Giemsa, toluidine blue, Gomori methenamine silver, and periodic acid Schiff.

Epizootiology

Although there have been widespread reports of Tyzzler's disease from many countries in mice, there have been only scattered reports in hamsters. There is a possibility of Tyzzler's disease occurring in hamsters whenever they are housed near susceptible species. It is possible that the prevalence of Tyzzler's disease in hamsters may be higher because some cases may go undiagnosed. For instance, gross lesions in a retail pet supplier colony included cecal distention and enlargement of mesenteric lymph nodes without gross hepatic lesions (Motzel and Gibson, 1990). Diagnosis may not be confirmed in some cases because lesions are not always characterized by focal hepatic necrosis, *C. piliforme* will not grow on artificial media, and special stains are needed to observe the organism histologically.

The transmission of *C. piliforme* from animal to animal is by the oral route. If a hamster in a cage contracts the disease, usually its cage mates become ill (Zook et al., 1977). Nakayama et al. (1975) exposed hamsters to contaminated bedding 3 months after the hamsters housed on it died of Tyzzler's disease. After a few days of exposure, all hamsters died with enteric lesions.

Clinical Manifestations

Tyzzler's disease outbreaks may be characterized by high mortality with the sudden unexpected loss of primarily weanling hamsters with few clinical signs. Hamsters may die within 24 hours to a few weeks after onset. Diarrhea is the most characteristic sign, however, it is not always observed. Affected hamsters have pale, yellow, watery feces which result in wet hairs in the perianal area. Other clinical signs include a ruffled or dry hair coat, lethargy, anorexia, and dehydration. The diseased hamster may appear hunched and can progress to a moribund condition.

Pathology

Lesions can vary in the hamster; in some outbreaks the predominant lesions involve liver (Takagaki et al., 1974), some involve enterocolitis without liver lesions (Motzel and Gibson, 1990; Nakayama et al., 1975), and others involve heart lesions (Zook et al., 1977). Typical liver

lesions consist of organ enlargement with white-yellow or white-gray plaques that are pinpoint to 2 mm in diameter. Gross lesions involving the intestinal tract most commonly occur in the ileum, cecum, and proximal colon. These regions show edema, hyperemia to hemorrhage, and dilatation with semi-liquid, foamy, yellow contents. Focal necrotic gray plaques, 1–3 mm in diameter, can be observed on the mucosal surface. Multiple white nodules, 2–5 mm in diameter, may extend the full thickness of the ventricle in the heart (Zook et al., 1977). Other less notable gross lesions include markedly enlarged and congested mesenteric lymph nodes and enlarged spleens.

Histologically, liver lesions consist of periportal foci of coagulative necrosis of variable size (Figure 30.9). There is infiltration of affected areas by neutrophils and lymphocytes. *C. piliforme* are observed primarily in viable hepatocytes immediately adjacent to necrotic foci with fewer numbers in necrotic hepatocytes or the center of necrotic foci (Figure 30.10).

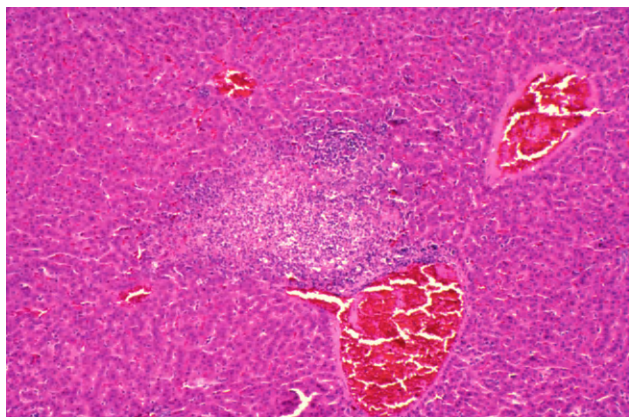


FIGURE 30.9 Focal area of coagulative necrosis in the liver of a hamster with Tyzzer's disease. Hematoxylin-eosin stain. Magnification: $\times 64$. Courtesy of Dr. K. S. Waggle.

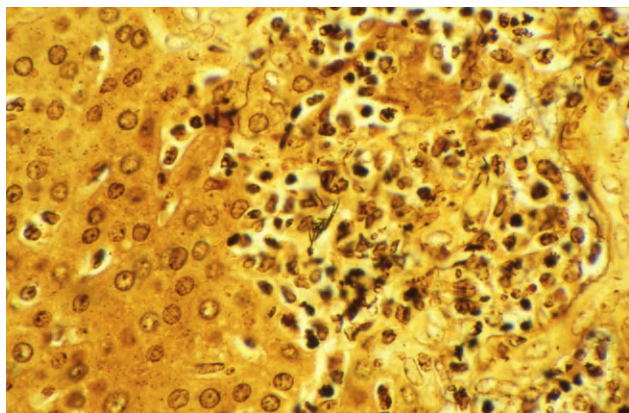


FIGURE 30.10 *Clostridium piliforme* organisms are present within an area of hepatic necrosis from a hamster with Tyzzer's disease. Warthin-Starry silver stain. Magnification: $\times 640$. Courtesy of Dr. K. S. Waggle.

Intestinal lesions can generally be classified as acute inflammation with focal mucosal erosions. The mucosa of the ileum, cecum, and colon shows degenerative and necrotic changes. There is edema, hyperemia, and inflammatory cell infiltration of the lamina propria. The most severe mucosal erosions include edema and inflammation of the submucosa and muscle layers. Organisms can be observed within the cytoplasm of mucosal epithelial cells in the intermediate zone of villi and less predominantly in crypts. Organisms may also be observed within smooth muscle cells bordering areas of necrosis within the tunica muscularis (Waggle et al., 1987).

Myocardial histopathologic lesions consist of ill-defined granulomas and focal areas of necrosis with accompanying inflammation (Zook et al., 1977). Organisms can be observed within these myocardial lesions.

Pathogenesis

Tyzzer's disease in hamsters has been studied by inoculating *C. piliforme* contained within liver homogenates, cecal contents or spore suspensions produced from embryonated chicken eggs. Although hamsters have been experimentally infected by many routes, oral inoculation produces results which are the closest to the naturally occurring disease.

The organ in which Tyzzer organism first grows is the cecum, its physiology being favorable to the proliferation of *C. piliforme* (Nakayama et al., 1976). Dissemination of the organism is rapid throughout the remainder of the intestinal tract, affecting all regions from duodenum to rectum by the third day post inoculation. Microscopic intestinal lesions were first detected 2–3 days after oral inoculation (Waggle et al., 1987). Foci of necrosis were present within the smooth muscle layers of the ileum, cecum, and colon 7 days after inoculation. Through cecal ligation experiments, it was demonstrated that the route of *C. piliforme* from the cecum to the liver was via portal circulation (Nakayama et al., 1976). *C. piliforme* were observed in hepatocytes on the periphery of necrotic foci 3–5 days after oral exposure (Waggle et al., 1987). Hepatic lesions showed a healing process involving granuloma formation containing giant cells 12–14 days post inoculation.

Diagnosis

The definitive diagnosis of Tyzzer's disease is dependent upon the demonstration of typical bacilli adjacent to regions of necrosis within the cytoplasm of hepatocytes, intestinal epithelial cells, or myocardial cells. Diagnosis is most commonly made with special stains of tissue sections. PCR assays for detection of *C. piliforme*, typically using feces or colon samples, are available commercially. Serologic methods, including indirect fluorescent antibody, enzyme-linked immunosorbent assays, and complement fixation testing, detect

exposure to *C. piliforme*. Cecal contents from hamsters suspected of Tyzzer's disease may be orally inoculated into gerbils, or cortisone-treated hamsters, or mice to reproduce the disease (Motzel and Gibson, 1990).

Prevention and Therapy

The most important factors in the control and prevention of Tyzzer's disease involve improved sanitation and isolation. Spores of *C. piliforme* are most likely the most important factor in maintaining an outbreak. Cleaning the environment to eliminate spores and preventing cage-to-cage spread with microisolation filter covers are helpful in controlling spread of the disease.

Treatment of hamsters with Tyzzer's disease was not described in most of the reported outbreaks. Oxytetracycline added to the drinking water of a pet store supplier of hamsters was attempted without success (Motzel and Gibson, 1990). This particular outbreak of Tyzzer's disease was believed to be precipitated by stressors including overcrowding, poor sanitation, high environmental temperatures and humidity, marginal diet, and parasitism.

Although there have been only sporadic outbreaks of Tyzzer's disease in hamsters, the disease can be significant because of high morbidity and high mortality. If an outbreak of Tyzzer's disease occurs in any species within an animal care facility, other susceptible species including the hamster may be at risk of infection. Outbreaks in hamsters have been reported associated with diseased gerbils in one case (White and Waldron, 1969) and a kitten concurrently infected in another (Takagaki et al., 1974). *C. piliforme* is not believed to be transmissible to humans.

Investigators who carry out experimental protocols that involve immunosuppression of hamsters, especially with cortisone or irradiation, should be observant for Tyzzer's disease. Cortisone administered subcutaneously at 2.5mg per hamster enhanced infection (Nakayama et al., 1976). Organisms were more abundant in affected tissues; and lesions involving the alimentary tract and liver were accentuated. Hamsters treated with cortisone died 4–7 days after inoculation as compared to 7–13 days if not given cortisone (Nakayama et al., 1976). Hamsters exposed to whole body irradiation (500r) at the time of inoculation with *C. piliforme* resulted in the production of much more severe lesions (Takagaki et al., 1966).

Antibiotic-Associated Enterocolitis (*Clostridium difficile*)

Background

Many antimicrobial agents can cause enterocolitis in hamsters including lincomycin, clindamycin, ampicillin, vancomycin, erythromycin, cephalosporins,

gentamicin, and penicillin (Percy and Barthold, 2001; Small, 1987). Because high mortality may occur following antibiotic administration, the risks of therapy with antibiotics in hamsters must be carefully considered. The hamster has been used as an animal model for antibiotic-associated enterocolitis in humans because of its sensitivity to antibiotic use and the resulting intestinal lesions. The literature regarding antibiotic-associated enterocolitis has been reviewed (Small, 1987).

Etiology

The enterocolitis that occurs following the use of antibiotics is caused by the toxins from *C. difficile*. It is believed that alterations to the hamster's intestinal microflora are also important in causing the disease. In hamsters treated with clindamycin, the cecal contents were shown to have toxic activity (Chang et al., 1978; Rifkin et al., 1978). Experimentally, the mortality rate for hamsters administered vancomycin may approach 100% (Wilson et al., 1981). However, when cecal contents from normal hamsters were administered orally to those animals receiving vancomycin, nearly all of the animals were protected from disease development. Therefore, it is believed that bacteria from the normal intestinal flora are necessary to suppress *C. difficile* growth. A yeast, *Saccharomyces boulardii*, has been found to prevent fatalities in hamsters following clindamycin treatment (Toothaker and Elmer, 1984).

Epizootiology

From reports regarding the use of antibiotics in hamsters, some have recorded toxicity while others indicated that no problems were experienced (Small, 1987). It is postulated that variations in toxicity are due to differences in intestinal microflora and/or the presence of *C. difficile* in the cecum.

Antibiotic-associated enterocolitis is an infectious disease. The sources of infection with *C. difficile* for hamsters after treatment with an antibiotic are believed to be carrier hamsters or the environment including contaminated technicians' hands, cage equipment, food, and contaminated air flow (Fekety et al., 1980). In a controlled experiment where a conventional room was compared with a clean room including the use of gloves, garbing, and sterile equipment; hamsters administered antibiotics survived twice as long in the clean room (Toshniwal et al., 1981). Therefore, it was postulated that antibiotic use causes hamsters to become susceptible to *C. difficile* that is usually acquired from the environment.

The route of administration that is used in treating hamsters is important with some antibiotics to determine if enterocolitis will develop (Small, 1987). If a route is used (for example subcutaneous with an antibiotic such as chloramphenicol) that is excreted through the urine, the cecal microflora are not affected because

the antibiotic is not present in the gastrointestinal tract (Fekety et al., 1979b). Thus, fatal enterocolitis will not develop.

In experiments investigating treatment of clindamycin-induced colitis in hamsters, disease was prevented by treating with orally administered vancomycin or metronidazole (Fekety et al., 1979a). When these antibiotics were discontinued, hamsters succumbed to fatal enterocolitis. This same type of relapsing syndrome after vancomycin treatment occurred in a group of hamsters with naturally occurring non-antibiotic-associated *C. difficile* enterocolitis (Boss et al., 1994). This high incidence of relapse in clindamycin-induced enterocolitis in hamsters is a model for humans with a similar syndrome occurring with *C. difficile*-associated diarrhea. Relapse enterocolitis may be associated with persistence of spores, reinfection from the environment, and/or failure to develop an effective immunity. In clindamycin-induced colitis of hamsters, relapse of enterocolitis did not occur after oral administration of either rifoximin or rifalazil for 5 days (Anton et al., 2004; Kokkotou et al., 2008).

Clinical Manifestations

Clinical signs and lesions of antibiotic-associated enterocolitis are similar in hamsters regardless of the antibiotic that induces the disease. Clinical signs include ruffled hair coat, dehydration, and diarrhea. Signs are usually observed within a few days, and death is observed 4–10 days post treatment.

Pathology

Characteristic gross lesions are cecal distention with fluid contents and hemorrhage of the distal ileum, colon, and cecum. Microscopically, there is acute to pseudomembranous typhilitis (Percy and Barthold, 2001). The lamina propria is edematous and infiltrated by neutrophils; and the mucosa may show hyperplasia.

Pathogenesis

Although alterations to the intestinal microflora following the use of antibiotics in hamsters have been shown to be an important component, the exact pathogenesis of antibiotic-associated enterocolitis remains uncertain. *Lactobacillus* and *Bacteroides* are the primary bacterial flora in the hamster intestine (Hagen et al., 1965). *C. difficile* can be cultured from the intestinal tract of a low percentage of normal hamsters; however its toxic effects are suppressed. The dysbiosis that may occur within 2–10 days following antibiotic administration allows toxigenic *C. difficile* to be established. It has been postulated that *C. difficile* colonization is suppressed by Gram-negative anaerobes and other clostridia in untreated hamsters (Wilson et al., 1981). The loss of these inhibitory organisms from antibiotic use allows colonization of *C. difficile* and its toxins

are released. These toxins cause the typical lesions of acute colitis, which leads to death. *C. difficile* can elaborate two antigenically distinct toxins (A and B) (Taylor et al., 1981). These toxins cause different inflammatory lesions. Enlarged moderately hemorrhagic cecitis is caused by toxin A; and normal-sized hemorrhagic cecitis is caused by toxin B (Libby et al., 1982).

Diagnosis

The diagnosis of antibiotic-associated enterocolitis is usually made based on the history of antibiotic use, clinical signs, and typical gross and microscopic lesions. *C. difficile* can be detected by PCR and enzyme immunoassay testing of feces, cecum, or colon. Isolation of the organism can be made using anaerobic techniques. Because hamsters can be carriers of *C. difficile*, isolation or detection of the organism is not diagnostic. Toxins of *C. difficile* can be detected by PCR, mouse inoculation, or by cell culture of large intestinal contents (Percy and Barthold, 2001). Differential diagnoses include any disease which can cause diarrhea, such as proliferative ileitis, enteropathogenic *E. coli* infections, campylobacteriosis, salmonellosis, and Tyzzer's disease.

Non-Antibiotic-Associated Clostridial Enteropathy (*Clostridium difficile*)

Background

C. difficile can cause disease in hamsters unrelated to a history of antibiotic use. Hamsters may unexpectedly die with or without signs of diarrhea and have lesions of cecitis. Affected hamsters may vary in age from juveniles to adults. Multiple hamsters died in an outbreak from a *C. difficile*-associated disease with a peracute clinical course (Hart et al., 2010). Most affected hamsters in this outbreak did not receive experimental manipulations. In a separate outbreak, *C. difficile* organisms were able to infect and cause enterocolitis in hamsters that were untreated with antibiotics, but were exposed to hamsters within the same room that did have antibiotic treatment (Rehg and Lu, 1982).

Etiology

C. difficile is a Gram-positive, anaerobic, spore-forming bacterium (Hart et al., 2010). Disease from this organism has been attributed to the production of two exotoxins, toxins A and B (Goulding et al., 2009). Hamsters are the most sensitive species to naturally acquired *C. difficile* infections compared to other common laboratory animals (Hart et al., 2010).

Epizootiology

The prevalence of asymptomatic carriers of *C. difficile* in hamsters has not been determined (Hart et al., 2010).

C. difficile may cause a potentially fatal diarrheal disease in humans. It is considered one of the most important nosocomial infections worldwide (Goulding et al., 2009). Experimental infection of hamsters with *C. difficile* serves as an important model for studying the human disease. Clinical signs of diarrhea, histopathologic lesions, and relapse of disease following removal of treatment are all important aspects of the human disease that can be produced in the hamster model (Goulding et al., 2009). Experimental studies using the hamster model have provided valuable information with regard to the role of toxins in the pathogenesis and potential treatments of the disease (Goulding et al., 2009).

Clinical Manifestations

C. difficile-associated cecitis in hamsters is characterized by sudden death. Hamsters of all ages in a colony may be affected and outbreaks may continue for up to 4 months. Clinical signs include primarily diarrhea which can be profuse and watery. In hamsters with hemorrhagic or watery diarrhea, mortality is high. Hamsters may also die peracutely without diarrhea having formed fecal pellets in the gastrointestinal tract with food in the stomach (Hart et al., 2010). Feces in chronic cases are semi-formed, which are matted in the perineal area. Signs may include dehydration, emaciation, rough hair coat, and hunched back.

Pathology

Gross lesions in hamsters are very similar to the ileocectitis that occurs after antibiotic use. The cecum is predominantly involved, showing enlargement with serosal hemorrhage varying in distribution from diffuse areas to multiple petechiae. Affected ceca are distended with odiferous contents. Intestines have a moderate amount of gas, are congested, and contain a yellow mucinous fluid. The cecum and colon may be thickened and firm (Chang and Rohwer, 1991; Ryden et al., 1991).

Microscopically, the cecum shows acute inflammation, submucosal edema, necrosis, loss of epithelium, and hemorrhage. A lymphoplasmacytic cellular infiltrate is present in the lamina propria of the cecum and colon. Chronically affected hamsters may have cecal mucosal hyperplasia (Ryden et al., 1991). Mucosal hyperplasia has also been reported in both the cecum and colon of affected hamsters (Chang and Rohwer, 1991; Hart et al., 2010). Hyperplastic lesions show increased height of villi and increased basophilia with many mitotic figures. In a group of hamsters fed an atherogenic diet, colonic hyperplasia was also present in most of the affected hamsters (Blankenship-Paris et al., 1995). Intracytoplasmic organisms are not observed in any of the hyperplastic epithelial cells.

In chronic cases, other organ systems may be involved. Livers may have cholangiohepatitis with biliary hyperplasia, and renal and hepatic amyloidosis.

Pathogenesis

It has been postulated that cecal dysbiosis may result in cecal hyperplasia which leads to overgrowth of *C. difficile* and the resulting necrotic cecitis (Ryden et al., 1991).

Acute toxigenic lesions from *C. difficile* have been attributed to toxins A and B acting synergistically (Hart et al., 2010). Toxin A is an enterotoxin which causes mucosal damage followed by toxin B, a cytotoxin affecting epithelial cells. High titers of *C. difficile* toxin B were detected in a hamster breeding colony with mortality due to cecocolitis (Ryden et al., 1991). Toxin B was also demonstrated in a group of aged hamsters that developed cecitis and colitis on a scrapie study (Chang and Rohwer, 1991).

Colitis caused by *C. difficile* can be caused by compounds other than antibiotics such as methotrexate (Cudmore et al., 1980). Non-antibiotic-associated enterocolitis developed in hamsters treated with fenazaquin, which is a miticide (Boss et al., 1994). *C. difficile* cecitis occurred in this group of hamsters that were being gavaged daily and fed a purified powdered diet (Boss et al., 1994). It was theorized that stresses associated with daily gavaging, a purified diet, and treatment with a miticide modified the intestinal flora which allowed proliferation of *C. difficile* that resulted in enterocitis. In this case, daily oral administration of vancomycin (20 mg/kg) for 3 months protected against the development of fatal *C. difficile* enteritis.

Diet has also been implicated to have a role in non-antibiotic-associated clostridial enteropathy. Hamsters administered neomycin on a purified diet developed enterocolitis due to *C. difficile*, where neomycin-treated hamsters on a commercial diet did not show disease (Micheli et al., 1981).

C. difficile infection occurred in hamsters fed an atherogenic (39% fat content) diet (Blankenship-Paris et al., 1995). The disease was characterized by a high mortality rate (50%). Acute hemorrhagic cecitis was evident at necropsy. The composition of the diet definitely was involved in the pathogenesis of the disease. The infection reoccurred in hamsters fed the high-fat diet, but was not present in hamsters on control diets housed within the same room. This reported case of *C. difficile*-associated enterocolitis occurring in hamsters on a high-fat diet may show that factors other than alteration of cecal flora are involved in the pathogenesis (Blankenship-Paris et al., 1995). It was postulated that slowed gastrointestinal motility in these animals may have been important to increase the colonization of *C. difficile*. The pathogenesis of this clostridial enteropathy could be due to disruption

of the gut flora and/or increased susceptibility of hamsters fed this atherogenic diet.

Diagnosis

C. difficile infection should be considered in hamsters with lesions of enteritis especially those affecting the large intestine (Hart et al., 2010). Diagnosis of *C. difficile* can be made by PCR testing on feces, cecum, and/or colon. The PCR test is very sensitive and, with specific primers, can detect toxins A and B (Hart et al., 2010). In a study involving detection of *C. difficile* from humans, PCR following combined antigen and toxin detection by enzyme immunoassay provided rapid turnaround time and reliable diagnostic accuracy (Swindells et al., 2010). Isolation of the organism from feces or large intestinal contents can be made using anaerobic culture techniques.

Prevention

Control of an outbreak of *C. difficile*-associated disease may be accomplished by depopulation, decontamination of animal holding rooms with chlorine dioxide, and repopulation (Hart et al., 2010).

Cecal Mucosal Hyperplasia

Background, Etiology, and Epizootiology

Cecal mucosal hyperplasia occurred in a colony of 800 breeding adult hamsters (Barthold et al., 1978). The disease affected suckling (8–14 days of age) and weanling hamsters with a mortality rate of approximately 80%. The disease was transmitted by close contact between hamsters, its incubation period being approximately 4 days.

The epizootiology of the disease was characteristic of an infectious organism, however none could be identified. Aerobic cultures of affected ceca revealed presumed non-pathogenic organisms from the families *Enterobacteriaceae* and *Pasteurellaceae*. Bacteria or viruses could not be demonstrated in cecal lesions by transmission electron microscopy. Hamsters experimentally inoculated with ground cecal lesions orally and by direct cecal injection did not develop the disease.

Clinical Manifestations and Pathology

The most prominent clinical sign was diarrhea evidenced by matted fur at the base of the tail by liquid feces. Other signs included runting and dehydration, the syndrome progressing to a moribund condition.

Affected ceca had thickened, opaque walls and hyperemic serosal vessels. Ceca were contracted containing a small amount of ingesta. Histologically, there was cecal mucosal hyperplasia. Crypts were two to three times normal height, mitoses were numerous and

extended onto villi, and cellular differentiation was diminished. Mucosal erosion with acute inflammation was also occasionally observed within the cecum and lower portions of the small intestine. Mild non-suppurative portal hepatitis was often observed.

Diagnosis and Prevention

The differential diagnosis for cecal mucosal hyperplasia should include any disease which causes diarrhea. Proliferative ileitis occurs in the same age group of hamsters and proliferation of the large intestine has been associated with typical ileal lesions (Frisk and Wagner, 1977b; Jackson and Wagner, 1970; Jonas et al., 1965). Intracytoplasmic organisms, however, could not be demonstrated in lesions of cecal mucosal hyperplasia nor were proliferative ileal lesions observed (Barthold et al., 1978). Also, hyperimmune serum to *L. intracellularis* from hamsters with proliferative ileitis was reacted with hyperplastic cecal lesions and was negative. It appears, therefore, that cecal mucosal hyperplasia is distinct from proliferative ileitis. Other diagnoses which can be differentiated by culture and lesions include non-antibiotic-associated cecitis (*C. difficile*), salmonellosis, Tyzzer's disease, and hymenolepiasis. This outbreak of cecal mucosal hyperplasia was eradicated by colony depopulation.

Salmonellosis

Background, Etiology, and Epizootiology

Salmonellosis was a significant disease of hamsters prior to the improvement of sanitation practices, and the commercial production of diets and bedding. In recent decades, the disease in laboratory hamsters is rarely diagnosed (Renshaw et al., 1975). However, *Salmonella* has been diagnosed in hamsters bred and distributed for retail pet stores (Swanson et al., 2007).

Salmonella enterica serovars *enteritidis* and *typhimurium* have been isolated from affected hamsters. Organisms have been cultured from heart blood, liver, spleen, lungs, intestines, and feces. Incubation period is usually 3–6 days and transmission is by the oral route. A carrier state may exist where apparently normal hamsters shed *Salmonella* organisms in the feces (Soave, 1963).

Hamsters have been shown to be more susceptible to *Salmonella* infection than mice (Innes et al., 1956). The mortality rate in affected hamsters in a retail pet distributor was approximately 60% (Smith et al., 2005).

Clinical Manifestations

The disease in hamsters produces symptoms and lesions which are very similar to salmonellosis of other rodents (Habermann and Williams, 1958; Soave, 1963). Clinical signs include lethargy, rough hair coat,

diarrhea, anorexia, weight loss, and increased respiratory rate. Feces may appear as normal pellets or may be lighter in color and soft. With chronic salmonellosis, there may be no observable signs or animals may show progressive cachexia. Hamsters that survive weeks may show distended abdomens from enlargement of the liver and spleen.

Pathology

Gross necropsy reveals small white foci in the liver, patchy hemorrhagic lungs, and reddened lymph nodes. Enteritis may be observed, however, it may not be apparent in all cases (Innes et al., 1956). Microscopically, there is focal hepatic and focal splenic necrosis. Lung sections show focal congestion, intra-alveolar hemorrhage, and focal interstitial pneumonitis. Bronchopneumonia is only rarely observed (Innes et al., 1956). Phlebothrombosis involving the venous side of pulmonary circulation was reported as a prominent lesion (Innes et al., 1956). Pulmonary veins and venules were partially occluded by septic thrombi. Venous thrombosis was also noted in the liver. Mediastinal lymph nodes may show congestion, hemorrhage, erythrophagocytosis, and foci of necrosis.

Pathogenesis

The pathogenesis of *Salmonella* infection of hamsters was studied by Innes et al. (1956). They found that the hamster was highly susceptible to infection causing death in all inoculated hamsters by the second day after intraperitoneal injection and by the ninth day orally. Histologically, embolic glomerular lesions, purulent pericarditis, and necrotizing placentitis occurred. Acute enteritis with necrosis of Peyer's patches developed after oral inoculation.

Diagnosis

The diagnosis of salmonellosis should be suspected whenever there are unexpected deaths in a hamster colony. Differential diagnosis can usually be easily made because the organism can be readily isolated from many parenchymal organs. Screening colonies of hamsters for *Salmonella* can be done with PCR assays of feces.

Prevention and Therapy

The most effective strategy to manage a *Salmonella* outbreak in hamsters is through preventive measures rather than treatment. Antibiotics and drug treatments have no lasting benefit (Frisk, 1987). Multidrug-resistant *S. enterica* serovar *typhimurium* were isolated from hamsters destined for the retail pet trade where there was widespread use of prophylactic antibiotics including spectinomycin and tetracycline (Swanson et al., 2007). Eradication of the disease from a colony, once it is established, is best done by depopulation, sanitizing all equipment and environment, and restocking

with unaffected hamsters. To prevent the introduction of *Salmonella* into an existing colony, incoming hamsters should be quarantined and their feces cultured. Species separation should also be practiced because other species such as mice, rats, and guinea pigs are susceptible to infection.

Good sanitation practices and the use of commercially prepared diets and bedding will reduce the chances of introducing salmonellosis. Bacteriological screening of food, bedding, and water can be useful in detection of contaminants. Stress, such as overcrowding or feeding an inadequate diet, can change unrecognized salmonellosis in a colony to an epizootic with high mortality (Soave, 1963).

Salmonellosis can be latent or produce high morbidity and high mortality in hamster colonies; it is more commonly a problem in distributors and breeders for the retail pet industry. Salmonellosis is transmissible to many species including man. Handling of pet hamsters should be considered a potential health risk. Cases of salmonellosis in humans caused by multidrug-resistant *S. enterica* serovar *typhimurium* were associated with exposure to pet hamsters, mice, or rats (Swanson et al., 2007).

Tularemia

Etiology and Epizootiology

There has been one reported outbreak of tularemia in hamsters (Perman and Bergeland, 1967). This highly fatal disease occurred in a closed hamster breeding colony over a 1-month period. *Francisella tularensis* can infect many species of animals including all wild rodents and lagomorphs (Frisk, 1987).

Clinical Manifestations and Pathology

The tularemia outbreak described in hamsters was depicted by sudden onset; the disease's course was less than 48 hours. Hamsters were unexpectedly found dead or moribund. In this outbreak, hamsters 4–6 weeks of age in different areas of the room were affected; however, breeding animals were unaffected. Gross pathological lesions included mottled lungs with subpleural petechial and ecchymotic hemorrhages. Livers were enlarged and pale, spleens were enlarged with miliary white foci, and lymph nodes were enlarged and mottled. Histopathologically, the most consistent lesion was necrosis of lymphoreticular tissue associated with large numbers of small Gram-negative bacteria contained within phagocytes.

Diagnosis

Tularemia in hamsters would be a very unexpected diagnosis; however, it should be included along with other differential diagnoses in any disease outbreak

which causes high mortality. A definitive diagnosis can be confirmed from bacteriological cultures.

Prevention

The source of the tularemia in this single reported outbreak was not definitely identified. Contaminated diet supplements were suspected as the source of the organism because when the supplements were eliminated, no additional cases of tularemia occurred within the colony. A tularemia outbreak, although very rare and unexpected, would be a very significant public health issue because the organism is infectious to humans.

Bacterial Respiratory Infections

Bacterial respiratory infections of hamsters do occur, but they have not been extensively studied. In the United States, pneumonia of hamsters was recognized by 43% of laboratories (six of 14) surveyed and was listed as the second most common clinical condition (Renshaw et al., 1975). In a German survey to determine the incidence of diseases of hamsters, respiratory infections were noted in 8% (ten of 121) of total clinical cases (Lindt, 1958). In both of these surveys, viruses as well as bacteria accounted for the respiratory infections that were reported. Bacterial respiratory infections occur with much less frequency in hamsters than in mice, rats, or guinea pigs.

Bacterial respiratory infections can be caused by several organisms, including *Pasteurella pneumotropica* (Brennan et al., 1965; Gleiser, 1974; Harkness and Wagner, 1995; Silverman and Chavannes, 1983), *Pasteurella* sp. (Castleman, 1983), *Streptococcus pneumoniae* (Frisk, 1987; Renshaw et al., 1975), *Streptococcus agalactiae* (Kummeneje et al., 1975), *Streptococcus* sp. (Frisk, 1987), and *Salmonella* sp. (Innes et al., 1956). These respiratory conditions in hamsters include pneumonia, conjunctivitis, otitis media, and otitis interna which can progress to encephalitis. Hamsters may also carry potentially pathogenic organisms in their respiratory tract without showing clinical signs. Organisms which are screened for in nasopharyngeal cultures of hamsters include *P. pneumotropica*, *P. multocida* (Orcutt, 1980), cilia-associated respiratory (CAR) bacillus (Shoji-Darkye et al., 1991), *S. pneumoniae*, *Staphylococcus aureus* (Castleman, 1983), *Klebsiella pneumoniae* (Castleman, 1983), *Bordetella* sp. (Castleman, 1983), and *Salmonella* sp.

Mycoplasma pulmonis was isolated from the respiratory system of hamsters that were exposed to aerosolized graphite (Battiegelli et al., 1971). *M. pulmonis* could not be isolated from control hamsters or hamsters in groups treated with low concentrations of graphite or calcium sulfate. The significance of the presence of *M. pulmonis* in this particular experimental group was not established. The possible role *M. pulmonis* has as an

etiologic agent in naturally occurring respiratory infections of hamsters is not well defined. From the lack of reported clinical cases, it appears that the occurrence of *Mycoplasma* infection in the hamster is extremely rare.

Apparent bacterial pneumonias based on gross and microscopic lesions have been reported in the past (Hagen et al., 1965; Kleinerman, 1972). Cultures from affected lungs were not reported, therefore, the exact etiology of the lesions in these reports is unknown.

Further discussions of specific bacterial respiratory infections are found in the sections on *Pasteurella* infections, CAR bacillus, streptococcal infections, and salmonellosis.

Pasteurella Infections

P. pneumotropica is commonly present in the nasal cavities of carrier hamsters that do not show clinical signs (Gleiser, 1974; Harkness and Wagner, 1995; Leshner et al., 1985). *P. pneumotropica* may occasionally cause pneumonia and rarely cause enteritis in hamsters. Stress, including parturition, may be associated with the precipitation of clinical disease. *P. pneumotropica* is a small, Gram-negative, non-motile, bipolar rod. Pneumonia, cutaneous abscessation, conjunctivitis, otitis interna, and enteritis have been reported by natural infection (Harkness and Wagner, 1995; Leshner et al., 1985). Clinical signs associated with pneumonia may include labored breathing, nasal exudate, and weight loss. Experimentally, hamsters inoculated with *P. pneumotropica* could not be infected nor were lesions produced (Jawetx, 1948, 1950). In a colony, the most effective control measure for *P. pneumotropica* is elimination of carrier animals by isolating and culturing new animals before introduction into the existing population.

Cilia-Associated Respiratory (CAR) Bacillus

Although natural infections have not been reported, hamsters are susceptible to experimentally induced infection with CAR bacillus (Shoji-Darkye et al., 1991). Hamsters were inoculated intranasally with CAR bacillus isolated from a spontaneously infected mouse. As compared with mice, histopathologic lesions in hamsters were milder. Eight weeks after inoculation, pathologic changes included mild submucosal mononuclear cell proliferation of bronchi and trachea with marked thickening of bronchial epithelium. After experimental infection, CAR bacillus antigen was detected by immunostains throughout the respiratory tract and could be reisolated from tracheal washes (Shoji-Darkye et al., 1991). Successful experimental infections of hamsters with CAR bacillus suggest that natural infections could occur (Shoji-Darkye et al., 1991). PCR assays for CAR

bacillus, typically done on tracheal washes or lung, are commercially available.

Corynebacterium

Corynebacterium kutscheri may be isolated from clinically and pathologically normal hamsters. *C. kutscheri* was isolated from oral cavities, esophagus, cecal contents, colon/rectum, submaxillary lymph nodes, nasal cavities, and trachea of aged hamsters (Amao et al., 1991). In this same survey, the organism could not be isolated from lung, stomach, kidney, spleen, and mesenteric lymph nodes. Experimentally, hamsters inoculated subcutaneously or intramuscularly with pure cultures of *C. kutscheri* did not result in systemic disease (Amao et al., 1995). Only local inflammatory lesions including abscesses were noted. Although hamsters may be asymptomatic carriers, *C. kutscheri* apparently is not a systemic pathogen for hamsters. However, because *C. kutscheri* may be pathogenic for mice and rats, hamsters carrying the organism in their intestinal or respiratory tracts for long periods of time may serve as a source of infection for other rodents. For hamster health-monitoring programs, selective media for isolation of *C. kutscheri* should be considered (Amao et al., 1991).

An isolated case of acute pneumonia in a hamster caused by *C. paulometabulum* has been reported (Tansey et al., 1995). A single hamster from a small colony showed rapid, shallow breathing which did not clinically improve with tetracycline treatment. At necropsy, lungs showed acute edematous pneumonia from which *C. paulometabulum* was isolated. Based on only a single animal within a colony with no other clinical cases, this organism is not a significant pathogen for hamsters.

Streptococcal Infections

Streptococci have been isolated from hamsters with pneumonia. Streptococcal pneumonias are relatively uncommon and oftentimes are associated with stress. In a survey of naturally occurring diseases of hamsters, diplococcus (*Streptococcus pneumoniae*) was the cause of pneumonia from one of 14 laboratories (Renshaw et al., 1975). *S. agalactiae* has been reported to cause acute pleuropneumonia and septicemia in hamsters (Kummeneje et al., 1975). Diplococci and *Streptococcus* sp. have been reported to cause pneumonia and cervical lymphadenitis in hamsters (Frisk, 1987). The course of the disease is short (3 days), the route of infection being aerosol. Clinical signs include depression, anorexia, nasal and ocular discharge, dehydration, and weight loss. Diagnosis is made by clinical signs, lesions, and culture. Treatment may be attempted with antibiotics that are specifically effective against the isolated organism.

Bacterial Mastitis

A beta hemolytic *Streptococcus* was isolated from two female hamsters with acute bacterial mastitis (Frisk et al., 1976). The infection occurred 7–10 days after parturition. Litters were noted to be runt until 1 week of age when they were cannibalized by the affected female. Affected mammary glands were hemorrhagic, firm, and contained suppurative exudate. Histopathologically, myriads of polymorphonuclear inflammatory cells had infiltrated glandular parenchyma. The frequency of occurrence of streptococcal mastitis in breeding hamsters is very low.

Multiple nodules were diagnosed in the caudal mammary gland of a hamster 10 days after parturition (Heurkamp and Dillehay, 1990). Two prominent firm subcutaneous masses which were discrete and pink-yellow in color were noted. The cut surface had many small cystic cavities that contained a caseous yellow exudate. *E. coli* was cultured from the affected mammary glands. Histopathology revealed focal areas of coagulative necrosis and neutrophilic infiltration. These histologic changes were felt to be caused by endotoxins. Mastitis in this hamster caused by coliforms was thought to be from an ascending infection from contamination of the teat rather than via a hematogenous route.

Post Irradiation Infections

Bacterial infections may occur in hamsters after whole-body irradiation. Smith et al. (1955) irradiated groups of hamsters with a range of 760–10000r. These animals had 67–90% positive heart blood cultures for *Pasteurella* and/or *Streptococcus* up to 28 days following exposure. Infections caused by *Pseudomonas* resulted in the shortest survival time post irradiation. Hamsters showed rapid deterioration 4–5 days after being exposed to between 700 and 2000r (Mayo et al., 1962). Abscesses that were midway between the eye and ear were reported in hamsters that received 500–5000r (McKenna et al., 1970). *Pasteurella pneumotropica* was cultured from these abscesses which appeared 2–3 weeks following exposure.

The portal of entry for these bacteria is most likely the nasopharynx. Clinical signs of post-irradiation infections of hamsters are diarrhea, weight loss, dehydration, and edema of the face. Lesions may include suppurative blepharitis, orbital abscesses, and foci of necrosis in the oral cavity.

Bacterial infections can alter the results of experiments utilizing hamsters in whole-body irradiation studies. The occurrence and severity of these infections may depend upon the bacterial microflora within the upper respiratory tract. Therefore, it may be prudent to screen a percentage of hamsters by culturing the nasopharynx for *P. pneumotropica*, *Pseudomonas* sp., and beta hemolytic *Streptococcus* prior to radiation exposure.

Cutaneous Bacterial Abscesses

Skin abscesses can occur in hamsters at any location, but are most prevalent near the head, sometimes involving the cheek pouch (Burke, 1979). Abscesses can contain a variable amount of purulent exudate. The most commonly reported organism isolated from skin abscesses is *Staphylococcus aureus*. Other organisms associated with cutaneous abscesses are *Streptococcus* sp. (Frisk, 1987), *Actinomyces bovis* (Gorlin and Chaudry, 1959) and *Pasteurella pneumotropica* (Harkness and Wagner, 1995; McKenna et al., 1970). Abscesses may be associated with superficial injuries caused by poor caging or fighting (Frisk, 1987; Gleiser, 1974).

The oral route of infection may be predominant when abscessed lesions involve the submandibular and cervical lymph nodes (Frisk, 1987). The most predominant clinical sign is a swelling which may rupture and cause matting of the fur. Cervical lymphadenitis takes weeks to develop and may become severe enough to cause anorexia and death (Frisk, 1987).

Treatment may be attempted through surgical drainage and flushing with an antiseptic. Topical and systemic antibiotic therapy may also be employed. Caging should be examined for sharp edges or protruding wire and affected hamsters should be isolated from others to prevent fighting.

Pseudotuberculosis

Infections of *Yersinia pseudotuberculosis* in hamsters are very rare and do not occur in well-managed colonies. The chronic form of pseudotuberculosis produces gastrointestinal lesions which resemble tubercular lesions (Handler, 1965). The clinical course of the disease progresses slower in hamsters than other species (Frisk, 1987). Clinical signs may include intermittent diarrhea and cachexia prior to death. The organism can be cultured from small necrotic nodules that may occur in the intestines, mesenteric lymph nodes, liver, spleen, and lungs. Introduction of *Y. pseudotuberculosis* into a hamster colony can usually be attributed to contamination of food or water with the feces of wild rodents or birds (Gleiser, 1974). Sources of food and water and husbandry practices should be critically reviewed. Control of pseudotuberculosis is best accomplished by depopulation followed by sanitation of all equipment prior to repopulation with unaffected hamsters.

Actinomycosis

The occurrence of *Actinomyces bovis* infection in hamsters appears to be extremely rare. A tumor in the submaxillary salivary gland of a hamster where the salivary gland duct had been previously ligated was

attributable to *A. bovis* infection (Gorlin and Chaudry, 1959). Infection with this organism should be considered whenever an abscess is found.

Miscellaneous Bacterial Infections

Several conditions and/or lesions of hamsters have been reported to have a bacteria associated with them; however, there is a paucity of specific information on each disease. An "unidentified coryneform organism" has been associated with vaginitis (Sebesteny, 1979). Kidney lesions were reported to be due to *Proteus vulgaris*, *Proteus mirabilis*, and *E. coli* (Sebesteny, 1979). *Pseudomonas aeruginosa* has been listed as a cause of generalized fatal septicemia in hamsters (Sebesteny, 1979). *Staphylococcus aureus* may be associated with ulcerative dermatitis as well as cutaneous abscesses (Gleiser, 1974).

FUNGAL DISEASES

Although the hamster has been used as an animal model for fungal infections, spontaneously occurring fungal diseases are extremely rare. *Encephalitozoon cuniculi* is presently classified as an atypical fungus.

Dermatomycosis

Although dermatomycosis caused by *Trichophyton mentagrophytes* has occurred in the Djungarian hamster, cases in Syrian hamsters are not well documented. Sebesteny (1979) includes *Trichophyton* and *Microsporum* as causes of skin lesions in hamsters. The report states that infections with these organisms may be asymptomatic or may cause dry scaly encrustations with broken hair. Diagnosis can be confirmed with culture. Treatment with antifungal agents is reported by Sebesteny (1979) as not reliable; however, topical fungicides and oral griseofulvin may be attempted. Incidence of infection in hamsters or individual case reports are not described by Sebesteny (1979). Ringworm infections may be transmissible to humans as evidenced by a case of *T. mentagrophytes* infection which occurred on the arm of an employee working with hamsters in Romania (Alteras, 1966). Although hamsters were reported as the source of this human infection, cultures from suspected carrier hamsters were not recorded.

Cryptococcosis

Cryptococcal infection occurred concurrently with tuberculosis in a group of hamsters that experimentally received cortisone and a tumor transplant from a

human with tuberculosis (Chesterman, 1972). Multiple nodules containing mycobacterium and fungi, "morphologically indistinguishable from *Cryptococcus neoformans*", were observed. Nodules occurred in peritoneum, spleen, mesentery, and lungs 4 months after the tumor transplant.

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Viral Diseases

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INTRODUCTION

Compared to other species, such as the mouse and the rat, there are only very few viruses that have definitively been associated with the hamster and those that are, generally, affect the Syrian hamster. While we can only speculate as to the reasons for this phenomenon two explanations come to mind. It seems that the number of viruses found in laboratory rodents seems to be directly proportional to their popularity as experimental animals. Most rodent viruses, and the hamster is no exception, only produce subclinical infections in their hosts and they generally were only discovered accidentally. It stands to reason that these accidental discoveries would be more frequent, the more frequently an animal species is used in research.

Another possible explanation may be found in the history of the hamster, again primarily the Syrian hamster, and how they became introduced into the laboratory. All Syrian hamsters used today in animal research are believed to be descendants of only a handful of animals captured in Syria in 1930. One could argue that if there indeed has not been any additional contact with the wild population all hamster-specific viruses we know today must have been present in the initially captured animals. It is therefore not surprising that most of the agents described in this chapter have a broader host range and naturally infect other rodent species as well.

As mentioned above most viral infections in hamsters are clinically silent with the notable exception of the hamster polyomavirus and to a lesser degree the hamster parvovirus. These seem to be the only agents that consistently produce clinical disease. Not surprisingly these viruses may actually not be natural pathogens of the Syrian hamster. However, despite the lack of clinical signs the agents described in this chapter may have a significant negative impact on the research that is conducted while using infected animals. As the number of viruses, the potential for confounding biomedical research is most likely underreported. The one hamster virus that arguably has the most significant impact on human medicine is lymphocytic choriomeningitis virus (LCMV) due to its zoonotic potential. While it also is primarily a mouse virus, the hamster, albeit the pet hamster, is the most common animal species to transmit the virus to humans.

DNA VIRUSES

Hamster Polyomavirus

Background

Hamster polyomavirus was first reported by Graffi et al. in 1967 in a colony of Z3/BlnA Syrian hamsters. The hamsters developed spontaneous skin epitheliomas

from which viral particles were later identified (Graffi et al., 1968a, 1968b, 1970). The same virus also causes lymphoma, which is atypical of a polyomavirus (Delmas et al., 1985). The agent was previously misidentified as a papillomavirus; however, DNA sequencing and analysis of genetic structure have confirmed the virus as a polyomavirus (Bastien et al., 1985; Delmas et al., 1985; Simmons et al., 2001). The agent has been rarely reported in laboratory hamsters in the US and Europe, has been reported in an 8-week-old pet Syrian hamster and may be common in certain colonies of pet hamsters (Foster et al., 2002; Percy and Barthold, 2007; Simmons et al., 2001).

Historically, hamster polyomavirus has been abbreviated to HaPV. This abbreviation is confusing since hamster parvovirus is also abbreviated as HaPV. The International Committee on Taxonomy of Viruses uses the abbreviation HaPyV in reference to hamster polyomavirus, therefore that abbreviation will be used for this text (ICTV, 2002).

Etiology

HaPyV is a member of the Polyomaviridae family in the *Polyomavirus* genus (ICTV 2009). HaPyV is a closed, circular, supercoiled double-stranded, non-enveloped DNA virus, which has a 5366-bp genome, which is larger than mouse polyomavirus and SV40 (Bottger et al., 1971; Delmas et al., 1985; Scherneck et al., 1979). The ability of HaPyV to undergo episomal amplification and successfully integrate into the host genome is a quality that distinguishes it from papillomaviruses, which only replicate as episomes (Delmas et al., 1985; Simmons et al., 2001). Sequence homology also indicates that HaPyV is evolutionarily related to murine and simian polyomaviruses (Scherneck et al., 1979; Vogel et al., 1986). Similar to mouse polyomavirus strain A2, HaPyV has coding capacities on both DNA strands for the early and late transcriptional units of viral replication and they are the only polyomaviruses that produce middle T antigens (Delmas et al., 1985; Simmons et al., 2001; Vogel et al., 1986). Small, middle, and large T antigens are early-phase transcripts that regulate replication of the viral genome, activate the late-phase transcriptional unit, autoregulate the early promoter, and induce tumor formation (Courtneidge et al., 1991; Pipas, 1992; Simmons et al., 2001). It is known that HaPyV and its cloned DNA can transform rodent cell lines (Scherneck et al., 1987). Of the primate polyomaviruses, monkey lymphotropic virus (LPV) shares the greatest base sequence homology with HaPyV and also shares its lymphotropism (Vogel et al., 1986).

Epizootiology and Pathogenesis

Although the exact origin of HaPyV has not been determined, the virus has been isolated from the spleen

and kidney of subclinically infected European hamsters and this suggests that they are the natural hosts of the virus (Hannoun et al., 1974; Mahy, 2001). It is likely that the organism was transmitted to laboratory strains after the two species co-mingled (Barthold, 1991; Percy and Barthold, 2007).

Transmission of HaPyV is likely horizontal through ingestion of virions in the environment or from contaminated fomites and less likely from aerosolization of dried dirty litter (Ambrose and Coggin, 1975; Coggin et al., 1983). The virus enters target cells by endocytosis and is transported to the nucleus where it is uncoated and begins to replicate (Simmons et al., 2001). There are three reported phases of viral replication that include acute infection, viral clearance, and viral DNA accumulation leading to tumor induction (Prokoph et al., 1996). Within epitheliomas, infected cells are permissive to viral replication resulting in cell lysis and release of viral particles. Alternatively, cells may be non-permissive to replication leading to either genomic integration of the virus or, more commonly, episomal amplification with non-random deletions in the late coding regions of the genome. Lymphocytes appear to be non-permissive to viral replication resulting in neoplastic transformation of the host cell (Mazur et al., 1995; Scherneck et al., 1987; Simmons et al., 2001).

HaPyV is a unique viral entity in the papovavirus family in that it displays tropism for both undifferentiated keratinocytes and lymphocytes. Viral DNA has been localized within transformed lymphocytes, lymph nodes, bone marrow, renal tubular epithelium of the papillary collecting ducts, uterus, amnion, embryos, and in large quantities in jejunal enterocytes (Prokoph et al., 1996; Simmons et al., 2001). Some papers have reported only rare observation of viral DNA in trichoepitheliomas (Mazur et al., 1994; Prokoph et al., 1996). After inoculation with cell-free tumor extracts the virus proliferates in secondary lymphoid tissues in the thymus, spleen, and lymph nodes, as well as non-hematopoietic tissues such as the liver and less commonly in the lung and kidney (Mazur et al., 1994; Prokoph et al., 1996; Scherneck et al., 1979). Experimentally, thymectomy greatly reduces the occurrence of lymphoma in HaPyV-infected hamsters and supports the notion of viral tropism for T lymphocytes (Graffi et al., 1970; Rudolph and Graffi, 1973). It is presumed that viral spread occurs hematogenously through infected circulating lymphocytes.

HaPyV infection may be subclinical or, due to its variable tissue tropism, it may cause two different disease syndromes. The syndrome observed depends upon the immunological status of the colony and the age of the animal when infected. The first disease course is noted in naïve juvenile hamsters resulting in epizootic multicentric lymphoma mainly forming in the

mesentery, intestines, and liver, and often other organs such as the kidney and thymus (Barthold et al., 1987; Graffi et al., 1969; Simmons et al., 2001). In one report approximately half of a colony of inbred LSH and random bred LVG hamsters developed epizootic lymphomas around 17–19 weeks old when there had been no occurrences of lymphoma noted in the colony over the previous 5 years (Ambrose and Coggin, 1975; Coggin et al., 1983). Graffi et al. (1969) hypothesized that HaPyV induced lymphoma formation via activation of a secondary virus of a C-particle type, hamster leukemia virus. However, this was later discounted when other groups did not observe C-type particles in lymphomas or observed them in non-infected animals. The observation of C-type particles in lymphomas is now considered an incidental finding (Coggin et al., 1983; Graffi et al., 1969; Percy and Barthold, 2007; Scherneck et al., 1987).

Lymphoma may be induced by injection of virus or viral DNA (Barthold et al., 1987; Graffi et al., 1969, 1970). The agent has been successfully isolated from homogenized lymphomas in cell-free media using protamine sulfate buffer and although transmission of lymphoma is possible, Coggin et al. (1981) were unable to detect any viral or viral-like particles in the lymphoma using electron microscopy. Cell-free tumor transmission of epithelioma extracts may result in a 30–80% incidence of leukosis in naïve neonatal hamsters post-injection in 1–2 months, but can vary from 3 weeks to 8 months (Graffi et al., 1968b, 1969, 1970). The induced tumors can then be transferred into hamsters of all ages and produce localized tumors (Graffi et al., 1968a).

The second syndrome is observed in older hamsters or in colonies of endemically infected animals and results in the formation of trichoepitheliomas on the face, feet, neck, back, flanks, and less commonly on the abdomen of animals ranging in age from 3 months to over a year (Coggin et al., 1985; Graffi et al., 1968a, 1970; Simmons et al., 2001). One colony of random-bred HaB hamsters developed multiple skin epitheliomas in 5–10% of animals 6–9 months old (Graffi et al., 1968a, 1970, 1972; Scherneck and Feunteun, 1990). Young hamsters typically do not develop lymphomas due to protection from maternal antibodies (Barthold et al., 1987).

Simultaneous formation of epitheliomas and lymphomas in a single animal is a rare occurrence and the life cycle of HaPyV does not require tumor formation (Barthold et al., 1987). In contrast to HaPyV, murine polyomaviruses result in disease only in neonatal or immunocompromised animals, making HaPyV unique in causing disease in enzootically infected hamster colonies. Another difference between the two agents is noted in the ability of murine polyomavirus to induce multiple tumor types with the exclusion of lymphomas and leukemia (Courtneidge et al., 1991).

It is believed that, similar to mouse polyomavirus, HaPyV persists in the renal tubular epithelium and sheds viral particles in the urine thus contaminating the environment although some reports have indicated only small amounts of viral DNA in the kidneys (Mazur et al., 1994; Simmons et al., 2001). Additional routes of viral shedding are through virally infected keratinocytes exfoliated during grooming or aggressive interactions and virus-containing enterocytes released in feces that contaminate the environment and consumed by coprophagic cage mates (Simmons et al., 2001).

Clinical Manifestations

Animals with lymphoma may have variable clinical signs depending on the region infiltrated by the neoplasm, but may include reduced appetite, weight loss, diarrhea, dehydration, poor grooming, dyspnea, and ataxia. On physical examination animals may have palpable abdominal masses or enlarged lymph nodes (Barthold et al., 1987; Coggin et al., 1978, 1983; Simmons et al., 2001). Numerous secondary disease conditions have been reported in hamsters with epizootic lymphoma including non-bacterial enteritis, ileitis, ulcerative bowel disease, multiple intussusceptions, pyelonephritis, and hepatic disorders (Coggin et al., 1978, 1983; Mancini et al., 1984).

Trichoepitheliomas are observed as miliary nodules that develop and progress to coalescing nodules within the cutis. Tumor growth may be gradual and masses may regress spontaneously (Graffi et al., 1968a, 1970). No other adverse clinical effects have been noted.

Pathology

Lymphomas can appear in almost any tissue, but are most common in the intestines, mesentery, and liver. Tumors are also frequently observed in the spleen, cervical and mesenteric lymph nodes (Figure 31.1), occasionally in the thymus, stomach, kidney, skin, lung, diaphragm, aorta, skeletal muscle, bone marrow, testes, perirenal and axillary lymph nodes, variably in the spleen, and rarely in the eye and inguinal lymph nodes. Tumors may be single or multiple, and may be primary or metastatic lesions (Ambrose and Coggin, 1975; Barthold et al., 1987; Coggin et al., 1983; Graffi et al., 1968a, 1970). Tumors are round, soft, yellow-white in color and may have centralized necrosis (Coggin et al., 1983). Multiple adhesions may also be noted within the abdominal cavity (Simmons et al., 2001).

The morphological appearance of lymphomas can vary from immunoblastic to plasmacytoid. Cells may have round to oval nuclei that may be occasionally cleaved with varying degrees of mitosis or lymphomas may show moderated pleomorphism with increased cytoplasm, fascicular nuclei, irregular nuclear

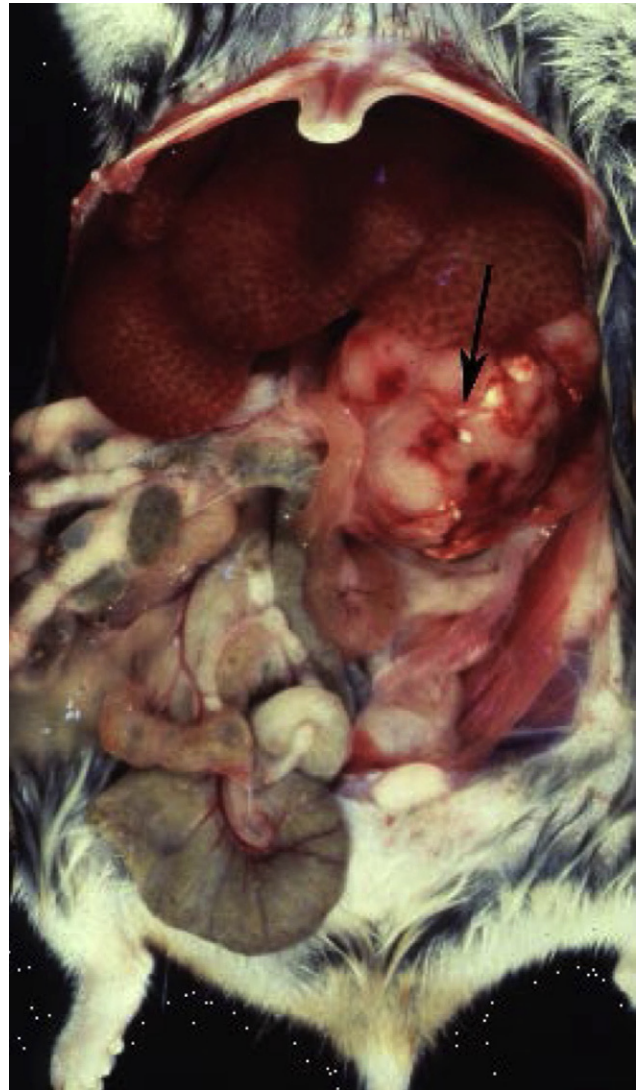


FIGURE 31.1 Abdominal viscera of a hamster infected with epizootic hamster polyomavirus resulting in the formation of a large mesenteric mass (arrow) and hepatic lymphoma (Barthold et al., 1987).

membranes, and multiple nucleoli (Ambrose and Coggin, 1975; Coggin et al., 1983). Tumors are typically lymphoid, with myeloid, reticular and erythroid cellular types occurring less frequently (Graffi et al., 1968b, 1969; Prokoph et al., 1996). The majority of lymphomas in a large outbreak were B-cell origin, but T-cell lymphomas have been identified in the thymus (Coggin et al., 1978, 1983; Mazur et al., 1994). Lymphomas, in contrast to skin epitheliomas, do not contain viral particles (Graffi et al., 1970). Non-randomly deleted extrachromosomal viral DNA has been detected in large quantities in the neoplastic cells of lymphomas (Scherneck et al., 1987). These deletions are unique to DNA molecules of the lymphoma and may lead to the inactivation of viral production due to defects in late



FIGURE 31.2 Multiple trichoepitheliomas on the chin of a hamster infected with polyomavirus (Foster et al., 2002).

protein synthesis; consequently, tumor induction may not require viral production and viral mutations with accumulation of viral DNA may be favorable for oncogenesis (Mazur et al., 1995; Scherneck et al., 1987).

Trichoepitheliomas are observed as multicentric to coalescing nodules (Figure 31.2) within the cutis and subcutis (Courtneidge et al., 1991; Graffi et al., 1968a, 1969, 1970). Histologically, the epitheliomas originate from the hair follicular epithelium (Figure 31.3) and form cyst-like structures containing cornified cells and occasionally melanin (Figure 31.3) (Graffi et al., 1968a, 1968b, 1970). HaPyV virions are easily identified as approximately 40-nm diameter viral particles in the nuclei of keratinized cells, but they are never observed within the cells of the stratum basale or spinosum (Graffi et al., 1968a, 1970). Large numbers of mitotic figures may be observed within the basal epithelium (Graffi et al., 1968a). HaPyV has tropism for the hair follicular keratinocytes, which additionally distinguishes it from papillomaviruses that localize within interfollicular epidermal keratinocytes (Scherneck and Feunteun, 1990).

Diagnosis

Diagnosis of lymphoma in a hamster less than 2 years of age is strongly suggestive of HaPyV since lymphoma is rarely observed in young HaPyV-free hamsters (Coggin et al., 1983; McMartin, 1979; Trentin, 1987). The observation of trichoepitheliomas has always been associated with HaPyV infection in hamsters and is therefore diagnostic (Percy and Barthold, 2007). Confirmatory laboratory tests including PCR, histology, immunohistochemistry, and electron microscopy have all been described (Simmons et al., 2001). The virus can also be successfully grown in GD36 cells resulting in virions that are indistinguishable, both structurally and functionally, from virions isolated from epitheliomas (de la Roche Saint Andre et al., 1990).

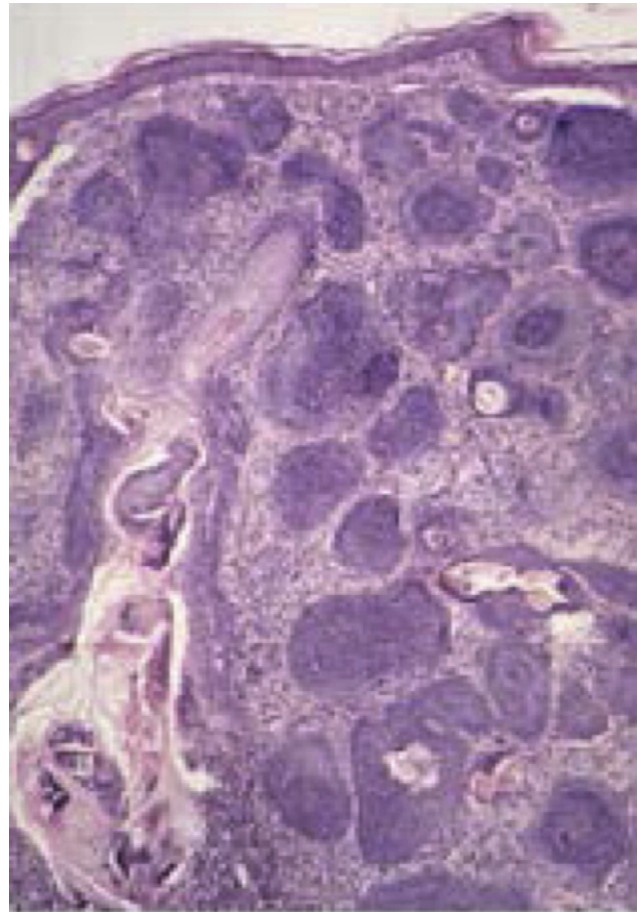


FIGURE 31.3 Follicular lesions with central keratinization from a contact-exposed hamster with HaPyV cutaneous trichoepithelioma (Barthold et al., 1987).

Sentinel hamsters may help to identify epizootics of lymphoma. It has been reported that non-contact sentinels can develop lymphoma and contact sentinels can develop lymphoma or cutaneous epitheliomas (Barthold et al., 1987).

Prevention and Control

Therapy is not recommended due to potential for contamination of the animal facility. Culling of the entire colony, aggressive decontamination of the facility and improved use of personal protective equipment has failed to prevent new outbreaks in some facilities. In these colonies, incoming hamsters had no history of infectious lymphoma (Ambrose and Coggin, 1975; Coggin et al., 1978). The virus is stable in the environment for prolonged periods of time, is resistant to UV light, formaldehyde vapor, RNase, proteinase K, chlorinated and iodinated disinfectants, but susceptible to DNase, phenols, and KOH (Ambrose and Coggin, 1975; Coggin et al., 1983; Mancini et al., 1984).

Hamster Parvovirus

Background

Hamster parvovirus is a relatively new viral agent affecting multiple organ systems resulting in high mortality. It was first described in 1982 in a colony of Syrian hamsters that experienced high mortality in suckling and weanling animals (Gibson, 1983). Rodent parvoviruses are common contaminants of laboratory animal colonies, tumor transplants, and cell cultures, and may have confounding influences on immune, transplant, and cancer research (Jacoby et al., 1995; Shek et al., 1998; Smith et al. 1993).

Etiology

Hamster parvovirus is a 20-nm diameter, non-enveloped single-stranded DNA virus with an icosahedral capsid in the family Parvoviridae (Cotmore and Tattersall, 1987; Soderlund-Venermo et al., 2001). Approximately 92.6% of the negative-sense DNA genome is encapsidated (Besselsen et al., 1996). Molecularly, HaPV shares 94.6% sequence homology with MPV-1 and over 98% homology with MPV-3. HaPV also shares great similarity with the NS1 proteins of MVMc, MVMm, and MPV-3 and has similar VP1 amino acid sequences as MPV-1a, MPV-1b, and MPV-1c (Besselsen et al., 1996, 2006).

Epizootiology and Pathogenesis

It is suspected that the hamster may not be the natural host for HaPV due to the observation that many rodent parvoviruses, such as MPV, are subclinical in their host species and yet multiple rodent parvoviruses cause a similar course of disease in young hamsters (Christie et al., 2010). However, it should be noted that minute virus of mice and rat parvovirus can cause disease in their natural hosts (Christie et al., 2010; Jacoby et al., 1991; Kimsey et al., 1986). The mouse has been implicated as the natural host of HaPV due to the discovery of MPV-3 in mouse colonies that is nearly genetically identical to HaPV (Besselsen et al., 2006, 2008; Christie et al., 2010). Recent research has shown the experimental infection of immunocompetent and immunodeficient mice of various ages with HaPV that had similar pathogenesis to mice infected with MPV-1 and therefore HaPV may more appropriately be called MPV-3/HaPV until further characterization clarifies the relationship of these two organisms (Christie et al., 2010). Infection of hamsters with mouse MPV-3 has yet to be demonstrated.

MPV-3/HaPV is most likely acquired through ingestion or inhalation (Jacoby et al., 1996). Hamster parvovirus is distributed systemically throughout the infected animal possibly due to persistent viremia or viral DNA infection within endothelial cells (Besselsen et al., 1999; Christie et al., 2010).

Clinical Manifestations

Two- to 4-week-old hamsters ranging in weight from 15–20 grams are most susceptible to MPV-3/HaPV infection (Besselsen et al., 1999). Affected neonatal and weanling hamsters are runted with incisor abnormalities, domed craniums, small testicles, and a potbellied appearance (Gibson, 1983). Infection may also cause diarrhea, ataxia, and mortality resulting from hemorrhagic disease (Besselsen et al., 1999). Surviving hamsters seroconvert in 1–3 weeks, but animals remain persistently infected for several weeks. It is unclear whether persistently infected animals shed viral particles. Humoral immunity protects animals from subsequent infections. Infection in adult hamsters is typically subclinical (Jacoby et al., 1988, 1996).

Pathology

An array of lesions may be noted grossly and include incisor root abscesses, periodontal disease, discolored, malformed or absent incisors, and facial bone deformities (Besselsen et al., 1999). Internal examination may reveal intestinal hemorrhage, renal petechiation, a pale liver and spleen, testicular atrophy, and cerebellar hypoplasia (Besselsen et al., 1999).

Histologically, there may be necrosis, hemorrhage, inflammation, and mineralization of the incisive dental pulp with enamel hypoplasia (Figure 31.4) resulting from ameloblast necrosis (Besselsen et al., 1999; Gibson, 1983). Periodontitis, monocytic infiltration of the dental lamina, and alveolar bone osteoclasts are additional lesions noted in the oral cavity (Gibson, 1983). Hemorrhage beneath the renal capsule, adjacent to the cerebral lateral ventricles, and in the granular layer of the cerebellum, gastrointestinal tract, testicles, uterus, and hippocampus may be noted (Besselsen et al., 1999). Other lesions include multifocal cerebral mineralization, testicular hypoplasia with multifocal epithelial necrosis, mineralization of the seminiferous tubules, and pampiniform plexus perivasculitis (Gibson, 1983; Jacoby et al., 1996). Low viral doses of MPV-3/HaPV inoculated into young hamsters result in a mild disease with incisor and testicular abnormalities, whereas high viral doses result in a multi-systemic hemorrhagic syndrome involving multiple organ systems (Besselsen et al., 1999). Basophilic intranuclear inclusion bodies have been observed in the endothelial and mesenchymal cells of the small intestine (Besselsen et al., 1999).

MPV-3/HaPV may be cultured in baby hamster kidney (BHK-21) cells and rat RN cells. Culture in human NB3224K cells is possible but with lower kinetics whereas murine cells have not been shown to be permissive to viral infection with MPV-3/HaPV (Besselsen et al., 1996; Soderlund-Venermo et al., 2001).

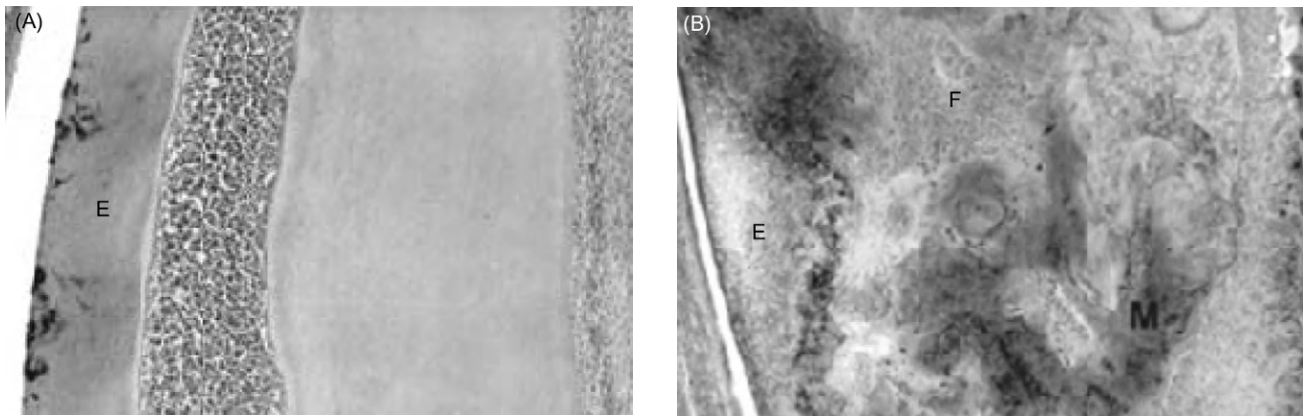


FIGURE 31.4 Longitudinal photomicrographs of incisors from (A) a 6-week-old sham-infected hamster and (B) an age-matched HaPV-infected animal with enamel hypoplasia. E, enamel hypoplasia; F, fibrosis; M, mineralization (Besselsen et al., 1999).

Diagnosis

Diagnosis may be made with PCR using MPV-3/HaPV-specific primers for viral DNA or with bead-based suspension microarray for MPV-3/HaPV antibody detection (Besselsen et al., 1999, 2006; Christie et al., 2010; Redig and Besselsen, 2001). MPV-3/HaPV may cross-react to MPV-1 in hemagglutination inhibition assays (Besselsen et al., 1996).

Prevention and Control

Infection of mice with MPV can have serious research implications that include tumor suppression, accelerated graft rejection, and alteration in interferon production and lymphocyte proliferation and activity; therefore, careful consideration is required when using hamsters infected with MPV-3/HaPV in these types of studies (Christie et al., 2010; Shek et al., 1998; Smith et al., 1993). Monitoring cell lines, serum, and transplantable tumors prior to introduction into a colony is critical to preventing introduction of MPV-3/HaPV. If the agent is inadvertently introduced into the colony then control measures similar to those employed for mice should be used and include quarantine, thorough facility disinfection, and restocking with non-infected animal. The agent may be killed by heat sterilization of caging and equipment (Jacoby et al., 1996).

Adenovirus

Background

Adenoviral inclusion bodies are commonly found in naturally infected hamsters but no specific hamster adenovirus has been isolated. Hamsters can be experimentally infected with adenoviruses from a variety of other species (ICTV, 2002).

Clinical Manifestations

Adenoviral infections are typically subclinical unless an animal is stressed or immunocompromised (Richter, 1986). Seroconversion to two strains of murine adenovirus, FL strain (MAdV-1) and K87 strain (MAdV-2), has been reported to naturally infect laboratory hamsters (Gibson et al., 1990; Reed et al., 1974; Schoonermark-van de Ven et al., 2006; Suzuki et al., 1982; Wigand et al., 1977). Hamsters infected naturally with adenovirus are typically subclinical (Gibson et al., 1990). Experimental inoculation of neonatal hamsters with adenoviruses from other species, including primates, mice, bovids, and poultry, has been used to induce oncogenesis in a variety of tissues (Trentin, 1987; Trentin et al., 1962).

Pathogenesis and Pathology

In mice, MAdV-1 infection is chronic with persistent shedding of virus in the urine for months, whereas MAdV-2 infection is shed in the feces from infected intestinal cells for 3–4 weeks (Richter, 1986). Naturally infected weanling hamsters with antibodies to enteric MAdV-2 displayed large amphophilic intranuclear inclusions in ileal enterocytes overlying gut-associated lymphoid tissue, goblet cells, and rarely crypt cells of the ileum and jejunum. The adenoviral particles formed paracrystalline arrays only in condensed nuclei of pyknotic intestinal cells (Gibson et al., 1990). Only sporadic evidence of hamsters with antibodies to MAdV-1 has been reported (Suzuki et al., 1982).

Diagnosis, Prevention, and Control

Adenoviral infections may be diagnosed using serology and electron microscopy (Gibson et al., 1990; Percy and Barthold, 2007). The significance of adenoviral infection in hamsters is unknown (Percy and Barthold, 2007).

Cricetid Herpesvirus

Cricetid herpesvirus (CrHV-1), also known as hamster cytomegalovirus, is a double-stranded, linear DNA virus that has been rarely reported in adult Chinese hamsters (*Cricetulus griseus*) (Davison et al., 2009; ICTV 2002; Kuttner and Wang, 1934). Infection in Chinese hamsters is clinically silent (Kuttner and Wang, 1934). Sialodacryoadenitis due to viral infection resulting in keratoconjunctivitis sicca, exophthalmia, and proptosis has been suggested in pet hamsters (Donnelly, 2003). Young hamsters inoculated intracerebrally with tissues from infected adult hamsters died within days after inoculation with meningitis and intranuclear inclusions (Kuttner and Wang, 1934). Eosinophilic intranuclear and intracytoplasmic inclusions in the acinar epithelium and mononuclear cell infiltrates have been observed in the submaxillary glands in adult hamsters naturally infected with the virus (Kuttner and Wang, 1934). The impact and significance of this virus on research in laboratory colonies of Chinese hamsters remains unknown.

Hamster Oral Papillomavirus

Background and Etiology

Virally induced oral papillomas were discovered in a model of lingual carcinoma in Syrian hamsters after tumor induction with a combination of 9,10-dimethyl-1,2-benzanthracene (DMBA) and wounding (Iwasaki et al., 1997; Maeda and Kameyama, 1986; Maeda et al., 1989). Episomal nucleic acid was identified and the genome of the supercoiled DNA virus was digested revealing a unique viral entity distinct from other rodent papillomaviruses and HaPyV. The agent was classified as hamster oral papillomavirus (HaOPV) (ICTV, 2002; Iwasaki et al., 1997).

Epizootiology and Pathogenesis

It has been reported that the formation of lingual lesions requires application of a chemical carcinogen in combination with wounding as either method alone is not sufficient in hamsters (Maeda et al., 1989). The administration of a chemical carcinogen possibly reactivates a latent virus that can be identified in large quantities by PCR and Southern blot hybridization. In the same study, small amounts of HaOPV DNA were identified only by PCR in normal, untreated lingual tissue (Iwasaki et al., 1997).

Clinical Manifestations and Pathology

Lingual lesions were small (~1 mm in diameter) with squamous epithelium, disoriented stratification, cellular and nuclear pleomorphism, aggregations of perinuclear hyalin, basal cell proliferation, koilocytotic changes that are consistent with papillomas observed in other species

and a mild inflammatory response (Iwasaki et al. 1997; Maeda et al. 1989). The significance of HaOPV in other models and research remains unknown.

RNA VIRUSES

Sendai Virus

Background

In 1952 an unrecognized infectious agent was isolated from a mouse that had been inoculated with a lung emulsion from the autopsied lung of a newborn child (Kuroya et al., 1953; Sano et al., 1953). Because the isolated virus was thought to have originated in the newborn child, Sendai was initially believed to be a human respiratory virus and was subsequently named "Newborn Virus Pneumonitis (Type Sendai)" (Sano et al., 1953). In 1954 it was proposed that the virus was not of human origin and may have been present in the mice used for the initial viral isolation (Fukumi et al., 1954). The close relationship between Sendai virus and human parainfluenza 1 may have created confusion regarding the host origin of the virus. Human parainfluenza 1 was isolated subsequent to Sendai in 1958 (Brownstein, 2007; Chanock et al., 1958).

Sendai virus is considered to be the prototype virus of the family Paramyxoviridae (Homma et al., 1999) and is an agent of significance because it is capable of causing morbidity and mortality in mice, rats, and hamsters. Sendai is an undesirable pathogen in rodent research colonies because of the gross and microscopic lesions created by the virus and the ability of the virus to alter humoral and cell-mediated immune responses (Kay, 1978; Warr and Jakab, 1979). Additionally Sendai viral infections may also influence experimental carcinogenesis and Sendai has been reported to alter the tumorigenicity of transplantable tumor lines (Aderka et al., 1986; Matsuya et al., 1978; Peck et al., 1983).

Etiology

Sendai virus belongs to the genus *Respirovirus* of the subfamily *Paramyxovirinae* in the family *Paramyxoviridae* and is considered a murine subtype of human parainfluenza type 1 (ICTV, 2009). Sendai virus morphologically appears as an enveloped spherical to filamentous virion. The virus is composed of single-stranded negative-sense RNA genome (Homma et al., 1999). Viral replication occurs exclusively in the cytoplasm of the host cell. One replication cycle takes approximately 12–15 hours with one cell yielding thousands of virions (Homma et al., 1999).

One of the three functional proteins associated with the virion envelope, the fusion (F) protein, needs to be cleaved into two disulfide subunits before becoming

biologically active. Specific proteases within the host are responsible for initiating cleavage. These “activating” proteases are primarily found within the respiratory tract. One serine protease, “Trypsin Clara”, is thought to be partially responsible for the respiratory tropism of the virus. Trypsin Clara is secreted by Clara cells located throughout the terminal conducting airways and is a protease capable of cleaving F, thereby activating the virus (Kido et al., 1992; Tashiro et al., 1992).

Epizootiology and Pathogenesis

The natural host range includes mice, rats, and Syrian and Chinese hamsters (Parker et al., 1966; Percy and Barthold, 2007; Percy and Palmer, 1997). Guinea pigs have been shown to be susceptible to experimental infection (Elwood et al., 1993). Population studies in the 1960s and 1970s varied widely suggesting that 50–83% of hamster colonies were affected (Ishida and Homma, 1978; Parker et al., 1987). Alternatively, a study done in 1974 testing 201 Syrian hamsters, reported only 2% to have antibody to Sendai virus (Reed et al., 1974). No further epidemiological data in hamsters have been published since then, but based on more recent reports in mice and rats suggest a decreasing overall prevalence in laboratory animals (Zenner and Regnault, 2000).

Transmission studies in mice have indicated that direct contact with infected rodents or contaminated fomites constitutes the primary route of infection. Aerosol inhalation has been successfully used to experimentally infect hamsters (Blandford and Charlton, 1977). Findings in mice and rats suggest vertical transmission can occur after experimental infection. A study in hamsters found no difference in susceptibility to Sendai virus based on age at the time of exposure, however, virus replication was found to be higher in younger animals (van Nunen and van der Veen, 1967).

It has been determined in studies done in the mouse that natural infection causes a descending infection which is usually restricted to the mucociliary epithelium of conducting airways. Sendai virus is capable of spreading to the alveolar epithelium, although there seems to be limited viral replication at this level (Brownstein, 2007). Viral infection promotes the secretion of trypsin Clara (located in non-ciliated secretory cells of terminal conducting airways) and assists in viral replication by cleaving the F glycoprotein (Kido et al., 1992; Tashiro et al., 1992). In male Syrian hamsters intranasally inoculated with Sendai, viral antigen was present postinoculation day (PID) 3 in the respiratory tract epithelium of the nasal passages and trachea with bronchiolar extension by PID 5. Antibodies were first detected PID 7 with antibody titers remaining high throughout the 21-day study (Percy and Palmer, 1997).

Clinical Manifestations

Sendai infection in Syrian hamsters typically is clinically silent with low mortality rates (Parker et al., 1987; Profeta et al., 1969). Occasional deaths in suckling hamsters were reported from one enzootically infected colony (Profeta et al., 1969). Chinese hamsters and immunosuppressed Syrian hamsters can succumb to the disease and are documented to have extensive pulmonary lesions (Blandford and Charlton, 1977; Parker et al., 1987). Sendai virus may exacerbate secondary bacterial or *Mycoplasma* infections in hamsters. Increased lesion severity has been reported in mice experimentally co-infected with Sendai virus, *Haemophilus influenza*, and *Pasteurella pneumotropica* infections (Degre, 1970; Jakab, 1974).

Pathology

Microscopic lesions associated with natural Sendai virus infection in hamsters typically consist of a focal to segmental rhinitis progressing to necrotizing tracheitis and multifocal bronchiolitis (Percy and Barthold, 2007). Secondary bacterial infection of the lung can complicate the pathologic picture (Parker et al., 1987). Lung consolidation has been noted grossly in newborn hamsters (Hankenson and Van Hoosier, 2002b). In male Syrian hamsters inoculated intranasally with Sendai virus, gross necropsy findings were restricted to PID 5–7 and were consistent with pneumonia, characterized as dark pink to tan mottling at the hilus of the lung extending into the adjacent parenchyma (Percy and Palmer, 1997). At PID 3 minimal histopathologic findings were present, confined to the trachea and epithelium of the nasal turbinates. Cellular changes varied from hyperplasia to cytoplasmic vacuolation and degeneration of respiratory epithelial cells. Lesions were histologically evident in the lower portion of the respiratory tract by PID 7. Hyperplasia of the epithelial cells with peribronchial edema and peribronchial lymphocytic infiltration was found in affected airways. Microscopic reparative lesions could still be detected at PID 16 and 21 in the nasal passages, trachea, and alveoli (Percy and Palmer, 1997). A transient renal immune complex deposition has been reported in a small percentage of experimentally infected hamsters (Blandford and Charlton, 1977).

Diagnosis

Due to the non-specific nature of the lesions, a diagnosis of Sendai virus generally cannot be made based on gross or histopathologic lesions, nor can a single test detect viral infection at all stages of the disease. During the acute phase of the infection, virus isolation and reverse-transcriptase polymerase chain reaction (RT-PCR) can be used to detect virus in lung tissue or nasal washes. However, virus may no longer be present when the animal is displaying clinical symptoms

(Brownstein, 2007). RT-PCR can also be used to detect viral RNA in environmental samples and contaminated biological materials (Wagner et al., 2003).

Enzyme-linked immunosorbent assay (ELISA), indirect immunofluorescence assay (IFA), and multiplexed bead-based immunoassays are available serologic methods of detection. Antibody to Sendai virus can be detected 10–12 days following infection, although some studies demonstrate that antibody can occasionally be detected as early as day 7 (Liang et al., 1999; Parker et al., 1979).

Prevention and Control

There are no current published reports that document the elimination of Sendai from a hamster population. The best approach is to prevent introduction by purchasing animals that are known to be free of the virus (Parker et al., 1987). A program for colony surveillance should be established. Dirty-bedding sentinel programs have been found to be less efficacious at detecting Sendai virus in a colony of mice (Compton et al., 2004). In mice, seroconversion among dirty-bedding sentinels is variable and appears to be both dose- and strain-dependent (Artwohl et al., 1994). A more effective means of colony monitoring could include the exposure of dirty-bedding sentinels to exhaust air from individually ventilated caging systems (Compton et al., 2004). Animal biological products should be screened by PCR or by hamster antibody production (HAP) test before use in colony animals. If populations of rodents are suspected to be harboring Sendai virus infections within the same vivarium, hamsters should be maintained under strict isolation or barrier conditions to prevent cross-infection (Hankenson and Van Hoosier, 2002; Parker et al., 1987). Though never documented, culling of the colony should be an effective method to eliminate the virus. Another option could be isolation and a strict breeding moratorium for 8 weeks, which has been shown to effectively eliminate the infection in mice (Parker et al., 1987).

Lymphocytic Choriomeningitis Virus

Background

Lymphocytic choriomeningitis virus (LCMV) was discovered by Armstrong and Lillie during a 1933 investigation of an outbreak of St. Louis encephalitis in humans. The virus was named after the brain lesions it induced when inoculated into mice and monkeys (Armstrong and Lillie, 1934). LCMV was found to cause an aseptic meningitis in humans (Armstrong and Wooley, 1937). It was later discovered that the immune system played a significant role in the pathogenesis of LCMV-related disease (Buchmeier and Oldstone, 1978).

LCMV has been studied extensively and is considered the prototype virus of the arenavirus family, most often used as a model for virus-induced immune complex disease (Buchmeier and Oldstone, 1978; Oldstone and Dixon, 1969). Syrian hamsters are highly susceptible to persistent natural infections with LCMV, and the virus poses a serious public health risk due to its zoonotic potential. Congenital infection in humans has been implicated in causing chorioretinitis, hydrocephalus, microencephaly, or macroencephaly, and mental retardation in infants (Barton and Hyndman, 2000; Barton and Mets, 2001; Barton et al., 1995; Bonthius et al., 2008). Transfer of the virus from Syrian hamsters, as well as their transplantable tumors, to laboratory animal personnel or pet owners has been documented (Armstrong et al., 1969; Biggar et al., 1975, 1977; Bowen et al., 1975; Deibel et al., 1975; Gregg, 1975; Hinman et al., 1975; Hotchin et al., 1974; Maetz et al., 1976; Skinner et al., 1976). In addition, LCMV can persistently infect standard hamster cell lines which remain capable of infecting susceptible species (Biggar et al., 1976; van der Zeijst et al., 1983). LCMV transmission has also been documented with solid organ transplantation. In December 2003 and April 2005 a cluster of transplant recipients whose donor had been exposed to LCMV died of fatal lymphocytic choriomeningitis (Amman et al., 2007; CDC, 2005). LCMV viral sequences from the 2005 outbreak were identical to those found in the donor's pet hamster and the infection could be traced back to a hamster breeding colony that distributed animals nationwide (Amman et al., 2007). Medical practitioners speculate that LCMV may be frequently overlooked and underdiagnosed in human patients with neurologic disease (Barton and Hyndman, 2000; Jamieson et al., 2006).

Etiology

LCMV belongs to the family *Arenaviridae*, genus *Arenavirus* (ICTV, 2009). Arenaviruses are divided into Old World and New World serocomplexes which share genetic and antigenic relationships with one another. LCMV is included in the Old World group of viruses along with Ipply, Mobala, Mopeia, and Lassa viruses (Auperin et al., 1982; Mason et al., 2005).

Arenavirus virions are composed of a lipid-containing envelope which is covered with glycoprotein spikes evenly distributed over the envelope surface (Neuman et al., 2005). The *Arenaviridae* genome consists of two single-stranded negative-sense RNA molecules designated L (large) and S (small), coding for non-structural and structural proteins, respectively (Romanowski and Bishop, 1985; Salvato and Shimomaye, 1989).

Laboratory strains of LCMV include among others the Armstrong (Arm) and several of its variants, WE, and the Fortner strain (Dutko and Oldstone, 1983; Parker et al., 1976). The biological behavior of each strain varies.

Strains that are passaged intracerebrally, such as Arm CA 1371, enhance the neurotropism of the virus and can cause severe illness and mortality. Wild-type strains, such as the Fortner, tend to be “docile” and are more likely to induce immunosuppression (Hotchin et al., 1962; Parker et al., 1976).

Epizootiology and Pathogenesis

Mus musculus is considered the primary reservoir for LCMV (Hankenson and Van Hoosier Jr., 2002). The hamster has been implicated as a secondary reservoir and is highly susceptible to persisting natural infections (Bonthius et al., 2008; Skinner et al., 1976; Smadel and Wall, 1942). Guinea pigs, non-human primates, and humans also demonstrate a natural susceptibility to infection and disease (Skinner and Knight, 1979; Traub, 1936). A host of other species have been experimentally infected with LCMV such as rabbits, rats, dogs, cats, birds, ferrets, voles, cotton rats, horses, cows, and pigs (Lehmann-Grube, 1972; Skinner and Knight, 1979).

LCMV is found worldwide. Seroprevalence in feral mouse populations ranges from 2.5% to 9.0% in the United States, 6.8% in northern Italy, and 7.0% in Japan (Childs et al., 1992; Morita et al., 1991; Tagliapietra et al., 2009). Approximately 3% of pet hamsters from a single rodent distributor implicated in the 2005 LCMV solid organ transplant outbreak were found to be seropositive for the virus (Jay et al., 2005). In a 1993 survey of biological contaminants, LCMV was isolated from 28% of hamster transplantable tumors (Nicklas et al., 1993). Serologic surveys performed in the 1990s on healthy men and women living in Birmingham, Alabama and in Baltimore, Maryland found that 5.1% and 4.7%, respectively, possessed antibodies to LCMV (Childs et al., 1992; Stephenson et al., 1992).

High concentrations of virus have been identified in the blood, organs, urine, and feces of Syrian hamsters (Skinner and Knight, 1979; Smadel and Wall, 1942; Thacker et al., 1982). Viral shedding occurs primarily in the urine and saliva, but also in feces, milk, semen, and nasal secretions (Skinner and Knight, 1979; Traub, 1936). LCMV can be transmitted vertically or horizontally. Direct contact has been found to be the most efficient mode of horizontal transmission, specifically when traumatized epidermis or mucous membranes were exposed to infectious urine or saliva (Skinner and Knight, 1973, 1979). Indirect contact has been described in mice (Traub, 1936). Humans can be infected either by direct contact or inhalation of infectious aerosolized rodent excretions or secretions (Amman et al., 2007; Bowen et al., 1975; Skinner and Knight, 1979).

The pathogenesis of an LCMV infection in hamsters depends on the age of animal, the virus strain, dose, and route of inoculation. In one study 50% of hamsters inoculated parenterally as neonates developed

a persistent infection with lifelong viremia and viruria. The rest of the animals cleared the infection after 6 months of viremia and 12 months of viruria. Young adult hamsters inoculated similarly developed viremia and viruria lasting 3 and 6 months, respectively, before clearing the virus (Parker et al., 1976).

In immunocompetent adult mice experimentally inoculated with virus, the host immune response results in a CD8+ T-cell-mediated disease involving multiple organs. How severely each organ is affected by CD8+ T-cell-mediated disease depends on the route of inoculation. For example, intracerebral inoculation results in more notable brain lesions, while intraperitoneal inoculation results in more notable liver lesions (Hotchin et al., 1962).

Clinical Manifestations

Disease manifestation is ultimately dependent on a variety of factors including virus strain or variant, dose of virus administered, route of infection, age of the host, strain of the host, and host immunocompetence (Barthold and Smith, 2007). The clinical manifestations of natural disease also vary from what is seen clinically with experimental disease.

Natural LCMV infection in adult hamsters generally causes an acute short-term infection that rarely causes clinical signs of illness. Infections in perinatally exposed hamsters remain subclinical for at least 6 months even though the animals are shedding large amounts of virus during this period (Skinner and Knight, 1979; Skinner et al., 1976; Smadel and Wall, 1942). However, animals that remained persistently infected past this time developed a progressive wasting disease characterized by reduced activity, ruffled fur, hunched posture, blepharitis, and weight loss. These clinical signs can persist for weeks or months until the animals become moribund (Parker et al., 1976; Skinner et al., 1976). Studies have found that all hamsters, regardless of age or strain, are uniformly susceptible to the fatal wasting disease caused by LCMV when they are treated concurrently with immunosuppressive agents (Genovesi et al., 1988).

Pathology

Pathologic lesions of LCMV vary based on the animal's age, virus strain, virus dose, and the route of administration. Hamsters infected as adults or perinatally infected animals that are able to clear the virus develop minimal to moderate transient lymphocytic infiltration of the visceral organs (Parker et al., 1976). However, perinatally infected animals that are unable to clear the infection develop chronic disease characterized by lymphocytic infiltration of liver, lung, pancreas, kidney, spleen, meninges, and brain (Genovesi et al., 1988; Oldstone and Dixon, 1969; Parker et al., 1976;) as well as a widespread vasculitis and progressive

glomerulonephritis (Genovesi et al., 1988; Parker et al., 1976). The progressive glomerulonephritis can be attributed to antigen–antibody complex deposition in the arterioles and glomerular basement membranes of infected kidney (Buchmeier and Oldstone, 1978; Oldstone and Dixon, 1969). Pancreatic lesions can also be found with subacute and chronic interstitial pancreatitis, acinar atrophy, and interstitial fibrosis and have been associated with hyperglycemia and abnormal glucose tolerance (Oldstone et al., 1984).

Diagnosis

Care must be taken when using dirty-bedding sentinels to screen hamster colonies for LCMV as the virus is best transmitted via direct contact and viral transmission through dirty bedding is limited (Ike et al., 2007). Contact sentinels should be considered if LCMV is suspected to be endemic in a colony or if the colony is considered to be a high exposure risk. Serologic screening for LCMV in enzootically infected colonies may be difficult. Studies in persistently infected mice have yielded mixed results. Some found that circulating antibody to LCMV was difficult to detect presumably due to the formation of antigen–antibody complexes with the high number of viral particles present in the bloodstream (Oldstone and Dixon, 1969). Others have found that the detection of serum antibodies to LCMV was possible in most of the animals tested by both ELISA and IFA (Homberger et al., 1995). An investigation during a recent outbreak of LCMV in pet hamsters found that most hamsters where viral RNA was detected in the kidney were negative by ELISA, while some were positive by IFA (Amman et al., 2007). The efficacy of serologic testing is thus most likely assay-dependent and should not be used as the sole means to determine the status of a colony but a combination of screening tests should be used. Alternatively HAP (hamster antibody production) or PCR (polymerase chain reaction) could be used. LCMV infection in symptomatic hamsters can be readily diagnosed by serology using IFA, ELISA, or multiplexed bead-based immunoassays (Homberger et al., 1995; Thacker et al., 1982).

Prevention and Control

When populating a facility, it is important to purchase LCMV-free hamsters from reputable vendors and perform regular serologic surveillance of hamster colonies. It is also important to include in the regular surveillance any mouse colonies which could potentially be a source of exposure for hamster colonies. A pest control program needs to be established to prevent direct or indirect contact with feral mice. In addition, screening of transplantable tumors and hamster- or mouse-derived cell lines is strongly advised (Biggar et al., 1976). Studies that are utilizing LCMV or LCMV-infected animals require CDC Biosafety Level 3 containment

(Jay et al., 2005). If an LCMV outbreak were to occur, quarantine, depopulation, and thorough decontamination are recommended (Hankenson and Van Hoosier, 2002). If attempting to rederive a colony, embryos should be washed prior to transfer. Progeny should then be monitored using a combination of screening tests to confirm elimination of the virus (Ike et al., 2007).

Pneumonia Virus of Mice

Background

Pneumonia virus of mice (PVM) was discovered in 1938 during an attempt to isolate virus from human nasopharyngeal washes by serially passaging them through mice (Horsfall and Hahn, 1940). Although closely related to Sendai virus, PVM is less pathogenic and is only known to cause disease in immunocompromised mice (Brownstein, 2007). It has not been as extensively studied as Sendai virus, however, PVM infection in mice is an important model for human respiratory syncytial virus, a Pneumovirus pathogen in children (Cook et al., 1998; Rosenberg and Domachowske, 2008; Rosenberg et al., 2005).

Etiology

PVM is classified as a *Pneumovirus* of the family *Paramyxoviridae* (ICTV, 2009). PVM is pleomorphic, capable of forming both filaments and spheres, with a single-stranded negative-sense RNA genome (Compans et al., 1967). Pneumoviruses differ from other parainfluenza viruses because they lack neuraminidase. There are two primary glycosylated proteins associated with the virion envelope, the G protein and the fusion glycoprotein (F). The G protein mediates viral attachment and hemagglutination (Levine et al., 1987; Ling and Pringle, 1989). The F protein mediates fusion of the viral envelope with the host cell. As in Sendai virus, the F protein of PVM requires cleavage by host proteases in order to be biologically activated (Brownstein, 2007). PVM has been reported to grow in hamster kidney cell cultures and chick embryo cultures (Harter and Choppin, 1967; Tennant and Ward, 1962).

Epizootiology and Pathogenesis

The natural host range of PVM consists of mice, rats, and hamsters (Brownstein, 2007). However, it should be noted that the only documented isolation of PVM from hamsters was performed from animals inoculated with lung tissue suspension from mice (Pearson and Eaton, 1940). Antibody to PVM has been frequently isolated from hamsters in the past in North America, Europe, and Asia (Carthew et al., 1978; Gannon and Carthew, 1980; Lussier and Descoteaux, 1986). Early reported prevalence rates in hamster colonies have varied from

as high as 83% to as low as 10%, and were frequently found to be higher than prevalence rates reported in mouse colonies (Lussier and Descoteaux, 1986; Parker et al., 1987; Reed et al., 1974). There appeared to be a lower prevalence of PVM antibody among Chinese hamster (*Cricetus griseus*) colonies (Schiff et al., 1973). More recent surveys indicate that the prevalence of this pathogen has declined (Brownstein, 2007).

There are no documented reports of naturally occurring clinical disease associated with PVM in the hamster and the epizootiology of PVM in the hamster remains unknown. Studies done in mice indicate that PVM is not easily transmitted and that it is most likely spread through direct contact with respiratory secretions (Brownstein, 2007; Horsfall and Hahn, 1940). It has been shown that vertical transmission does not occur because antibody titers can be eliminated through cesarean derivation in successive generations of hamsters (Parker et al., 1987; Van Hoosier et al., 1970).

In pathogenesis studies performed in mice, PVM infection is restricted to the respiratory tract. Natural infection or intranasal dosing of immunocompetent mice with low viral loads appears to result in disease that is restricted to the nasal epithelium (Smith et al., 1984). Experimental inoculation of high viral loads has led to evidence of viral replication in the lower respiratory tract (Volkert and Horsfall, 1947).

Clinical Manifestations

There are no documented reports of naturally occurring clinical disease or mortality from PVM in hamsters (Parker et al., 1987). Hamsters inoculated with PVM intranasally by Horsfall and Hahn in 1939 did not display any signs of clinical disease (Horsfall and Hahn, 1940). Hamsters experimentally inoculated with mouse-passaged lung suspension showed signs of sneezing, dyspnea, and weakness with high mortality, however, retrospectively it is unclear whether PVM had been the only pathogen present (Pearson and Eaton, 1940).

Pathology

Experimental inoculation of hamsters with mouse lung suspension containing PVM has been shown to cause interstitial pneumonia with moderate to severe lung consolidation in a very early study (Pearson and Eaton, 1940). Swollen hyperemic nasal turbinates with a transient mild erosive rhinitis has been reported in both experimentally inoculated hamsters and mice (Pearson and Eaton, 1940; Smith et al., 1984).

Diagnosis

The detection of serum antibodies is the most commonly used diagnostic technique for routine monitoring. ELISA, IFA, and multiplexed bead-based immunoassays are available and show high specificity

and sensitivity. Viral isolation and RT-PCR have been described, however, due to the low prevalence of the virus and the absence of clinical signs in the infected animals these techniques are not useful as screening tools (Brownstein, 2007; Wagner et al., 2003).

Prevention and Control

The best form of control is prevention through the purchase of hamsters that are free of the virus and by performing regular serologic screening. Cesarean derivation and foster rearing eliminated infection in one hamster colony (Van Hoosier et al., 1970). Natural infection is thought to result in immunity (Tennant et al., 1966).

MISCELLANEOUS VIRUSES

A number of viruses have historically been associated with hamsters based on the detection of antibodies that cross-react with viral antigens used in mouse and rat serology. These agents include reovirus 3 (Reo-3), Theiler's meningoencephalitis virus of mice (TMEV), simian virus 5 (SV-5), and Toolan's H-1 virus (Parker et al., 1976; Percy and Barthold, 2007). Reo-3 is a virus with a broad host range infecting more than 60 animal species, however, the virus has never been isolated from a hamster and the presence of serum antibodies has never been correlated with clinical disease (Parker et al., 1987). The presence of TMEV and SV-5 antibodies could potentially be due to the hamsters being infected with closely related viruses. However, the presence of these viruses has never been confirmed in hamsters, the agents have not been isolated and again there is no clinical disease associated with them (Percy and Barthold, 2007). There is a single report of an enzootic outbreak of H-1 in a hamster breeding colony causing clinical disease in suckling and weanling hamsters (Gibson, 1983) but this is considered to be part of the hamster parvovirus cluster and is discussed there. Finally there is histological evidence of C-type particles associated with an endogenous retrovirus that was briefly thought to be involved in lymphoma formation during a hamster polyomavirus infection (Graffi et al., 1969). This is discussed in the polyomavirus section.

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Parasitic Diseases

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INTRODUCTION

There is a dearth of information on naturally occurring parasitism of the Syrian hamster (*Mesocricetus auratus*). This is somewhat surprising considering this species has never been successfully rederived by cesarean section. In this chapter, we provide information specific to the Syrian hamster when it is available. Information is also drawn from the literature pertaining to the same or similar parasites of other rodent species when it is applicable and relevant to the hamster, especially with respect to prevention, treatment, and

eradication. The limited available information on parasites of other hamster species is also presented.

Commercially reared Syrian hamsters available today for use in the laboratory harbor a plethora of non-pathogenic commensal protozoa and often *Demodex* spp. mites as they have never been successfully cesarian rederived. Interestingly, a protocol for generating protozoa-free hamsters using carbasone and quinacrine was reported by Saxe in the early 1950s (Wagner, 1987). Although there is limited literature pertaining to the indigenous parasites of the Syrian hamster, this species has historically and continues to serve as an important

model for a variety of diseases caused by various protozoal and helminth species including *Leishmania* spp., *Schistosoma* spp., *Fasciola* spp., and *Ancylostoma* spp., among others.

There are numerous treatment regimens published for eradication of parasitic diseases from laboratory rodents, each with associated challenges and the potential to confound research. Colony size, age, strain, and immune status may impact successful treatment. Inadequate treatment of all animals greatly increases the possibility of treatment failure. Dosing regimens extrapolated from other rodent species may also be inadequate and there is a potential for the development of resistance. Lastly, as with avermectins, treatment may present a health risk to the animal.

PROTOZOA

Microsporidia

Encephalitozoon cuniculi

BACKGROUND Minimal information is available regarding *Encephalitozoon cuniculi* infections in hamsters. Much debate over the correct taxonomy of this species has seen it classified at different times as either a fungus or a protozoan (Hankenson and Van Hoosier Jr., 2007). The current classification of this organism is, in fact, a fungal agent (Didier et al., 2000). Throughout the literature microsporidia are often referred to as protozoal species and thus are included in this section.

Microsporidia were first discovered in 1857 by Nägeli as the etiological agents responsible for pébrine disease in silkworms (Didier, 2005). *E. cuniculi* was subsequently recognized as infecting mammalian species by Wright and Craighead in 1922 when they discovered the infection in rabbits (Didier et al., 2000). Historically, the organism was commonly diagnosed in laboratory rodents and was a confounding factor in experimental models of infectious disease, altering immunological responses. As sanitary standards have improved within the laboratory setting, microsporidia no longer present a significant threat to laboratory rodents (Mathis, 2000). *E. cuniculi* is the microsporidial species most often associated with the Syrian hamster. It is an obligate intracellular parasite (Noble et al., 1989; Percy and Barthold, 2007).

ETIOLOGICAL AGENT The current classification of microsporidia as fungi is based on certain shared characteristics such as a chitinous spore wall, presence of the mitochondrial HSP70 gene, comparable gene sequences for α -tubulin, β -tubulin, large subunit RNA polymerase II, TATA box binding protein, glutamyl synthase, EF-1 α , and EF-2 (translocation elongation factors) (Didier, 2005).

EPIZOOTIOLOGY *E. cuniculi* infects an extensive range of mammalian hosts including mice, guinea pigs, squirrel monkeys, cats, dogs, and the "classical host" rabbit (Wagner, 1987). Studies in the 1970s analyzing the ultrastructural and serological properties of isolates from different animal species demonstrated that the isolates are identical (Pakes et al., 1975). This was confirmed by Shadduck et al. (1979) when they titrated rabbit, mouse, and hamster isolates of *E. cuniculi* in cultures of rabbit choroid plexus. They postulated that any differences in observed lesions caused by the organism are due to differences in host response to infection between animal species. In contrast, Didier et al. (1995) described the existence of three defined strains of *E. cuniculi*, strains I, II, and III using Western blot, PCR amplification and sequencing of the small subunit ribosomal RNA (SSU-rRNA) gene unique to microsporidial organisms. They suggested that strain variation may explain differences in host response. Strains I and III (the rabbit and dog strains, respectively) have been confirmed as zoonotic; however, strain II (the "mouse strain") is yet to be described as infecting humans (Mathis, 2000). The strain of *E. cuniculi* affecting hamsters remains "unclassified" (Didier et al., 2000).

CLINICAL MANIFESTATIONS *E. cuniculi* is not recognized as a significant cause of clinical disease in the hamster. Encephalitozoonosis tends to cause clinical disease in large animals such as non-human primates and canines (Percy and Barthold, 2007). Microsporidia cause opportunistic infections in people suffering from AIDS and manifest as a diarrheal and systemic disease (Didier, 2005). *E. cuniculi* has also been implicated as a cause of neurological disease in AIDS patients (Tosoni et al., 2002; Weber et al., 1997).

PATHOLOGY In mammals, *E. cuniculi* infections are generally localized to the brain and renal tubules. Infection can also occur within the liver, adrenal glands, myocardium, optic nerve, and retina (Noble et al., 1989). Minimal information is available regarding specific pathologic findings in the hamster.

PATHOGENESIS/LIFE CYCLE The spore wall is composed of three layers, the outermost being proteinaceous and the innermost comprised of chitin. The mature spore has a single coiled polar filament (Percy and Barthold, 2007) which is thought to play a role in infecting host cells by acting as a kind of "needle". An increase of internal pressure causes the filament to extrude and the sporoplasm is then expelled via the filament lumen, allowing it to penetrate the gut epithelium and travel to tissues elsewhere in the body via the blood stream (Noble et al., 1989; Percy and Barthold, 2007). Once within the gut epithelium, the sporoplasm matures into

a meront and enters the phase of merogony, undergoing two cycles to become a sporont. In a process called sporogony, the sporont undergoes meiotic division to form two mononucleate sporoblasts. The life cycle is complete when the sporoblasts undergo sporogenesis to become spores (Noble et al., 1989). The infective stage of the microsporidia spores is extremely small ($1.0\text{--}3.0\mu\text{M} \times 1.4\text{--}4.0\mu\text{M}$) (Didier, 2005). The host cell eventually ruptures, releasing the mature spores for spread to neighboring cells (Percy and Barthold, 2007).

Transmission is primarily via ingestion or inhalation of the spores. The initial tropism for the organism tends to be reflective of the mode of transmission and therefore commonly the epithelium of the gastrointestinal or respiratory tracts is affected (Didier et al., 2004). Later in the course of infection, the organism disseminates to other organs within the body, localizing in organs such as the brain and kidney (Didier et al., 2004).

DIAGNOSIS Definitive diagnosis of *E. cuniculi* may be achieved using transmission electron microscopy to detect microsporidia at any life stage in tissue biopsies, or spores in bodily fluids or feces (Didier et al., 2000). This method is not ideal due to the fact that tissue biopsy is invasive, testing bodily fluids or feces lacks sensitivity and the procedure is labor-intensive. Modified trichrome stain and fluorescent brighteners may be used to histochemically stain microsporidia in respiratory secretions, urine, and feces (Didier et al., 2000). Modified trichrome stains spores pink with a transparent posterior vacuole and a pink diagonal line is visualized within the spore against a blue-green background. Fluorescent staining of microsporidial spores binds chitin, resulting in the spores appearing as blue-green oval halos when visualized under ultraviolet light (Didier et al., 2000). Stained tissue sections reveal Gram-positive organisms. A Warthin-Starry silver stain may also be used to stain the organisms gray-black (Didier et al., 2000). The use of histological staining does not allow for speciation of the organism. Premortem diagnosis of *E. cuniculi* can be achieved via serological tests such as indirect fluorescent antibody and ELISA to detect anti-parasite antibody. These tests are fairly reliable in laboratory animals as they have limited environmental exposure (unlike humans, for example) and titers only remain high in chronically infected animals (Didier et al., 2000). Molecular techniques carried out on fecal samples, such as PCR amplification of primers complementary with the SSU-rRNA gene are gaining favor in the laboratory setting (Fedorko and Hijazi, 1996).

PREVENTION AND THERAPY As *E. cuniculi* rarely poses a clinical threat to hamsters, treatment has not been specifically described for this species. The latter, whilst effective against a greater spectrum of

microsporidial species, is toxic to mammals. Albendazole can be used as a treatment for affected animals and is part of the benzimidazole drug family and works by inhibiting tubulin polymerization (Didier et al., 2004). A dose of 400 mg of albendazole given twice daily for 2–4 weeks is described, however the paper does not specify whether this is a human or animal dose (Didier et al., 2000). Boiling water for a minimum of 5 minutes kills any spores present in water used for drinking or cleaning equipment/caging (Waller, 1979). A number of disinfectants, such as, quaternary ammonium, 70% ethanol, formaldehyde, phenolic derivatives, 1% hydrogen peroxide, sodium hydroxide or amphoteric surfactants applied for a period of 30 minutes at 22°C are also effectively sporicidal (Santillana-Hayat et al., 2002; Shadduck and Polley, 1978).

Flagellates

A plethora of flagellates have been identified in the hamster cecum. These protozoa are more often than not, non-pathogenic. The more commonly found and studied protozoa are described below.

SPIRONUCLEUS MURIS

BACKGROUND Historically this organism was referred to as *Hexamita muris*, but this non-host-specific flagellate has since been renamed. In general, immunocompetent adult animals are asymptomatic when infected with this parasite. Rats and hamsters seem to be particularly resistant to infection, whereas certain inbred mouse strains are more susceptible.

ETIOLOGIC AGENT *Spiroonucleus muris* are small, bilaterally symmetrical pyriform parasites ($7\text{--}9 \times 2\text{--}3\mu\text{m}$). The organism has two nuclei positioned at the anterior segment. Just anterior to this, six long flagella extend from the organism. Two additional flagella extend backwards parallel to two short axostyles. The organisms swim in a “torpedo-like” fashion. Binary fission of the organism results in the production of ovoid cysts measuring $7.4 \times 4.0\mu\text{m}$ (Owen, 1992).

EPIZOOTIOLOGY Schagemann et al. (1990) demonstrated that *Spiroonucleus* spp. isolates from the rat are highly host-specific and do not seem to cross-infect other rodent species. *S. muris* clones from mice, on the other hand are capable of infecting other mice and Syrian hamsters. *Spiroonucleus* spp. derived from Syrian hamsters also showed no host specificity, however cyst excretion was poor in rats and mice infected with the golden hamster isolate (Schagemann et al., 1990).

A study conducted by Kunstyr et al. (1993) demonstrated that *Spiroonucleus* spp. isolated from the European hamster (*Cricetus cricetus*) was ineffective at infecting rats and mice. Heterologous experimental

infection in the Syrian hamster was significantly less intense than spontaneously occurring infections in the European hamster (Kunstyr et al., 1993).

CLINICAL MANIFESTATIONS *S. muris* does not cause disease in the hamster (Barthold, 1985a).

PATHOLOGY Trophozoites may be observed extracellularly in between villi, within crypts and the central lumen but generally do not cause noticeable damage to the mucosa (Barthold, 1985a). On post-mortem, histological preparation of the intestine reveals mucoid hyperplasia of the mucosa and an inflammatory reaction characterized by mononuclear cells (Schmidt, 1995).

PATHOGENESIS/LIFE CYCLE *Spiroplasma* spp. cysts are ingested in contaminated water and/or feed (Schmidt, 1995). The prepatent period is generally 2–3 days but may take up to 6 days. Peak shedding of cysts occurs at 1–2 weeks (Barthold, 1985a).

DIAGNOSIS Trophozoites may be found in wet mounts of the duodenum and can be differentiated from *Giardia* by their smaller size and rapid, torpedo-like movement in comparison to *Giardia*'s slow, tumbling motion (Wagner, 1987).

PREVENTION AND THERAPY Prevention of *S. muris* outbreaks can be achieved by housing mice separately, as cross-contamination is known to occur between the species (Schagemann et al., 1990). Wagner suggests decontamination by pasteurization may be more effective than using disinfectants (Wagner, 1987). As this parasite does not cause clinical disease in the hamster, treatment may not be warranted. In mice, treatment of *S. muris* requires the addition of 0.1% dimetridazole to the drinking water for 2 weeks (Schmidt, 1995).

GIARDIA MURIS

BACKGROUND *Giardia muris* affect a wide host-range globally, including laboratory rodents, non-human primates, and man. The organism found in hamsters is morphologically indistinguishable from that found in mice (Hankenson and Van Hoosier Jr., 2007). *Giardia* is an opportunistic pathogen of the duodenum (Patterson et al., 2000).

ETIOLOGIC AGENT Trophozoites are the motile form of *Giardia* and are classically pear-shaped, possessing a flat ventral surface with an adhesive disc composed of microtubules and ribbons, allowing it to grasp onto the host's epithelial cells (Figure 32.1). Trophozoites range in size from $7\text{--}13 \times 5\text{--}10\mu\text{m}$ (Barthold, 1985b).

Four pairs of flagella confer motility to the organism. These flagella are classified as anterior, posterior,

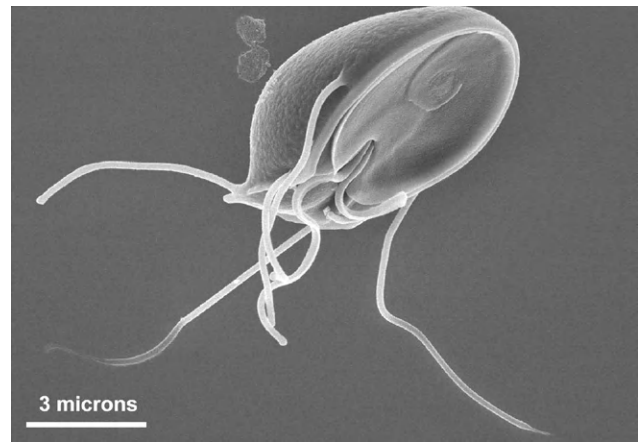


FIGURE 32.1 A scanning electron micrograph (SEM) of the ventral surface of a *Giardia muris* trophozoite. The ventral adhesive disk resembles a suction cup, where overlapping microtubules in the cytoplasm form a number-6-shaped figure. *Giardia muris* has four pairs of flagella that are responsible for the organism's motility. The adhesive disk facilitates adherence of the protozoan to the intestinal surface. Reproduced from the Centers for Disease Control and Prevention/Dr. Stan Erlandsen (<http://phil.cdc.gov/phil/details.asp>) with permission.

ventral, and caudal pairs. The flagella originate from kinetosomes deep within the cytoplasm and extend extracellularly. The ventral flagella lie within a dark-staining ventral groove which is comprised of fibers called axonemes or axostyles. *Giardia* possess two nuclei with an eccentrically located endosome (Gardiner et al., 1988). Claw-like median bodies of unknown function are present within the organism, crossing the axonemes in the middle of the body (Noble et al., 1989). The cyst form of *Giardia* usually contains two or four nuclei and measures $8\text{--}11 \times 7\text{--}19\mu\text{m}$. The cysts do not possess an adhesive disc or exterior flagella. Cysts are also devoid of cellular machinery such as mitochondria, Golgi apparatus, lysosomes, and endoplasmic reticulum. Cysts require moist conditions to survive for a period of weeks to months (Noble et al., 1989).

EPIZOOTIOLOGY Host specificity of *G. muris* was analyzed by inoculating mice, rats, and hamsters with isolates from both naturally infected mice and hamsters (Kunstyr et al., 1992). The results showed that rats were not susceptible to infection from either isolate. Hamsters seemed to be more sensitive to infection from mouse isolates than homologous isolates. Mice were sensitive to heterologous isolates but to a lesser degree than hamsters. Kunstyr, Schoeneberg, and Friedhoff (1992) conclude that mice present a greater threat to hamsters in terms of infection with *Giardia* spp. than vice versa (Kunstyr et al., 1992).

CLINICAL MANIFESTATIONS While the prevalence of *G. muris* in the hamster is very high, the likelihood

that clinical signs or pathological lesions accompany the organism is very low (Barthold, 1985b). A self-limiting condition exists in weanling hamsters in which the animal presents with a pot belly and large numbers of both *G. muris* and *S. muris* (Owen, 1992). A number of hamsters with *Helicobacter aurati* infections culminating in gastritis and intestinal metaplasia were found to have *Giardia* and *Trichomonas* within the gastric pits (Figure 32.2) (Patterson et al., 2000). The authors suggest that decreased acidity may account for the migration of *Giardia* to the unusual location within the gastric mucosa.

PATHOLOGY Infections are most often subclinical (Schmidt, 1995). In animals clinically affected by giardiasis, the small intestine is often distended with yellow-white watery contents. Histologically, the parasite generally resides on the brush border of the small intestine but can also be found within the lumen. The oval cysts are found mostly within the large bowel (Barthold, 1985b). A lymphoplasmacytic infiltrate may be seen (Schmidt, 1995).

PATHOGENESIS/LIFE CYCLE Cysts represent the infective stage of the organism. Hosts are infected via the fecal-oral route. *Giardia* excysts in the duodenum to produce two trophozoites. In hamsters and other rodents, the organism may infiltrate the intestinal epithelium and submucosa traveling to the lungs and other organs via the lymphatics and blood stream. Gallbladder penetration may result in cholestasis (Noble et al., 1989). While tissue invasion in hamsters by *Giardia* is rare, it has been described within the duodenum and a renal blood vessel of mice (el-Shewy and Eid, 2003). The pre-patent period is generally 3–5 days and peak cyst excretion occurs at 7–14 days (Barthold, 1985b).

DIAGNOSIS Phosphate-buffered saline wet mount preparations from the duodenum can be examined to confirm the presence of *Giardia*. The characteristic tumbling motion of trophozoites can be visualized under a microscope ($\times 100$). Hematoxylin-eosin-stained sections of small intestine also reveal the organism adhered to the villous epithelium. The cystic form can be found in fecal smears stained with Lugol's iodine or in tissue sections of the large intestine stained with hematoxylin-eosin (Wagner, 1987). The identification of cysts using zinc-sulfate centrifugation flotation remains the gold standard for diagnosis. *Giardia* may be detected in pet cats and dogs using a SNAP Ag test (Payne and Artzer, 2009).

PREVENTION AND THERAPY Strict quarantine practices and testing of new animals coupled with high levels of sanitation can be used to prevent *Giardia* infection

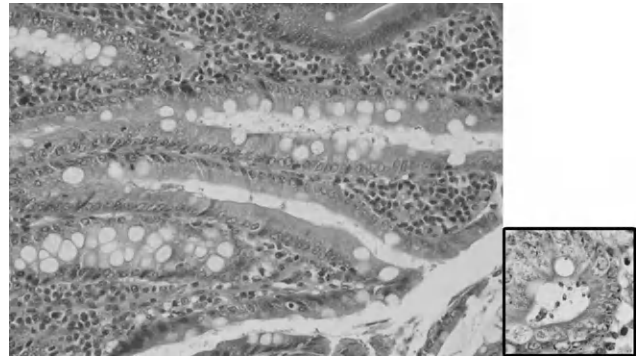


FIGURE 32.2 Area of intestinal metaplasia in hamster antrum. Lumen of gastric pit contains many protozoa. HE stain. Bar = 50 μ m. Inset: Demonstrates ovoid shape and two nuclei consistent with *Giardia*. Warthin-Starry stain. Bar = 10 μ m. Reproduced from Patterson et al. 2000 with permission.

within a colony. Cleaning surfaces and equipment with boiling water is effective at killing cysts, however chlorination does not tend to destroy cysts successfully (Noble et al., 1989). Quinacrine, amodiaquin, and chlorocrine have been described as potentially effective drugs for the treatment of giardiasis (Schmidt, 1995). Metronidazole, while effective at eradicating *Giardia* in mice, is not useful in treatment of hamsters, even at doses 16 times higher than those used in mice (Hankenson and Van Hoosier Jr., 2007).

TRICHOMONAS MURIS

BACKGROUND The names *Trichomonas cricetus* and *T. criceti* were previously used to describe the trichomonads found in hamsters. These species are synonymous with *Tritrichomonas muris* (Hankenson and Van Hoosier Jr., 2007). Other trichomonads found in Syrian hamsters include *Trichomonas wenyoni*, *Tritrichomonas minuta*, *Tetratrichomonas microti*, and *Pentatrichomonas hominis* (Hankenson and Van Hoosier Jr., 2007). *Trichomonas* spp. are non-pathogenic and are commonly found in the cecum (Owen, 1992; Patterson et al., 2000).

ETIOLOGIC AGENT The morphologic characteristics of this protozoa include multiple anterior flagella conferring motility to the organism, a crescent-shaped shielding structure at the anterior margin of the body called the pelta (Tasca and De Carli, 2003), an undulating membrane at the base of which lies a deep staining ribbon-like structure called the costa which is thought to provide structural support (Gutierrez, 2000), an axostyle, and a single nucleus (Figure 32.3). The term trichomonad is a misnomer for the order as it was originally thought that this group of organisms had three flagella, when in fact various species possess from three to five flagella. *Trichomonas* actually possess four flagella, whereas *Tritrichomonas* possess three flagella.

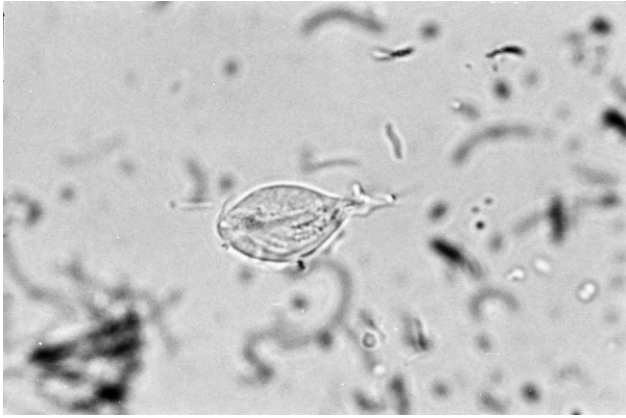


FIGURE 32.3 Trophozoite of *Tritrichomonas muris* detected in cecal contents from a protozoa-free mouse transfaunated with Armenian hamster feces. Note its characteristic pyriform appearance, the undulating membrane, the three anterior flagella, recurrent flagellum and axostyle.



FIGURE 32.4 Round refractile body (*T. muris* pseudocyst) detected in the feces from an Armenian hamster. Lugol's iodine preparation. Reproduced from Lipman et al., 1999 with permission.

Trichomonads are anaerobic parasites that can grow under low oxygen tensions. They rely on the glycolytic pathway as a means of producing metabolic energy which involves the breakdown of glucose to either pyruvate or succinate. Pyruvate may also be used as an energy source to produce acetate within hydrogenosomes via hydrogen production (Noble et al., 1989). These organisms do not appear to encyst, rather they form pseudocysts (Figures 32.4 and 32.5). These structures are simply less-motile forms of the organism with internalized flagella. They appear like cysts but lack a true cyst wall, hence the name "pseudocyst". The organism is thought to transform into this shape as a survival mechanism in response to environmental stress (Martin, 2008).

Trichomonads have been implicated in barrier breaches within SPF mouse colonies established from

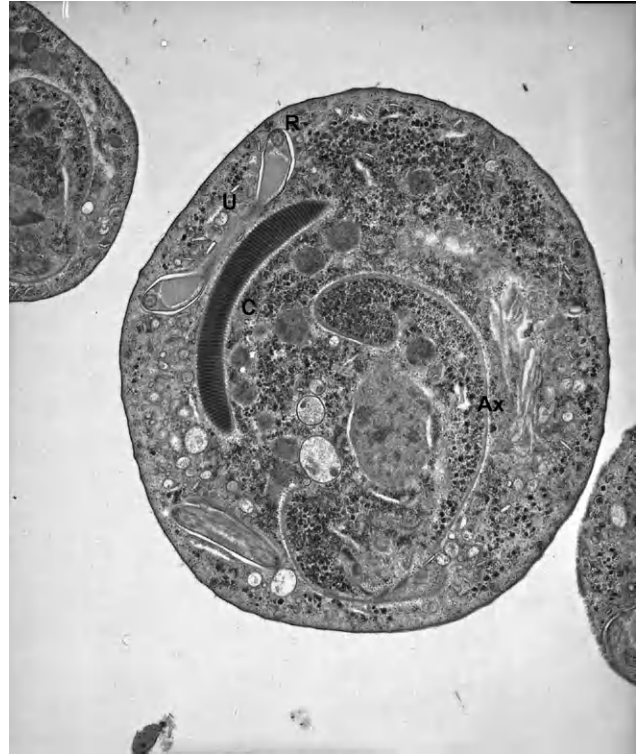


FIGURE 32.5 Electron micrograph of a *T. muris* pseudocyst. Notice the presence of the costa (C), recurrent flagellum (R), undulating membrane (U), and axostyle (Ax). Magnification, $\times 7500$. Reproduced from Lipman et al., 1999 with permission.

cesarean-derived stock. Because it has such a low infective dose (five pseudocysts) in mice (Stachan and Kunstyr, 1983), it is a good indicator of microisolation technique failure (Owen, 1992).

EPIZOOTIOLOGY *T. muris* was found to form pseudocysts in Armenian hamsters. Cross-infectivity was established when it was discovered that immunocompetent outbred mice were subsequently infected by the hamster (Lipman et al., 1999).

CLINICAL MANIFESTATIONS Trichomonads are not considered pathogenic in the hamster. They are often found in the presence of enteric disease as an incidental finding (Wagner, 1987).

PATHOLOGY This organism is found in the cecal lumen, however as it is non-pathogenic, no gross or microscopic pathology accompanies infection (Hankenson and Van Hoosier Jr., 2007).

PATHOGENESIS/LIFE CYCLE Trichomonads reproduce asexually by binary fission. They form pseudocysts which will eventually excyst to produce the motile trophozoite. Feco-oral transmission occurs via

ingestion of either the pseudocyst or trophozoite stage of this parasite (Wagner, 1987).

DIAGNOSIS Pseudocysts can be found microscopically in fecal flotations using Lugol's iodine staining (Lipman et al., 1999) or trichrome stain (Hankenson and Van Hoosier Jr., 2007). Both trophozoites and pseudocysts may be identified in the cecum and colon (Wagner, 1987).

PREVENTION AND THERAPY The glycolytic pathway is blocked by metronidazole and is therefore the treatment of choice for infections with trichomonads (Noble et al., 1989). Metronidazole may be administered intragastrically at 80 mg/day for 6 days (Hankenson and Van Hoosier Jr., 2007).

MISCELLANEOUS FLAGELLATES

The following organisms are mentioned as either examples of relatively common parasites or those which have, at the very least, been described over the years. Wantland (1955) examined numerous hamsters and found *Chilomastix bettencourti* in the cecum of approximately 9% of his subjects. These pyriform trophozoites measure 10–15 μm , are asymmetrical and possess a short, pointy tail. The organism has three anterior flagella which confer motility and a fourth flagellum which acts as a prehensile organ for feeding. *Chilomastix* move with jerky, spiral movements. The cystic form of this parasite is lemon-shaped and typically measures 6–9 μm (Wantland, 1955). Griffiths (1971) observed various cecal protozoa including *Monocercomonoides* sp. and *Octomitus* sp., both of unknown prevalence.

Sporozoa

Cryptosporidium spp.

BACKGROUND Numerous studies have proposed that *Cryptosporidium* spp. acts as an opportunistic pathogen in immunocompromised hosts. In general, cryptosporidial species are thought to play a minor to insignificant role in disease of hamsters.

ETIOLOGICAL AGENT It remains unclear which cryptosporidial species is responsible for naturally occurring infections in the hamster (Davis and Jenkins, 1986; Orr, 1988). The two most commonly discussed cryptosporidial species in hamsters are *C. parvum* (Rasmussen and Healey, 1992a, 1992b) and *C. muris* (Rhee et al., 1999). Both the sexual and asexual stages of these coccidia attach to the microvilli of epithelial cells (Gardiner et al., 1988). The oocysts typically measure $7 \times 5 \mu\text{m}$ and have a smooth wall (Owen, 1992).

EPIZOOTIOLOGY Rasmussen and Healey (1992a) evaluated whether a difference in susceptibility to *C. parvum*

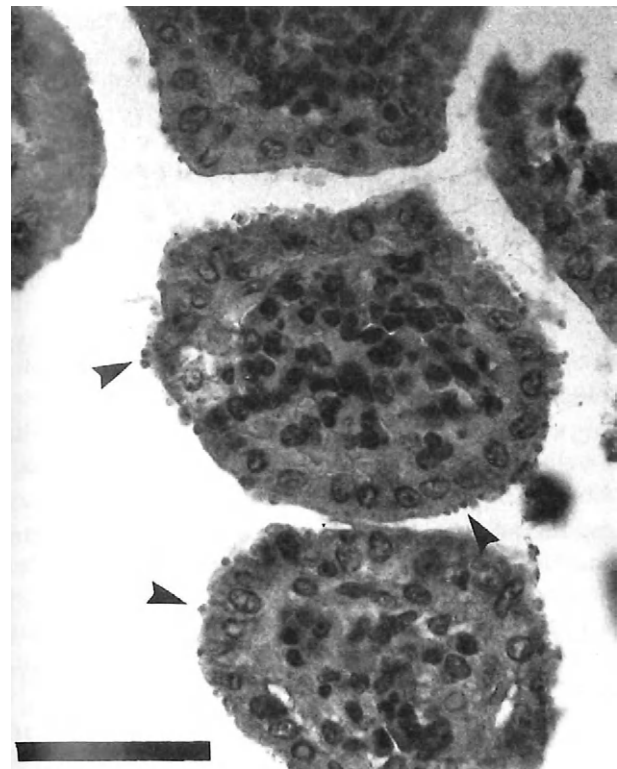


FIGURE 32.6 Histologic section of ileum from aged hamster showing numerous *Cryptosporidium parvum* (arrowheads) associated with villous brush border. Bar = 70 μm . Reproduced from Rasmussen and Healey 1992 with permission.

exists between aged and young hamsters. Aged hamsters (20–24 months old) shed significantly more oocysts for a longer period of time than did young hamsters (8–12 weeks old). Histologically, the organism adhered to the villous enterocytes of the small intestine of aged hamsters (Figure 32.6), whereas no parasites were found in tissue sections of the juveniles. While there were no histologic lesions in young hamsters, widespread villous atrophy and hyperplastic crypts were seen in aged animals. The aged hamsters were found to have decreased T and B cell response to mitogens and reduced NK cell activity in contrast to the young hamsters. These findings supported the theory that immune competency is thought to be a significant factor in host susceptibility to cryptosporidiosis (Rasmussen and Healey, 1992a).

In a study conducted by Rhee et al. (1999), the prepatent period for *C. muris* (10–13 days) remained similar regardless of the animal's age at infection. However, an inverse relationship was found between age at infection and the length of the patent period. These authors also found that hamsters inoculated at 1 week of age shed higher oocyst numbers than did those infected at 5 and 10 weeks old. These findings suggested that innate immunity to *C. muris* is independent of age whereas acquired immunity is age-dependent (Rhee et al., 1999).

CLINICAL MANIFESTATIONS/PATHOLOGY Davis and Jenkins (1986) described a case of cryptosporidiosis (species unidentified) in a weanling male Syrian hamster with concomitant proliferative ileitis. Aside from poor body condition and a dehydrated state, the animal showed no clinical signs prior to death. On necropsy, the hamster was found to have gross lesions consistent with ileitis and colitis. Examination of the intestinal lesions via light microscopy revealed lesions consistent with proliferative ileitis. These lesions include significant hyperplasia of the enterocytes lining both crypts and villi, fusion of the villi, crypt abscessation, necrosis, hemorrhage, and granulomatous inflammation. In addition to the numerous bacteria infiltrating the enterocytes, many organisms identified as *Cryptosporidia* spp. were found along the microvillus borders of the enterocytes. While *Cryptosporidia* spp. were definitely present, it remained unclear whether the organism had contributed to the lesions as the prominent lesions were attributed to infection with the intracytoplasmic bacteria (subsequently recognized as *Lawsonia* spp.) (Davis and Jenkins, 1986). Similarly, Orr (1988) described several cases of enteritis in Syrian hamsters which on necropsy had histopathological lesions consistent with proliferative enteritis as well as various cryptosporidial stages within the epithelial brush border and intestinal crypts. The author did not draw any conclusions about the significance of the presence of cryptosporidia.

PATHOGENESIS/LIFE CYCLE Unsporulated oocysts are shed within feces where they eventually sporulate, becoming infective. Cryptosporidia are directly transmitted via the feco-oral route and occasionally via the respiratory route. Sporozoites are released upon entry into the body and invade the gastrointestinal epithelium. Rarely, invasion of the respiratory epithelium may occur, in hosts with a compromised immune system. The sporozoite matures to a trophozoite, which in turn forms into a meront. Several cycles of merogony result in the release of merozoites which become macrogametocytes and microgametocytes containing macrogametes and microgametes respectively. Once the macrogametes become fertilized, a thick-walled oocyst is formed (Gardiner et al., 1988; Owen, 1992).

PREVENTION AND THERAPY *Cryptosporidium* species are known to infect humans and, as such, represent a public health risk to laboratory personnel (Fayer et al., 2004; Xiao et al., 2004). Apart from adhering to hygienic practices such as hand washing and utilizing personal protective equipment, it is important to sanitize the laboratory environment. Unfortunately, *Cryptosporidium* spp. are notoriously resilient organisms and resistant to many commonly used disinfectants. Oocysts are

thought to be sensitive to heat shock (King and Monis, 2007); therefore cleaning surfaces and equipment with boiling water may be effective at rendering them incapable of infection. Cryptosporidia are transmitted via water sources and therefore water treatment is a critical control point. Recent studies indicate that UV disinfection may be effective at destroying cryptosporidial organisms (Le Goff et al., 2010; Xiao et al., 2004).

Very few studies discuss treatment of *Cryptosporidium* infections in hamsters. One study by Rasmussen and Healey (1992b) tested the hypothesis that pre-treatment of aged hamsters with exogenous dehydroepiandrosterone (DHEA) would lead to a reduction in susceptibility to *C. parvum* infection compared to aged hamsters that were left untreated. This hypothesis was based on a previous observation that the steroid hormone (naturally produced by the adrenal cortex) when administered exogenously was shown to increase lifespan and boost immunity in animals. Shedding of *C. parvum* oocysts was monitored and found to be significantly reduced by 75% in DHEA-treated hamsters when compared to the untreated group. They concluded that DHEA treatment prior to infection reduced susceptibility to cryptosporidiosis in aged Syrian hamsters. Presumably this is due to an increase in IL-2 production, which plays a critical role in clonal expansion of antigen-activated T cells and alteration of the intestinal flora, impairing the organism's ability to colonize the gut (Rasmussen and Healey, 1992b). It is questionable whether this treatment would have any practical application.

Amoebae

Entamoeba muris

BACKGROUND The most common species of amoebae found in hamsters, rats, and mice is *Entamoeba muris*. It is a non-pathogenic, large, slow-moving organism found in the lumen of the large intestine and its motility is conferred by pseudopodia. Pseudopodia also act as prehensile limbs for feeding on bacteria. The trophozoites are 8–30 μ m in diameter and the cysts are sizeable, measuring 9–20 μ m in diameter. Cross-infectivity between rats, mice, and hamsters can occur (Wagner, 1987).

ETIOLOGIC AGENT *Entamoeba* is an obligate parasite with a direct life cycle. The infective stage is a resistant cyst which is excreted in feces. The cyst is prone to desiccation yet it can withstand temperature extremes and is relatively resistant to disinfectants (Owen, 1992). Once ingested, development into a trophozoite occurs within the intestinal lumen. On rare occasions this stage can invade the intestinal wall and migrate to other organs (Gardiner et al., 1988).

EPIZOOTIOLOGY *E. muris* is a non-pathogenic organism, commonly found in the cecum of hamsters. The organism bears similarities to other amoebae affecting other laboratory animal species such as *E. caviae* in the guinea pig and *E. cuniculi* in rabbits.

CLINICAL MANIFESTATIONS/PATHOLOGY *E. muris* does not cause disease in hamsters.

PATHOGENESIS/LIFE CYCLE *E. muris* is directly transmitted via the feco-oral route. Resistant cysts are shed in feces and upon ingestion the nuclei of the cysts divide into eight, followed by the cytoplasm. As a result, eight trophozoites excyst into the small intestine where they can feed and travel to the large intestine where they can multiply by binary fission. Trophozoites may also migrate to other vital organs (Gardiner et al., 1988; Owen, 1992).

DIAGNOSIS Diagnosis of *E. muris* is by identification of the trophozoites within the intestinal lumen or feces or cysts in the feces. The cyst contains eight small nuclei that can be visualized in both unstained and iodine-stained fecal preparations. Other features of the *E. muris* cyst include chromatoid bodies described as having a "splinter-like appearance" and a distinct glycogen granule (Owen, 1992).

PREVENTION AND THERAPY Research on the treatment of this parasite is scarce and as this organism is not considered clinically significant in laboratory hamsters, its prevention and treatment are not discussed.

NEMATODES

Oxyuriasis

Syphacia spp., Aspiculuris spp., and Dentostomella spp.

BACKGROUND Oxyurids, or pinworms, are routinely detected in mice and rats from modern vivaria (Carty, 2008; Jacoby and Lindsey, 1998; Pritchett, 2007). There are far fewer reports of pinworm infection in hamsters, but an understanding of the disease is important due to potential research complications and transmission of infection to other rodents (Kellogg and Wagner, 1982; Ross et al., 1980). *Syphacia*, *Aspiculuris*, and *Dentostomella* are genera of nematode worms of the order Oxyurida, superfamily Oxyuroidea that parasitize rodents worldwide. *Syphacia* spp. belong to the family Oxyuridae and *Dentostomella* spp. and *Aspiculuris* spp. belong to the family Heteroxyematidae (Adamson, 1989; National Center for Biotechnology Information, 2009).

ETIOLOGY Hamsters have been reported to harbor many species of pinworms including *Syphacia mesocriceti*, *Syphacia criceti*, *Syphacia stroma*, *Syphacia peromysci*, *Syphacia obvelata*, *Syphacia muris*, *Aspiculuris tetraptera*, and *Dentostomella translucida* (Table 32.1). *S. mesocriceti* (Figures 32.7 and 32.8) is reported to be host-specific to the hamster and was originally described by Quentin (1971) from female worms collected from lab-reared Syrian hamsters in Anchorage, Alaska in 1953. The nematode was further described from specimens collected from Ontario, Canada and Japan (Dick et al., 1973; Hasegawa, 1981). Hosts include *Mesocricetus auratus* (Dick et al., 1973; Hasegawa, 1981; Quentin, 1971) and the desert (Roborovski) hamster, *Phodopus roborovskii* (Hasegawa et al., 2008).

Syphacia criceti (Figure 32.9) was originally described from nematodes collected from *Oryzomys subflavus* and *Calomys callosus* in Brazil (Quentin, 1971). The nematode was isolated from lab-reared Syrian hamsters in Brazil (Pinto et al., 2001), though a subsequent publication has questioned the identification suggesting that the pinworms were actually *Syphacia stroma* based on the morphology of the male's tail (Hasegawa et al., 2008). *S. stroma* infects *Apodemus* spp. in the Palaearctic region and *S. peromysci* is native to the deer mouse, *Peromyscus maniculatus*, in North America (Quentin, 1971). Both were recovered concomitantly with *S. mesocriceti* in Syrian hamsters that had been imported to Japan for the pet trade. It is believed that captive conditions provided a suitable environment for infection (Hasegawa et al., 2008).

Syphacia obvelata, the common mouse pinworm, has been reported in Syrian hamsters worldwide as early as 1946 (Watson, 1946) with transmission of infection possible from infected mice to hamsters (Kellogg and Wagner, 1982). *Syphacia muris*, the rat pinworm, has also been reported in Syrian hamsters with transmission of infection possible between Sprague-Dawley rats and hamsters (Ross et al., 1980). The mouse pinworm, *Aspiculuris tetraptera*, has been reported in pet desert (Roborovski) hamsters and Siberian dwarf hamsters (*Phodopus sungorus*) imported to Japan (Hasegawa et al., 2008), though infection in Syrian hamsters has not been reported (Wagner, 1987). Lastly, *Dentostomella translucida*, common to several cricetid rodents including *Meriones unguiculatus* (Mongolian gerbil), *M. meridanus*, *Rhombomys opimus*, *Dipus sagitta*, and *Mastomys fumatus*, has been isolated from the stomach of a Syrian hamster from a breeding colony in Minnesota, with an earlier suspect case reported in Iowa (Greve, 1985).

EPIZOOTIOLOGY Oxyurid nematodes are usually host-specific since the environmental phase in their monoxenous life cycle is reduced or absent (Hasegawa et al., 2008; Okamoto et al., 2007). Pinworm species

TABLE 32.1 Comparison of Rodent Pinworms Isolated from Hamsters^{a,b,c,d}

	<i>S. mesocriceti</i>	<i>S. criceti</i>	<i>S. stroma</i>	<i>S. peromysci</i>	<i>S. obvelata</i>	<i>S. muris</i>	<i>D. translucida</i>	<i>A. tetraptera</i>
Natural Host	<i>Mesocricetus auratus</i>	<i>Oryzomys subflavus</i> ; <i>Apodemus</i> spp. <i>Calomys callosus</i>		<i>Peromyscus maniculatus</i>	<i>Mus musculus</i>	<i>Rattus norvegicus</i>	<i>Rhombomys opimus</i> ; <i>Meriones unguiculatus</i>	<i>Mus musculus</i>
EGG								
Location	Perianal skin				Perianal skin	Perianal skin	Colon & Fecal pellet; Intermittent shedding	Colon & Fecal pellet; Intermittent shedding
Shape	One side flattened				One side flattened; crescent-shaped	Slight asymmetry; One side flattened	Ovoid, asymmetrical	Ovoid, symmetrical
Length (μm)	130–140	111–120	123–150	80–89	118–153	72–82	89–150	89–93
Width (μm)	40–50	43–48	41–64	23–31	33–55	25–36	30–60	36–42
Time to Infectivity	5–20 hours after released from female				5–20 hours after released from female	5–20 hours after released from female	5–10 days after released from female	5–8 days after released from female
WORM								
Location	Cecum; Small Intestine	Small Intestine	Small Intestine		Cecum; Colon	Cecum; Colon	Proximal third of Small Intestine; Stomach	Proximal Colon; Cecum
Color	White	White			White	White		White
Adult Female								
Length (mm)	3.2–6.9	3.4–3.8	2.0–4.11		3.4–5.8	2.5–4.0	9.63–30	2.6–4.7
Width (μm)	150–180	210	172		240–400		380–1000	190–275
Adult Male								
Length (mm)	1.1–1.5	1.4–1.6	1.53–1.75	0.8–1.3	1.1–1.5	1.2–1.3	6.14–15.3	2.0–4.0
Width (μm)	80–90	90–100	91–115	120–214	120–140	100	270–530	120–190
Cervical alae	Prominent	Absent	Absent	Subtle (narrow)	Subtle	Prominent	Subtle	Prominent

Mouth	Simple, reduced lips around triangular stoma		3 distinct lips around triangular stoma	Distinct lips	3 distinct lips	3 distinct lips	Lips absent	Lips present
Esophagus	190–210 µm long; Round bulb; Nerve ring 82–90 µm & Excretory pore 280–324 µm from cranial end	250–260 µm long; Round bulb; Nerve ring 110–144 µm & Excretory pore 460–470 µm from cranial end		200–350 µm long; Round bulb; Nerve ring 90–97 µm & Excretory pore 172 µm from cranial end	Round bulb	Round bulb	270–350 µm long; Nerve ring 130–250 µm from cranial end; Excretory pore not observed	Oval bulb
Vulva	700–830 µm from cranial end	840 µm from cranial end		507–700 µm from cranial end	Cranial 1/6 of body	Cranial 1/4 of body	9.5–13 mm from cranial end	Middle of body
Spicule	Single; 50–65 µm long	Single; 78–80 µm long	72–90 µm long	Single; 55–70 µm long	Single; 68–90 µm long	Single	Single; 280–340 µm long	Absent
Gubernaculum	29–32 µm long with hook-shaped distal process	30–32 µm long with hook-shaped distal process	25–47 µm long	26–37 µm long	28–34 µm long	Present		Absent
Ventral male mamelons	3 – prominent	3 – cranial less prominent than 2 caudal		3	3	3		Absent
Caudal papillae	3 pairs	3 pairs					4 pairs	
Tail	Conical		Stout		Pointed	Sharply pointed	Conical	Conical
Female length (µm)	870–960			525–700	530–675			445–605
Male length (µm)	155–207				130			117–169
Life cycle	Direct	Direct	Direct	Direct	Direct	Direct	Direct	Direct
Infection route	Ingestion of embryonated eggs	Ingestion of embryonated eggs	Ingestion of embryonated eggs	Ingestion of embryonated eggs	Ingestion of embryonated eggs	Ingestion of embryonated eggs	Ingestion of embryonated eggs	Ingestion of embryonated eggs
Prepatent period	8–15 days				11–15 days	7–8 days	25–29 days	21–25 days
Zoonosis	Not reported	Not reported	Not reported	Not reported	1 report in child (1919)	1 report in 2 children and 2 rhesus monkeys (1965)	Not reported	Not reported

^aFrom Anya, 1966a, 1966b; Chan, 1952; Dick et al., 1973; Greve, 1985; Hasegawa, 1981; Hasegawa et al., 2008; Hsieh, 1952; Pinto et al., 2001; Pritchett et al., 2002; Quentin, 1971; Taffs, 1976; Wightman et al., 1978.

^bFrom Kruidenier, F.J., Mehra, K.N., Harkema, R., 1961. Comparative studies of *Syphacia peromysci* and *S. samorodini* (Nematoda: Oxyuridae). J. Parasitol. 47, 47–51; Newcomer, C.E., Fox, J.G., 2007. Zoonoses and Other Human Health Hazards. In: Fox, J.G., Barthold, S.W., Davisson, M.T., Newcomer, C.E., Quimby, F.W., Smith, A.L. (Eds.), *The Mouse in Biomedical Research*, second ed., vol. Diseases. Academic Press, New York; Pinto, R.M., Gomes, D.C., Menezes, R.C., Muniz-Pereira, L.C., Noronha, D., 2003. First natural helminth infection in the Mongolian gerbil *Meriones unguiculatus* (Rodentia, Muridae), parasitized with *Dentostomella translucida* (Nematoda, Heteroxynematidae) in the neotropical region. Braz. J. Biol. 63, 173–175; Riley, W.A., 1919. A mouse oxyurid, *Syphacia obvelata*, as a parasite of man. J. Parasitol. 6, 89–93; Wiger, R., Barus, V., Tenora, F., 1978. Scanning electron microscopic studies on four species of the genus *Syphacia* (Nematoda, Oxyuridae). Zoologica. Scripta. 7, 25–31.

^cUnable to obtain complete descriptions of all pinworms.

^dThis table modified from Wagner, J.E. (1987) Parasitic Diseases. In “Laboratory Hamsters,” (eds. Van Hoosier Jr., G.L. & McPherson, C.W.), pp 145–146, Copyright Academic Press, Inc. with permission.

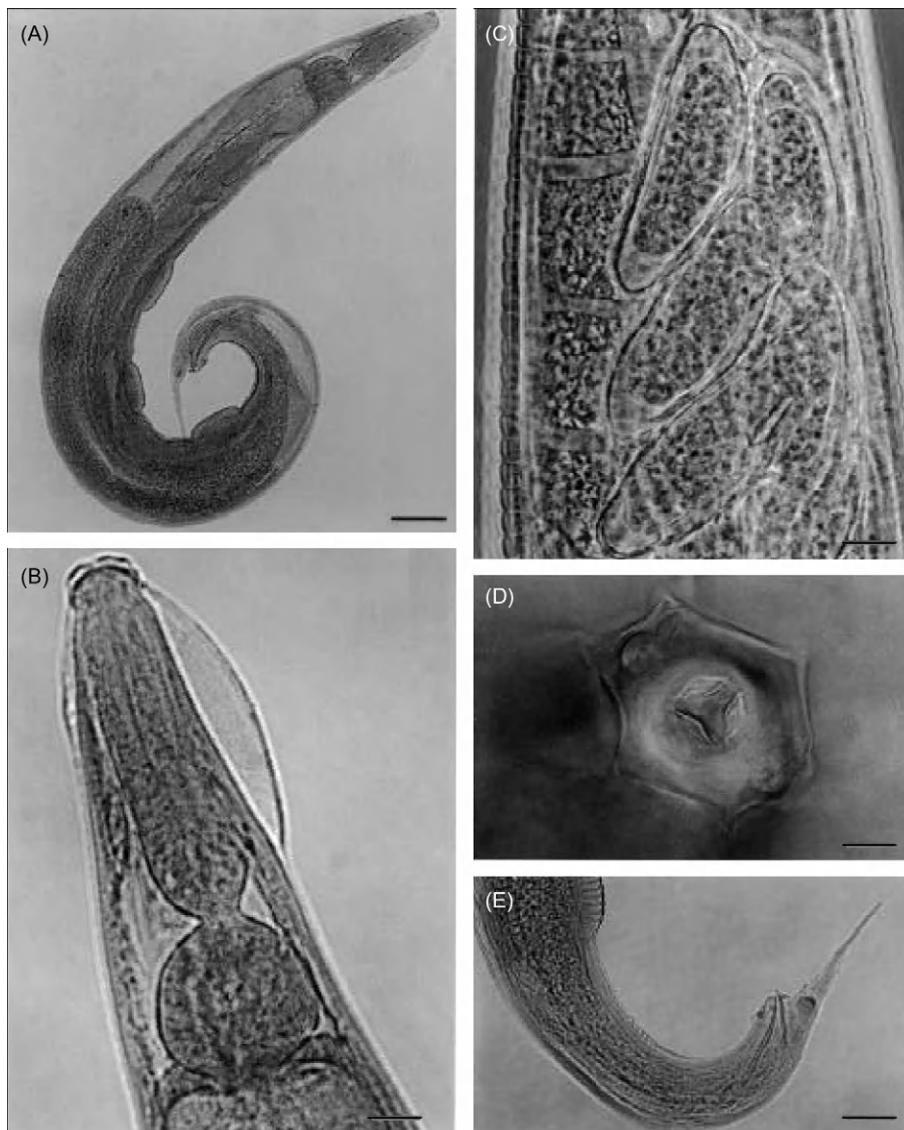


FIGURE 32.7 *Syphacia mesocriceti*. (A) Male, whole body, lateral view. Bar = 0.1 mm. (B) Cranial portion of female, lateral view. Bar = 0.03 mm. (C) Ova in utero. Bar = 0.02 mm. (D) Head of female, en face view. Bar = 0.01 mm. (E) Caudal portion of male, lateral view. Bar = 0.2 mm. Bar of A common to B–E. Reproduced from Pinto et al. 2001 with permission.



FIGURE 32.8 *Syphacia mesocriceti* ova from perianal tape test of Syrian hamster. Reproduced from the University of Missouri Research Animal Diagnostic Laboratory (<http://www.radil.missouri.edu/pinworms.html>) with permission.

specificities are found to be predilections, not obligate associations (Pritchett and Johnston, 2002), as demonstrated by the various pinworm infections in hamsters. Analysis of mitochondrial CO1 gene sequences suggests that the co-evolutionary relationship between pinworms and rodent hosts may not be completely rigid and host switching has likely occurred during evolution (Okamoto et al., 2007).

Pinworm infections typically result from contact with fomites contaminated with embryonated eggs (Pritchett, 2007). *Syphacia* spp. can spread among rodents via grooming and ingestion of embryonated eggs directly from the perineum (Prince, 1950; Taffs, 1976). Since *Syphacia* spp. ova are rapidly infective within 5–20 hours (Chan, 1952; Taffs, 1976), retroinfection with larval migration into the colon via the anus

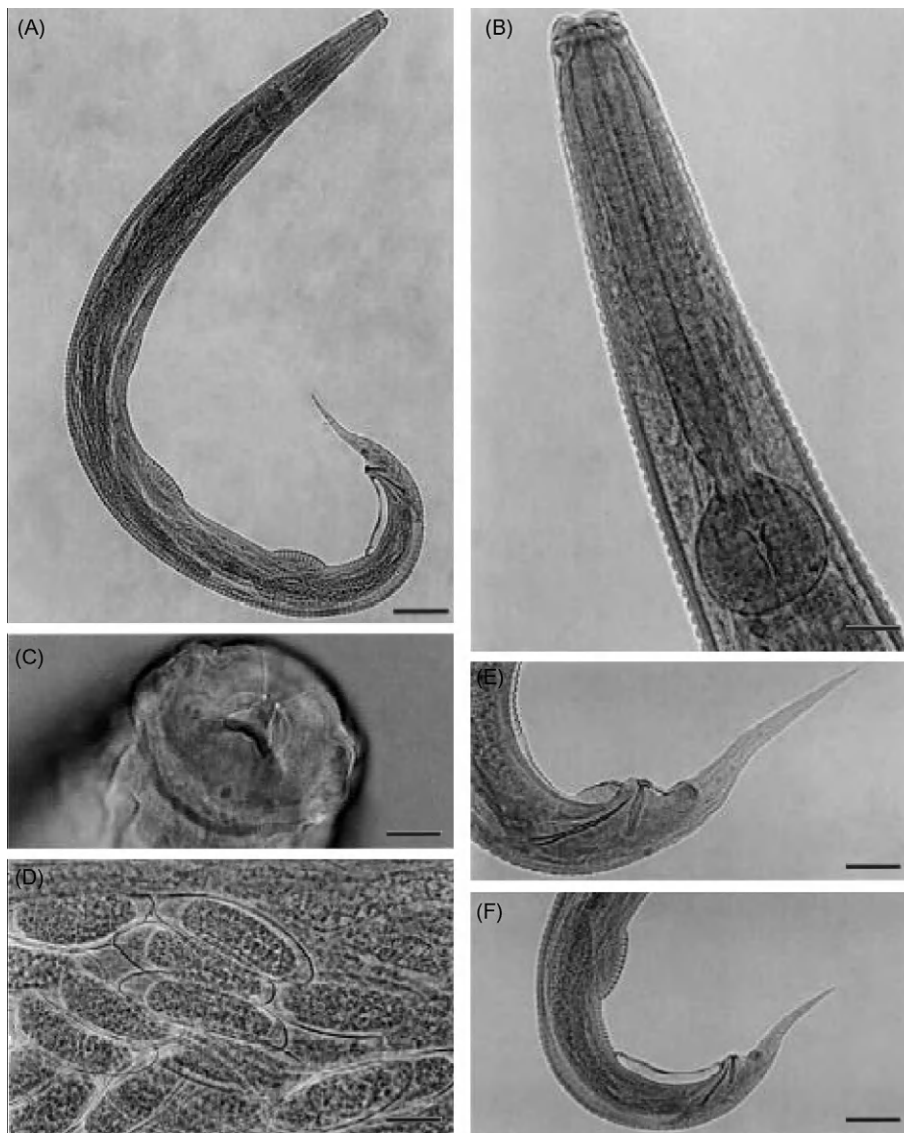


FIGURE 32.9 *Syphacia criceti*. (A) Male, whole body, lateral view. Bar = 0.08mm. (B) Cranial portion of male, lateral view. Bar = 0.02mm. (C) Head of female, en face view. Bar = 0.01mm. (D) Ova in utero. Bar = 0.03mm. (E) Caudal extremity of male, lateral view. Bar = 0.02mm. (F) Caudal portion of male, lateral view. Bar = 0.07mm. Bar of A common to B–F. Reproduced from Pinto et al. 2001 with permission.

has been proposed (Prince, 1950). Retroinfection does not occur with *A. tetraptera* or *D. translucida* due to the location of egg release and the prolonged time to egg infectivity, approximately 5–10 days (Any, 1966b; Hsieh, 1952; Pritchett and Johnston, 2002).

CLINICAL MANIFESTATIONS In immunocompetent animals, pinworm infections are usually clinically silent (Taffs, 1976). Clinical signs are not observed following natural infection with *S. criceti* and *S. mesocriceti* in hamsters (Hankenson and Van Hoosier Jr., 2007). *D. translucida* infections typically are asymptomatic in Mongolian gerbils (Wightman et al., 1978; Wilkerson et al., 2001) and parasitism is considered incidental in Syrian hamsters (Greve, 1985). *S. obvelata*, *A. tetraptera*, and *S. muris* infections in mice and rats generally cause no clinical signs but may be associated with non-specific

signs of illness such as poor body condition, roughened hair coat, reduced exploratory behavior (McNair and Timmons, 1977), rectal prolapse (Hoag, 1961), and intestinal impaction or intussusception (Taffs, 1976). *Citrobacter rodentium* and *Helicobacter* spp., however, are known to cause similar signs in mice and may have contributed to these generalized conditions (Clifford and Watson, 2008; Pritchett and Johnston, 2002). *S. muris* has been found to have a shortened patent period in Syrian hamsters suggesting that the clinical effects would be minimal (Ross et al., 1980).

PATHOLOGY *S. criceti* and *S. mesocriceti* are considered non-pathogenic in hamsters (Hankenson and Van Hoosier Jr., 2007). *D. translucida* caused no pathological lesions in Mongolian gerbils (Wightman et al., 1978) and adults were found unattached to the stomach

mucosa of a Syrian hamster with no gastric lesions noted at necropsy (Greve, 1985). *S. muris* and *S. obvelata* infections in rats and mice are generally non-pathogenic, but infections may impact intestinal electrolyte transport (Lubcke et al., 1992), growth rate, and the hepatic enzyme system (Mohn and Philipp, 1981), and most well documented, the immunological response (Agersborg et al., 2001; Michels et al., 2006). In hamsters, *S. obvelata* is reported as non-pathogenic but the possibility of mild enteritis has been suggested (Unay and Davis, 1980) and immature forms of the nematode have also been isolated from the brain of an adult Syrian hamster (De Roeвер-Bonnet and Rijpstra, 1961).

PATHOGENESIS/LIFE CYCLE Pinworm infections are generally non-pathogenic in immunocompetent rodents (Taffs, 1976) and rarely penetrate the mucosa of the gastrointestinal tract (Anya, 1966a; Pritchett, 2007).

The life cycle of *S. mesocriceti* in hamsters is incompletely described, but information can be extrapolated from other species in the genus. *Syphacia* spp., *Aspiculuris tetraptera*, and *Dentostomella translucida* all have direct life cycles with varying prepatent periods (Anya, 1966a; Chan, 1952; Hsieh, 1952; Taffs, 1976; Wightman et al., 1978) (Table 32.1). Upon ingestion of infective embryonated eggs, *Syphacia* spp. larvae hatch in the small intestine and migrate to the cecum to feed on bacteria. In mice, *S. obvelata* reaches the cecum within 24 hours and within 6 days post-hatching, two-thirds of the females are fertilized and the males die (Chan, 1952). Similar to other *Syphacia* spp., gravid *S. obvelata* females then migrate to the anus to lay their eggs on the perineum of the host and die (Chan, 1952). In contrast, *A. tetraptera* ova hatch in the cecum and larvae reside in the crypts of Lieberkühn of the mid-colon for approximately 5 days before migrating to the proximal colon (Behnke, 1974; Chan, 1955). Adult females migrate to the distal colon to lay their eggs which are excreted in the mucus layer of the feces (Behnke, 1974; Taffs, 1976). Females live another 21–24 days after first egg release for a lifespan of 45–50 days (Hsieh, 1952). *D. translucida* resides in the proximal third of the small intestine and occasionally the stomach of gerbils. Like *A. tetraptera*, the eggs of *D. translucida* are shed in the fecal pellet (Wightman et al., 1978).

DIAGNOSIS Common diagnostic techniques in rodents include the perianal cellophane tape test, anal swab, fecal flotation, necropsy with microscopic examination of cecal and colonic contents, and histologic examination (Hill et al., 2009; Pritchett and Johnston, 2002). Demonstration of adult worms in cecal and colonic contents has been reported as the most sensitive diagnostic technique for the detection of pinworms in mice (West et al., 1992). *S. obvelata* ova have

been detected in Syrian hamsters with the perianal tape test technique (Taylor, 1992; Unay and Davis, 1980). This technique has a reported sensitivity of 100% for detection of *S. muris* in rats and 85.5% for detection of *S. obvelata* in mice (Hill et al., 2009). A modified anal swab technique has been used to detect *S. mesocriceti* and *S. criceti* in hamsters (Pinto et al., 2001). The technique involves inserting a plastic rod that is moistened in 0.85% NaCl into the anus; the rod is rotated and the collected feces are then transferred to a glass slide with saline and examined under light microscopy (Goncalves et al., 1998).

Due to shedding of ova in the feces, the perianal tape test will not detect *Aspiculuris* spp. and *Dentostomella* spp. infections (Pritchett and Johnston, 2002). As with other pinworms, evaluation of colonic and cecal contents for adult worms at necropsy is the most effective way to diagnose *A. tetraptera* and *D. translucida* infections (West et al., 1992; Wightman et al., 1978). Ova may also be found on fecal flotation and the anal swab technique described above has recovered *A. tetraptera* (Goncalves et al., 1998; Pritchett, 2007; Wightman et al., 1978).

A PCR-based method for identification of *S. muris*, *S. obvelata*, and *A. tetraptera* has also been described that differentiates the species based on their rDNA sequences and subsequent analysis of the amplicons with restriction endonucleases (Parel et al., 2008). A PCR assay to identify the three pinworm species is newly offered; the technique is reported to be more sensitive than either the tape test or fecal float and nearly as sensitive as post-mortem direct exam (University of Missouri Research Animal Diagnostic Laboratory, Columbia, MO, 2009).

PREVENTION AND THERAPY Preventive measures include exclusion of feral rodents from vivaria, the use of filter tops or microisolator caging, strict sanitation, and sterilization of feed and bedding (Farrar et al., 1994; Jacoby et al., 2002). Appropriate biosecurity measures should be employed when importing rodents, including the purchase of rodents from commercial vendors with well-established health monitoring programs that are free of parasites. Rodents imported from non-commercial research colonies should be quarantined and evaluated for parasite infection; preemptive treatment with an appropriate anthelmintic may also be employed.

Environmental decontamination of vivaria is often employed alongside anthelmintic treatment of rodents. Pinworm eggs are considered to be persistent environmental contaminants based on characteristics of the human pinworm, *Enterobius vermicularis*, including the ability to aerosolize (Pritchett and Johnston, 2002; Wantland, 1955). Pinworm eggs have been found in dust, on equipment, and in ventilation air-intake ducts

of animal-holding rooms housing infected mice (Hoag, 1961). *S. muris* eggs have been isolated from equipment and shelving in a room housing infected rats in individually ventilated cages (Huerkamp, 1993). Despite this, tape impressions from the interior surfaces of filter-top cages housing *S. obvelata*-infected mice failed to isolate eggs (Lipman et al., 1994). *S. muris* eggs remain infective in the laboratory environment for at least 4 weeks (Dix et al., 2004), while *S. obvelata* eggs may only remain viable for 42 hours at 30°C (Grice and Prociw, 1993). *A. tetraptera* is also thought to be long-lived in the environment (Clifford and Watson, 2008). With pinworm eggs resistant to most common disinfectants (Dix et al., 2004), physical methods such as scrubbing with detergent, steam cleaning, or painting are likely to be most effective for environmental decontamination (Clifford and Watson, 2008). In modern rodent vivaria with individually ventilated caging systems, failure to eradicate pinworm infections may be due less to environmental contamination than to ineffective anthelmintic regimens and compliance (Pritchett, 2007). Environmental decontamination may be unnecessary if all rodents are effectively treated to eradicate pinworm infection and for a duration that exceeds persistence of the eggs in the environment (Clifford and Watson, 2008).

Treatment strategies for pinworms in mice and rats are well documented (Pritchett and Johnston, 2002). Avermectins and benzimidazoles are currently the most widely used anthelmintics for pinworm eradication (Pritchett, 2007; Pritchett and Johnston, 2002). Avermectins, which include ivermectin, paralyze parasites by increasing chloride permeability in the cell membranes of peripheral nerve tissues and possibly potentiate gamma aminobutyric acid (GABA)-gated sites. Benzimidazoles, including fenbendazole, bind nematode B-tubulin and inhibit microtubule formation (Hill et al., 2006) and have adulticidal, larvicidal, and ovicidal actions (Pritchett and Johnston, 2002). Fenbendazole-medicated diet (150 ppm fenbendazole) fed for a total of 5–6 weeks in rats and mice has eradicated *S. muris* and *S. obvelata*, in some instances without environmental decontamination (Barlow et al., 2005; Hill et al., 2006; Huerkamp et al., 2004). Fenbendazole-medicated diet (150 ppm fenbendazole) fed for a total of 5 weeks with or without ivermectin-supplemented drinking water (ivermectin dose 0.005 mg/ml) has also been effective at eliminating *Dentostomella translucida* from Mongolian gerbils without additional husbandry measures (Wilkerson et al., 2001).

Studies detailing treatment strategies in Syrian hamsters are limited and strategies can be adapted from the treatment of other rodents. *S. obvelata* has been eradicated in Syrian hamsters via two treatment courses of 10 mg of piperazine citrate per ml of drinking water for 7 days each, separated by 5 days without treatment

(Unay and Davis, 1980). A combination of 0.1% thia-bendazole in the diet for 6 weeks and 0.6% piperazine hydrate in drinking water for 8 weeks, beginning 2 weeks before the thiabendazole, has eradicated *S. obvelata* from six Syrian hamsters that could not be transferred to a clean environment (Taylor, 1992).

Miscellaneous Nematodes

Trichosomoides nasalis

Adult *Trichosomoides* have been identified in decalcified parasagittal sections of the nose of an approximately 5-week-old male Syrian hamster. Ova with polar plugs were detected in the feces of hamsters (37/185) from the same colony. *Trichosomoides nasalis* was suspected (Chesterman, 1972; Chesterman and Buckley, 1965) and *T. nasalis* was subsequently reported in the nasal cavity of a golden hamster in Switzerland (Redha and Horning, 1980). *T. nasalis* was first described in the nasal cavities of *Rattus norvegicus* with infection spread via the ingestion of embryonated eggs (Biocca and Aurizi, 1961). Based on other Trichosomoidinae, the life cycle is direct and *T. nasalis* is presumed to have a migratory phase which is presently undetermined (Diagne et al., 2004) and may produce lesions in the lungs or other organs (Wagner, 1987).

A recent histological study of the nasal cavities of the African grass rat, *Arvicanthis niloticus*, naturally infected with *T. nasalis* shows female worms to reside in the nasal epithelium. Ova, mixed with mucus and polymorphonuclear cells, were found in the nasal lumen and one male was found within a female's uterus (Diagne et al., 2004) as is common for Trichosomoidinae (Wagner, 1987). Significant inflammatory lesions in the lamina propria resulted in rhinosinusitis with a lymphocytic infiltrate causing nasal obstruction (Diagne et al., 2004).

Mastophorus spp. and Protospirura spp.

Mastophorus muris has been reported in a variety of rodents, including *Graomys griseoflavus*, *Apodemus sylvaticus*, *Clethrionomys glareolus*, *Rattus* spp., and marsupials including *Trichosurus vulpecula* and *Hypsiprymnodon moschatus* (Pisanu et al., 2009; Rojas Mdel and Digiani, 2003). *Protospirura muricola* has also been isolated from many Muridae (Smales et al., 2009). Confusion regarding classification of *Mastophorus* spp. and *Protospirura* spp. is well documented (Smales et al., 2009). *Mastophorus muris* has also been cited as *Spiroptera obtusa* or *Protospirura muris* (Milazzo et al., 2003).

Reports specifically of *Mastophorus* spp. in hamsters are vague (Hankenson and Van Hoosier Jr., 2007; Wagner, 1987). *Protospirura muris* has been recovered from a single hamster exhibiting a heavy worm burden resulting in distention at the cardiac end of the esophagus and stomach (Mauer et al., 1953). The authors

suspected that the hamster ate a heavily infected insect which accounted for the parasitism (Wantland, 1955). Adults reside in the esophagus and stomach and infection is acquired through ingestion of the intermediate host, American and Madiera cockroaches (Brenner, 2002; Wagner, 1987; Wantland, 1955). Beetle larvae and meal worms can also serve as intermediate hosts (Pierce, 1921). Parasitism has not been reported as a natural occurrence in laboratory rodents (Hankenson and Van Hoosier Jr., 2007; Wagner, 1987).

Gongylonema neoplasticum

G. neoplasticum has been reported in rodents and lagomorphs worldwide. Infection is spread through ingestion of intermediate hosts, including cockroaches, mealworms, and fleas. The nematode occurs in the esophagus and stomach and can cause gastric ulcers. Natural infection has not been reported in laboratory rodents and there are only limited reports of the parasite in hamsters (Hankenson and Van Hoosier Jr., 2007; Wagner, 1987).

Heligmosomum juvenum

H. juvenum has been isolated from the small intestine of hamsters in western Asia. Infection is thought to occur through the ingestion of infectious larvae. Pathologic effects are unknown and zoonosis is not reported (Hankenson and Van Hoosier Jr., 2007; Wagner, 1987). Current infections in hamsters have not been reported.

CESTODES

Hymenolepiasis

BACKGROUND Hamsters are susceptible to a number of cestode infections of the family *Hymenolepididae*, including *Rodentolepis nana*, *Hymenolepis diminuta*, and *Rodentolepis microstoma*. Historically, *R. nana* (formerly *Hymenolepis nana*) was one of the most important internal parasites of hamsters (Renshaw et al., 1975; Soave, 1963). Recent infections have been detected in laboratory animal suppliers (Pinto et al., 2001). *R. nana* is also the most common cestode infecting humans world-wide (Macnish et al., 2003). It is frequently used in biologic research to study tapeworm biology and its pathologic effects.

ETIOLOGY/ETIOLOGIC AGENT *R. nana* is known as the dwarf tapeworm. Table 32.2 provides the average morphological measurements for this small, white tapeworm. The anterior portion has a rounded, inverted scolex with four unarmed suckers and a prominent rostellum (Habermann and Fletcher, 1958). This rostellum has a single layer of 20–27 hooks forming an anterior crown (Figure 32.10). The wider, posterior strobila

TABLE 32.2 Comparison of Cestode Physical Characteristics^a

	<i>Rodentolepis nana</i>	<i>Hymenolepis diminuta</i>	<i>Rodentolepis microstoma</i>
Body length	25–40 mm	20–60 cm	205 mm
Body width	1 mm	3–4 mm	2 mm
Scolex	4 unarmed suckers	4 unarmed suckers	
Rostellum	20–27 hooks	No hooks	<30 hooks
Ova	Oval, transparent	Spherical, transparent, yellow tint	Oval, transparent
Length (μm)	50–55	70–85	75–90
Width (μm)	37–47	60–80	51–75
Onco sphere	6 hooks	6 hooks	
Length (μm)	24–30	24–30	27–48
Width (μm)	16–25	16–23	29–54
Polar bodies	2 with 4–8 attached filaments	No polar filaments	Slight polar bodies, 3–5 polar filaments
Life cycle	Direct; indirect; autoreinfection	Indirect	Indirect
Location of adult worm in host	Ilium	Anterior small intestine	Duodenum; pancreatic ducts; biliary ducts; mesenteric lymph nodes

^aFrom Dvorak et al., 1961; Larsh, 1946; Litchford, 1963; Macnish et al., 2003; Wagner, 1987.

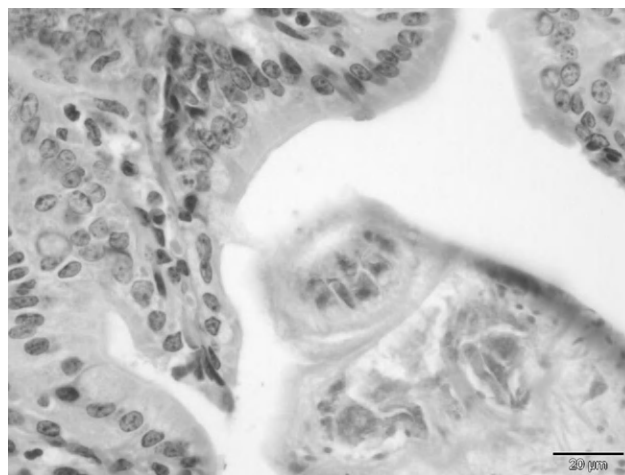


FIGURE 32.10 A section of small intestine showing the head of *R. nana*. Note the prominent rostellum.

consists of numerous, transversely elongated proglottids (Pinto et al., 2001). Gravid proglottids release the transparent, ovoid eggs of *R. nana*. The ovum consists of an outer membrane encircling an enveloped



FIGURE 32.11 A photo of an egg of *Rodentolepis nana* demonstrating the three pairs of hooks.

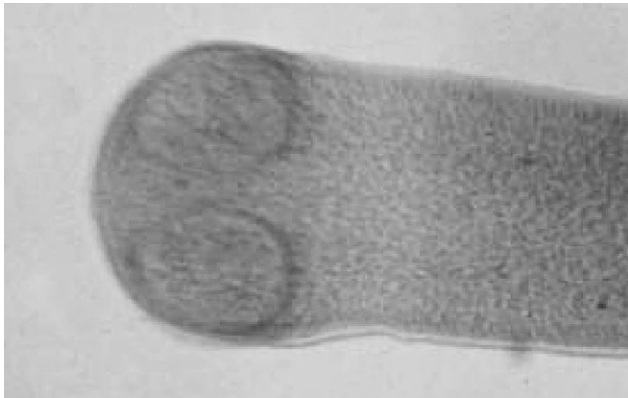


FIGURE 32.12 A photo of a *Hymenolepis diminuta* scolex. Note the lack of rostellar hooks. Reproduced from Centers for Disease Control and Prevention (http://phil.cdc.gov/PHIL/Images/370/370_lores.jpg) with permission.

oncosphere (hexacanth embryo). The oncosphere membrane has two polar thickenings from which 4–8 attached filaments arise. These are characteristic of *R. nana* and *R. microstoma* ova (Morrissey, 1996). These filaments extend into the space between the oncosphere and outer membranes. The oncosphere possesses three pairs of small hooks (Figure 32.11).

Hymenolepis diminuta is significantly larger than *R. nana* and has a pear-shaped scolex (Table 32.2). It has no rostellar hooks which further differentiates it from *R. nana* (Figure 32.12). The proglottids of *H. diminuta* are similar in shape to those of *R. nana*, but larger in size. The yellow-tinted, transparent ova of *H. diminuta* are spherical in shape, approximately twice the size of those of *R. nana* (Habermann and Fletcher, 1958) (Figure 32.13). These hardy ova are capable of



FIGURE 32.13 A photo of a *Hymenolepis diminuta* egg. Reproduced from Centers for Disease Control and Prevention/Dr. Mae Melvin (<http://phil.cdc.gov/phil/details.asp>) with permission.

surviving in feces for up to 6 months (Baker, 2007). The oncosphere of *H. diminuta* also contains three pairs of hooklets.

R. microstoma's developmental stages are similar in appearance to *R. nana* as adults possess rostellar hooks and the ova contain polar filaments (Table 32.2). Adult *R. microstoma* are generally larger in size, although an increased worm burden will negatively affect adult body length (Dvorak et al., 1961).

EPIZOOTIOLOGY The host specificity of *R. nana* is uncertain. Sources cite humans, wild or feral rodents, hamsters, and other lab rodents as definitive hosts (Larsh, 1946). *R. nana* may be found within non-human primates such as Rhesus macaques, squirrel monkeys, and chimpanzees (Wagner, 1987). Although *R. nana* is a prevalent human pathogen, a recent study indicates that the human strain of *R. nana* may be non-infective to rodents (Macnish et al., 2002). Attempts to infect rodent species such as rats with *R. nana* isolated from another rodent species have frequently failed, leading to the hypothesis that there are host-adapted strains of *R. nana* (Macnish et al., 2002; Vianna and de Melo, 2007). This is supported by evidence from experimental infections with *H. nana* which found the Syrian hamster to be the most permissive host when compared to experimental infections in other rodents (Larsh, 1946).

Definitive hosts for *H. diminuta* include hamsters, mice, and rats. Human infection with *H. diminuta* is rare and is most likely found in children (Macnish et al., 2002). These infections usually result from ingestion of dried fruits or cereals which are contaminated with infected intermediate hosts. The primary host of *R. microstoma* is the mouse, but cotton rats and hamsters

are also permissive hosts (Litchford, 1963). *R. microstoma* has also recently been detected as an infection in humans (Macnish et al., 2003).

CLINICAL MANIFESTATIONS Infection with *R. nana* rarely results in clinical signs (Morrisey, 1996). A small parasite load is relatively non-pathogenic, but increased intestinal burden may lead to clinical disease. In severe infections, decreased growth and emaciation have been observed (Wagner, 1987). Other sequelae include mechanical obstruction by worms leading to constipation, impaction, and eventually death (Soave, 1963). Infected young hamsters can have diarrhea and a pot-bellied appearance (Hankenson and Van Hoosier Jr., 2007). Poor husbandry has been cited as the major contributing factor in the development of clinical signs (Morrisey, 1996). *H. diminuta* causes clinical disease less frequently than *R. nana* (Morrisey, 1996). Experimental infections of Syrian hamsters with *R. microstoma* have resulted in significant clinical signs including emaciation, jaundice, and death (Dvorak et al., 1961).

PATHOLOGY Pathologic findings associated with *R. nana* infection include chronic catarrhal enteritis (Habermann and Fletcher, 1958). Severe cases may include the destruction of the intestinal mucosa. Abscesses of the mesenteric lymph nodes as well as hyperplasia of the Peyer's patches have been reported (Simmons et al., 1967; Wagner, 1987). One report demonstrated *Hymenolepis* in the duodenum, pancreatic ducts, bile ducts, and mesenteric lymph nodes, possibly contributing to liver cirrhosis in the infected animals (Chesterman, 1972; Chesterman and Pomerance, 1965). It is conceivable that these findings are due to a *R. microstoma* infection based on the location of the adult worm.

PATHOGENESIS/LIFE CYCLE *R. nana* is the only cestode with both direct and indirect lifecycles. The direct life cycle predominates for *R. nana*, lasting 14–16 days (Heyneman, 1963). The cycle begins with a definitive host's ingestion of embryonated eggs contained within feces. Eggs hatch in the anterior small intestine and the hexacanth larvae migrate into the local villi. Mucosal penetrance by the developing worm results in immunity which can prevent autoinfection (Heyneman, 1963). Once in the villi, the larvae develop into cysticercoids over the next 4–5 days. The developing worms exit the villi to attach to the lower ileal mucosa via the suckers and hooks of their scolices. Development into a mature worm takes 10–11 days. Adult worms survive for only a few weeks within the host. During this time, an adult worm may produce eggs which are expelled through the feces of its host.

Both *H. diminuta* and *R. microstoma* have obligate indirect lifecycles. Intermediate hosts are similar for

R. nana, *H. diminuta*, and *R. microstoma*. These include insects within the family Tenebrionidae, such as the confused flour beetle, *Tribolium confusum*. *R. nana* and *H. diminuta* can develop in other intermediate hosts such as moths, earwigs, fleas, and dung beetles. Intermediate hosts ingest cestode eggs within rodent feces. It takes approximately 2 weeks for the oncospheres to differentiate into cysticercoids within the hemocoel of *T. confusum* (Ito, 1997). However, the time to complete the indirect life cycle varies as the rate of larval development in intermediate hosts changes with environmental temperature (Wagner, 1987).

A definitive host is infected after ingesting an infected intermediate host. The indirect life cycle does not stimulate a protective immune response in the host due to the lack of a mucosal phase of parasite development (Heyneman, 1963). The ultimate locations of *H. diminuta* and *R. microstoma* adults within the final host vary from that of *R. nana*. *H. diminuta* adult worms are found predominantly within the upper small intestine. *R. microstoma* cysticercoids excyst within the duodenum and migrate to the bile duct within the next 5–7 days to mature. However, within hamsters they may remain solely within the duodenum (Macnish et al., 2003).

A unique feature of *R. nana* is the potential for autoreinfection. Autoreinfection occurs when eggs hatch within the host in which they were produced, rather than are excreted through the feces. Eggs then undergo development into mature worms within the intestinal tract of the original host. Evidence supports autoreinfection as a means for massive clinical infection in humans as well as potentially causing clinical disease in rodents (Heyneman, 1961). As stated above, induced immunity through the direct life cycle can prevent autoreinfection.

DIAGNOSIS There are several methods by which a cestode infection can be detected. Ova or proglottids (*H. diminuta*) can be observed directly in the feces or via crush preparations of intestinal contents. Adult worms may be visualized by inspection of the intestine at necropsy. Worm segments may also be seen on histologic preparations of host tissues. The ova of *R. microstoma* are very similar in appearance to *R. nana* and are not reliable for differentiation via light microscopy alone (Macnish et al., 2003). *R. microstoma* are also identifiable by finding adult worms within the bile duct (Baker, 2007). Molecular techniques such as PCR-RFLP or protein electrophoretic methods exist for species identification (Casanova et al., 2001; Macnish et al., 2003).

PREVENTION AND THERAPY There are several pharmaceutical and non-pharmaceutical methods for preventing and eliminating cestodes. The most effective method for control is maintaining a high level of

equipment and room sanitation along with wild rodent and arthropod control. Quarantine of incoming animals will aid in preventing the introduction of disease into animal facilities. Pet hamsters frequently carry *R. nana* and serve as a source of eggs which may be introduced into animal facilities by animal users (Pinto et al., 2001). Therapeutic measures to treat *R. nana* include the use of praziquantel, thiabendazole, and niclosamide (0.33% in feed for 7 days) (Ronald and Wagner, 1975). One study using *H. diminuta* as a model for testing anticestode medications derived oral, single administration doses for several agents, including praziquantel (3.125 mg/kg), bunamidine (100 mg/kg), niclosamide (200 mg/kg), cambendazole (50 mg/kg), and mebendazole (50 mg/kg) (Ostlind et al., 2004). It is reasonable to believe that these doses would be effective for treating *R. nana* and *R. microstoma*. One caveat exists regarding benzimidazole anthelmintics. *R. microstoma* is less susceptible to benzimidazole anthelmintics, such as albendazole and mebendazole, than other hymenolepidid tapeworms (McCracken et al., 1992).

Miscellaneous Cestodes

Taenia crassicollis

The cat cestode, *T. crassicollis* (= *Taenia taeniaeformis*) larval fragments were noted in hamster livers in one report (Chesterman and Negroni, 1961). Rodents are the intermediate host of this cestode, with its larval form previously known as *Cysticercus fasciolaris* (Irizarry-Rovira et al., 2007). Later experimental infections with this cestode failed to reproduce the reported natural infections (Olivier, 1962).

ARTHROPODS

Demodicosis

BACKGROUND *Demodex* spp. are in the class Arachnida along with ticks. These mites are further classified into the family Demodicidae within the suborder Prostigmata. *Demodex* mites are generally recognized to be host-specific. *Demodex* spp. have been reported in numerous species of hamsters including the Syrian hamster, the Siberian dwarf hamster, the Armenian hamster (*Cricetulus migratorius*), and the desert (Roborovski) hamster (*Phodopus roborovskii*) (Karaer et al., 2009; Tani et al., 2001). They are the most common ectoparasites of hamsters. Not all *Demodex* spp. have been definitively identified. Demodectic mites are still prevalent as health reports from a hamster vendor indicate an infection rate of approximately 5% (Charles River Laboratories International, Inc., 2009).

ETIOLOGY/ETIOLOGIC AGENT The identified mites include *Demodex criceti* and *D. aurati* of the Syrian hamster, *D. cricetuli* of the Armenian hamster, and *D. sinocricetuli* of the Chinese striped hamster (*Cricetulus griseus*). *D. criceti* has been described as the “stubby” mite due to its rounded body throughout all stages of development (Hasegawa, 1995) (Figure 32.14). There is sexual dimorphism for hamster *Demodex* spp. with the female mite typically being larger than the male (Table 32.3). In *D. criceti* the greater length of the female is due to a longer opisthosoma, the posterior portion of the body (Nutting and Rauch, 1958). The ova of *D. criceti* are thin-shelled and transparent. The larval form of *D. criceti* has six plates which appear similar to legs, though

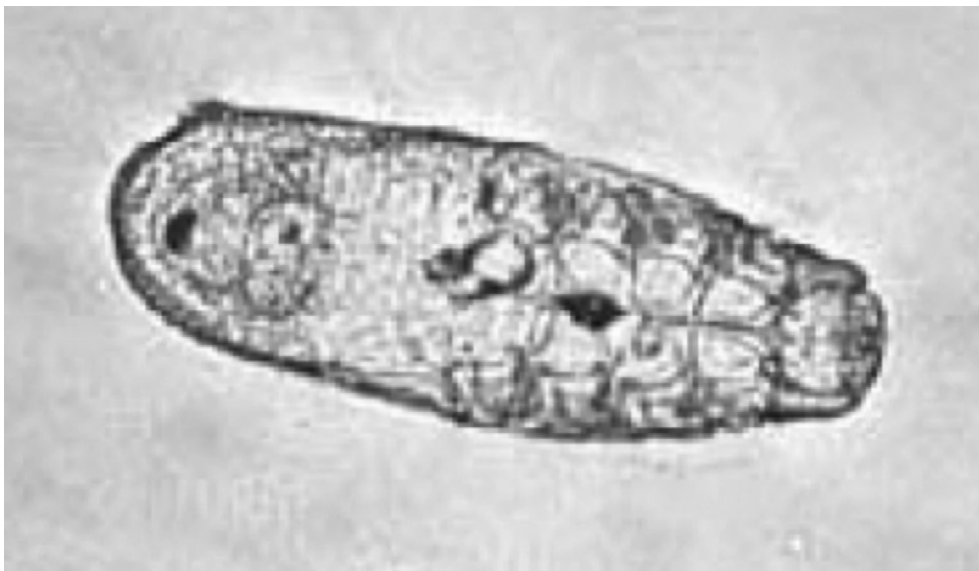


FIGURE 32.14 A photo of *Demodex criceti*, a mite of hamsters and gerbils. Note that this mite has similar short stubby legs but its body is shorter than that of *D. aurati*. Reproduced from University of Missouri Research Animal Diagnostic Laboratory (<http://www.radil.missouri.edu/ectoparasites.html>) with permission.

TABLE 32.3 Comparison of Mite Physical Characteristics^a

	<i>Demodex criceti</i>	<i>Demodex aurati</i>	<i>Demodex cricetuli</i>	<i>Demodex sinocricetuli</i>	<i>Notoedres spp.</i>	<i>Ornithonyssus bacoti</i>	<i>Speleorodens clethrionomys</i>
Ova							
Width (μm)	20	17	18	17			
Length (μm)	38	65	104	67		300–400	
Larval stage							
Width (μm)	29	17	18	14			
Length (μm)	56	104	140	74		300–400	
Nymph stage							
Width (μm)	30	25	30	29			
Length (μm)	84	207	228	120		500–700	
Adult stage – male							
Width (μm)	32	21–23	37	20			
Length (μm)	87	183	173	120	100–200	500	135
Adult stage – female							
Width (μm)	34	21–23	35	23			
Length (μm)	103	192	192	139	300–400	700–1000*	336

^aFrom Bornstein and Iwarsson, 1980; Desch and Hurley, 1997; Hurley and Desch, 1994; Hurley et al., 1992; Nutting, 1961; Nutting and Rauch, 1958; Nutting and Rauch, 1963; Uzal et al., 2007; Watson, 2008.

*In engorged state.

they have no capability for movement. There is size and morphologic variation within larval and nymph forms because of the multiple molts. The early nymph stages have four pairs of plate legs which most likely serve to hold the mite in its location. During later nymph and adult stages these develop into eight functional legs with two terminal claws (Nutting and Rauch, 1958).

D. aurati differs from *D. criceti* as it has a thin, elongate body terminating in a sharp opisthosoma (Figure 32.15). Adult *D. aurati* average almost twice the length of adult *D. criceti* (Table 32.3). The transparent, thin-shelled ova of *D. aurati* are spindle-shaped, with the length being approximately four times the width. The acutely tapered end of the ovum will become the posterior end of the adult (Nutting, 1961). Larvae are variable in length and also have six plate legs with two small claws. There is no determination of the difference between deutonymphs from protonymphs (Nutting, 1961). Nymphs have eight plate legs with two small claws.

Originally identified as *D. aurati*, *D. cricetuli* is a separate species which infects the Armenian hamster (Hurley and Desch, 1994). It is similar in size and appearance to *D. aurati* (Table 32.3). The opisthosomal terminus of *D. cricetuli* adults is more rounded than that of *D. aurati* adults (Hurley and Desch, 1994). The ova are spindle-shaped. The larval and nymph stages are

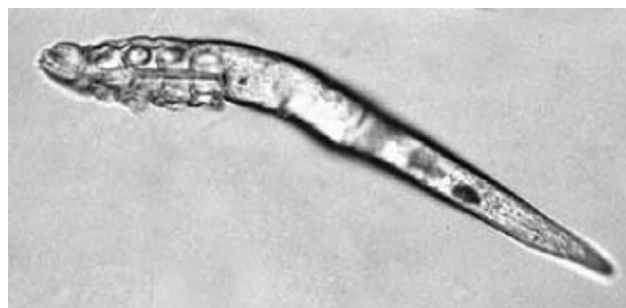


FIGURE 32.15 A photo of *Demodex aurati*, the mange mite of hamsters and gerbils. Note the vermiform shape and 4 pairs of short stubby legs. Reproduced from University of Missouri Research Animal Diagnostic Laboratory (<http://www.radil.missouri.edu/ectoparasites.html>) with permission.

similar in appearance to those of *D. aurati*. The legs of the *D. cricetuli* larvae have only one small claw per leg.

D. sinocricetuli is also similar to *D. aurati* in appearance. The adult males are approximately two-thirds the length of *D. aurati* males, and adult females are approximately three-quarters the length of *D. aurati* females (Table 32.3). Differentiating it from the previous demodectic mites discussed, *D. sinocricetuli* does not have uniform spacing between its legs. The opisthosoma of the female is more rounded at the terminus than that of the

male. The eggs are ovoid, with a posterior tapering. The fusiform larvae lack ventral scutes, which the immature stages of *D. aurati* possess (Desch and Hurley, 1997).

EPIZOOTIOLOGY Most *Demodex* spp. are normal fauna, found in small numbers on healthy animals causing no overt disease. They may become pathologic under situations of high stress, illness, or immune suppression. Transmission of mites is by direct contact or vertical transmission at the time of suckling. Demodectic mites have been recovered from juvenile hamsters as young as 5 days of age (Nutting and Rauch, 1958). Due to host specificity, hamster *Demodex* mites are not zoonotic.

CLINICAL MANIFESTATIONS Even with high infestation numbers, *D. criceti* rarely causes clinical signs (Nutting and Rauch, 1958). *D. aurati* infections are more likely to result in clinical signs than *D. criceti*. Clinical signs vary from rough hair coats to dermatitis with moderate to severe dorsal alopecia extending from the neck to the hind quarters. Alopecic areas are dry with excoriations and scales (Estes et al., 1971; Heath and Bishop, 1984; Karaer et al., 2009). Abdominal skin may be affected (Tani et al., 2001). Pruritus is uncommon. *D. cricetuli* does produce a mild to moderate degree of pruritus along with a scaling dermatitis and alopecia (Hurley et al., 1992). Epidermal hyperemia may be seen along with a mild serous exudate. No clinical signs have been reported for *D. sinocricetuli*.

PATHOLOGY *D. criceti* is generally of low to no pathogenicity. Microscopically, *D. criceti* mites are found in epidermal pits which extend from the epidermal surface to the *stratum basale* (*stratum germinativum*), rarely entering into the dermis. All life stages of *D. criceti* lie within the pits with their mouth parts facing towards the dermis. They are not found within normal hair follicles.

D. aurati can distort the normal morphology of hair follicles. Normal-appearing or dilated hair follicles may contain up to five mites of either gender along with debris (Nutting, 1961). Hair shafts from infected hair follicles are variably lost. *D. aurati* causes little inflammation, but the *stratum corneum* may show mild to moderate hyperkeratosis (Estes et al., 1971).

D. cricetuli also dilate the lumen of hair follicles, as up to six mites may live within a single follicle (Hurley and Desch, 1994). Mite opisthosomas have been observed to protrude from hair follicles (Hurley and Desch, 1994). Histopathology may include hyperkeratosis, parakeratosis, epidermal hyperplasia with lengthening of the rete pegs, and some areas of suppurative inflammation (Hankenson and Van Hoosier Jr., 2007; Hurley et al., 1992). Pathologic effects are not reported for *D. sinocricetuli*.

PATHOGENESIS/LIFE CYCLE The direct life cycle of *Demodex* spp. requires approximately 24 days to complete. Following hatching, *Demodex* mites undergo several stages of development beginning with the larval stage and progressing through the protonymph and deutonymph stages to eventually molt into an adult. Research has not elucidated the number of molts undertaken by the larval and nymph forms (Nutting and Rauch, 1958).

D. criceti and *D. aurati* complete their life cycles within different ecological niches of the Syrian hamster's epidermis, permitting dual-species infection. *D. criceti* creates pits in the epidermis of its host. Eggs deposited into epidermal pits hatch and develop into larvae and nymphs. The mite spends its entire life within these pits, apparently feeding on epidermal cells (Nutting and Rauch, 1958). Rarely, *D. criceti* exist upon the host epidermis (Nutting, 1961).

All stages of *D. aurati* are found within the pilosebaceous system. There is no preference by the mite to hair type; mites were found in all hair follicle types or adherent to all hair types (Nutting and Rauch, 1963). Eggs are laid singly within the hair follicle at a level adjacent to the sebaceous canal. After hatching, larvae migrate deeper into the hair follicle below the sebaceous canal opening. Larvae and nymphs feed on epithelial cells as they molt. Following development into adults, the mites return to the level of the sebaceous canal to mate and lay eggs. Adult mites appear to eat follicular epithelial cells and sebaceous gland cells. *D. aurati* have also been found within Meibomian and perineal glands (Nutting and Rauch, 1963).

D. cricetuli are found within hair follicles throughout the body, but the greatest densities are found on the dorsum and face (Hurley and Desch, 1994). Similarly to *D. aurati*, adult *D. cricetuli* may feed on sebaceous gland cells along with follicular epithelium.

Clinical disease has been associated with immunosuppressive factors such as underlying disease, advanced age, malnutrition, and the presence of tumors (Estes et al., 1971; Tani et al., 2001). *Demodex* mites possess a lipase which hydrolyzes triglycerides in the sebum leading to the release of fatty acids which have an irritant quality (Jimenez-Acosta et al., 1989). This may explain why *D. aurati* has been reported as more pathogenic than *D. criceti*, as *D. aurati* are found within the pilosebaceous system. Gender differences possibly exist as male hamsters are reported to carry heavier infestations than female hamsters (Nutting and Rauch, 1963; Tani et al., 2001).

DIAGNOSIS Deep skin scrapes from the dorsum or from alopecic areas are the recommended method for detection of *D. criceti*, *D. cricetuli*, and *D. sinocricetuli* (Hurley and Desch, 1994; Nutting, 1961). Skin scrapes

and hair plucks are also useful for *D. aurati* detection; hair plucks will identify the adult form of *D. aurati* (Nutting and Rauch, 1963). Skin scrapes should be deep enough to cause erythema (Hankenson and Van Hoosier Jr., 2007). To increase the probability of mite detection, skin scrapings can be cleared with 5–10% sodium hydroxide or potassium hydroxide. Different life stages of mites may be seen in histologic preparations. By 16 hours after the death of the host, mites will move from their epidermal locations allowing for collection from the skin surface (Nutting and Rauch, 1963). Radiant heat applied to excised skin samples will also incite *Demodex* mites to leave the epidermis (Nutting and Rauch, 1963). Male hosts should be tested preferentially due to their higher mite burdens. Even if *Demodex* mites are noted, other causes of dermatitis such as bacterial infections, bite wounds, dermatophytes, or other ectoparasites need to be ruled out (Wagner, 1987). The presence of *Demodex* could be incidental within a lesion.

PREVENTION AND THERAPY Prevention of disease due to *Demodex* requires maintenance of high standards for animal health and well-being. Stressful situations should be minimized through proper husbandry techniques including appropriate housing and sanitation. Incoming animals should be properly quarantined and tested. Although treatment regimens exist as described below, identification and culling of infected animals is recommended (Hankenson and Van Hoosier Jr., 2007).

Application of topical miticidal amitraz (0.0013%) is a standard treatment for demodicosis. Treatment regimens vary from once weekly to once every 14 days. It is recommended that treatment continues for at least 4 weeks following a negative skin scraping (Hasegawa, 1995; Meredith, 2006). The efficacy of and tolerance to amitraz is animal-dependent; following the first dose the animal should be monitored carefully for adverse effects (Tani et al., 2001). Coumaphos (0.017%), another topical miticide, can be applied every 4 days for 2 weeks, then every 7 days for 2 weeks (Hasegawa, 1995). Repeated application of topical medications is necessary as immature mites hide deep within the epidermis where medication may not reach (Tani et al., 2001). Topical miticides should be applied with a cotton-tipped applicator or toothbrush. Once-weekly selenium sulfide or benzoyl peroxide shampooing can be used in conjunction with topical miticide treatment (Hasegawa, 1995). Oral ivermectin has proven efficacious at a dose of 0.3 mg/kg daily; however, the authors report that this may not be the optimal dosing regimen (Tani et al., 2001). For *D. cricetuli*, topical ivermectin at a dose of 0.3 mg/kg for 10 days has been recommended (Hankenson and Van Hoosier Jr., 2007). The above treatment regimens are assumed effective for *D. sinocricetuli* as adequate treatment information is not available.

Notoedric Acariasis

BACKGROUND Two species of *Notoedres* capable of infecting hamsters have been identified, *Notoedres cati*, the cat mange mite, and *Notoedres notoedres*. These mites fall within the family Sarcoptidae, along with *Sarcoptes scabiei*. These mites, causes of notoedric mange are no longer common parasites of laboratory animals. Literature of the 1940s and 1950s repeatedly reported cases within animal facilities (Wantland, 1955). Today it exists as a pet hamster disease, but without cumulative data on its true prevalence (Beco et al., 2001).

ETIOLOGY/ETIOLOGIC AGENT *Notoedres* spp. are small, round mites closely resembling *Sarcoptes* (Figure 32.16). Female mites are larger than male mites (Table 32.3). *Notoedres* adults have posterior limbs which do not protrude past the body margins (Leone, 2007). They also possess blunt dorsal spines and a dorsal anus which aids in differentiating them from *Sarcoptes* (Uzal et al., 2007). Males and females can be differentiated by the lack of a mid-dorsal shield in females.

EPIZOOTIOLOGY Transmission is by direct contact between animals and presumably by contact with infected bedding (Beco et al., 2001). The nymph and larval stages are the predominant transmissible stages of this highly contagious disease. It will spread rapidly within a colony if proper treatment strategies are not initiated. Unlike *Sarcoptes*, *Notoedres* appears to have limited zoonotic potential.

CLINICAL MANIFESTATIONS The clinical signs of *Notoedres* infections are severe pruritus, erythema, crusts, erosions, and hyperpigmentation (Beco et al., 2001). These lesions may be generalized, but are more often found on ear pinnae, face, genitalia, tail, and limbs (Beco et al., 2001) (Figure 32.17). These lesions may result in death within 2–3 months if left untreated (Wagner, 1987).

PATHOLOGY Infections with *N. cati* cause chronic inflammatory changes in the epidermis. Extrapolating from *N. cati* infections in other species, potential changes include hyperkeratosis and acanthosis along with inflammatory cells, cellular debris, and mites in the epidermis (Uzal et al., 2007). One or more mites are found within tunnels in the stratum corneum.

PATHOGENESIS/LIFE CYCLE The life cycle of *Notoedres* is not clearly understood (Uzal et al., 2007). Ova are deposited within epidermal tunnels. These ova hatch into six-legged larvae within 3–4 days. These larvae undergo several more molts, transforming into eight-legged nymphs in approximately a 3-day period. These nymphs continue to molt as they burrow within the skin,



FIGURE 32.16 A photo of *Notoedres* mite characterized by a dorsal anus. Skin scraping; chloral lactophenol. Reproduced from Beco et al. 2001 with permission.



FIGURE 32.17 A photo of a hamster with lesions of *Notoedres* infestation on its face and feet. Severe crusting can be observed on the muzzle, ears, and extremities. Reproduced from Beco et al. 2001 with permission.

eventually becoming adults. The entire cycle is estimated to take 10–20 days (Baker, 2007; Wantland, 1955). All three mobile life stages can be found on the skin.

Epidermal burrowing by mites causes pruritus and increases potential for secondary bacterial infections following mechanical damage to the skin by mites and the host's attempts to alleviate discomfort through scratching.

DIAGNOSIS Diagnosis is made by observation of mites and fecal pellets on skin scrapings using light microscopy (Beco et al., 2001).

PREVENTION AND THERAPY Along with proper sanitation and cage changing, several methods for elimination of *Notoedres* infections are reported. Whole body lime-sulfur dips can be done once a week and continued for 2 weeks after a negative skin scrape. One study compared treatment of infected hamsters using ivermectin and moxidectin (Beco et al., 2001). Once-weekly subcutaneous ivermectin (400 µg/kg) or once- or twice-weekly oral moxidectin (400 µg/kg) were each given for 8 weeks. Both reduced clinical signs dramatically, but neither treatment led to complete remission. Repeat skin scrapings are therefore necessary to ascertain the effectiveness of these drugs. Selamectin has also been used successfully to treat notoedric mange in cats (Itoh et al., 2004).

Ornithonyssus spp.

BACKGROUND *Ornithonyssus* spp. are mesostigmatid mites of the family Macronyssidae. The two *Ornithonyssus* species reported to infect hamsters are *Ornithonyssus bacoti*, the tropical rat mite or red mite, and *Ornithonyssus sylviarum*, the northern fowl mite. Although *O. bacoti* has a wide host range, the primary hosts are the brown Norway rat (*Rattus norvegicus*) and the black roof rat (*Rattus rattus*) (Fox, 1982). Its common name is also a misnomer, as it is found in tropical and moderate climates (Beck and Folster-Holst, 2009). Historical reports of severe *O. bacoti* infections in hamsters exist, and although no hamster-specific outbreaks have been reported in the last 10 years, outbreaks within animal facilities have occurred infrequently in this

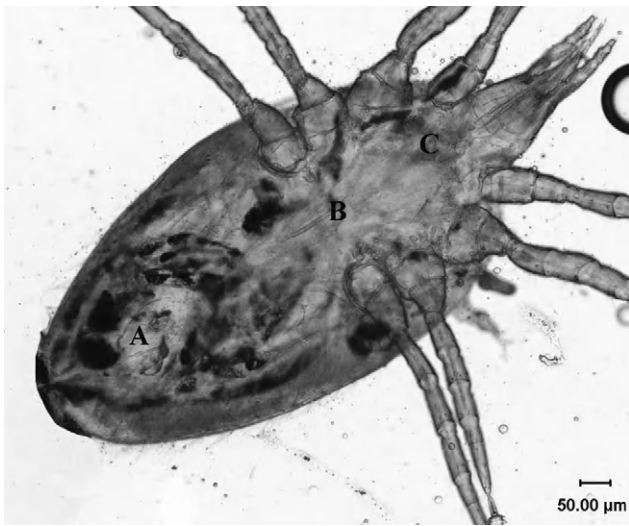


FIGURE 32.18 A photo of ventral view of *Ornithonyssus bacoti* with anal (A), genital (B) and sternal (C) shields labeled. Reproduced from Hill et al. 2005 with permission.

time (Watson, 2008). *O. sylviarum* is a common mite of chickens in temperate climates. The hamster may serve as an incidental host (Miller and Price, 1977).

ETIOLOGY/ETIOLOGIC AGENT *O. bacoti* have dorsoventrally flattened bodies (Figure 32.18). It has four pairs of jointed legs arising from the cranial half of the body. Males and females can be distinguished by the number of chitinous thickenings on their ventrums, the ventral shields. Males have one ventral shield and females have three. *Ornithonyssus* appear white prior to feeding, when they then become a red-brown following engorgement (Watson, 2008). Female mites will double in size following engorgement (Table 32.3). The mite is visible to the naked eye at this time. *O. sylviarum* resembles *O. bacoti*. These can be differentiated as *O. bacoti* has a greater number of hairs and an anal plate with a cranial anus as compared to few hairs and an anal plate with a caudal anus of *O. sylviarum* (Beck and Folster-Holst, 2009).

EPIZOOTIOLOGY If it does not find a suitable rodent host, *O. bacoti* will feed upon humans causing pruritic papules on the skin. Pet hamsters are documented as sources of human disease (Creel et al., 2003). As well as causing discomfort, *O. bacoti* has the capability to carry pathogens such as *Litomosoides carinii*, a rodent filarial nematode, and other diseases such as *Francisella pestis*, *F. tularensis*, and *Trypanosoma cruzi*. Whether they serve as vectors for human infection is uncertain (Watson, 2008).

CLINICAL MANIFESTATIONS *O. bacoti* does not typically cause clinical signs in hamsters. Heavier

infestations may lead to severe anemia and death (Creel et al., 2003). In humans, cutaneous lesions such as pruritic papules result as a response to mite saliva (Fox, 1982).

PATHOGENESIS/LIFE CYCLE *O. bacoti* females will feed on a host for up to a day. Two to three days following this blood meal, females will lay 90–120 eggs in different environmental locations such as cage nesting material. These eggs hatch and undergo four more developmental stages (Fox, 1982). Non-feeding, six-legged larvae hatch within 1–4 days. These molt into an eight-legged protonymph which requires a blood meal. The nymph has up to 3 weeks to find a suitable host upon which to feed or it will die (Wagner, 1987). The nymph undergoes another molt to a non-feeding deutonymph stage 1–2 days prior to molting into an adult. The life cycle is generally completed within 11–16 days (Beck and Folster-Holst, 2009).

DIAGNOSIS Often detection of the mite results from laboratory animal facility staff complaining of insect bites (Watson, 2008). The mites can be visualized as small black to red dots moving upon the animals or with cages. Application of adhesive tape to the pelage of the animal followed by microscopic evaluation may reveal nymphs or adults (Beck and Folster-Holst, 2009).

PREVENTION AND THERAPY Repeated animal and facility treatments will be required to eliminate mites for several reasons. Mites are capable of surviving off of a host for extended periods of time, they may lay large numbers of eggs which are hidden throughout the laboratory environment and it has a rapid reproduction rate (Creel et al., 2003; Hill et al., 2005). *O. bacoti* can enter individually ventilated cages, making exclusion of this pest difficult once an infestation is present (Watson, 2008). Infestation can spread through the nocturnal journey of mites searching for new hosts. Environmental treatment should be the first action step followed by individual animal or cage treatments. Synthetic pyrethroids have been used successfully for environmental decontamination (Hill et al., 2005). Pest eradication professionals should be consulted to determine a proper treatment plan which will prevent unnecessary exposure of personnel to insecticides (Watson, 2008). At the cage and animal level, there are several medications which have shown efficacy. Permethrin-impregnated cotton balls (7.4%) were placed in cages weekly for 5 weeks to eliminate infection from mouse cages (Hill et al., 2005). Ivermectin has not been reported as a treatment for *O. bacoti*, but a single application of selamectin to infected gerbils eliminated infection within a week (Fisher et al., 2007).

Speleorodens clethrionomys

S. clethrionomys is a nasal mite. A single report details the infection of laboratory hamsters with this mite (Bornstein and Iwarsson, 1980). These mites do not appear to cause clinical signs. Mites are most readily identified living on the mucosa of the posterior nasal cavity (Bornstein and Iwarsson, 1980). Mites appear oval in shape and milky white in color. Only female mites of this species have been described (Table 32.3). Treatment suggestions have not been proposed, however ivermectin and other types of avermectins may be effective.

Radfordia cricetuliphila

The identification of fur mites in laboratory hamsters is not well documented. The importance of these reported mites in a laboratory animal setting is undetermined at this time. Several species of Myobiid mites are reported in feral hamsters. Subspecies of *Radfordia cricetulus* have been collected from *Cricetulus migratorius* in Iran (Fain and Hyland, 1980). A myobiid mite, *Radfordia cricetuliphila* sp. n., has been identified on the striped hamster, *Cricetulus barabensis*, in a Russian Republic (Bochkov, 1999).

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Non-Infectious Diseases

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INTRODUCTION

Hamsters are generally hardy animals when housed under optimal conditions. As with many species, however, even animals free of infectious pathogens develop disease associated with conditions such as advancing age, heredity, and environment. This chapter summarizes such conditions as recorded in the published literature.

DISEASES ASSOCIATED WITH AGING

Amyloidosis

Background

Amyloidosis is the non-infectious disease of primary importance in the aging Syrian hamster (Franks and Chesterman, 1957). The incidence of amyloidosis varies

by hamster colony (Pour et al., 1976a, 1979), and there is a higher incidence of amyloidosis in females compared to males (Coe and Ross, 1990; Tennent et al., 1993). Amyloidosis results from the extracellular deposition of highly ordered proteinaceous material in tissues (Eanes and Glenner, 1968; Gruys et al., 1979; Kisilevsky and Young, 1994; Mezza et al., 1984).

Etiology

The amyloid fibril is the major component of amyloid. Amyloid forms from the straight, non-branching arrangement of fibrils which contain a high amount of beta-pleated sheet secondary structure (Eanes and Glenner, 1968; Glenner, 1980a, 1980b). Amyloid fibrils are derived from precursor proteins, and other proteins that can be transformed into fibrils (Kisilevsky and Young, 1994). In the Syrian hamster, amyloid fibrils are typically composed of Amyloid A and Amyloid P proteins (Coe et al., 1981; Linke et al., 1984). As in other species, there is a growing list of other distinct proteins that can be recruited into amyloid fibrils.

Epizootiology and Clinical Manifestations

Spontaneous age-related systemic amyloidosis is associated with early mortality in Syrian hamsters (Anderson et al., 1978; Chesterman and Pomerance, 1972; Gleiser et al., 1971; McMartin, 1979; Pour et al., 1976a, 1979; Redman et al., 1979; Schmidt, 1983). Amyloidosis can be detected in hamsters as early as 5 months of age, but there was a documented increase in mortality in 15–20-month-old hamsters when the incidence of amyloidosis of the liver and kidney was the highest (Schmidt, 1983).

The occurrence of amyloidosis differs by sex. There is a higher incidence, increased severity, and earlier onset of amyloidosis of the liver, kidney, stomach, adrenal, thyroid, and spleen of female hamsters (Coe and Ross, 1990; Schmidt, 1983; Tennent et al., 1993). The increased incidence is related to a higher expression of “Female Protein” compared to males. The “Female Protein” is a plasma protein with similar biochemical and functional characteristics compared to Amyloid P, and is under sex hormone control (Coe and Ross, 1990; Coe et al., 1977, 1981, 1997; Pepys et al. 1982; Tennent et al., 1993).

Pathology and Pathogenesis

Amyloid may be diagnosed in histological sections using characteristic colors imparted by special stains: green birefringence when stained with Congo red, metachromasia when stained with crystal or methyl violet, and secondary fluorescence when stained with thioflavin S and thioflavin T (Glenner, 1980a, 1980b; Husby and Sletten, 1986; Jakob, 1971). As seen with the electron microscope, amyloid is composed of non-branching fibrils approximately 100 Å in diameter, which are

composed of two or more twisted subfilaments about 40 Å diameter and separated by a 20-Å gap, forming a double helix of 100-Å periodicity (Jones et al., 1997).

Hepatic amyloidosis was detected as early as 5 months of age in one Syrian hamster colony (Schmidt, 1983). Eosinophilic, homogeneous deposits appear to develop first in the vascular walls and portal triads. With advanced disease, the portal areas and adjacent parenchyma fill with amyloid. The accumulation of amyloid accentuates the lobular architecture of the liver. These histologic changes typically promote the appearance of a swollen liver with accentuated lobes.

Renal amyloidosis typically presents primarily as glomerular depositions; but interstitial and combined amyloidosis can also occur (Schmidt, 1983). Kidneys often appear pale tan, enlarged, and/or misshapen. Amyloid deposition occurs in the glomerular and tubule basement membranes, but with advanced disease amyloid can disrupt the ultrastructure of the kidney (Percy and Barthold, 2007). Glomerular amyloidosis was reported to be the main cause of death in Syrian hamsters (Redman et al., 1979), and has been associated with atrial thrombosis (McMartin and Dodds, 1982). In another study, amyloidosis was observed in the liver, kidney, and adrenal gland, with 88% of hamsters greater than 18 months of age experiencing amyloidosis (Gleiser et al., 1970). In that study, amyloidosis was first detected in the kidneys and was accompanied by a rise in serum globulins.

Adrenal amyloid was observed to deposit initially in the zona fasciculata and zona reticularis, with eventual collection throughout the cortex. This deposition was seen as early as 5 months of age and became more frequent after 15 months (Schmidt, 1983).

Amyloid deposition may also occur in the salivary glands, stomach, small intestine, thyroid gland, parathyroid gland, spleen, meninges, trachea, lung, gallbladder, and pancreas (Chesterman, 1972; Frenkel, 1958; McMartin, 1979; Percy and Barthold, 2007; Pour et al., 1976b; Schmidt, 1983).

Diagnosis

Microscopic examination of affected tissues is the most reliable method of diagnosis. Special histochemical staining procedures as described above are useful for verifying the presence of amyloid in tissues. Use of the potassium permanganate method with Congo red stain can be used to distinguish types of amyloid fibrils in tissues (Mezza et al., 1984).

Prevention and Therapy

Amyloidosis occurs spontaneously in the aged Syrian hamster and there is no treatment for this disease (Anderson et al., 1978; Schmidt, 1983). The incidence of amyloidosis in female hamsters may be reduced through

feeding a diet high in fat and protein, but this does not completely prevent the disease (Birt and Pour, 1985). Since the incidence of amyloidosis varies based on colony source (Pour et al., 1976a, 1979), selecting the appropriate source of hamsters may be another approach to reducing the occurrence of amyloidosis when establishing a colony.

Glomerulonephropathy

Epizootiology and Pathogenesis

Among those diseases associated with the aging hamster, glomerulonephropathy remains an important cause of morbidity and mortality. Schmidt (1983) described progressive and degenerative kidney disease affecting both the tubules and connective tissue. In severe cases, areas of tubular collapse and connective tissue proliferation resulted in gross manifestations, visible on the capsular surface. The etiology and pathogenesis of this disease process remains unclear (Schmidt, 1983).

Doi et al. (1987) described a systematic pathological study comparing age-related, non-neoplastic lesions in the heart and kidneys of APA strain Syrian hamsters. This colony was developed and maintained as a closed breeding colony in Japan. In this colony, renal lesions developed more rapidly in females and were found more frequently and with greater severity than in males. In general, the renal lesions closely resembled glomerulonephropathy (chronic progressive nephrosis) of aged rats. Further characterization using electron microscopy of the same colony of APA strain Syrian hamsters revealed the lesion to be progressive and associated with advancing age (Han et al., 1992).

Pathology

Schmidt et al. (1983) described early kidney lesions as minimal and displaying areas of radial tubular atrophy, with minimal amounts of connective tissue. Severe lesions had variable numbers of linear or wedge-shaped areas of tubular collapse and increased amounts of connective tissue, which resulted in gross changes to the renal capsule. Most glomeruli in affected areas appeared normal, while tubular dilatation was common throughout much of the kidney. Most of the tubular luminae were empty, indicating functional nephrons, though some contained proteinaceous fluid and the glomeruli had changes to the mesangium and basement membranes but did not stain positive for amyloid. Inflammatory cells in affected kidneys were minimal or absent, and vascular lesions were not observed.

Doi et al. (1987) graded renal lesions in APA strain hamsters as mild, moderate, or severe based on the number of nephrons involved in each cross-section of tissue. Mild cases demonstrated focal changes consistent with tubular atrophy and/or tubular dilatation with proteinaceous casts scattered throughout

the tissue section. Segmental thickening of the basement membrane, mesangium, and Bowman's capsule was also observed in some nephrons. Moderately diseased kidneys demonstrated more injured nephrons and wedge-shaped or linear lesions extending from the subcapsular zone to the inner medulla. Injured nephrons were characterized by tubular dilation and thickening of peritubular basement membranes with a mild increase in interstitial connective tissue. Dilated tubules commonly contained proteinaceous material, while glomeruli demonstrated, on occasion, eosinophilic and PAS-positive deposits in the capillary walls and mesangium, resulting in segmental thickening. Severely affected kidneys, in which over half of the nephrons were involved, typically had large numbers of proteinaceous casts. The presence of interstitial connective tissue around the atrophied tubules was also a common finding. Despite many of the glomeruli showing increased intercapillary PAS-positive deposits and other glomerular changes, no amyloid deposits were detected.

Kamino et al. (2001) investigated age-related spontaneous non-neoplastic processes in a colony of Han:AURA hamsters. Interestingly, calcification, cystic change, and infarction, were frequently found as concurrent lesions in the kidneys and were considered as evidence of chronic progressive nephropathy.

Atrial Thrombosis

Background and Epizootiology

Thrombosis of the heart chambers is a common finding in aged Syrian hamsters but the incidence varies widely by colony. Pour et al. (1976a) reported an incidence of 16% in one colony versus 35% in another. McMartin (1977) reported a 73% incidence in yet a different colony of aged hamsters. Males and females were equally affected but females developed the condition at an earlier age. In a study of 750 control, male hamsters on long-term carcinogenicity studies, atrial thrombosis was the most frequently observed cardiovascular lesion (Schmidt, 1983). The condition was first seen at 13 months of age, with a peak incidence at 27 months of age. According to Doi et al. (1987), cardiac thrombosis was commonly seen in the APA strain of Syrian hamsters. It affected primarily the left atrium and was diagnosed in 71% of males and 50% of females over 16 months of age that died spontaneously. Of interest is that of the 16 male hamsters that were euthanized per protocol at 18 or 24 months, only three showed evidence of cardiac thrombosis.

Clinical Signs

Clinical signs, when reported, were consistent with heart failure, including hyperpnea, tachycardia, and cyanosis (Sichuk et al., 1965).

Pathogenesis

Changes in coagulation and fibrinolytic parameters suggest a consumption coagulopathy (McMartin and Dodds, 1982). The authors theorized the thrombi occurred as a result of local blood stasis, secondary to heart failure.

Pathology

Grossly, thrombi were present primarily in the left atrium, and usually filled the atrium (McMartin and Dodds, 1982). The authors also noted bilateral ventricular hypertrophy in thrombosed hearts and myxomatous thickening of the atrioventricular valves. However the AV valve lesions were found in most aged hamsters, regardless of whether or not thrombi were present. Myocardial degeneration was also a common finding in aged hamsters (McMartin and Dodds, 1982). Doi et al. (1987) reported that the absolute and relative heart weights increased with age, suggesting cardiac hypertrophy in older APA hamsters.

Prevention

In one study, the incidence of cardiac thrombosis was reduced to zero when hamsters were fed a low-fat/high-protein diet during the first 3–7 weeks of life (Birt and Pour, 1985). However, the incidence in hamsters fed other diets (control and varying levels of fat and protein) was only 6%, which is significantly less than seen in other colonies of Syrian hamsters.

Polycystic Disease

Background

Polycystic disease is an incidental finding typically affecting the liver in older Syrian hamsters (Gleiser et al., 1970).

Etiology

The cause of polycystic disease in hamsters is not fully understood. There appears to be a genetic predisposition for cyst formation since the incidence of cysts varies based on the colony under study (Gleiser et al., 1970; Kaup et al., 1990; Somvanshi et al., 1987). Congenital abnormalities of hepatic or bile duct development have also been implicated in polycystic disease (Somvanshi et al., 1987).

Epizootiology

Polycystic disease is a common postmortem finding in Syrian hamsters between 6–24 months of age (Gleiser et al., 1970; Kaup et al., 1990; Pour et al., 1976a; Schmidt, 1983; Somvanshi et al., 1987). Cysts are typically found in the liver, but cystic dilations may also be present on the epididymis, seminal vesicle, pancreas, endometrium, ovary, esophagus, and adrenal gland.

Clinical Manifestations

Polycystic disease is an incidental finding, and Syrian hamsters typically do not develop clinical signs associated with this condition. However, one study reported abdominal enlargement in an affected hamster due to ascites (Somvanshi et al., 1987).

Pathology

On gross examination, cysts vary in size, but may be as large as 3 cm in diameter (Somvanshi et al., 1987). Hepatic cysts have thin walls and contain clear to straw-colored fluid, and may occupy the parenchyma or be located on the capsule (Gleiser et al., 1970; Kaup et al., 1990; Pour et al., 1976c; Somvanshi et al., 1987).

Microscopically, cysts are present as small unilocular or large multilocular cystic areas. Low cuboidal or flattened epithelial cells line the cysts, and these thin-walled structures contain homogeneous eosinophilic material (Gleiser et al., 1970; Kaup, 1990; Somvanshi et al., 1987). Pressure necrosis of the adjacent liver parenchyma, hemosiderin deposition, and lymphocytic infiltrations are variable pathologic changes associated with polycystic disease of the liver (Somvanshi, 1987).

Pathogenesis

The exact mechanism of cyst formation in the hamster is unknown. Histopathology and epidemiology suggest a congenital condition resulting from abnormal hepatic and bile duct development (Somvanshi et al., 1987). Further investigation is necessary to substantiate these findings.

Diagnosis

Polycystic liver disease of the Syrian hamster is an incidental finding that is easily diagnosed on necropsy and confirmed with histopathology.

Prevention and Therapy

There is no treatment or proven method of prevention for polycystic disease.

Hepatic Cirrhosis

Epizootiology and Pathogenesis

Pathological lesions in the liver including hepatic cirrhosis have been reported as incidental findings (Fortner, 1957; Frenkel, 1963; Galton, 1964; Ising, 1956; Klement and Svoboda, 1963). Chesterman and Pomerance (1965) reported finding pathological features of hepatic cirrhosis and liver tumors affecting mostly their inbred breeding stock and experimental study animals. The incidental finding of cirrhosis increased from less than 1% to greater than 20% during a 5-year period. Interestingly, the increased incidence

in cirrhosis was mirrored by an increased incidence of hepatic tumors. Both of these findings were more common in female hamsters and the number of lesions in both sexes increased as the hamsters aged. Of the experimental animals identified as having cirrhosis and hepatic tumors, most were over 6 months of age. It was speculated that dietary contamination, infectious agents, and/or genetics could have played a role in the development of these diseases; however, the etiology is still unknown (Chesterman and Pomerance, 1965).

Pathology

The dominant microscopic feature of the cirrhotic livers was periportal fibrosis and bile duct proliferation (Chesterman and Pomerance, 1965). Also noted were small groups of hepatic lobules separated by broad bands of tightly arranged regular acini and bile duct proliferation. Most of the hepatic tumors seen were cholangiocarcinomas, described as groups of firm gray nodules distributed in the subcapsular surface of the liver.

NUTRITIONAL DISORDERS

Spontaneous Hemorrhagic Necrosis (SHN) of the Central Nervous System

Spontaneous hemorrhagic necrosis has been reported in neonatal hamsters and fetuses during the last trimester of pregnancy. While the etiology is unknown, vitamin E deficiency has been implicated in this condition. In one study, supplementing the dam on day 7 of gestation with low levels of vitamin E significantly decreased the incidence and severity of lesions, while supplementation with high levels of vitamin E eliminated the condition entirely (Keeler and Young, 1979). In affected hamsters, hemorrhage and edema are seen initially in the forebrain, progressing to intraventricular hemorrhage and necrosis of portions of the axial nervous system (Young and Keeler, 1978). Clinical signs include stillbirths and/or weak neonates that fail to survive.

GENETIC, TRAUMATIC, ENVIRONMENTAL AND OTHER NON-INFECTIOUS DISEASES

Hereditary Hydrocephalus

Epizootiology and Pathogenesis

The prevalence of hereditary hydrocephalus in the Syrian hamster varies based on the colony under study. Yoon et al. (1972) reported a sporadic and lethal case of hydrocephalus, which resulted in the death of most

of the affected animals prior to reaching breeding age. All of the affected animals had a characteristic dome-shaped head. A more recent report described a high incidence of hydrocephalic animals in a colony of Swiss golden hamsters (Edwards et al., 2006). In contrast to the first report, these hamsters did not have the characteristic phenotype or the behavioral or neurological deficits despite 71% of the animals having severe gross lesions and 81% showing both gross and microscopic evidence of hydrocephalus.

Pathology

Hydrocephalic features included greatly distended lateral ventricles, which were fused together forming a large space filled with cerebral fluid. The third ventricle was less affected, but combined with the weight of the lateral ventricles compressed the fourth ventricle. Massive degeneration of the ependyma in the lateral ventricles was also described (Yoon et al., 1972).

Edwards reported that affected study animals only had dilation of the lateral ventricles, and further histologic examination revealed the third and fourth ventricles were not affected. This suggests the condition is caused by aqueductal stenosis (Edwards et al., 2006).

Congenital Heart Valve Anomalies

A family of Syrian hamsters, subjected to systematic inbreeding for over 25 generations, was noted to have a high incidence of aortic and pulmonary valve anomalies (Fernandez et al., 1998; Sans-Coma et al., 1996). Bicuspid aortic valves as well as bicuspid and quadricuspid pulmonary valves are common in this line. In addition, cartilaginous foci were commonly found in the aortic valves of these hamsters (Sans-Coma et al., 2005). These anomalies have no apparent clinical implications.

Spontaneous Seizures

Spontaneous motor seizures were reported in a colony of BIO[®] 86.93 Syrian hamsters, which had been inbred for 23 generations (Yoon et al., 1976). The seizures were manifested as tonic muscle spasms and severe paralysis, and which were distinct from audiogenic seizures. The seizures occurred in animals between 30–60 days of age, lasted from 2–5 hours, and were easily precipitated by mild stress. Breeding tests confirmed a simple recessive gene (*sz*) which was proposed as the symbol for this condition.

More recent studies using this colony described a condition more similar to dystonia (Loscher et al., 1989). Placement of the hamsters in a novel environment resulted in immobility lasting for hours. Based on these studies and the nature of disease, the authors proposed

that the hamster mutation be renamed “dystonic,” and designated using the new gene symbol, *dt^{sz}*.

Traumatic Disease

Syrian hamsters avoid social interactions and prefer a private existence unless breeding. Hamsters are easily agitated and have a tendency to fight (Harkness and Wagner, 1995). Cannibalism is a problem in stressed or young hamsters and is best prevented through improved husbandry (clean bedding, proper nutrition, and noise reduction) (Magalhaes, 1968; Ponsford and Whyley, 1954; Pour et al., 1976a). In one retrospective study, 97.5% of total preweaning mortality was due to cannibalism (Renshaw et al., 1975). The frequency of maternal cannibalism is more severe with malformed hamsters (Harkness and Wagner, 1995). Hamsters that destroy all or parts of a litter should not be used as breeding stock (Magalhaes, 1968).

NEOPLASMS

Spontaneous neoplasms are uncommon in the Syrian hamster, especially when compared to mice and rats. There have been a number of papers published documenting the tumor rates and types (Homburger and Russfield, 1970; Pour et al., 1976a; Schmidt, 1983; Van Hoosier and Trentin, 1979). There are differences in the reported frequencies of certain tumors. This is likely due to a number of factors including genetic differences in hamster colonies; different methods of tumor detection; and gender and age differences. In general, there is agreement that tumors occur more frequently in females than males (Homburger and Russfield, 1970; Pour et al., 1976a; Van Hoosier and Trentin, 1979).

Van Hoosier reported an overall incidence of spontaneous malignant neoplasms in the Syrian hamster as 3.7%. Pour et al. (1976c) studied two different colonies and noted an overall incidence for all tumors as 32% in one colony versus 42% in another. The most commonly reported group of primary tumors in hamsters are lymphomas. Endocrine tumors, especially adrenal gland tumors, are also very common (Homburger and Russfield, 1970; Pour et al., 1976a; Schmidt, 1983; Van Hoosier and Trentin, 1979). Intestinal tumors, both benign (polyps) and malignant (adenocarcinomas) are commonly reported (Van Hoosier and Trentin, 1979). However, to underscore differences between hamster colonies, Pour et al. (1976a) reported no tumors, either benign nor malignant, in the intestines of one hamster colony, while a different colony developed a significant number of malignant intestinal neoplasms. Melanomas are also reported with some frequency. Tumors of the nervous system, thymus, and the mammary glands are uncommon.

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The Experimental Use of Syrian Hamsters

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INTRODUCTION

The Syrian hamster (*Mesocricetus auratus*) is a widely used experimental animal model. A recent fiscal-year report from the USDA indicates that 172498 hamsters were used in 2007. Although the hamster still makes a significant contribution to research and teaching, the number of hamsters utilized currently is well below their peak usage of over 500000 achieved during the 1980s (Hankenson and Van Hoosier, 2002). Much of this decline is likely attributable to the availability and widespread use of genetically engineered mice. Despite this decline, the hamster still provides key data for a variety of research areas. As such, this chapter will focus primarily on the most current research uses of the hamster. More classical uses will be covered only as they pertain to these current uses.

Hamsters possess unique anatomical and physiological features which make them desirable research models. Unlike other commonly used laboratory rodents, hamsters possess a cheek pouch which can be easily everted and examined in situ at both the gross and microscopic level (Hankenson and Van Hoosier, 2002). The hamster's relative size also allows for better visualization of certain biological systems including the respiratory and reproductive systems when compared to the mouse (Magers et al., 1995). Additionally, hamsters are relatively free of pathogens yet they are naturally susceptible to a wide range of experimental pathogens (Hankenson and Van Hoosier, 2002). Further, laboratory hamsters develop a variety of inherited diseases which display similarities to human conditions. Hamsters possessing some of these inherited traits are commercially available (Biobreeders Inc. Watertown, MA; Table 34.1). In addition to these benefits, hamsters are susceptible to a variety of carcinogens and develop tumors that other research animals less commonly develop. Furthermore, hamsters are susceptible to the induction of a variety of metabolic disorders through the use of dietary manipulations. Finally, the antagonistic nature of hamsters is utilized to study the effect of treatment on male aggressive and defensive behaviors.

TABLE 34.1 Inbred and Spontaneous Mutant Hamsters Currently Available and Their Uses in Research

Name	Research Use
Bio 1.5	Carcinogenicity and dental caries
Bio 14.6	Cardiomyopathy and muscular dystrophy
Bio 15.16	Carcinogenicity studies
Bio F1B	Diabetes and atherosclerosis
Bio HT	Hypertension
Bio To-2	Cardiomyopathy and muscular dystrophy

CANCER RESEARCH

Syrian hamsters display several unique characteristics that make them desired models for carcinogenesis studies. Specifically, they develop tumor types that other models, particularly the mouse, are less apt to develop. Further, they can be reliably infected with oncogenic and oncolytic viruses. This section details the most commonly used hamster models of cancer and carcinogenesis and highlights the key findings discovered in these models.

Pancreatic Adenocarcinoma

Pancreatic tumors of non-endocrine origin are particularly aggressive tumors with a poor prognosis in humans (Uchida et al., 2008). The most common anatomic form of this tumor is ductular adenocarcinoma. The Syrian hamster is used extensively to study this tumor. Most commonly, the tumors are induced by the administration of nitrosamines (particularly *N*-nitrosobis amine) as a single subcutaneous injection. The resulting tumor is both morphologically and biologically similar to human pancreatic ductal adenocarcinoma (Uchida et al., 2008). One of the limitations of this model is the prolonged time-course for tumor induction (as long as 51 weeks). This time-course can be expedited by giving multiple nitrosamine injections (Konishi et al., 1998). Additional limiting factors of the model are the incomplete penetrance of tumor formation even with multiple injections and the low rate of tumor metastasis (Konishi et al., 1998; Mizumoto et al., 1990). Environmental factors, including a choline-deficient diet, given in combination with nitrosamines produce tumors more rapidly and with a greater prevalence in both male and female hamsters (Mizumoto et al. 1988, 1989a, 1989b).

Current studies utilizing the carcinogen injection model focus on treatment and reduction of tumor development. Specifically, chemicals and biologics with the potential to prevent cancer can be given synchronously or shortly after carcinogen administration, and effectiveness in cancer reduction (both tumor size and prevalence) can be assessed phenotypically at specified time points. Utilizing this general scheme several potential anti-cancer compounds were identified, including dimethylamino-parthenolide, celecoxib, iNOS inhibitors, and melatonin (Ruiz-Rabelo et al., 2007; Takahashi et al., 2008; Yip-Schneider et al., 2008). Alternatively, studies can be carried out after induction to determine the efficacy of tumor treatment and metastasis prevention. This approach recently demonstrated that somatostatin analogs are able to decrease metastasis of pancreatic cancers (Kilian et al., 2009).

More recently, a transplantable cell line, PGHAM-1, was isolated from the pancreata of hamsters with

induced pancreatic cancer (Fukuhara et al., 2005). A variety of other biological behaviors can be induced with this transplantable model. Specifically, if the cell line is injected into the pancreas, hamsters develop pancreatic cancer which metastasizes to the liver whereas splenic injection induces direct metastasis to the liver, and intraperitoneal injection induces dissemination to the entire peritoneal cavity (Uchida et al., 2008). Therefore, this model can be used to study the primary disease, metastasis, and dissemination in a rapid fashion. An additional benefit of the transplant model is that it is characterized molecularly and is known to contain mutations of *k-ras*, *Vegf*, and matrix metalloproteinases similar to human pancreatic adenocarcinoma cells (Matsushita et al., 2001).

Respiratory Tract Tumors

Primary Lung Tumors

The two predominant types of lung cancer in humans are small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC) (Koletsis et al., 2009; Powell et al., 2009). NSCLC are lung tumors which originate from diverse cell types but behave and are treated in similar fashions. In contrast, SCLC originates primarily from neuroendocrine cells of the respiratory tract (Lee et al., 1992). Both tumor types are associated with tobacco smoking in humans.

In hamsters, lung tumors are induced by carcinogen treatment and typically result in NSCLC. One method to induce lung tumors is to inject the carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) subcutaneously (Sunday et al., 1995). Multiple injections must be given over a prolonged (6 months or longer) time course. Modifications to this induction protocol include the use of hyperoxia in conjunction with the carcinogen treatment (Oreffo et al., 1993). Presumably, the addition of hyperoxia increases the potential for free-radical damage to DNA of the lung tissue. The NNK model displays molecular characteristics of human NSCLC including mutations in the *k-ras* gene (Oreffo et al., 1992, 1993). A thorough immunohistochemical characterization of both the chemical model and the chemical plus hyperoxia model indicates that both experimental paradigms induce similar lung lesions that display strong similarity to human NSCLC at the protein level (Sunday et al., 1995).

Upper Airway Tumors

Upper airway tumors occur in the larynx, trachea, or pharynx. The primary risk factors for development of these tumors are smoking and chronic alcohol consumption; however, other environmental and genetic factors may contribute to the disease (Spitz, 1994).

The hamster is susceptible to tumors of the upper airways. These tumors are induced by inhalation or topical application of carcinogens with or without the addition of co-carcinogenic stimuli (Estensen et al., 2007; Homburger et al., 1979; Moon et al., 1992a, 1992b). A reported limitation of topical carcinogen application models is the lack of reproducibility among different labs (Estensen et al., 2007). It is unclear why various labs are unable to faithfully reproduce the cancer phenotype using the same protocols; however, one possibility is that because the hamsters came from different sources, they may be genetically different. To overcome this limitation, a recent publication describes combining the administration of N-methylnitrosourea (MNU) with direct wounding of the tissue (Estensen et al., 2007). The experimental design includes two tracheal wounding procedures done under general anesthesia. The trachea is gently abraded using a curette and this abrasion is coupled with six applications of MNU using a gavage needle. The induction of the wound induces epithelial proliferation which acts epigenetically in conjunction with the genotoxic mutagen to promote carcinogenesis. A benefit of this model is the coinduction of esophageal tumors which mimics the epidemiological linkage of these two tumor types in human smokers (Wattenberg et al., 2004). The model can be altered to incorporate preventative and post-induction treatments. Utilizing this paradigm, difluoromethylornithine (DFMO; a mitosis inhibitor) and 5-fluorouracil (5-FU; a DNA replication inhibitor) treatments were both found to reduce the prevalence of tumor formation in hamsters (Wattenberg et al., 2004).

Oral Carcinogenesis

Oral squamous cell carcinoma (OSCC) is a common cancer of humans and generally carries a poor prognosis (Vairaktaris et al., 2008a, 2008b). Primary risk factors for the development of OSCC include tobacco and alcohol use, but other genetic and environmental factors contribute as well (Williams, 2000). The hamster cheek-pouch carcinogenesis model is one of the most accepted animal models to study oral tumor formation and progression (Vairaktaris et al., 2007, 2008a, 2008b). Carcinogens, most notably 7,12-dimethylbenz(a)anthracene (DMBA), administered by repeated topical application to the buccal pouch, induces carcinomas rapidly (~14 weeks) (Vairaktaris et al., 2008b). The DMBA administration technique is simple and involves anesthetizing the hamsters and brushing the desired carcinogen onto the buccal surface of the cheek pouch. Importantly, oncogenesis can be assayed in vivo or the transformed cells can be grown ex vivo (Wong et al., 1988).

Tumor formation and progression in the hamster cheek pouch occurs in a predictable, reproducible stepwise fashion. First, hyperkeratosis develops followed

rapidly by hyperplasia and dysplasia. Finally, these lesions progress to invasive carcinomas (Vairaktaris et al., 2008b). Cellular and molecular alterations that occur during these predictable stages of tumorigenesis are extensively characterized (Vairaktaris et al., 2007, 2008b). The cellular and molecular alterations in the hamster display many similarities to human OSCC. Early in the disease, tyrosine kinase receptors are highly expressed in hamsters similar to the increase of the ErbB2 tyrosine kinase noted in humans (Werkmeister et al., 2000). Additionally, the oncogenes p53 and c-myc are highly expressed early in hamster tumorigenesis and there is a loss of expression of the p16 tumor suppressor gene during the early dysplastic stage (Papakosta et al., 2006; Vairaktaris et al., 2007). More recently, the microRNA expression profile of the DMBA-treated hamster cheek pouch has been analyzed and compared to normal cells. Treated cells display a unique microRNA profile distinct from normal tissue (Yu et al., 2009). These data imply that microRNA may play a role in the formation and progression of hamster cheek pouch tumors. The similarities of these new data to the human disease are not yet known.

A key advantage of the hamster cheek pouch carcinogenesis model is the predictable nature of tumor progression. Because of this predictability, the hamster model is also utilized to analyze various treatments and preventatives which may be given either concurrently with induction or at specified points during tumorigenesis (Shklar, 1999; Sood et al., 2005). Since the hamster cheek pouch undergoes a predictable pathway of induced oncogenesis, these studies can be accurately timed to the appropriate tumor stage or pre-cancerous lesion. A more recently described advantage of the hamster model is the isolation of buccal pouch stem cells, which can be studied in vitro and may provide data on novel treatments (Huang et al., 2009).

SV40 Tumor Induction

Simian vacuolating virus 40 (SV40) is a polyoma-virus naturally occurring in macaques that was introduced into humans via contaminated polio vaccines (Pershouse et al., 2006; Rollison et al., 2004). The role of SV40 in human cancers has a long and controversial history. Large epidemiological studies tracking infected humans indicate no link between the agent and cancer (Rollison et al., 2004). Proponents of the link between SV40 and cancer state that the true prevalence of SV40 in the human population is not known, and therefore, an accurate epidemiological assessment cannot be made (Pershouse et al., 2006). Detailing the controversies surrounding the role of the SV40 virus in human tumors is beyond the scope of the current chapter. However, the hamster figures prominently into the data detailing the oncogenic potential of SV40.

Hamsters injected with SV40 develop a variety of different tumors depending on the route of inoculation and the age of hamsters at the time of inoculation. Tumor formation is highest in newborn hamsters and decreases progressively with age (Cicala et al., 1993). Local fibrosarcomas develop with subcutaneous injection of virus, even when given at a low dose in newborn hamsters. In contrast, adults develop fibrosarcomas only when given a high dose of virus (Allison et al., 1967; Cicala et al., 1993). Intravenous inoculation of young hamsters produces a variety of tumors including leukemia, lymphoma, and a variety of sarcomas (Cicala et al., 1993; Diamandopoulos and McLane, 1972). Finally, mesotheliomas develop when hamsters are injected with virus into the heart, peritoneum, or pericardium (Cicala et al., 1993). SV40 viral infection can therefore be utilized to analyze a variety of tumor types using the hamster model.

Recent studies utilizing the hamster lend further evidence to support the hypothesis that SV40 may play a role in human carcinogenesis. Hamsters that are infected with a non-oncogenic SV40 virus strain or a low dose of asbestos, fail to develop tumors. In contrast, when sub-oncogenic doses of the two agents are given together a large percentage of hamsters (90%) develop tumors (Kroczyńska et al., 2006). Additionally, these results are reproducible in in vitro cell-culture systems. These data imply that, at least in the hamster, SV40 and asbestos can act as co-carcinogens.

The role of SV40 in human cancers remains highly controversial. However, the hamster model provides an excellent way to prospectively test these hypotheses. Even if SV40 plays no role in human tumorigenesis, much can still be learned from the hamster model regarding how viral agents transform mammalian cells. For example, a recent study demonstrates that differences in the SV40 regulatory region lead to differences in tumor induction in the hamster model (Sroller et al., 2008). These new data provide evidence that various strains of virus possess pro-oncogenic factors and these data argue that epidemiological studies in humans should account not merely for the presence or absence of SV40, but rather for the type of SV40 present.

Estrogen-Dependent Renal Tumors

Exogenous estrogen therapy in post-menopausal women decreases the risk of developing both osteoporosis and coronary artery disease; however, it also increases the risk for development of breast cancer and endometrial adenocarcinoma in this population (Sartwell et al., 1977; Stampfer et al., 1991). The benefits and limitations of the use of estrogen therapy in postmenopausal women remains an active area of research and debate. Animal models of estrogen-induced tumorigenesis

provide valuable mechanistic data to optimize estrogen therapy.

The Syrian hamster is a valuable model to study the effects of exogenous estrogenic compounds on tumor development. The administration of estrogens induces renal tumors in 100% of male hamsters in a predictable fashion (Li and Li, 1996; Liehr, 1997). Most commonly, estrogens are administered to hamsters in the form of slow-release subcutaneous implants which maintain constant levels in the blood (Debauve et al., 2006). In addition to *in vivo* studies utilizing the estrogen injection or implantation hamster model, HKT-1097 cell-lines generated from this model can be studied *in vitro* (Laurent et al., 1999).

A wealth of mechanistic data on the tumorigenic potential of estrogenic compounds has been generated utilizing the hamster model. Specifically, administration of a variety of natural and synthetic estrogenic compounds demonstrates that tumorigenesis occurs with some estrogenic compounds but not others (Liehr, 1997; Liehr et al., 1983, 1987a, 1987b). These data led to the hypothesis that tumorigenic estrogens mediate carcinogenicity, at least in part, through their metabolic breakdown products (Liehr, 1997). In particular, estrogen is broken down to 4-hydroxyestrogen, a catecholesterogen, in the hamster kidney (Dwivedy et al., 1992; Liehr, 1997). This intermediate can be further broken down into quinones which cause DNA damage by forming DNA adducts. This pathway, initially characterized in the hamster, was later identified in women with breast cancer. Specifically, the "normal" breast tissue of women with breast cancer displays higher levels of quinones when compared to unaffected controls (Li et al., 1994; Rogan et al., 2003). The estrogen receptor may be actively involved in tumor formation by shuttling quinone directly to nuclear DNA (Bolton and Thatcher, 2008).

Aside from the ability of quinones to directly damage DNA, quinine production results in the formation of reactive oxygen which can also cause DNA damage (Li et al., 1994; Liehr, 1997). Since the breakdown products of estrogen can damage DNA (mutation), and estrogen itself can promote cell growth (proliferation), all of the necessary components of carcinogenesis are present in the estrogen-treated hamster kidney (Liehr, 1997). The administration of some estrogenic compounds does not damage DNA because toxic metabolic byproducts are not generated (Bhat et al., 2003). Cancer induction with these compounds can be rescued by inducing oxidative stress while administering these normally non-oncogenic estrogens. This finding highlights the need for both the proliferative and the DNA-damaging aspects of estrogen to produce tumors. The hamster model may facilitate development of novel, non-DNA-damaging, estrogens which could provide the benefits of estrogenic therapy without the increased risk of cancer.

Oncolytic Viruses

The use of oncolytic adenoviruses holds promise as a future cancer treatment in humans. These adenoviruses kill tumor cells as part of their normal lytic replication cycle (Thomas et al., 2006a). The prospective *in vivo* study of these viruses is hampered by the lack of animal models that permit the replication of human adenoviruses (Hjorth et al., 1988; Thomas et al., 2006a). However, the hamster can be infected with human adenoviruses via intranasal instillation (Hjorth et al., 1988). In addition, permissive hamster cancer cell lines are available for assessing tumor suppression (Bortolanza et al., 2007; Thomas et al., 2006a). Notably, the Syrian hamster pancreatic carcinoma cell line (SHPC6) is highly permissive for human adenovirus replication (Spencer et al., 2009).

Thomas and colleagues recently developed a step-wise, reproducible hamster model to study the efficacy of adenoviral vectors in tumor therapy (Thomas et al., 2006b). The investigators injected immortalized tumor cells into the flanks of hamsters. After tumors developed, hamsters were inoculated with adenovirus into the respiratory tract. Tumor reduction at both the primary site, and the site of metastasis, was then analyzed. The investigators found that adenovirus inhibits primary tumor growth and metastasis and may promote survival (depending on tumor type injected). The virus is capable of replicating in the liver, lungs, and tumor tissues of the hamster following instillation and distribution in the blood (Thomas et al., 2006b). This experimental paradigm can be expanded to examine the treatment of a variety of tumors in the hamster and potential toxicities associated with the use of these viruses (Dhar et al., 2009; Lichtenstein et al., 2009). A further modification of this method is immunosuppression of hamsters with cyclophosphamide which allows for sustained adenoviral replication and oncolysis (Thomas et al., 2008). The hamster provides a valuable experimental model to evaluate the potential benefits, limitations, and strategies of this novel treatment modality.

METABOLIC DISEASES

Metabolic diseases are an area of great biomedical concern. Although all these diseases may occur independently of one another, they often present with a variety of overlapping clinical signs. Like humans, the hamster is highly susceptible to metabolic diseases and there are similar overlaps in the signs that they develop (Ginsberg, 1996; Gotto, 1992; Laakso, 1996; Lamarche and Lewis, 1998). Therefore, the hamster is an excellent animal model to study metabolic diseases of humans.

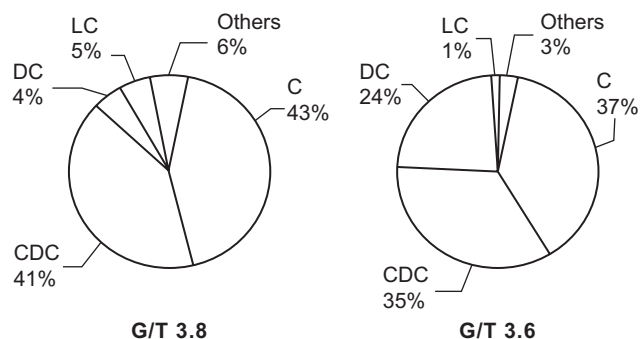


FIGURE 34.1 Distribution of different bile acids in vesicular bile of hamsters (left) and humans (right). The ratio of glyco- and tauro-conjugated bile acids is very similar in both species. C, cholic; CDC, chenodeoxycholic; DC, deoxycholic; LC, lithocholic acid. Modified from Combettes-Souverain et al. 2002, with permission from Elsevier Limited.

Cholesterol Gallstones

Cholesterol cholelithiasis is a significant health problem worldwide. The prevalence of this condition is three times higher in women than in men and increases regularly with age (Capron, 1994). The Syrian hamster is commonly used as a model for this condition which can be induced by excess dietary cholesterol (Cohen et al., 1989; Trautwein et al., 1999) or sucrose (Khallou et al., 1991a). The metabolism of cholesterol in the hamster is similar to humans (Khallou et al., 1991b). The nature and composition of hydrophobic bile salts and the ratio of taurine- and glycine-conjugated bile salts are similar in hamsters and humans (Figure 34.1) (Combettes-Souverain et al., 2002; Hardison, 1983; Singhal et al., 1983). In earlier studies, gallstones were induced in Syrian hamsters by feeding a fat-free glucose-rich diet (Dam, 1971). Diet models have evolved to include elevated levels of butterfat (Cohen et al., 1992; Hayes et al., 1992; Holzbach, 1984). In hamsters, the butterfat-diet induces a high cholesterol/phospholipid ratio in bile which promotes cholesterol cholelithiasis. In contrast, diets rich in safflower oil induce phospholipid secretion and reduce the lithogenicity of bile, thereby inhibiting cholesterol gallstones (Ohshima et al., 1996a).

The Syrian hamster displays significant gender differences in the basal rate of hepatic sterol synthesis which is several-fold higher in females fed a regular chow diet as compared to age-matched males on the same diet (Spady et al., 1983). In contrast to humans, female hamsters are less susceptible to gallstones because they maintain more favorable biliary lipid and bile acid profiles (predominantly glycocholate) characterized by lower molar percentages of cholesterol and chenodeoxycholate (Ayyad et al., 1993; Trautwein et al., 1999). Dietary administration of androgens to female hamsters on a lithogenic diet (0.3% cholesterol) induces

cholesterol gallstones at a comparable rate to induction in males fed a similar diet. In these androgen-treated, cholesterol-fed female hamsters, chenodeoxycholate increases while cholate decreases (Ayyad et al., 1995). Furthermore, castrated male hamsters on the lithogenic diet do not form cholesterol gallstones, and maintain a higher ratio of biliary cholate to chenodeoxycholate as compared to uncastrated males (Ohshima et al., 1996b).

Male LPN hamsters (bred at Laboratoire de Physiologie et de la Nutrition, France) are highly susceptible to cholesterol gallstones (~70%) induced by a low-fat high-sucrose diet, whereas commercial Janvier (JAN) hamsters on a similar diet do not develop gallstones (Boehler et al., 1999; Combettes-Souverain et al., 2002; Ferezou et al., 2000). In LPN hamsters, the sucrose-rich lithogenic diet causes an increase in biliary cholesterol and phospholipid. A cholesterol-enriched diet results in hypercholesterolemia, increased liver cholesterol, and reduced cholesterologenesis along with expression of the LDL receptor in both LPN and JAN hamsters (Souidi et al., 2001). In both strains, the cholesterol-enriched diet increases the cholesterol content in both the LDL and HDL fractions of sera (Souidi et al., 2001; Woollett et al., 1997). Further, excess dietary cholesterol leads to a reduction of CYP7A (the rate-limiting enzyme in the classical pathway of cholesterol breakdown to bile salts) activity and transcription in both LPN and JAN hamsters (Souidi et al., 2001). There is a concurrent stimulation of sterol 27-hydroxylase (CYP27) and oxysterol 7 α -hydroxylase (CYP7B), enzymes involved in the alternative bile-salt pathway of cholesterol conversion to bile salts in both LPN and JAN hamsters. Compared with LPN hamsters, hypercholesterolemia in JAN hamsters is associated with hepatomegaly and fatty liver but only modest hypertriglyceridemia. Interestingly, after cholesterol feeding for 5 weeks, neither LPN nor JAN hamsters develop cholesterol gallstones, perhaps because of the upregulation of the alternative pathway of bile-salt synthesis (Souidi et al., 2001).

Differences in gallstone formation also exist between commercially available hamsters. Charles River Laboratory hamsters display the highest cholesterol saturation of bile on low-cholesterol diets and display a correspondingly high prevalence of stones (64%) when challenged with a high-cholesterol diet (Trautwein et al., 1993). Biobreeder F1 hamsters also commonly form gallstones (58%) when challenged with cholesterol-enriched diets. In contrast, Harlan Sprague-Dawley hamsters display a low prevalence (23%) of gallstones when fed the same diet (Trautwein et al., 1993). These findings highlight the role of genetics in both hamsters and humans in this complex polygenic disease. One drawback of this study is that the sex of these animals was not described and therefore the data are difficult to evaluate thoroughly.

Diabetes Mellitus

Diabetes mellitus is a disease of metabolic dysregulation including abnormal glucose metabolism due to derangement of insulin secretion and development of insulin resistance. Diabetes is often accompanied by dyslipidemia (high LDL, VLDL, and chylomicron levels, and a low level of HDL) and elevated risk for the development of atherosclerosis (Ginsberg, 1996; Gotto, 1992; Laakso, 1996; Lamarche and Lewis, 1998).

An animal model of pre-diabetic insulin resistance, the fructose-fed Syrian hamster, is employed to investigate mechanisms mediating overproduction of VLDL in the insulin-resistant state. Feeding fructose for 7 days causes hyperinsulinemia and hyperlipidemia in hamsters with absence of overt hyperglycemia (Taghibiglou et al., 2002). Fructose feeding for a 2-week period induces significant hypertriglyceridemia and hyperinsulinemia (Taghibiglou et al., 2000). Plasma hepatic lipase activity, and hepatocyte hepatic lipase concentration and mRNA levels are increased in these hamsters (Lewis et al., 2004). Activation of farnesoid X receptor (FXR) by the addition of chenodeoxycholic acid in high-fructose-diet-fed hamsters reduces plasma triglyceride and cholesterol concentrations, mainly by decreasing de novo lipogenesis and hepatic secretion of triglyceride-rich lipoproteins (Bilz et al., 2006). These studies provide evidence that stimulation of FXR could decrease the risk of hyperlipidemia in the diabetic state.

Diabetes can be experimentally induced in hamsters by different approaches, such as dietary modifications or use of chemical agents. Chemical agents such as alloxan or streptozotocin (STZ) are commonly used to induce diabetes in animal models. To induce chronic diabetes in hamsters, STZ is more effective and reliable when compared to alloxan (Phares, 1980). STZ depletes nicotinamide adenine dinucleotide (NAD) in pancreatic β -cells leading to necrosis (Ar'Rajab and Ahren, 1993). Furthermore, type 2 diabetes can be induced by administration of STZ to hamsters on a high-fat diet, resulting in an increase in serum lipid profile, fasting blood glucose, and fasting plasma insulin level (Zhu et al., 2007). Another described method to induce type 2 diabetes in hamsters is to inject nicotinamide intraperitoneally 15 minutes before administration of STZ (Fararh et al., 2002). Nicotinamide provides partial protection against the beta-cytotoxic effect of STZ resulting in partial (40%) preservation of pancreatic insulin stores (Masiello et al., 1998). Dietary modifications alone, such as feeding high-fat (15%) diets containing modest cholesterol (0.12%) to hamsters, induce type 2 diabetes as well as obesity, hyperinsulinemia, hyperleptinemia, hypercholesterolemia, and hypertriglyceridemia (van Heek et al., 2001). A combined hyperlipidemia and diabetes hamster model is induced by feeding a fat-rich diet (3% cholesterol and

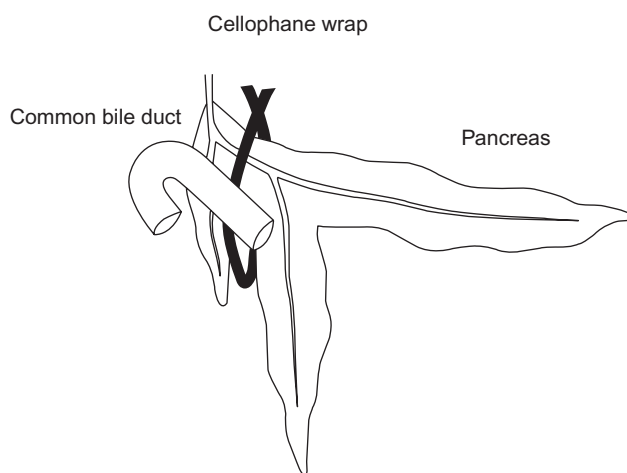


FIGURE 34.2 Displayed is the pancreas and common bile duct. Cellophane (black) is wrapped around the head of the pancreas to induce islet neogenesis. Modified from Rosenberg 1998, with permission from Wiley InterScience.

15% butter fat) and a single IP injection of STZ. These animals exhibit pathobiochemical changes characteristic of both diabetes and hyperlipemia and show an earlier and more severe induction of diabetic and hyperlipidemic changes compared to either STZ or high-fat diet alone (Simionescu et al., 1996).

Albino-panda-albino (APA) hamsters develop diabetes with nephropathy following STZ injections (Inenaga et al., 2002). The STZ-induced diabetic APA hamster is a good model of human diabetic nephropathy because progressive renal lesions are a common component of this model. In addition to renal lesions, the diabetic APA hamster also develops coronary lesions and is an excellent model to investigate the etiology and pathogenesis of aortic dissection accompanying diabetes mellitus in humans (Horiuchi et al., 2005).

Hamsters can be utilized to study islet neogenesis, which is the new formation and differentiation of pancreatic islets. This process is induced in Syrian hamsters by wrapping the head of the pancreas with cellophane tape and is efficient in reversing induced diabetes in hamsters (Figure 34.2) (Rosenberg et al., 1983, 1988). Islet neogenesis-associated protein (INGAP) is involved in the control of islet development and growth (Pittenger et al., 1992). INGAP is identified in both endocrine and exocrine pancreatic cells of normal hamsters, consistent with findings in humans (Flores et al., 2003). The hamster provides a model to understand the potential therapeutic application of islet neogenesis in the treatment of diabetes in humans.

Atherosclerosis

Atherosclerosis is a fibroproliferative disease of the arterial intima caused by the retention of modified

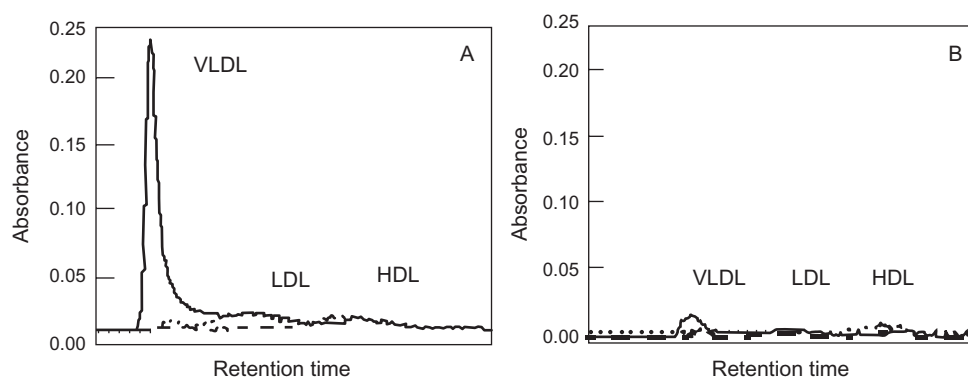


FIGURE 34.3 Distribution of lipoproteins (by FPLC system) in fasting plasma samples from F1B (A) and Syrian (B) hamsters. The low-density fractions of plasma cholesterol in F1B hamsters on a fish oil diet (–) was significantly higher than the Syrian hamsters on a similar diet. Both groups of hamsters had a comparable lipoprotein profile when fed monounsaturated-fatty-acid-rich (---) and N6:N3 fatty acid ratio of ~5 (...) diets. From Cheema and Cornish, 2007, with permission from BioMed Central.

low-density lipoproteins and by hemodynamic and redox stresses (Hayden and Tyagi, 1998, 2000). Hamsters are a good animal model to investigate cholesterol metabolism and atherosclerosis because they possess similar lipid metabolism to humans (Asami et al., 1999; Foxall et al., 1992). As in humans, the main plasma cholesterol carrier in hamsters is LDL (Sullivan et al., 1993). The hamster LDL receptor gene shows strong sequence and structural similarities to the human gene (Bishop, 1992). Unlike mice and rats, which lack cholesterol ester transfer protein, hamsters exhibit all enzymatic pathways in lipoprotein and bile metabolism that are present in humans (Bishop, 1992). The low basal rates of bile acid and cholesterol synthesis, coupled with a lack of CYP7A induction by cholesterol, render the hamster much more sensitive than the rat or mouse to the cholesterolemic effects of excess dietary cholesterol (Horton et al., 1995). In contrast to mice and rats, apolipoprotein (apo) B-48 is exclusively of intestinal origin in hamsters and humans and apoB-100 is exclusively of hepatic origin (Arbeen et al., 1992; Ebara et al., 1994).

Hamsters develop atherosclerosis in a predictive manner in response to dietary manipulation and the vascular changes accompanying the development of atherosclerotic plaques are similar to those encountered in humans (Mitchell and McLeod, 2008; Simionescu et al., 1993; Wissler, 1991). Hypercholesterolemic (0.05% cholesterol and 10% coconut oil) diet-induced atherosclerosis in susceptible hamsters is confined initially to the inner curvature of the aortic arch. Atherogenesis in this region is characterized by the infiltration of monocytes, which become lipid-filled foam cells (Kowala et al., 1991a). Male hamsters on a hyperlipidemic diet (3% cholesterol and 15% butter fat) develop coronary atherosclerotic lesions which are very similar to lesions seen in humans (Sima et al., 1990). Interestingly, hyperglycemia alone in hamsters induces atherosclerotic

lesions in the coronary arteries, aortic arch, and aortic valves, implying that the hyperglycemic hamster could provide a model of diabetes-induced atherosclerosis (Sima et al., 1997).

Albino-panda-albino (APA) hamsters (previously described in the diabetes section of this chapter) develop atheromatous lesions in the aortic arches and show signs of hypercholesterolemia and hypertriglyceridemia with an increased LDL fraction and a decreased HDL fraction following injection of STZ (Han et al., 1992; Kume et al., 1995; O'Brien et al., 1994). The deposition of ApoE and advanced glycation end-products in the atheromatous lesions of these hamsters are similar to humans with atherosclerosis (Kume et al., 1995; O'Brien et al., 1994). The APA hamster, therefore, is a good animal model to study both diabetes and atherosclerosis. Since these conditions are often co-morbidities in humans, this model could provide valuable data to understand the pathogenesis of these diseases and test novel treatments.

The Bio F1B hamster is highly susceptible to diet-induced atherosclerosis. This strain develops aortic atherosclerotic lesions even at low (0.05%) dietary cholesterol concentrations (Kowala et al., 1991b). F1B hamsters on a 20% fish oil diet developed markedly elevated plasma cholesterol and cholesterol ester concentrations compared to wild-type Syrian hamsters (Figure 34.3) (Cheema and Cornish, 2007). Wild-type Syrian hamsters typically display increases in both LDL and HDL cholesterol in response to an atherogenic diet (Hayes et al., 1992). In contrast, F1B hamsters develop highly elevated LDL-cholesterol concentrations without appreciable alterations of HDL-cholesterol (Kowala et al., 1991a).

Ovariectomized hamsters are used to study postmenopausal hypercholesterolemia (Sohn et al., 1999). In humans, there is an association between postmenopausal hormone deficiency and the increased risk for

cardiovascular disease (Sohn et al., 1999). Similar to postmenopausal women, ovariectomized hamsters experience an increase in serum cholesterol and atherosclerosis. This increase occurs even without feeding a high-cholesterol or high-fat diet (Lucas et al., 2003; Sohn et al., 1999).

Hypercholesterolemic hamsters are also a good model of peripheral arterial disease. Angiogenesis in response to femoral artery-induced hindlimb ischemia is reduced in hypercholesterolemic hamsters (Caron et al., 2004). Feeding a cholesterol-rich diet to hamster causes a chronic angiogenic disorder both at the macro- and micro-vascular levels, mimicking the conditions seen in humans with peripheral arterial disease (Caron et al., 2004). This model is also utilized to study the potency of therapeutic gene transfer to overcome revascularization defects in these patients (Caron et al., 2004).

NON-CANCEROUS RESPIRATORY DISEASES

Syrian hamsters possess characteristics that make them ideal models for studying human non-cancerous respiratory diseases. Specifically, researchers can induce respiratory diseases in hamsters that closely resemble similar human diseases. This section outlines common inducible hamster models for human non-cancerous respiratory disease and describes scientific and medical advances made using these models.

Smoke Inhalation

The negative effects of cigarette smoking on the cardiovascular and respiratory systems in humans are well known (Alberg, 2008; Fagerstrom, 2002). Over the past 10 years, an active area of research focuses on the consequences of smoking on the female reproductive tract (Rogers, 2008, 2009). Epidemiological studies demonstrate that women who smoke cigarettes experience increased rates of reproductive problems, including delay to conception, ectopic pregnancies, lower birth weights, and spontaneous abortions (DiCarlantonio and Talbot, 1999; Magers et al., 1995). Syrian hamsters are a prominent rodent used in female reproductive toxicology inhalation studies including mainstream (MS) smoke (active inhalation of a bolus of smoke) and side-stream (SS) smoke (passive inhalation of smoke). Advantages of hamsters in these studies are that they adapt well to smoking machines and they have reproductive tracts that are large enough to allow thorough evaluation (Magers et al., 1995).

To test the effects of smoke inhalation on reproductive parameters, female Syrian hamsters in various stages of estrous or pregnancy are anesthetized, placed in dorsal

recumbency, and exposed through a nose cone to smoke released from the burning end of a cigarette (SS smoke) or to a puff of MS smoke with a puffer box (Magers et al., 1995). A machine of equivalent design that pumps fresh air is used for control animals. Exposure to doses of MS and SS smoke equivalent to those in women who smoke actively or passively (second-hand smoke) is ensured by quantifying serum levels of cotinine, a metabolite of nicotine (DiCarlantonio and Talbot, 1999). The major downfall of this technique is that it exposes females to either MS or SS smoke, but not to both, as would be the case for an active human smoker (Magers et al., 1995). Female hamsters that inhale either MS or SS smoke twice per day for 7 days, starting 1 month prior to mating and ending at day 7 of pregnancy, develop fewer corpus lutea (CL) with accompanying decreases in CL vascularity (Magers et al., 1995). Additional changes in these hamsters include blebbing of ciliated epithelial cells in oviducts, increases in secretory cells of the ampulla, decreases in the ability of the uterus to stretch, and crowding of fetuses. Functionally, the transport of pre-implantation embryos through the oviduct is retarded, and oviduct muscle contraction rate is greatly decreased by smoke inhalation (DiCarlantonio and Talbot, 1999). Transport rate is more significantly retarded in the SS exposure group, suggesting that SS smoke exposure is more detrimental than MS smoke exposure with regard to embryo transport. Inappropriate transport and implantation could explain the increase in ectopic pregnancy in human smokers. Interestingly, when mating is delayed for 1 month following smoke inhalation, all deleterious effects of MS and SS exposure are ablated (Magers et al., 1995). This indicates that the female reproductive tract can quickly recover from the negative effects of smoke inhalation.

COPD and Emphysema

Chronic obstructive pulmonary disease (COPD) is a chronic, progressive airflow obstruction with concurrent inflammation of the small airways and emphysema of lung parenchyma (Cosio Piqueras and Cosio, 2001; Petty, 2006). Emphysema contributes to airflow obstruction by reducing the elasticity of the lung, increasing alveolar size, and increasing airway resistance (Cosio Piqueras and Cosio, 2001). COPD is the fourth most common cause of death in the United States, with a prevalence of approximately 16 million (Petty, 2006). Animal models yield clues to the pathophysiology and therapeutic approaches for COPD (Petty, 2006).

The Syrian hamster is a useful model to study the emphysema component of COPD because hamsters develop severe diffuse emphysema resembling human panacinar emphysema following a single intratracheal dose of porcine pancreatic elastase (Borzone et al., 2007;

Hayes et al., 1977; Snider and Sherter, 1977). Elastase is used as an induction agent because it destroys or impairs the formation of elastin, resulting in emphysema (Soskel et al., 1984). The elastase induction model reproducibly results in alveolar damage and destruction of alveolar septa resulting in airway enlargement, and causes lung hyper-inflation, all of which are seen in humans with COPD (Borzone et al., 2007; Hayes et al., 1977). Additionally, elastase-treated hamsters do not gain weight at a normal rate, which is analogous to the weight loss seen in humans with emphysema (Snider and Sherter, 1977). Syrian hamsters are also used as a model for emphysema treatment. Intratracheal instillation of hyaluronic acid in hamsters with elastase-induced emphysema reduces emphysema by binding with and protecting elastic fibers from elastase-induced injury, and may be useful in preventing emphysema (Cantor et al., 1997, 2002).

Emphysema can also be induced in hamsters by feeding diets deficient in copper (Soskel et al., 1984). This model requires chronicity (8–10 weeks), thereby resembling the time course of human emphysema development (Soskel et al., 1984). Copper deficiency results in the inhibition of lysyl oxidase-mediated elastin cross-linking, causing impaired elastin formation and emphysema (Soskel et al., 1984). Also, since superoxide dismutase requires copper, copper deficiency leads to free radical accumulation which can cleave elastin bonds and attract inflammatory cells to the lung exacerbating emphysema.

A popular hypothesis regarding the pathogenesis of emphysema is that an imbalance between proteases and antiproteases plays a major role in disease development (Borzone et al., 2007). When compared to Syrian hamsters, rats develop only mild emphysema when given similar doses of elastin. This could be due to a 60% reduction in serum levels of the antiprotease α_1 -antitrypsin in Syrian hamsters compared to rats, which leads to low elastase inhibitory capacity, thereby predisposing hamsters to elastase-induced emphysema.

Pulmonary Fibrosis

Interstitial pulmonary fibrosis (IPF) is a debilitating disease characterized by proliferation of lung fibroblasts, collagen accumulation, loss of alveolar space, reduction in lung volume and compliance, and impairment of gas exchange (Giri et al., 1994, 1997; Lazo et al., 1990; Wang et al., 2002). These changes cause hypoxemia and, if the fibrosis is severe, pulmonary insufficiency and fatal hypoxemia (Lazo et al., 1990). Currently, there are no adequate treatments for IPF and mortality in humans is high (Giri et al., 1994; Iyer et al., 1999; Wang et al., 2002). Bleomycin, a DNA-interactive antineoplastic drug is a known cause of dose-dependent pneumonitis that progresses to IPF in humans (Giri et al., 1994, 1997; Lazo

et al., 1990; Thrall et al., 1979). The prevalence of bleomycin-induced toxicity is reported to be 10%, with many of these cases resulting in death (Lazo et al., 1990).

Bleomycin is one of the most commonly used agents to induce IPF because it causes a similar, reproducible condition in the Syrian hamster (Lazo et al., 1990). A single intratracheal installation of approximately 5mg/kg body weight produces pulmonary fibrosis within 7 days (Lazo et al., 1990). Importantly, other organs are unaffected by this treatment and the treatment induces biochemical, inflammatory, and histological changes similar to those seen in the lungs of humans with IPF (Giri et al., 1994; Thrall et al., 1979; Wang et al., 2002). The bleomycin model of IPF involves an initial injury to the lung, followed by an influx of inflammatory cells that release cytokines (i.e. IL-1, TNF- α , PDGF, and TGF- β), leading to increased collagen synthesis and deposition (Iyer et al., 1999). A drawback of this model is that it produces high mortality in Syrian hamsters (Giri et al., 1994; Lazo et al., 1990). A refinement is the three-dose bleomycin hamster model where IPF is produced by the intratracheal instillation of three lower doses of bleomycin into the lungs of anesthetized hamsters at weekly intervals (Giri et al., 1994; Wang et al., 2002). This model uses repeated insults on the lung to initiate and sustain the fibroproliferative stage of fibrosis, and is the closest model to human IPF because it provides a multi-hit stimulus for lung injury, causing the slow development of IPF (Wang et al., 2002). There is age-selective resistance to bleomycin in young hamsters due to enhanced antioxidant defenses and bleomycin hydrolase activity in neonates (Lazo et al., 1990).

After induction, the hamster model can be used to study antifibrotic compounds (Giri et al., 1994, 1997; Iyer et al., 1999; Wang et al., 2002). Transforming growth factor- β (TGF- β) plays a central role in the pathophysiology of excessive extracellular matrix (i.e. collagen) production resulting in IPF (Iyer et al., 1999; Lazo et al., 1990). TGF- β mRNA and protein levels are increased in fibrotic hamster lungs; therefore, suppressing its production constitutes a rational therapeutic approach (Iyer et al., 1999; Lazo et al., 1990; Wang et al., 2002). Decorin, a proteoglycan component of collagen fibrils, binds and inactivates all isoforms of TGF- β , offering a potential treatment for IPF (Giri et al., 1997). The antifibrotic drug pirfenidone down-regulates the bleomycin-induced overexpression of TGF- β in the lungs, and pirfenidone is now a clinical therapeutic agent for IPF in humans (Antoniou, 2006; Bhatt et al., 2006; Iyer et al., 1999). Further, a TGF- β antagonist, TGF- β chimeric soluble receptor, causes significant reductions in fibrosis in the hamster model (Wang et al., 2002).

Another area of IPF treatment research focuses on the elimination of reactive oxygen species (ROS) and increasing NAD and ATP needed for the repair

of injured pulmonary tissue (Giri et al., 1994). Dietary supplementation with taurine and niacin inhibits lung fibrosis in the bleomycin induced hamster model (Giri et al., 1994). This defense is provided by the ability of taurine to scavenge ROS and the ability of niacin to replenish NAD and ATP levels (Giri et al., 1994).

CARDIOVASCULAR

Syrian hamsters exhibit characteristics that make them desirable models for cardiovascular research. Specifically, spontaneous genetic mutations cause diseases that closely resemble human cardiovascular disease. Further, Syrian hamsters display unique anatomical characteristics, most notably a readily evertible cheek pouch, that facilitate cardiovascular studies.

Cardiomyopathy

Cardiomyopathy is a primary degenerative disease of the myocardium and is characterized as various forms, such as hypertrophic or dilated, based on gross appearance (Sakamoto et al., 1997). Dilated cardiomyopathy (DCM) is characterized by atrial and ventricular dilation with ventricular wall thinning, severe systolic and diastolic left ventricular dysfunction, and heart failure (Ikeda and Ross, 2000). DCM is one of the main causes of congestive heart failure in humans, is the leading cause of hospitalization of older patients in the United States, and is hereditary in 25% of affected patients (Ikeda and Ross, 2000). Hypertrophic cardiomyopathy (HCM) is characterized by increased ventricular wall thickness, generally involving the interventricular septum, and normal or reduced left ventricular volume (Richardson et al., 1996; Rodriguez et al., 2009). HCM is the most common, heritable cardiovascular disease in humans, affecting one in 500 people (Bos et al., 2009; Rodriguez et al., 2009). Approximately 10% of humans with HCM eventually develop left ventricular dilation and DCM (Ikeda and Ross, 2000). The cardiomyopathic Syrian hamster is a naturally occurring, inherited, established animal model for both dilated and hypertrophic cardiomyopathy. In the hamster, both DCM and HCM are caused by a defect in the δ -sarcoglycan gene, a component of the dystrophin complex, which is inherited in an autosomal recessive fashion (Bajusz et al., 1969; Escobales and Crespo, 2006, 2008; Goineau et al., 2001; Ikeda and Ross, 2000; Lipskaia et al., 2007; Ryoike et al., 1999; Sakamoto et al., 1997). The Syrian hamster model shares many features with human DCM and HCM and is used to study the pathophysiology, prevention, and treatment of cardiomyopathy (Escobales and Crespo, 2008; Ryoike et al., 1999).

All cardiomyopathic Syrian hamsters were originally derived from the polymyopathic line 1.50, providing an

example of a single mutation causing different types of cardiomyopathy (Goineau et al., 2001; Sakamoto et al., 1997). Hamster strains Bio 14.6, UM-X7.1, CHF 146, and CHF 147 are characterized by significant hypertrophy of cardiac muscle, while strains 53.58 and Bio TO-2 are characterized by ventricular dilation without hypertrophy (Goineau et al., 2001; Ikeda and Ross, 2000). Some strains, such as the Bio 14.6, exhibit compensatory hypertrophy progressing to left ventricular dilation, thinning and impaired function, while strains such as TO-2 develop dilated cardiomyopathy without an initial hypertrophic response (Goineau et al., 2001; Ikeda and Ross, 2000). These various strains provide diverse models which can be used to study various aspects of pathogenesis.

Many features of the TO-2 dilated cardiomyopathy strain closely resemble those of human DCM so this strain is used commonly to characterize the pathophysiology and hemodynamic profiles of DCM in humans (Escobales and Crespo, 2006; Goineau et al., 2001). TO-2 strain cardiomyopathy is progressive, beginning with myolysis, then developing fibrosis followed by hypertrophy and dilation, and culminating in congestive heart failure (Cruz et al., 2007; Escobales and Crespo, 2006). These stages represent the majority of phases seen in humans with cardiomyopathy, including heart dilation, ventricular hypertrophy, fluid congestion, and death (Cruz et al., 2007; Escobales and Crespo, 2006). Acute myocarditis is also observed in humans and hamsters (Escobales and Crespo, 2006). The common hemodynamic features include low cardiac output, elevated left ventricular end-diastolic pressure, delayed left ventricular relaxation rate, increased peripheral resistance, and reduced renal blood flow.

In the TO-2 Syrian hamster, abnormal sarcoplasmic reticulum function results in calcium sequestration and overload, and high ATPase activity results in decreased contractility. These features are partially responsible for decreased cardiac function in this strain, and these abnormalities are hypothesized to be a pathogenic mechanism in human DCM (Goineau et al., 2001). Deficiency of the δ -sarcoglycan gene also affects peripheral and coronary vasculature, ultimately contributing to heart failure in cardiomyopathic hamster models (Escobales and Crespo, 2006, 2008; Lipskaia et al., 2007). Angiotensin II promotes increased oxidative stress and decreased peripheral vasorelaxation, leading to high systemic blood pressure and increased cardiac work load in cardiomyopathic prone TO-2 hamsters (Escobales and Crespo, 2006). Angiotensin-II-dependent oxidative stress influences coronary vasculature by causing endothelial dysfunction which predisposes hamsters to the development of ischemic heart disease and cardiomyopathy (Escobales and Crespo, 2008). Based on the similarities between cardiomyopathic Syrian hamsters and humans,

these results associate angiotensin II with the development of cardiomyopathy in humans with hereditary sarcoglycanopathies (Escobales and Crespo, 2008).

Current medical treatment for human dilated and hypertrophic cardiomyopathy is only palliative (Sakamoto et al., 1997). Syrian hamster cardiomyopathic strains are used in research targeting prevention and treatment of the condition. The treatment of cardiomyopathic hamsters with angiotensin-converting enzyme (ACE) inhibitors improves left ventricular contractility and coronary flow, providing evidence that Syrian hamsters are useful models in studying drug treatment interventions. Coronary vasculature renin-angiotensin system (RAS) plays a role in generating increased coronary reactivity and resistance in young Syrian hamsters that have yet to develop cardiomyopathy. Early RAS blockade may prevent and improve the clinical signs of cardiomyopathy (Escobales and Crespo, 2008). Finally, the administration of carvedilol, a non-selective β -blocker with α_1 -blocker properties, to 6-month-old TO-2 hamsters, improves cardiac function due to decreased peripheral resistance (Cruz et al., 2007).

Microcirculation

The bilateral cheek pouches of the Syrian hamster are invaginations of the oral mucosa which extend to the shoulder region and possess a retractor muscle (Svensjo, 1990). These anatomical structures are highly vascular, thin, easily transilluminated, and can be everted with blood flow intact, making the hamster cheek pouch ideal for intravital microscopy and microcirculation research (Davis and Gore, 1985; Kerger et al., 1995; Klitzman et al., 1983; Svensjo, 1990). Numerous preparations are described in the literature to observe the hamster cheek pouch microscopically. These methods perfuse the tissue with a bicarbonate-buffered saline solution and maintain physiological temperature, oxygen tension, and pH in an anesthetized hamster (Davis and Gore, 1985; Greenblatt et al., 1969; Svensjo, 1990; Yamaki and Kritchman, 1981).

With the everted method, the cheek pouch is grasped with forceps, pulled through the mouth, and pinned over a specialized Lucite support pedestal (Davis and Gore, 1985; Persson et al., 1985). Longitudinal and transverse incisions allow exposure of the pouch in a single-layer preparation with high optical clarity, making this method one of the standards for microcirculation studies (Davis and Gore, 1985; Svensjo, 1990). The disadvantages of the everted preparation are restricted blood flow due to tension on the retractor muscle, and the requirement of pouch incisions. Refined everted techniques eliminate the disadvantage of restricted blood flow. Following a skin incision to expose the pouch the retractor muscle is removed and a thin glass

support plate is positioned in the cheek pouch which allows unrestricted blood flow. The everted pouch can then be observed in a chamber attached over the microscope condenser system. This preparation decreases surgical trauma and preserves the entire cheek pouch vascular supply (Davis and Gore, 1985).

An alternative method is the use of a skin incision in the neck followed by ligation and cutting of the retractor muscle at the point of insertion and retraction of the cheek pouch through the skin incision (Yamaki and Kritchman, 1981). This preparation eliminates tension on the retractor muscle, but requires more surgical manipulation than the everted technique.

A third method, the chamber method, involves incising the skin over the cheek pouch, inserting a translucent chamber through the hamster's mouth into two cheek pouch incisions, and illuminating the chamber with a Lucite rod inserted into the mouth (Greenblatt et al., 1969). This method can be used for acute and chronic experimentation, and does not place excessive tension on the retractor muscle. The primary disadvantage of this method is limited transillumination (Davis and Gore, 1985).

A detailed three-dimensional intravital microscopic view of hamster microcirculation can be visualized using one of the above-described preparations, and intravenous injection of fluorescein-labeled dextran (Endrich et al., 1980; Svensjo, 1990). Intravital microscopy is also used for observation of the microcirculation of non-anesthetized Syrian hamsters using the dorsal skinfold chamber method (Endrich et al., 1980; Lehr et al., 1991; Menger et al., 2002; Nolte et al., 1995). After implantation of two catheters into the jugular vein and carotid artery, two titanium frames are implanted into the dorsal skinfold to extend the double layer of skin. One layer is completely removed and the remaining layer is covered with a cover slip. This preparation allows for chronic studies of microcirculation (Menger et al., 2002).

Ischemia Reperfusion

The hamster cheek pouch is used to study ischemia-reperfusion injury. Ischemia-reperfusion injury is important because it contributes to tissue damage during myocardial and cerebral infarction, and following surgical manipulations that interrupt and then restore blood flow, such as during vascular and organ transplantation and reconstructive surgery (de With et al., 2005; Nolte et al., 1995). Ischemia-reperfusion injury is caused by several factors including the generation of reactive oxygen and nitrogen species and the resulting lipid peroxidation; cytokine production following leukocyte margination, adherence, and infiltration; and activation of the complement system (de Groot and Rauen, 2007; de With et al., 2005; Kaminski et al., 2002;

Yasuhara et al., 1991). The Syrian hamster is extensively used to reveal the pathophysiological mechanisms of ischemia–reperfusion injury, as well as therapeutic options to preserve tissue and vascular viability.

Numerous models of ischemia–reperfusion injury are described using the Syrian hamster cheek pouch. In the model of pressure-induced ischemia a translucent pressure chamber is pressed against the cheek pouch for variable amounts of time and then released to produce reperfusion (Romanus et al., 1977; Yasuhara et al., 1991). The disadvantage of this model is that the results are influenced by the pressure applied to the tissue. In a second model, ischemia is produced by tightening a latex cuff around the neck of an everted cheek pouch for up to an hour followed by cuff release (Persson et al., 1985; Yasuhara et al., 1991). The disadvantage of this model is that tension on the retractor muscle is produced by the everted preparation. Alternatively, ischemia can be induced by compressing the feed arteries and collecting veins of the pouch with a piece of silastic tubing or atraumatic microvascular clips (Bertuglia and Reiter, 2007; de With et al., 2005).

In the incomplete ischemia model, ischemia of the cheek pouch is produced by occluding the ipsilateral common carotid artery, its external branch, and the contralateral external carotid artery (Erlansson et al., 1987; Yasuhara et al., 1991). Partial ischemia better reproduces ischemia as seen in the clinical setting, when compared to models of global ischemia. The disadvantages of this model are that the degree of ischemia is not easily evaluated, and occlusion of the carotid arteries reduces cerebral blood flow (Yasuhara et al., 1991). A further refinement of the incomplete ischemia model is produced by selecting two sections of cheek pouch and placing a triangular chamber made of polyester/plastic film in each area. Ischemia is induced by applying a set amount of pressure to a cover slip over one of the chambers, while the other area acts as a control. Increased pressure is avoided by using a 1.0-mm aperture at one of the chamber corners. Reperfusion is produced when pressure is released after 1 hour (Yasuhara et al., 1991). The advantages of this method include decreased trauma to the pouch microcirculation, the ability to select various sizes of tissue, the maintenance of blood flow in surrounding microvasculature, and the ability to study both ischemic and non-ischemic areas simultaneously. In addition to the cheek pouch, the dorsal skin-fold chamber method can be used in ischemia reperfusion research utilizing an O-shaped silicone ring pressed into the tissue with an adjustable screw clamp, leading to cessation of blood flow into and out of the chamber (Pickelmann et al., 1999).

While Syrian hamster ischemia–reperfusion models are broadly used for elucidating the pathophysiology of the condition, the models are also utilized in the

development of prevention and treatment (Bertuglia and Reiter, 2007; Pickelmann et al., 1999). Melatonin, a potent antioxidant, effectively preserves microcirculation in the hamster cheek pouch during ischemia reperfusion, making melatonin administration a possible preventative measure during situations when ischemia reperfusion injury is a possibility (Bertuglia and Reiter, 2007). Another possible preventative intervention is daflon, a highly purified flavonoid with anti-inflammatory and antioxidative properties. Daflon blocks immigration and accumulation of leukocytes in perivascular tissue during ischemia reperfusion in the Syrian hamster (Pickelmann et al., 1999).

INFECTIOUS DISEASE RESEARCH

Syrian hamsters were introduced as a laboratory animal species in 1930 due to their susceptibility to *Leishmania* spp. and ease of breeding in captivity (Adler, 1948). Prior to the use of Syrian hamsters, Chinese hamsters were the only suitable models for *Leishmania* research but they did not breed well in captivity and had to be imported from the Far East (Adler, 1948). Early on, Syrian hamsters were also found to be useful models for tuberculosis and brucellosis. Syrian hamsters remain a valuable tool for studying many infectious diseases due to their relative lack of spontaneous diseases compared to other rodent models, and their susceptibility to many experimentally induced infectious diseases (Hankenson and Van Hoosier, 2002).

Viruses

Hantavirus

Hantavirus pulmonary syndrome (HPS) is a severe infectious disease caused by numerous members of the *Bunyaviridae* family (Jonsson et al., 2008; Wahl-Jensen et al., 2007). HPS can range from a typical flu-like disease with fever to fatal pneumonia. Unlike other diseases caused by Hantavirus, HPS is transmitted by aerosol and wild rodents in the family *Cricetidae* are the natural hosts of these infectious agents (Hooper et al., 2001). Research conducted on HPS agents must be conducted at ABSL-4 facilities.

The hamster serves as an experimental model to study the pathogenesis of HPS. Specifically, the Syrian hamster is highly susceptible to Andes and Maporal viruses which cause fatal pneumonia and edema in experimentally infected hamsters (McElroy et al., 2004; Milazzo et al., 2002; Wahl-Jensen et al., 2007). In contrast, Sin Nombre virus readily infects the hamster but does not cause disease. Further, Choclo virus is not fatal in the hamster (Eyzaguirre et al., 2008). These varying

results create an understanding of how these agents produce disease. Specifically, Choclo virus damages the endothelium of the lung without significantly inciting inflammation (Eyzaguirre et al., 2008). In contrast, Andes virus targets endothelium and induces a proinflammatory response (Eyzaguirre et al., 2008; Hooper et al., 2001, 2008). This same proinflammatory response is noted in humans with severe HPS. It therefore seems likely that more pathogenic viruses produce disease by both directly damaging lung endothelium and inducing a potent proinflammatory response (Eyzaguirre et al., 2008; Hooper et al., 2001, 2008). Based upon these data a potential treatment of HPS would be the initiation of anti-inflammatory therapy.

Coronavirus (SARS)

In 2003, an outbreak of a previously unknown condition, severe acute respiratory syndrome (SARS), affected more than 8000 people and resulted in 774 deaths across 30 countries (Schaecher et al., 2008). The etiological agent of SARS was identified as a novel coronavirus, SARS-CoV (Drosten et al., 2003). The outbreak was controlled through quarantine of infected individuals and travel advisories (Subbarao and Roberts, 2006). The possibility of future SARS outbreaks has led to the search for an appropriate animal model to study the pathogenesis and potential therapeutics for SARS (Subbarao and Roberts, 2006).

In the hamster model, a high virus titer develops in the respiratory tract following intranasal inoculation of SARS-CoV (Roberts et al., 2005). In the lungs, peak viral replication occurs two days post-infection and is cleared by day 7; however, low levels of virus are present in the nasal turbinates for up to 14 days (Roberts et al., 2005). SARS-CoV elicits a robust neutralizing antibody response in the hamster which can be detected in sera at day 7 post infection and hamsters are protected from re-challenge by 28 days after initial infection (Roberts et al., 2005). In hamsters, viral replication occurs in epithelial cells of the respiratory tract and causes interstitial pneumonia, pulmonary consolidation, and diffuse alveolar damage. A positive correlation exists between pulmonary viral titer and the extent of pneumonia, making the hamster an excellent model for studying the efficacy of potential vaccines and immunotherapy for SARS (Subbarao and Roberts, 2006). Despite the high level of viral replication and associated histopathological lesions, hamsters exhibit no overt clinical signs (Roberts et al., 2005).

Immunotherapy with anti-SARS-CoV monoclonal antibodies (MAbs) has been tested in the hamster model (Roberts et al., 2006). In these studies, the virus is administered intranasally and 24 hours post-infection hamsters are treated with MAbs by intraperitoneal injection. Treatment with MAb 201, a SARS-CoV-specific MAb,

significantly reduces viral replication and the severity of pulmonary lesions in hamsters (Roberts et al., 2006). There is a 1000-fold reduction in viral titer when MAb 201 is given at a dose of 40mg/kg and a 10000-fold reduction when given at a dose of 80mg/kg.

Cyclophosphamide-induced immunosuppression increases the severity of SARS in hamsters when given prior to and during infection (Schaecher et al., 2008). In these studies, hamsters are first given a loading dose of cyclophosphamide (140mg/kg) 2–5 days prior to infection, and then a maintenance dose (100mg/kg) every 3–4 days throughout the course of the study. In treated animals the virus displays a more expansive tissue tropism and high titers are present in the lung, liver, spleen, kidney, and heart and treated animals exhibit weight loss and mortality. This condition is similar to humans with SARS who develop extrapulmonary lesions including splenic atrophy, acute tubular necrosis in the kidneys, neuronal edema and degeneration, heart edema, and myocardial fiber atrophy (Gu and Korteweg, 2007). While the immunosuppressed hamster model does not replicate all of the extrapulmonary lesions in humans it more closely mimics the human disease presentation when compared to the immunocompetent hamster model (Schaecher et al., 2008).

Bacteria

Leptospira spp.

Leptospirosis, caused by organisms in the genus *Leptospira*, is a potentially fatal disease affecting humans and animals worldwide (Guerra, 2009). In humans, clinical signs range from asymptomatic to high fever, severe headache, chills, muscle aches, vomiting, diarrhea, jaundice, abdominal pain, and a rash (Arean, 1962). If the disease is left untreated, kidney damage, meningitis, liver failure, respiratory distress, and death are possible. Leptospirosis is contracted by ingestion or contact with contaminated water, food, or soil (Guerra, 2009).

Two strains of *Leptospira interrogans* (L1–130, Kito) and three strains of *Leptospira noguchii* (Cascata, Hook, and Bonito) produce an acute lethal infection when inoculated intraperitoneally into Syrian hamsters (Silva et al., 2008). Leptospirosis in hamsters is characterized by hepatic and renal complications similar to those seen in acute lethal infection in humans (Silva et al., 2008). Clinical signs that may be observed by the fourth day post-infection include hemorrhage, dehydration, ruffled fur, decreased activity, and isolation from cohorts. Hamsters infected with L1–130 or Kito strains die 7–14 days after inoculation, while hamsters infected with Cascata, Hook, or Bonito die 7–22 days after inoculation. The reproducible lethal nature of these isolates in hamsters, coupled with lesions in target organs that

resemble leptospirosis in humans, provides a useful animal model.

Through the use of this model, new treatment and prophylactic modalities can be tested. For example, azithromycin is an effective treatment for acute leptospirosis in hamsters, with 100% survival, following only two doses initiated 2–4 days post infection (Moon et al., 2007). Azithromycin provides an alternative to the standard doxycycline or penicillin treatment because it lacks contraindications in young children and pregnant women (Jain and Danziger, 2004; Moon et al., 2007).

Clostridium difficile

Clostridium difficile, a Gram-positive spore-forming bacillus, is the leading cause of antibiotic-associated diarrhea in humans (Poutanen and Simor, 2004). Disease occurs when the normal colonic flora is disrupted typically due to clindamycin, ampicillin, or cephalosporin administration and overgrowth of toxin-producing strains of *C. difficile* (Poutanen and Simor, 2004). Pathogenic strains of *C. difficile* produce two toxins, enterotoxin A and cytotoxin B, which are the major virulence factors for *C. difficile* (Razaq et al., 2007). Outbreaks of *C. difficile*, beginning in 2000, are attributed to a newly recognized strain, known as the BI strain (McDonald et al., 2005). In addition to the other toxins mentioned the BI strain of *C. difficile* produces a binary toxin, has an 18-bp deletion in the *tcdC* locus (a negative regulator of toxin A and B), and is resistant to fluoroquinolone antibiotics (McDonald et al., 2005). Patients that acquire *C. difficile* may remain asymptomatic carriers or develop diarrhea, pseudomembranous colitis, or toxic megacolon (Al-Eidan et al., 2000). Treatment consists of stopping the initiating antibiotics and beginning treatment with metronidazole or vancomycin for 10 days. Relapse after completion of treatment occurs in about 20% of cases (Pepin et al., 2005).

The Syrian hamster model for *C. difficile* closely mimics several important aspects of human infection (Kokkotou et al., 2008). Hamsters are orally gavaged with clindamycin followed by gastric inoculation of *C. difficile* 24 hours later (Kokkotou et al., 2008). Hamsters exposed to this experimental paradigm develop hemorrhagic typhlitis, similar to antibiotic-associated pseudomembranous colitis in humans, followed by death within 3 days of infection (Kokkotou et al., 2008). Clinical signs of infection may include diarrhea, weight loss, ataxia, dyspnea, and death (Babcock et al., 2006). Highly virulent strains may cause death without any premonitory signs (Razaq et al., 2007).

Hamsters, like humans, may succumb to a relapse after completing treatment of the primary disease (Kokkotou et al., 2008). Hamsters receiving vancomycin, the historical treatment for human *C. difficile*-associated colitis, display a 75% recurrence rate by 28 days post-initial-infection (Kokkotou et al., 2008).

However, treatment with Rifaximin, a non-absorbable oral antibiotic, results in a 0% recurrence rate, potentially providing refinement to therapy in humans (Kokkotou et al., 2008).

The hamster model of *C. difficile* is also used to test the use of human monoclonal antibodies (HuMAbs) directed against either toxin A (CDA1) or toxin B (MDX-1388) (Babcock et al., 2006). Intraperitoneal administration of CDA1 and MDX1388 daily for 4 days prior to inoculation with *C. difficile* protects against mortality both in the primary disease and in the relapse model of infection (Babcock et al., 2006). The use of HuMAbs may provide a useful therapeutic option either alone or in combination with antibiotics for treatment of humans with *C. difficile* colitis (Babcock et al., 2006).

Mycoplasma pneumoniae

Mycoplasma pneumoniae is the leading cause of pneumonia in children and young adults (Denny et al., 1971). A majority of the cases result in upper respiratory tract infections, with fever, cough, sore throat, headache, and malaise, but some cases may progress to tracheobronchitis or pneumonia (Denny et al., 1971). Antibiotics may reduce the severity of disease, but do not eliminate the infection (Ford et al., 1980). Recovery from *M. pneumoniae* infection protects the individual from reinfection, suggesting that the development of a vaccine is the most promising approach to controlling *M. pneumoniae* infections (Barile et al., 1988).

Syrian hamsters, which do not harbor *Mycoplasma* spp. as part of their indigenous microbiota, provided an early model for studying *Mycoplasma* and potential vaccines and therapeutics (Barile et al., 1988). In this model, *M. pneumoniae* is inoculated into hamsters via either the aerosol, intranasal, or intratracheal routes, and infection results in changes that are consistent with natural human disease (Clyde, 1971). The intratracheal route consistently produces pulmonary disease of greater severity with greater colonization than the other routes of exposure (Barile et al., 1988). In general, peak disease severity occurs 2 weeks post inoculation with hamsters over 6 weeks of age becoming most severely affected (Barile et al., 1988). Assessment of experimental vaccines and other therapeutics can be accomplished by assessment of lung lesion scores. Specifically, lung lesions are scored microscopically by evaluating the degree of leukocyte cell infiltrates, luminal exudate, and parenchymal pneumonia in three cross-sections of the entire lung (Barile et al., 1988). Alternatively, following treatment with a potential chemotherapy agent, viable mycoplasmas in the lung can be quantitated by homogenizing the lungs in mycoplasma liquid medium and plating serial dilutions on agar medium. The use of the hamster in *M. pneumoniae* research has decreased in recent years (2000 and beyond). This reduction

likely stems from the wide availability of mice free of *Mycoplasma* spp.

Treponema pallidum

Two forms of syphilis occur in humans: the venereal form with primary, secondary, and tertiary manifestations and possible congenital transmission, and the endemic form that is usually seen in childhood and is transmitted between children by close contact. In humans, *Treponema pallidum* subsp. *pallidum* is the etiologic agent for venereal syphilis and *T. pallidum* subsp. *endemicum* is the etiologic agent for the endemic form (Kajdacsy-Balla et al., 1993). In the venereal form, primary lesions present as small painless firm raised sores at the site of contact that heal in 3–6 weeks (McAdam and Sharpe, 2010). Secondary lesions occur about 2–10 weeks after the primary lesions and are characterized by widespread cutaneous and mucocutaneous lesions with lymphadenopathy that lasts for several weeks (McAdam and Sharpe, 2010). The tertiary stage occurs in about a third of people that do not receive treatment and appears after a latent period of 5 or more years. Internal organs are the target for the tertiary stage of syphilis, primarily causing cardiovascular or neurologic damage.

When Syrian hamsters are infected with *T. pallidum* subspecies *endemicum*, a disease process similar to

human venereal syphilis develops (Kajdacsy-Balla et al., 1993). Intradermal injection of *T. pallidum* into the inguinal regions of hamsters results in primary lesions at the site of inoculation. The primary lesions form about 3 weeks after inoculation as erythematous papules, which enlarge and become ulcerated by 4 weeks (Figure 34.4A). The lesions continue to expand until the sixth to eighth week post-inoculation when they begin to heal slowly (Kajdacsy-Balla et al., 1993). Around 24 weeks post-infection, secondary lesions develop as perioral ulcers (Figure 34.4B) and an erythematous rash on the paws and cranial trunk (Kajdacsy-Balla et al., 1993). Most infected hamsters die within 28–32 weeks following inoculation due to malnutrition from the presence of severe perioral ulcers. If the hamster survives, the perioral ulcers will resolve. Tertiary lesions in hamsters are not documented; however, this could be due to the rarity of survival past the secondary stage.

Gross pathologic features of hamster syphilis include lymphadenopathy and thymic atrophy (Kajdacsy-Balla et al., 1993). Histologic features include generalized follicular hyperplasia of lymph nodes, hepatic periportal foci of mononuclear cells, and a diffuse increase in mononuclear cells in the sinusoids. The perioral ulcers are characterized by an intense polymorphonuclear infiltrate at their base. Treponemes are visualized in the lymph nodes and perioral ulcers by dark-field

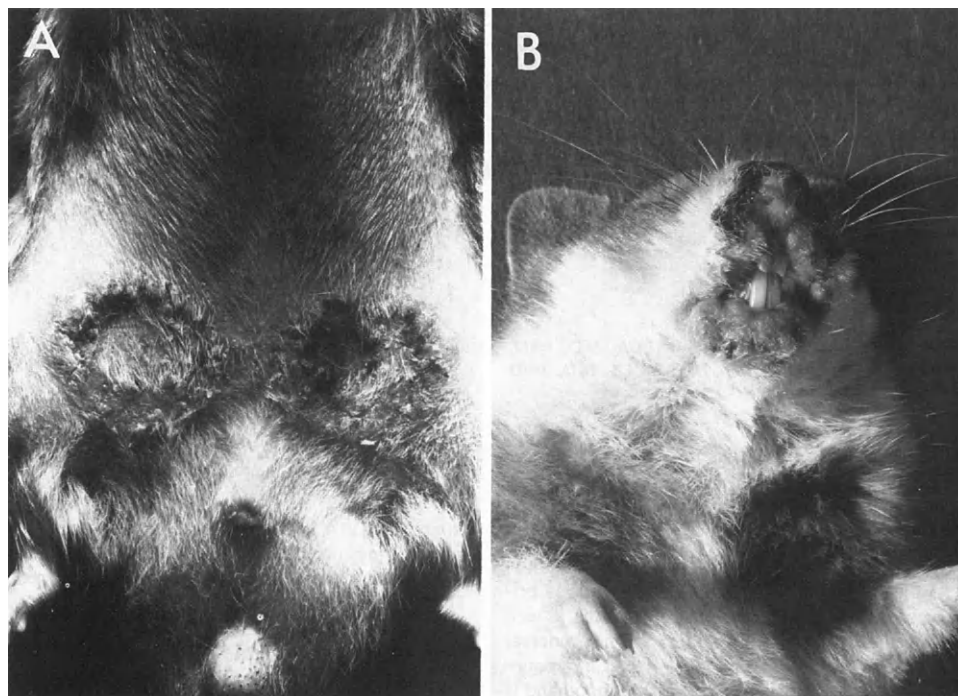


FIGURE 34.4 Hamster with syphilitic lesions. (A) Primary cutaneous lesions form at the site of inoculation as erythematous papules which enlarge and become ulcerated with raised indurated borders. (B) Secondary lesions spontaneously develop approximately 24 weeks after infection in the form of perioral ulceration. From Kajdacsy-Balla et al. 1993 with permission from the American Society for Investigative Pathology.

microscopy. Persistent latent infection is demonstrated in these animals by transfer of lymph node material into naïve hamsters.

Hamsters infected prior to pregnancy transmit syphilis to their offspring congenitally. A majority of the offspring exhibit rhinitis, about a third develop perianal and perigenital lesions, and a quarter are stillborn or die shortly after birth (Kajdacsy-Balla et al., 1993). Human congenital syphilis also results in about 25% of prenatal infections resulting in stillbirth, while 25–30% of newborn infants die shortly after birth and 40% develop syphilis later in life (Crissey and Denenholz, 1984).

Experimental infection of hamsters with *T. pallidum* provides a model to study syphilis infection and drug therapy. Daily clarithromycin given over 7 days successfully eliminates infection in hamsters (Alder et al., 1993). Success is confirmed by failure to infect susceptible hamsters following inoculation with lymph node material from treated hamsters (Alder et al., 1993). These findings may prove useful in establishing alternatives to penicillin treatment, which is the drug of choice for treating human infections.

Hamsters infected with *T. pallidum* for 8 weeks then treated with 4000 IU of penicillin to clear the infection are susceptible to reinfection (Schell et al., 1980). However, hamsters infected with *T. pallidum* for 10–16 weeks and treated with 4000 IU of penicillin are resistant to reinfection (Schell et al., 1980). Following re-inoculation with *T. pallidum*, hamsters do not develop lesions and treponemes are not detectable in the lymph material. The hamster's ability to develop resistance to *T. pallidum* may prove useful in studying human syphilis vaccine development.

While many features of the hamster *T. pallidum* subsp. *endemicum* model make it an excellent model for studying congenital syphilis, it does not utilize *T. pallidum* subsp. *pallidum*, which is the agent that causes congenital syphilis in humans. Hamsters do not develop any lesions in response to *T. pallidum* subsp. *pallidum*. In contrast guinea pigs do develop congenital syphilis in response to *T. pallidum* subsp. *pallidum*, and are therefore more popular current models in the study of congenital syphilis.

Parasites

Toxoplasma gondii

Toxoplasma gondii is an intracellular parasitic protozoan widely distributed in nature. Cats are the definitive host, but all mammals may serve as intermediate hosts. In all hosts, the parasite forms cysts within various tissues (Kean, 1972). Approximately 23% of adolescents and adults in the United States have anti-*T. gondii* antibodies (National Center for Health Statistics, 1994). In some areas of the world, up to 97%

of people are positive for *T. gondii* antibodies (Santos et al., 2009). Infection is usually asymptomatic; however, infection during pregnancy may lead to abortions or to offspring that develop mental retardation, epilepsy, or toxoplasmic retinochoroiditis (Lopez et al., 2000). Ocular disease resolves spontaneously, but reoccurs frequently, and may result in episodic blindness (Pavesio et al., 1995).

The hamster is a reliable model of toxoplasmic retinochoroiditis. Hamsters inoculated intraperitoneally with cysts of *T. gondii* strain ME 49 develop ocular disease without systemic illness (Pavesio et al., 1995). Retinal lesions develop in both eyes by 2–3 weeks after inoculation and cysts form as white pinpoint to optic disc-sized lesions, primarily at the posterior pole of the inner retina. Four weeks post-infection, the lesions enlarge and develop diffuse indistinct edges with vasculitis and occasionally vitritis. Regression typically occurs by 8–9 weeks, but retinal atrophy may persist (Pavesio et al., 1995).

Oral administration of *T. gondii* ME 49 results in a comparable outcome with no systemic signs of illness, and similar but less severe ocular lesions (Gormley et al., 1999). The lesions appear similar in color and position in the retina, however, they are smaller in size, fewer in number, associated with a milder inflammatory reaction, and first appear approximately 1 week later than with the intraperitoneal route.

During the past decade, mice have replaced hamsters as the model of choice for studying ocular toxoplasmosis. Mouse models offer the advantages of a completed genome, the wide availability of knock-out strains, and the ability to reproduce many features of human ocular toxoplasmosis.

Babesia microti

Babesia spp. are protozoal pathogens that infect red blood cells. Many *Babesia* spp. are found in animals, but *Babesia microti* is the main cause of infection in humans and is transmitted by *Ixodes scapularis* ticks (Vannier et al., 2008). Babesiosis in humans can range from asymptomatic to life-threatening. Hemolytic anemia and flu-like symptoms are possible, especially in people without a functional spleen or in people that are otherwise immunocompromised (Vannier et al., 2008).

Syrian hamsters provide a reliable model of babesiosis through intraperitoneal inoculation with *B. microti*-infected red blood cells (Wozniak et al., 1996). Without treatment, infection is patent for 3–5 weeks, and hamsters develop severe anemia and marked parasitemia with low mortality. Parasitemia is evident by day 7 post-inoculation, with a peak parasitemia occurring by day 14. This is followed by a carrier state with low or undetectable levels of parasites.

Because Syrian hamsters are highly susceptible to *B. microti* infection they are commonly utilized to

maintain *B. microti* within the laboratory. *B. microti*-infected hamster blood can be used to infect other animals or they may be used for in vitro studies of *Babesia* during infection of erythrocytes.

The hamster model also provides a valuable tool for assessing new therapeutic options for human babesiosis. Drug treatments are initiated once parasitemia reaches 3–7% of the total red blood cell population (Marley et al., 1997). To determine whether a treatment elicits a complete cure, blood is drawn from a treated hamster 14 days after the last drug dose and administered by intraperitoneal injection into a naïve sentinel hamster. The sentinel hamster is then monitored weekly for 6 weeks for development of parasitemia by examination of thin blood smears (Marley et al., 1997).

Utilizing this experimental approach, clindamycin consistently eradicates *B. microti* in hamsters (Rowin et al., 1982). The combination of quinine and clindamycin results in a more significant and faster therapeutic response and is now the treatment of choice for severely ill human patients with babesiosis (Rowin et al., 1982; Wittner et al., 1996). The combination of atovaquone and azithromycin also eliminates *B. microti* in hamsters and is an alternate therapy for human cases of babesiosis that are resistant to clindamycin and quinine (Falagas and Klemperer, 1996; Wittner et al., 1996).

Leishmania donovani

Visceral leishmaniasis is caused by *Leishmania donovani*, an intracellular protozoan transmitted by sandflies (Chappuis et al., 2007). Visceral leishmaniasis is a progressive disease characterized by prolonged fever, hepatosplenomegaly, anemia, leukopenia, severe weight loss, and ultimately death (McAdam and Sharpe, 2010). Pentavalent antimonial drugs, such as sodium stibogluconate, are the treatment of choice, despite harmful side effects and significant levels of drug resistance (Sundar et al., 2000). Identifying more effective chemotherapeutics is the focus of research for this disease (Wyllie and Fairlamb, 2006).

Hamsters can be experimentally infected with *L. donovani* by intracardiac injection under anesthesia or intraperitoneal injection of amastigotes (Dea-Ayuela et al., 2007; Wyllie and Fairlamb, 2006). Intracardiac injection results in a more rapid infection than the intraperitoneal route; however, the intraperitoneal route results in a greater parasite burden in the spleen (Wyllie and Fairlamb, 2006). Systemic *L. donovani* infection results in clinicopathological features that closely mimic the human disease (Melby et al., 2001). Infection of the hamster results in a persistent increase in parasite burden, progressive cachexia, hepatosplenomegaly, pancytopenia, hypergammaglobulinemia, and ultimately death (Melby et al., 2001).

Early detection of infection can be accomplished using a latex agglutination test developed to diagnose

visceral leishmaniasis in dogs (Wyllie and Fairlamb, 2006). The assay is based on detection of a low-molecular-weight glycoconjugate in the urine. Hamster urine must be diluted 1:10 in phosphate-buffered saline before testing due to its concentrated nature, which creates a vast excess of antigen in the test sample that can result in false-negative results.

Weight loss is the most commonly used ante-mortem indicator of infection in live hamsters (Wyllie and Fairlamb, 2006). Early detection of infection can be accomplished by comparing body weights between inoculated and control juvenile hamsters where control hamsters gain weight significantly faster than age-matched infected hamsters (Wyllie and Fairlamb, 2006). Weight loss of 25% is used as the end-point for tissue collection and post-mortem analysis and parasite burden can be purified and quantified from a homogenate of the spleen at this endpoint (Dea-Ayuela et al., 2007).

Trypanosoma cruzi

Chagas disease is caused by the flagellate protozoan *Trypanosoma cruzi* (Kirchhoff, 1993). In Chagas-endemic areas of Central and South America, the most common route of infection is from the triatomine insect, which shed *T. cruzi* in their feces. Chagas disease is comprised of two phases, acute and chronic. The acute phase lasts for the first few weeks or months following infection and symptoms include fever, fatigue, body aches, headache, rash, anorexia, diarrhea, and vomiting (Kirchhoff, 1993). Gross lesions may include splenomegaly, hepatomegaly, lymphadenopathy, and local swelling at the site of parasite entry. Rarely, young children or immunocompromised people develop fatal myocarditis or meningoencephalitis (Kirchhoff, 1993). During the chronic phase, infection may remain dormant for decades; however, up to 30% of infected people will develop life-threatening inflammatory cardiomyopathy 15–30 years following infection (Koberle, 1968).

Syrian hamsters, following intraperitoneal injection of *T. cruzi*, develop a *T. cruzi*-induced cardiomyopathy resembling human Chagas cardiomyopathy, and display high levels of anti-*T. cruzi* IgG antibodies (Bilate et al., 2003). *T. cruzi* infection results in the death of some hamsters during the acute phase, within the first 4 months of infection, and during the chronic phase, after 8 months of infection (Bilate et al., 2003). All infected animals develop myocarditis, interstitial fibrosis, and mild-to-severe left ventricular dysfunction (Bilate et al., 2003). In the chronic phase, hamsters also develop ventricular dilation and a decrease in ventricle wall thickness (Bilate et al., 2003). Hamsters do not develop apical or segmental wall hypcontractility, which are additional features of human Chagas cardiomyopathy (Bilate et al., 2003).

The Chagas disease hamster model allows quantitative evaluation of left ventricular function and

myocardial lesions throughout progression of the disease. Novel therapeutics and their effect on the progression of cardiac disease can therefore be evaluated rapidly using the acute and chronic hamster model (Bilate et al., 2003).

Opisthorchis viverrini

Epidemiological studies reveal a high prevalence of cholangiocarcinoma in northeast Thailand (Sriamporn et al., 2004). In this region, the traditional diet exposes the local population to infection with the liver fluke *Opisthorchis viverrini* and nitrosamines through consumption of contaminated water and salt-fermented fish (Pairojkul et al., 1991). The use of a Syrian hamster model established a connection between *O. viverrini* infection, consumption of nitrosamine, and the development of cholangiocarcinoma (Pairojkul et al., 1991).

Syrian hamsters can be readily infected with *O. viverrini* by oral administration of metacercariae. Infection results in inflammation and fibrosis in the periductular region of the liver, similar to human infection (Chaimuangraj et al., 2003; Pairojkul et al., 1991). Feeding *O. viverrini*-infected hamsters subcarcinogenic doses of dimethylnitrosamine results in the development of cholangiocarcinomas in up to 93% of hamsters (Thamavit et al., 1987). In contrast, administration of subcarcinogenic doses of dimethylnitrosamine alone or infection with *O. viverrini* alone does not cause cancer in this animal model, suggesting that these factors interact to produce cholangiocarcinoma (Thamavit et al., 1987, 1996).

After experimental infection with *O. viverrini*, liver procollagen prolyl hydroxylase activity increases, reflecting increased collagen synthesis that is proportional to the intensity of liver fluke infection (Hutadilok et al., 1983). Liver fluke infestation likely acts as an epigenetic promotor of tumor formation, rather than a frank genomic mutagen, by inducing inflammatory and fibrotic changes in the liver (Pairojkul et al., 1991). This is supported by similar studies that utilize complete bile duct ligation in hamsters which results in biliary inflammation and proliferation. If this procedure is performed in hamsters treated with subcarcinogenic doses of dimethylnitrosamine, promotion of cholangiocellular lesions develop similar to the findings in *O. viverrini* infection (Thamavit et al., 1993).

Taenia spp.

Humans are the natural definitive host for *Taenia solium* and *Taenia saginata* (Avila et al., 2002). Human taeniosis is acquired by ingestion of cysticerci in raw or undercooked pork or beef. In the human small intestine, the cysticerci attach to the intestinal wall and develop into adult tapeworms which become gravid approximately 4 months after infection (Avila et al., 2002; McAdam and Sharpe, 2010). Taeniosis is usually

asymptomatic but may result in mild gastrointestinal disturbances (McAdam and Sharpe, 2010). In contrast, when humans ingest embryonated eggs through ingestion of food or water contaminated with human feces, they may serve as intermediate hosts (cysticercosis) (McAdam and Sharpe, 2010). The larvae hatch, penetrate the intestinal wall and disseminate to other organs where they encyst (McAdam and Sharpe, 2010). Cysticerci develop in the central nervous system, heart, eyes, subcutaneous tissue, and skeletal muscle. Clinical manifestations depend on where the cysticerci develop and may include neurologic, cardiac, or ocular deficits, or even death (Avila et al., 2002, 2006).

Hamsters can successfully become infected with adult *Taenia* spp. by being fed cysticerci encysted in rodent muscle (Wang et al., 1999). Oral gavage of cysticerci without muscle encasement results in digestion of the cysticerci and no colonization with adult tapeworms (Wang et al., 1999). Tapeworms grow better in hamsters that are immunosuppressed with steroids; however, it is unclear if this is due to a direct effect of the steroids on the tapeworm or due to depression of the host immune response (Avila, 2006). Tapeworms reach lengths in excess of 30 cm after 30 days, but do not form gravid proglottids in the hamster model (Allan et al., 1991).

Utilizing the hamster model, an ELISA, which detects a *Taenia* spp.-specific fecal antigen, was developed and standardized (Allan et al., 1990). In contrast to identification of eggs in the feces, this test can be used to diagnose infection even during the prepatent period. Additionally, the hamster model can be used to study the morphological and ultrastructural attachment of adult tapeworms to the intestinal wall, the results of which may contribute to a better understanding of the biology of tapeworm infections (Merchant et al., 1998).

Ancylostoma ceylanicum

Hookworm infection affects hundreds of millions of people living in tropical and subtropical climates around the world. Adult hookworms attach to the intestinal mucosa and feed on blood from lacerated capillaries. Chronic hemorrhage from the site of attachment leads to iron-deficiency anemia, serum protein loss, and intestinal inflammation (Held et al., 2006).

Unlike mice, Syrian hamsters are fully permissive hosts for the development of adult *Ancylostoma ceylanicum* hookworms (Bungiro et al., 2003; Carroll et al., 1983). Hamsters can be inoculated with infective *A. ceylanicum* third-stage larvae via oral gavage (Mendez et al., 2005). Hookworm eggs can be detected in the feces 14 days following infection and live adult hookworms can be recovered from the small intestine and colon (Mendez et al., 2005). Infected hamsters experience anemia, weight loss, growth delay, and chronicity of infection, just like children that are infected with

hookworms, making them an excellent model for the study of human hookworm infection (Held et al., 2006; Mendez et al., 2005).

Hamsters can also be orally inoculated with adult *A. ceylanicum* (Bungiro et al., 2003). This method allows for the specific study of host responses to bloodfeeding adult hookworms without the confounding influence of prior larval exposure. This model provides the added benefits of allowing for manipulation of adult hookworms by immunologic, genetic, or pharmacologic methods prior to inoculation into the hamsters and for the study of single-sex infections.

Schistosoma spp.

Schistosomiasis is one of the most prevalent parasitic diseases affecting humans in tropical and subtropical climates, with an estimated 779 million people at risk and over 200 million infected (Steinmann et al., 2006). Following percutaneous invasion flukes mature and lay eggs within hepatic or pelvic blood vessels and eggs migrate to the liver, intestine, or bladder, causing inflammation and scarring possibly leading to hepatic cirrhosis.

Syrian hamsters can be readily infected with *Schistosoma* spp. cercariae via percutaneous or subcutaneous inoculation (Botros et al., 2004). Chemotherapeutic agents can be tested for effectiveness against the juvenile stage of infection by administration at 21 days post inoculation and against adult worms by administration at 49 days post inoculation.

Prion Diseases

Transmissible spongiform encephalopathies (TSE), such as Creutzfeldt-Jakob disease in humans, bovine spongiform encephalopathy in cattle, or scrapie in sheep are fatal neurodegenerative diseases of the central nervous system (Kratzel et al., 2007a, 2007b). A common feature of TSE is that normal host cellular prion protein (PrP^c), which is strongly expressed by cells of the nervous and lymphoreticular systems, converts into an abnormal disease-specific prion protein (e.g. PrP^{Sc} in scrapie) (Kratzel et al., 2007b). This abnormal prion protein is a biochemical marker for TSE and is linked with infectivity.

TSE can be transmitted by oral, intracerebral, intravenous, intraperitoneal, and subcutaneous routes, but the most common route is through ingestion (Kratzel et al., 2007b). To study the course of disease following the oral route of infection, hamsters can be infected with scrapie by housing them with other hamsters that were intracerebrally inoculated with scrapie prions as weanlings (Prusiner et al., 1985). The experimental hamsters cannibalize the infected hamsters when they become terminally ill. Approximately 120 days after

consuming scrapie-infected brain, progressive neurologic signs including ataxia, tremors, and head bobbing develop, and death occurs about 20 days later (Prusiner et al., 1985).

Injection of scrapie-infected brain homogenate into the foot pad using a 27-gauge needle provides an alternative route of experimental infection (Kratzel et al., 2007b). Clinical signs, starting with muscle tremors, begin approximately 78 days following unilateral foot pad injection (Kratzel et al., 2007b). This method of inoculation provides a shorter incubation time than oral inoculation and allows the distribution of scrapie prion protein in nerve tissue to be assessed at various time points. Utilizing this method, scrapie prion protein is detected in the ipsilateral sciatic nerve 60 days post infection and 80 days post infection in the contralateral nerve (Kratzel et al., 2007b). Within the brain, the red nucleus correlating with the ipsilateral sciatic nerve produces stronger prion protein immunoreactivity compared to the contralateral nucleus. These findings suggest centripetal propagation to the CNS and subsequent centrifugal spread to other nerve projections (Kratzel et al., 2007b).

The role of the lymphoreticular system on the pathogenesis of TSE is also studied using the unilateral footpad infection model (Kratzel et al., 2007a). Scrapie prion protein is consistently detected in the ipsilateral popliteal lymph node as early as 2 days post-inoculation. In contrast, the contralateral popliteal lymph node remains negative throughout the study, indicating that systemic spread of scrapie prion protein through the lymphoreticular system does not occur (Kratzel et al., 2007a). Removal of the ipsilateral popliteal lymph node 4 weeks prior to infection with scrapie does not affect the incubation period or mean survival time at high or medium doses; however, at low doses, hamsters remain free of clinical symptoms for 314 days post inoculation (duration of experiment) (Kratzel et al., 2007a). These data suggest that very low doses of scrapie may require replication in the lymphoreticular system prior to invasion of the nervous system (Kratzel et al., 2007a).

OTHER MODELS

Amyloidosis

Amyloidosis is an abnormal deposition of highly ordered proteinaceous material in various organ systems of humans and animals (Coe and Ross, 1990). There are currently 20 clinical syndromes in humans associated with amyloid deposition and each syndrome is classified into primary or secondary amyloidosis based on chemical composition (Hukkanen et al., 2006). Primary amyloidosis is the result of overproduction of immunoglobulin light chains and is principally of

neoplastic or genetic origin. Reactive or secondary amyloidosis is the result of the accumulation of β -pleated fibrils composed of serum amyloid A (SAA), an acute-phase protein associated with inflammation (Hukkanen et al., 2006). In addition to SAA, in reactive amyloidosis, amyloid deposits contain a minor protein component called amyloid P (AP) derived from serum AP (SAP) (Coe and Ross, 1990). Amyloidosis occurs both spontaneously and after induction with amyloid-inducing substances in Syrian hamsters. The spontaneous disease is more prevalent in females compared to males and spontaneous occurrence is promoted by fight wounds, stress, high-protein diets, and various infectious agents (Gruys and Snel, 1994). Syrian hamsters are commonly used as a model of induced amyloidosis, using casein or lipopolysaccharide (LPS) subcutaneous injections (Hol et al., 1986; Niewold et al., 1987). The lag or pre-amyloid phase associated with these amyloid-inducing substances can be accelerated by intraperitoneal or subcutaneous injection with amyloid-enhancing factors (AEF), which are small fragments of amyloid fibrils (Hol et al., 1986; Niewold et al., 1987). AEF injection shortens the pre-amyloid phase for splenic, hepatic, and renal amyloid deposition (Hol et al., 1986).

Syrian hamsters are unique when compared to other animal models of secondary amyloidosis because they have an SAP homolog called female protein (FP) which is under estrogenic control (Coe and Ross, 1990). The hamster amyloid model provides the unique opportunity to hormonally manipulate the synthesis of FP and study concurrent amyloid deposition. Female hamsters, when compared to males, possess higher serum concentrations of FP, acquire more amyloid at an earlier age and die at an earlier age due to amyloid accumulation (Coe and Ross, 1990). In the Syrian hamster, FP rather than amyloid fibril, plays a predominant role in the sex-limited expression of amyloidosis (Coe and Ross, 1990). Male Syrian hamsters that are deprived of testosterone by castration or administered diethylstilbestrol (DES) acquire amyloid deposition at an earlier age similar to female hamsters (Coe and Ross, 1990). The amyloidogenic effect of DES in male hamsters is inhibited by testosterone injections, and female hamsters given testosterone are also protected from amyloid deposition (Coe and Ross, 1990). These studies established the importance of FP in this disease and it is hypothesized that FP undergoes calcium-dependent binding of amyloid fibrils which stabilizes and protects the amyloid fibrils after formation (Coe and Ross, 1990).

Sex Behavior and Steroids

Sexual behavior of male humans and most other male mammals is dependent upon gonadal androgen secretion (Meisel and Sachs, 1994). Using a hamster

model, Schulz and colleagues described the two-stage model of sexual behavior development (Schulz and Sisk, 2006). The first stage is the perinatal period when neural circuits become sexually differentiated and the initial programming of adult responses to hormones occurs. The second stage is adolescence, when the structural and functional organization of behavioral circuits are refined and completed, resulting in the expression of sex-typical social behaviors. The presence of testosterone during adolescence results in adults who more readily display male-typical social behaviors compared to adults that matured in the absence of testosterone (Schulz and Sisk, 2006). The testosterone exposure during adolescence defeminizes the brain and behavioral responses to estradiol and progesterone (Schulz and Sisk, 2006).

Castrated male Syrian hamsters that are treated daily with 1000 μ g of dihydrotestosterone (DHT) do not display copulatory behavior or interest in female hamsters in estrus (Arteaga-Silva et al., 2005). In contrast, castrated males treated with 50 μ g of estradiol (E2) show sexual motivation but do not display the ejaculation reflex (Arteaga-Silva et al., 2005). Combined treatment with DHT and E2 restores all components of male sexual behavior in castrated males (Arteaga-Silva et al., 2005). Castrated male Syrian hamsters that receive brief infrequent elevations of testosterone display male sexual behavior (Piekariski et al., 2009). This provides a useful model to assess the neuroendocrine basis of male sexual behavior and raises the possibility that infrequent low-dose androgen replacement may restore sexual behavior in hypogonadal men without inducing negative side-effects.

Flank marking, like reproductive behavior, is activated by testosterone in adulthood (Albers et al., 2002; Johnston, 1981). In contrast, attack behavior toward another male in a resident-intruder paradigm is significantly higher prior to puberty when compared to adulthood, indicating that attack behavior is not under the influence of physiologic testosterone (Romeo et al., 2003). Therefore, exposure to gonadal hormones during adolescence and adulthood facilitates aggression towards an intruder and flank-marking behavior, respectively (Romeo et al., 2003).

Anabolic steroids are abused by humans for their tissue-building potency and performance-enhancing effects. These steroids induce a variety of negative effects including heightened aggression, changes in sexual libido, testicular atrophy, baldness, cancer, and cardiovascular disease (Leshner, 2000). Hamsters are used to study the deleterious behavioral effects of exogenous steroid administration. Specifically adolescent (post-pubertal) male hamsters exposed to anabolic steroids for 2–4 weeks show an increase in aggression (DeLeon et al., 2002; Grimes and Melloni, 2002; Melloni and

Ferris, 1996). In addition, when adolescent hamsters are given exogenous anabolic steroids they do not express submissive behaviors such as defensive posturing, escape dashes, or tail-up walking (Salas-Ramirez et al., 2008). Both adolescent male and adult male hamsters treated with anabolic steroids display increased flank marking. In adolescent hamsters, exposure to anabolic steroids results in levels of sexual behavior comparable to adult hamsters. In contrast, steroid administration in adult hamsters results in decreased sexual behavior compared to untreated controls. These studies suggest that the adolescent brain is more vulnerable than the adult brain to the adverse behavioral consequences of exogenous steroids (DeLeon et al., 2002; Grimes and Melloni, 2002; Melloni and Ferris, 1996; Salas-Ramirez et al., 2008).

The addiction potential of anabolic steroids is evaluated using a classical self-administration paradigm of drug abuse (Madden et al., 1980; Panlilio et al., 2003). Castrated male hamsters that are supplemented with testosterone self-administer additional testosterone at all doses tested (DiMeo and Wood, 2004). In contrast, when testosterone is not exogenously administered, castrated males do not self-administer testosterone (DiMeo and Wood, 2004). These findings indicate that circulating androgens enhance the desire to self-administer testosterone. This study also provides strong evidence that exogenous testosterone administration, particularly in individuals that possess endogenous testosterone, poses a strong potential for addiction.

Nutritional Infertility

Limited food availability diminishes fertility in mammals, particularly in females (Bronson, 1989). The hamster provides a model to evaluate the deleterious effects of inadequate nutrition on fertility. A negative energy balance in hamsters suppresses ovulatory cycles and estrus behavior (Wade et al., 1996). Food deprivation decreases estrogen receptor immunoreactivity in the ventromedial hypothalamus, implying a direct neurological effect of food deprivation in sexual behavior. Treatment with leptin promotes female sexual behavior in ad libitum-fed hamsters; however, in food-deprived hamsters, leptin treatment fails to overcome the loss of estrus behavior associated with poor nutrition. Interestingly, leptin-induced changes in female sexual behavior are not accompanied by changes in estrogen receptor immunoreactivity of the hypothalamus, implying that leptin acts via another mechanism (Wade et al., 1997). Neuropeptide Y (NPY) may play a role in nutritional infertility. Injections of NPY into the central nervous system stimulate robust and long-lasting increases in food intake in hamsters while simultaneously reducing estrus behavior (Corp et al., 2001; Kulkosky et al.,

1988). These studies provide strong evidence that multiple pathways (leptin and NPY) control alterations in female nutrition-associated fertility.

Generalized Dystonia

Dystonia, characterized by involuntary twisting movements and abnormal postures caused by co-contractions of antagonistic muscle groups, is the third most common movement disorder in humans (Raike et al., 2005). The disease is a heterogeneous disorder because patients display clinical signs associated with many different brain regions. Primary dystonia is inherited or arises spontaneously, and secondary dystonia follows brain trauma or insult. These disorders can be further classified, based on affected muscle groups, as focal (involving a small number of muscles) or generalized dystonia (involving all of the muscles throughout the body). The dt^{sz} disorder exhibited in the Bio 86.93 inbred line of Syrian hamsters is an animal model of generalized dystonia (Loscher et al., 1989; Raike et al., 2005). The disorder in hamsters, originally designated sz because of the seizure-like presentation, is inherited in an autosomal recessive fashion (Loscher et al., 1989). The mutation was later renamed dystonic and symbolized as dt^{sz} . In hamsters, dystonic attacks last several hours, vary in severity, progress from the head to the extremities, and are partially age-dependent with signs mostly disappearing after 8 weeks of age (Raike et al., 2005). The dt^{sz} phenotype is modified by hormonal fluctuations and late-term pregnant and nursing dt^{sz} hamsters experience attacks (Raike et al., 2005). Attacks begin with rapid vibrissae twitching, flattening of the ears, and facial contortions, followed by stiffening of the hindlimbs, gait abnormalities, and falling episodes, ending with limb hyperextension, severe truncal torsion, and immobility (Loscher et al., 1989; Raike et al., 2005). The dystonic attacks are stress-induced and initiated by placing the hamsters in a new environment. The motor disturbances can also be pharmacologically initiated with subconvulsant doses of pentylenetetrazol (PTZ), picrotoxin, or inverse benzodiazepine receptor agonists (Gernert et al., 1999; Loscher et al., 1989). The generalized syndrome in humans shares many phenotypic features with hamster dystonia including prolonged attacks lasting up to 4 hours, onset in infancy or childhood, and attacks initiated by stress or excitement (Loscher et al., 1989).

Wound Healing

Traumatic damage to tissue is followed by a complex cascade of tissue-repair events that involve overlapping inflammatory, proliferative, and remodeling phases. Research into understanding the process of tissue repair

focuses on the role of epithelial cells, fibroblasts, endothelial cells, and inflammatory cells (Wong et al., 1993).

Hamsters provide an excellent model for elucidating the role of eosinophils in wound healing (Wong et al., 1993). The induced model involves creating a circular transdermal wound between the shoulder blades and leaving the wound open to heal by secondary intention (Wong et al., 1993). In normal hamster skin, eosinophils are rarely found; however, a prominent eosinophilic infiltrate occurs during cutaneous wound healing. The eosinophils, and other inflammatory cells, provide a cellular source of two cytokines, transforming growth factor- α (TGF- α) and TGF- β_1 , both important regulators of wound healing.

These two cytokines are expressed from eosinophils in a temporal fashion. Eosinophil expression of TGF- α peaks 5 days after cutaneous injury (Wong et al., 1993) followed by a gradual decline. In contrast, expression of TGF- β_1 gradually increases throughout wound healing. The early expression of TGF- α is implicated in epithelial migration, proliferation, and angiogenesis (Barrandon and Green, 1987; Schreiber et al., 1986). Later expression of TGF- β_1 is believed to contribute to the down-regulation of epithelial migration and proliferation, as well as to granulation tissue formation (Quaglini et al., 1990).

Yang et al. (1997) developed an eosinophil-deficient hamster model by treating with anti-interleukin-5 monoclonal antibody (TRFK-5), which inhibits the IL-5-dependent pathway of eosinophil differentiation and maturation. TRFK-5 is injected intraperitoneally 7 and 4 days before and on the same day as wound creation. An absence of eosinophils results in accelerated re-epithelialization of cutaneous wounds, with wound closure occurring 4 days faster than in non-TRFK-5-treated animals. One mechanism which could explain the accelerated wound healing is a reduction in TGF- β_1 , which is antiproliferative for keratinocytes. Eosinophil depletion may be clinically relevant for the treatment of wounds to accelerate wound closure.

Due to the hamster's relatively large oral cavity, an 8-mm circular wound can be created in the oral mucosa overlying the masseter muscle and allowed to heal by secondary intention, providing an excellent model for comparing oral and cutaneous wounds. Similar to cutaneous wounds, eosinophils infiltrate oral wounds and express TGF- β_1 , however, they do not express TGF- α (Yang et al., 1996). The lack of TGF- α expression by eosinophils in oral wounds may reflect the presence of TGF- α and epidermal growth factor (EGF) in hamster saliva. Oral eosinophils can be induced to express TGF- α by first performing sialoadenectomy of the submaxillary and sublingual complex to knock-out the salivary-associated TGF- α and epidermal growth factor (EGF) (Yang et al., 1996). Furthermore, oral eosinophils cease producing TGF- α when EGF is supplemented in

the drinking water. This suggests that EGF and TGF- α in the saliva are responsible for the decreased production of eosinophil-associated TGF- α in oral wounds.

NSAID Gastropathy

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most frequently used group of drugs in medicine (Roth, 1988). Although these drugs are extensively utilized, they may result in significant gastrointestinal injury (Tamblyn et al., 1997). The ability of NSAIDs to inhibit prostaglandin synthesis leads to anti-inflammatory and pain reduction, but also compromises the protective properties of the gastric mucosa, potentially leading to gastric mucosal erosions and ulcers (Roth, 1996).

The hamster model of NSAID-induced gastric ulceration is created by subcutaneously injecting indometacin into hamsters (Kolbasa et al., 1988). Indometacin produces gastric antral ulcers in hamsters within 1–5 hours post-administration in a dose-dependent fashion. With repeated administration of indometacin, the gastric lesions become more severe and most hamsters die with perforated antral ulcers after 2–5 days. Indometacin reduces the antral mucosa production of prostaglandin E₂, prostaglandin F_{2 α} , and 6-keto prostaglandin F_{1 α} and increases gastric acid output more than twofold. The ulcers created in this model may be prevented by anti-secretory agents such as cimetidine, methscopolamine bromide, and omeprazole (Kolbasa et al., 1988).

To create a chronic NSAID-induced gastropathy model in hamsters which more closely mimics the disease process in humans, naproxen can be administered twice daily by oral gavage (Fitzpatrick et al., 1999). Gastric ulceration occurs almost exclusively in the antrum and increases in size as a function of dose and time for the first 3 days of naproxen treatment. At a dose of 80 mg/kg given twice a day, naproxen can be used for chronic anti-ulcer drug studies with a low (<5%) mortality rate (Fitzpatrick et al., 1999). Gastric ulcer area decreases in naproxen-treated hamsters by the fourth day of administration, suggesting adaptation of the gastric mucosa. The process of adaptation involves stimulation of gastric mucosal healing and the creation of a gastric mucosa that is more resistant to future injurious agents (Levi and Shaw-Smith, 1994).

The naproxen-induced model provides a useful system to study anti-ulcer drugs. Misoprostol, a prostaglandin analog, when administered in conjunction with naproxen, results in a significant reduction in number and size of gastric ulcers without modifying gastric acid secretion (Fitzpatrick et al., 1999). This same protection is noted in humans (Dajani and Agrawal, 1995). Famotidine, an H₂-receptor antagonist that inhibits stomach acid production, does not reduce the prevalence or number of gastric ulcers in the hamster

(Fitzpatrick et al., 1999). Similarly, H₂-receptor antagonists do not consistently reduce NSAID-induced gastric ulceration in humans (Dajani and Agrawal, 1995). These results suggest that gastric acid secretion is not the primary mechanism of naproxen-induced ulceration in hamsters or humans and that the naproxen hamster model accurately models NSAID-induced antral ulceration in humans (Fitzpatrick et al., 1999).

Gastric ulcer models have also been developed in rats and rabbits using subcutaneous administration of indometacin. Limitations of the rat model include the addition of profound intestinal ulceration and high mortality (Satoh et al., 1981). The rabbit antral ulcer model displays ulcers that are morphologically similar to human ulcers without the high mortality or intestinal injury noted in rats (Wallace and McKnight, 1993); however, the relatively large size of rabbits may make them less desirable when compared to hamsters.

Human Fertility

The hamster zona-free oocyte penetration assay is used to examine human male fertility (Aitken, 2006). This assay was first described in 1980 and involves the incubation of human sperm with hamster eggs that have undergone zona removal (Overstreet et al., 1980). The assay can be utilized both clinically and experimentally to determine human sperm fertility and it analyzes the ability of sperm to capacitate eggs, undergo the acrosome reaction, and fuse with the oocyte. Limitations of the assay are that it is labor-intensive, and difficult to standardize. Despite these limitations, results obtained in the hamster assay correlate well with human in vitro fertilization. Since intracytoplasmic sperm injection (ICSI) is replacing standard IVF in many settings the utility of the zona-free hamster assay from a clinical perspective may be waning.

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The Chinese or Striped-Back Hamster

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INTRODUCTION

The Chinese hamster used in biomedical research is traditionally classified as *Cricetulus griseus* (Honacki et al., 1982), but some taxonomists (Nowack and Paradiso, 1983) do not recognize this species and classify these animals as *Cricetulus barabensis*. For the purpose of this chapter, the Chinese hamster will be

referred to as *Cricetulus griseus* the classification used by most authors. The Chinese hamster, also known as the striped-back hamster (Figure 35.1), is indigenous to Northern Asia, with a range extending from southern Siberia, Mongolia, and northeastern China, to the northern regions of North Korea. The Chinese hamster has had an important and much storied role in biomedical research. However in recent years, this hamster's



FIGURE 35.1 An adult Chinese hamster, *Cricetus griseus*. Note the dark stripe on the dorsal midline. Photo courtesy of Dr. Don Holmes, Department of Veteran Affairs.

contributions as a laboratory animal have been largely overshadowed by the focus on its cell lines and the role they play in scientific research and biotechnology.

The Chinese hamster has several biological features that have helped promote its use in biomedical research. These attributes include its small size, polyestrous cycle, short gestation period and low chromosome number (Chang et al., 1987). In addition, the Chinese hamster has a low incidence of spontaneous and endogenous viral infections. This species has been shown to be susceptible to a number of experimentally induced viral, bacterial, and parasitic infections (Diani and Gerritsen, 1987).

The Chinese hamster was first used as a laboratory animal to type pneumococci obtained from human patients (Hsieh, 1919). Subsequently, the Chinese hamster became a valuable animal model in other infectious disease and epidemiology research studies. This hamster is a known carrier of the protozoal parasite *Leishmania* that causes the often deadly human disease leishmaniasis, also known as Kala-azar or black fever.

Efforts at domestication, in the mid-20th century, resulted in the serendipitous finding that some inbred lines of Chinese hamsters develop spontaneous hereditary diabetes mellitus. This finding fostered interest in the Chinese hamster as an animal model for diabetes and helped spur research interest in hamster genetics (Yerganian, 1959, 1985).

The Chinese hamster has been used as an animal model in radiobiological research and has been shown to be more resistant to the effects of radiation exposure than many other common laboratory rodents (Corbascio et al., 1962). In addition, the Chinese hamster has been utilized for the experimental induction of gastric and esophageal tumors (Baker et al., 1974).

Tijo and Puck (1958) isolated Chinese hamster ovary (CHO) cells (Figure 35.2) which have since been commonly used as an in vitro research tool for mutagenesis and carcinogenesis studies. Subsequent laboratory investigations have led to a series of elegant research studies directed at utilizing CHO cells for the

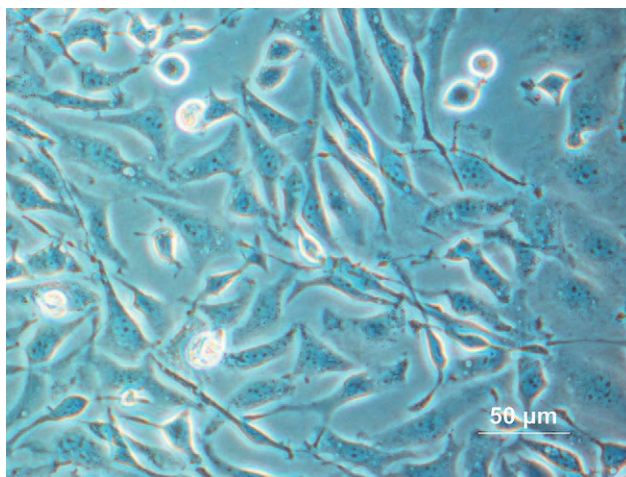


FIGURE 35.2 Chinese hamster ovary cell culture, phase contrast (positive) microscopy. Photo courtesy of Wikimedia Commons and User Alcibindes.

production of recombinant therapeutic proteins (Oka and Rupp, 1990). During the past 20 years, CHO cells have been used to synthesize a wide array of recombinant proteins that have found clinical application in the treatment of a variety of human diseases (Chin, 2007; Jayapal et al., 2007).

BIOLOGY

General

Chinese hamsters are small rodents with a grayish black coat and a black dorsal stripe. Adult animals weigh approximately 39–46 grams, and measure approximately 9 cm in length. The average gestation length is 20.5 days, with a litter size of approximately 4–5 offspring. Newborn pups weigh approximately 1.5–2.5 grams. The average life span under laboratory conditions is 2.5–3.0 years (Harkness, 1984; Harkness and Wagner, 1985). An overview of physiological reproductive data is provided in Table 35.1.

The Chinese hamster, like the Syrian hamster, has a cheek pouch that can be used as an immunologically privileged site. Normal hematological parameters for the Chinese hamster are shown in Table 35.2. In terms of distinctive anatomical features that have been quantitatively assessed, the brain and spleen in both sexes are relatively larger, with respect to body size, than those of the Syrian hamster (Festing, 1972). In addition, adult male Chinese hamsters have exceptionally large testicles.

Behavior

Chinese hamsters, particularly sexually mature females, are bellicose and frequently fight with one

TABLE 35.1 Normal Hemogram of the Chinese Hamster^{a,b}

Parameter	Mean $\pm$ SD
Erythrocytes ($10^6/\mu\text{l}$)	7.1 $\pm$ 0.01
Packed cell volume (%)	42.1 $\pm$ 5.6
Hemoglobin (g/dl)	12.4
Leukocytes ($10^3/\mu\text{l}$)	5.5
Neutrophils (%)	19.3 $\pm$ 2.2
Bands (%)	0.2 $\pm$ 0.1
Lymphocytes (%)	76.1 $\pm$ 7.8
Monocytes (%)	2.1 $\pm$ 0.3
Eosinophils (%)	1.7 $\pm$ 0.7
Basophils	0.1 $\pm$ 0.04
Sedimentation rate (mm/h)	3.5 $\pm$ 1.7
Bleeding time (s) ^b	55

^aFrom Moore (1966).^bFrom Yerganian et al. (1955).**TABLE 35.2** Reproductive Physiological Data for the Chinese Hamster^a

Weaned	21–25 days ^b
Sexually mature	8–12 weeks ^b
Type of estrous cycle	Polyestrus ^c
Duration of estrous cycle	4 days ^b
Length of estrus	6–8 hours ^b
Ovulation time	Immediately before estrus ^c
Copulation	2–4 h after start of dark period ^d
Implantation	5–6 days ^c
Gestation	20.5 days ^b
Average litter size	4.5–5.2 ^c
Number of mammae	8 ^c
Postpartum estrus	4 days ^b

^aTable from Hankenson and Van Hoosier (2002); reprinted with permission from Academic Press.^bFrom Yerganian (1958).^cFrom Festing (1972).^dFrom Moore (1965).

another. Fights erupt frequently between females, and least often among males. The presence of females usually suppresses fighting behavior in males, whereas the presence of males has no effect on female aggression and fighting behavior.

The marking pattern used by the Chinese hamster involves vigorous scratching of the flank gland with a hindfoot, followed by pressing the anogenital region onto a substrate to transfer the scent-bearing secretion of the flank gland onto the substrate. In female–female encounters, marking frequency correlates with behavioral dominance. In female–male interactions, the male marks more frequently although the females are usually dominant in fights or chases. In typical female–female and male–male encounters, marking behavior follows

rather than precedes engagement in fighting behavior. The lack of warning behaviors and the high level of food caching behavior observed in the Chinese hamster are suggestive that in their natural habitat this species lives a relatively solitary existence with infrequent encounters with conspecifics (Skirrow and Rysan, 1976).

CARE AND HUSBANDRY

Housing

The Chinese hamster can be purchased commercially or bred under laboratory conditions. It does well under standard laboratory animal housing conditions. Since the Chinese hamster is comparable in size to the mouse, a similar type of caging and husbandry practices are commonly used, and have proven adequate for general housing. Hamsters with metabolic disorders such as diabetes are more susceptible to stress and special precautions must be taken in the provision of routine husbandry to optimize colony management for diabetic animals.

Feeding

There are no unique dietary requirements for the Chinese hamster. A standard, commercially available laboratory rodent diet provides adequate nutrition. Wheat germ may be used as a dietary supplement for breeders. Average daily water intake is 11.4 ml and 12.9 ml per 100 g body weight for male and female hamsters, respectively (Thompson, 1971).

Breeding

Early attempts to breed Chinese hamsters under laboratory conditions were unsuccessful. However, a reversed illumination schedule has been used to successfully establish a production colony for research purposes (Yerganian, 1958). Subsequently, several other schemes have been utilized to manage Chinese hamster breeding colonies due to the aggressiveness of the animals, particularly the females. Consequently, females with non-aggressive behavior (Calland, 1986) or sexually aggressive males (Moore, 1965) are selected as breeders. Modified test mating and monogamous mating schemes are used to breed the Chinese hamster (Camden et al., 1968, 1969). In one approach, a female is placed with a male for 7 days or longer, but the female is removed prior to delivery of the litter. In a second scheme, the male and female are left together during the entire reproductive life of the pair. Hand-mating and artificial insemination have been used with moderate success (Avery, 1968). A more practical and efficient

approach to produce Chinese hamsters on a commercial scale has been reported (Cisar et al., 1972). In this system, three males are placed in a breeding cage with three to five female littermates at weaning; the authors reported a 3.5% loss of males per month.

Progesterone levels are markedly different during estrus and pregnancy. During the Chinese hamster's 4-day estrous cycle, maximal progesterone synthesis occurs on day 3, in contrast to the low levels observed on day 3 of the Syrian hamster estrous cycle (Sato et al., 1984). During pregnancy, progesterone blood levels increase through day 12, with a general stabilization through day 18 of gestation. Progesterone blood levels peak on day 19 with a dramatic drop immediately prior to parturition (Sato et al., 1984). Breeding is confirmed by the presence of a vaginal plug. Pregnancy is indicated by a closed vagina, with dry, pale, and scaly perineal tissue at 4 days post-mating. In one study, an increase in ambient room temperature (above 82°F) has been shown to increase the number of runts in a litter (Yerganian, 1958).

CYTOGENETICS AND FETAL DEVELOPMENT

The Chinese hamster has been frequently selected for studies of chromosomal abnormalities because of a low spontaneous mutation rate and a low diploid chromosome number ($2N = 22$). The ten large pairs of autosomes and the two sex chromosomes can be readily differentiated. Chinese hamster chromosomes can be divided into four morphologically distinct subgroups (Vistorin et al., 1977). Research investigations suggest that cleavage of embryos with chromosome abnormalities is delayed compared with karyotypically normal zygotes (Sonta et al., 1984). A survey of 226 Chinese hamster preimplantation embryos indicates a low incidence (0.9%) of genome mutations (Binkert and Schmid, 1977). The low spontaneous chromosome mutation rate has made the Chinese hamster a good animal model for mutagen testing.

Characterization of the gametes and in vivo fertilization of the Chinese hamster have been reported (Yanagimachi et al., 1983). Fertilization begins approximately 2–3 hours after ovulation and is completed within the next 4–5 hours. At 20–26 hours following ovulation, the first three cleavages occur. Four days post-ovulation, the fertilized egg reaches the blastocyst stage and enters the uterus. Implantation occurs at approximately day 5 or 6 post-ovulation (Pickworth et al., 1968). Embryonic and fetal development of the Chinese hamster have been extensively characterized. Prenatal developmental stages are similar to the developmental stages in the mouse (ten-Donkelaar et al., 1979).

DISEASES

Infectious Diseases

A Chinese hamster colony was evaluated for the presence of murine viral antibodies and intestinal/cecal microflora by Schiff et al. (1973). The presence of antibodies against several murine viruses was reported including: Pneumonia virus of mice (RNA virus, family *Paramyxoviridae*), Kilham rat virus (DNA, family *Parvoviridae*), Theiler's murine encephalomyelitis virus (GDVII; RNA virus, family *Picornaviridae*), H-1 (DNA virus; family *Parvoviridae*), and Reovirus-3 (RNA virus, family *Reoviridae*). No animals were found to have detectable antibody levels to Sendai virus (RNA virus, family *Paramyxoviridae*), Polyoma virus (DNA virus; family *Papovaviridae*), mouse adenovirus (DNA virus; family *Adenoviridae*), mouse hepatitis virus (RNA virus, family *Coronaviridae*), or lymphocytic choriomeningitis virus (RNA virus, family *Arenaviridae*). On assessment of 30 Chinese hamsters aged 2, 3, or 4 months from a colony of 200, *Lactobacillus* predominates as the major bacterial group identified following quantitative recovery from the intestinal tract. On examination of the ceca, several bacterial genera have been routinely identified including: *Bacillus*, *Bacteroides*, *Escherchia*, *Lactobacillus*, *Micrococcus*, and *Streptococcus*.

A *Mycoplasma* species (*M. cricetula*) was isolated from the conjunctiva and nasopharynx of 55 animals in one Chinese hamster colony by Hill (1974). The pathogenic significance of this organism has not been determined.

Zook et al. (1977) diagnosed Tyzzer's disease in a colony of Chinese hamsters. Affected animals exhibit clinical signs of lethargy, diarrhea, rough hair coat, and hunched posture. Gross necropsy findings include multiple, disseminated white foci in the liver and distension of the large intestine with semi-liquid yellow feces. On microscopic examination, there is focal necrosis of hepatic cells with no significant cellular inflammatory response. Giemsa, toluidine blue, and methenamine silver stains reveal clusters of pleomorphic bacilli in the cytoplasm of hepatic cells adjacent to necrotic foci. The histopathological lesions are consistent with a diagnosis of Tyzzer's disease (*Clostridium piliformis*).

There are few reports of spontaneous parasitic diseases in the Chinese hamster. In one survey, Schiff et al. (1973) consistently found *Trichomonas* spp. in 2-, 3-, and 4-month-old animals from a group of 200. An investigation by Benjamin and Brooks (1977) suggests a low incidence of demodectic mange in the Chinese hamster. Desch and Hurley (1997) identified a new species of mite, *Demodex sinocricetuli*, in the Chinese hamster. In their study, histopathologic evidence of demodectic mange is found in only two of 157 animals. The clinical significance of this organism has not been determined.

Neoplastic Diseases

Chinese hamsters have a low incidence of spontaneous tumors; when tumors occur the most common association is involvement with the liver or reproductive tract. The rarity of spontaneous and induced tumors has been postulated to be attributable to the absence of tumor-associated viruses in this species.

Uterine and Ovarian Tumors

Ward and Moore (1969) described spontaneous uterine adenocarcinomas in 30 of 120 aged female Chinese hamsters; approximately 10% of the affected hamsters had lung metastases. Benjamin and Brooks (1977) found similar uterine neoplastic lesions in 11 of 77 animals, however, no pulmonary metastases were observed. Brownstein and Brooks (1980) characterized and classified uterine neoplasms in 21 of 93 multiparous, non-inbred Chinese hamsters ranging in age from 3 to 4 years.

Animals with malignant uterine neoplasms frequently exhibit clinical signs of vaginal hemorrhage. Gross necropsy findings include hemorrhage within the peritoneal cavity and tumor spread by direct extension to other organs. On gross examination, the tumor tissue is usually white to yellow with a nodular firm surface. Brownstein and Brooks (1980) developed a histological classification system for uterine neoplasms of the Chinese hamster (Table 35.3). The most frequently observed uterine tumor in the aged Chinese hamster is endometrial adenocarcinoma. The Chinese hamster might be a useful model for studying endomyometrial neoplasms of women since the relative frequency and classification of uterine tumors in the two species is similar.

Kohn and Guttman (1964) report that the incidence of ovarian tumors is significantly increased following X-ray irradiation under several different exposure regimens, however tumors are rarely observed in control animals.

Hepatocellular Hyperplasia and Adenoma

Kohn and Guttman (1964) describe a benign liver lesion, hepatocellular adenoma, in five of 112 non-irradiated control animals but did not report any non-neoplastic liver lesions. Ward and Moore (1969) observed hepatocellular adenoma in 18 of 50 non-irradiated control animals; they found a similar incidence in animals receiving ^{131}I . They also reported hepatocellular hyperplasia in some animals which they postulate might be a pre-neoplastic lesion. Nodular hepatic hyperplasia, a non-neoplastic lesion, is reported in 111 of 157 aging animals in a survey conducted by Benjamin and Brooks (1977). They found evidence

TABLE 35.3 Classification of Endomyometrial Neoplasms in 21 Chinese Hamsters^a

Site	Type	Occurrence ^b	Metastasis
Endomyometrium	Adenocarcinoma	13	Abdominal spread (10)
	Mixed adeno-squamous carcinoma	3	Abdominal spread (3)
	Mixed Mullerian carcinosarcoma	2	Abdominal spread (1) Lung metastasis (1)
	Mixed mesodermal	1	Abdominal spread
Myometrium	Leiomyoma	1	
	Leiomyosarcoma	1	Abdominal spread

^aFrom Brownstein and Brooks (1980); reprinted with permission from the Journal of the National Cancer Institute.

^bFrom a group of 93 nulliparous non-inbred females with a median survival time of 1040 days.

of hepatic adenoma in only one of the 157 animals. Grossly, the two conditions appear similar with multiple nodules ranging in size from less than 1 mm to greater than 1 cm in diameter, and lighter in color than the surrounding tissue. Histologically, these are nodular hyperplastic lesions ranging from small nodules with normal sinusoid and central vein structure to coalescing masses with disrupted tissue architecture. Parenchymal cells are often enlarged with increased eosinophilic and sometimes vacuolated cytoplasm. Cell nuclei are enlarged but otherwise had a benign appearance. Nodular hyperplasia was distinguished from hepatocellular adenoma by the appearance of abundant hyperchromatic nuclei containing multiple nucleoli. Ward and Moore (1969) also distinguished between nodular hyperplasia and hepatic adenomas. In their investigation they found a much higher incidence of hepatic adenomas. Since the Chinese hamster is used in toxicology studies, it is important to distinguish spontaneous hepatic lesions from experimentally induced lesions.

Myeloproliferative Disease and Leukemia

Benjamin and Brooks (1977) report hematopoietic hyperplasia in 49 of 157 Chinese hamsters. Bone marrow hyperplasia occurred in 36 animals. In most cases, the hyperplasia is granulocytic. In approximately half the cases, the hematopoietic hyperplasia is associated with degenerative or inflammatory changes; in the other cases no underlying lesions are observed. Splenomegaly and a pale bone marrow are observed grossly in all clinical cases. On microscopic examination, the marrow is

hypercellular and granulocytic cells replace erythroid cells. A diagnosis of leukemia is made in three cases; myelocytes and myeloblasts with leukemic infiltrates are found in liver, kidney, brain, spinal cord, and spleen. The Chinese hamster might provide a useful model for the study of myelogenous leukemia.

Other Neoplasms

Table 35.4 provides a summary of some infrequently occurring, spontaneous malignancies that have been reported for the Chinese hamster.

Metabolic and Genetic Disease

Spontaneous Diabetes Mellitus

Spontaneous diabetes mellitus was observed in Chinese hamsters during early attempts to establish inbred breeding colonies. This metabolic condition is a suitable model for studying various aspects of the human disease, and will be discussed in more detail in the section on research uses. Since diabetes is a spontaneous disease in the Chinese hamster, the occurrence of this metabolic disorder might potentially confound experimental results in animals used for research protocols unrelated to diabetes. In addition, the use of Chinese hamsters with spontaneous diabetes mellitus in cytogenetic experiments might have a confounding influence on experimental results due to metabolic aberrations associated with the diabetic state.

TABLE 35.4 Infrequently Occurring Spontaneous Malignancies of the Chinese Hamster^a

Tumor type	Occurrence	Reference
Adrenal cortical carcinoma	1/157	Benjamin and Brooks (1977)
Pancreatic adenocarcinoma	4/112	Kohn and Guttman (1964)
Sweat gland adenocarcinoma	2/3	Poel and Yerganian (1961)
Squamous cell carcinoma	1/157	Benjamin and Brooks (1977)
Subcutaneous fibrosarcoma	1/157	Benjamin and Brooks (1977)
Undifferentiated sarcoma	1/157	Benjamin and Brooks (1977)
Gastric adenocarcinoma	1/112	Kohn and Guttman (1964)
Bile duct carcinoma	1/112	Kohn and Guttman (1964)
Ovarian adenoma	1/77	Benjamin and Brooks (1977) ^b

^aFrom Ladiges (1987); reprinted with permission from Elsevier.

^bKohn and Guttman (1964) reported the occurrence of ovarian tumors in 18 of 74 irradiated females, but none in 69 control animals.

Primary Chromosomal Abnormalities

Mikamo and Kamiguchi (1983) studied the occurrence of spontaneous chromosomal aberrations in Chinese hamster oocytes and zygotes, and classified the lesions according to their causal mechanism. A summary of this classification is provided in Table 35.5. The overall incidence of chromosomal anomalies has been determined to be 8%. This incidence is lower than that estimated for the human population; the lower incidence has been postulated to be due to the lack of mutagen influence in a controlled laboratory environment. Since the Chinese hamster is frequently used as an animal model for cytogenetic studies, it is important to be cognizant of the incidence of spontaneous chromosomal defects in this species.

Traumatic Diseases

Female littermates can become very aggressive as they reach sexual maturity. Fighting and severe bite wounds may lead to death. In addition, females can be very aggressive following mating with resultant death of male breeders. Separation of litters before fighting occurs, and removal of the male following mating are among the strategies used to prevent serious injury and death. Selection of non-aggressive female breeders through genetic selection has also been utilized as a strategy to reduce fighting and traumatic injuries in Chinese hamster breeding colonies.

TABLE 35.5 Primary Incidence of Spontaneous Chromosomal Anomalies, Classified According to Their Origins and Causal Mechanisms in the Chinese Hamster^a

Chromosomal Anomaly	Origin and Causal Mechanism	Incidence (%)
Aneuploidy	Non-disjunction and anaphase lagging	
	During oogenesis	2.1
	During spermatogenesis	0.7
Mosaic	Non-disjunction and anaphase lagging	
	During first somatic division	0.5
Triploidy	Diandry	0.9
	Digyny	0.3
	Uncertain	0.3
Haploidy	Parthenogenesis	0.5
Structural anomaly	Breakage during oogenesis	1.3
	Breakage during spermatogenesis	1.4
Total		8.0

^aFrom Mikamo and Kamiguchi (1983); reprinted with permission from Elsevier.

Reproductive Disorders

Avery (1968) reported infertility in young female Chinese hamsters housed in open-bottom cages. Infertility was believed to be attributable to excess hair growth around the vulva that prevented penile penetration during copulation. Infertility has also been associated with diabetic females in inbred colonies. Parkening (1982) studied aging changes and the effect on reproductive performance. Clinical signs include failure to conceive or decreased conception rates. Cage type and colony health status have been shown to have an impact on conception rates. Progesterone administration, immediately following mating, has been used with some success to increase conception in inbred and/or diabetic breeders, and in aged animals (Avery, 1968; Parkening, 1982). Testicular degeneration and atrophy has been observed in 14 of 80 male Chinese hamsters in an aging colony of outbred hamsters (Benjamin and Brooks, 1977).

Miscellaneous Diseases

Pulmonary Granulomas

Pulmonary granulomas have been observed in 54 of 157 aging Chinese hamsters (Benjamin and Brooks, 1977). On gross examination, the lesions are subpleural, 1–3-mm yellow-gray foci with variable degrees of lung involvement. Histologically, mild lesions consist of lipid-filled macrophages, while more severe lesions include well-developed granulomas containing lipid-filled macrophages, inflammatory cells, focal septal fibrosis, and occasional cholesterol clefts. Several animals exhibit severe alveolar and bronchiolar epithelial hyperplasia. The cause of the pulmonary granulomas has not been determined. The significance of this spontaneous lesion should be differentiated from pathology attributable to experimentally induced causes.

Cerebral Hemorrhage

Cerebral hemorrhage is observed in 20% of both control and experimental animals in a ¹³¹I chronic toxicity study (Ward and Moore, 1969). Sporadic deaths are observed in 1–2-year-old animals. The cause of the cerebral hemorrhage has not been determined but is postulated to involve an inflammatory or degenerative change in the anterior cerebral artery. On gross examination, hemorrhage is observed between the cerebral hemispheres with blood often present in the lateral ventricles. On histological examination, the lesions are restricted to the anterior cerebral artery. In acute cases, the vascular changes include an increased arterial lumen diameter, and thickened arterial wall with the presence of inflammatory cells. An amorphous eosinophilic material is often present in the media of the arterial wall. The eosinophilic material is periodic

acid-Schiff (PAS)-reagent-positive but fibrin-negative. In chronic cases, the arterial wall is greatly thickened with numerous macrophages surrounding the lesions. In affected animals, no vascular changes are found elsewhere in the brain or in other organs. The incidence of cerebral hemorrhage is not known since this clinical condition has not been reported in other Chinese hamster colonies.

Degenerative Renal Disease

INTERCAPILLARY GLOMERULOSCLEROSIS

Intercapillary glomerulosclerosis has been reported in Chinese hamsters in association with diabetes mellitus (Meier and Yerganian, 1961). It has also been reported as a progressive condition associated with aging (Guttman and Kohn, 1960; Kohn and Guttman, 1964).

NEPHROSCLEROSIS

Nephrosclerosis is described in 46 of 157 Chinese hamsters in one colony of animals older than 600 days (Benjamin and Brooks, 1977). Incidence and severity of the condition are age-dependent. Early changes include tubule degeneration, mild interstitial fibrosis, and cortical atrophic foci. Progressive pathologic changes consist of glomerular sclerosis, severe interstitial fibrosis, and severe tubular degeneration and atrophy. Severely affected kidneys are small in size, pale tan, and have a pitted surface.

Periodontitis

Cohen et al. (1961) describe periodontitis in an inbred strain of Chinese hamsters with hereditary diabetes mellitus. Incidence correlate with the presence and severity of the underlying metabolic disorder. The periodontitis in the Chinese hamster corresponds closely to the development of the clinical condition in humans affected by diabetes mellitus.

Spondylosis

Silberberg and Gerritsen (1976) report an increased incidence of spondylosis in an inbred strain of Chinese hamsters with spontaneous diabetes mellitus compared to non-diabetic control animals.

RESEARCH USES

Infectious Diseases

There are few reports of spontaneously occurring infectious diseases in Chinese hamsters. However, this species has been experimentally shown to be susceptible to a variety of different infectious agents and has been used extensively in viral, bacterial, and parasitological disease investigations. Table 35.6 provides a list of some

TABLE 35.6 Representative Experimental Infectious Diseases in the Chinese Hamster

Disease agent	Reference
Viral	
Rabies virus	Yen (1936)
Influenza	Yen (1940)
Viral encephalitis virus	Chang et al. (1951)
Scrapie virus	Chandler and Turfrey (1972)
Rous sarcoma virus	Hillova et al. (1985)
Transmissible mink encephalopathy virus	Kimberlin (1987)
Corona virus	Luo (2007)
Bacteria	
<i>Pneumococcus</i>	Hsieh (1919)
<i>Corynebacterium diphtheria</i>	Lu and Zia (1935)
<i>Mycobacterium tuberculosis</i>	Wang (1951)
<i>Leptospira</i> spp.	Plesko (1977)
Parasites	
<i>Leishmania</i> sp.	Hindle et al. (1926)
<i>Monila</i> sp.	Kurotchkin and Lin (1930)
<i>Trichinella spiralis</i>	Ritterson (1968)
<i>Hymenolepis microstoma</i>	Ritterson (1971)
<i>Babesia microti</i>	Krampitz and Baumler (1978)
<i>Toxoplasma gondii</i>	Fuji et al. (1983)
<i>Schistosoma mansoni</i>	Crabtree and Wilson (1984)
<i>Trichinella pseudospiralis</i>	Stewart and Larsen (1989)
<i>Acanthamoeba</i> sp.	Van Klink et al. (2007)

infectious disease agents that have been studied experimentally using the Chinese hamster as an animal model.

Metabolic Disorders

Spontaneous Diabetes Mellitus

Spontaneous hereditary diabetes mellitus, with similarities to the human disease, was first described in Chinese hamster inbred lines by Meier and Yerganian (1959). The diabetic Chinese hamster has been studied extensively to elucidate the pathophysiology associated with the disease and to develop effective clinical therapies for treatment of the human disease. Numerous reviews of the spontaneously diabetic Chinese hamster model have been published (Chang, 1978, 1981; Diani and Gerritsen, 1987; Gerritsen, 1982; Hunt et al., 1976; Sirek and Sirek, 1967, 1971; Wappler and Fiedler, 1972).

PATHOPHYSIOLOGY

The diabetic Chinese hamster has been described as an inbred, lean animal that exhibits glucose intolerance, mild to severe hyperglycemia, hypoinsulinemia, polyuria, glycosuria, possible ketonuria, and high free fatty acid levels (Dulin and Gerritsen, 1967; Dulin and Soret, 1972; Gerritsen and Blanks, 1974; Gerritsen and Dulin, 1967).

Several genetic and environmental factors have been demonstrated to play a role in this metabolic disturbance in the hamster and influence the extent of beta cell death. These factors include the amount of food consumed, dietary composition, and uterine environment of the dam (Dulin and Gerritsen, 1967, 1972; Gerritsen and Dulin, 1967, 1972; Gerritsen et al., 1970, 1974, 1976, 1981). Plasma insulin and glucagon levels have been shown to be altered in chronically diabetic animals. In general, diabetic Chinese hamsters exhibit low, non-fasting plasma insulin levels (Gerritsen, 1982; Gerritsen and Blanks, 1974; Gerritsen and Dulin, 1967). Plasma insulin levels of newly diagnosed diabetic Chinese hamsters are usually within normal limits or slightly elevated (Gerritsen, 1982; Gerritsen and Blanks, 1974). Plasma glucagon levels are usually elevated with chronic disease (Chang et al., 1977a). The chronic absolute deficiency of plasma insulin and the high plasma glucagon levels in the diabetic Chinese hamster are similar to endocrinologic changes observed for human type I diabetes. Pancreatic hormone levels are markedly altered in the chronic diabetic state. Pancreatic insulin levels are decreased, whereas pancreatic glucagon levels are generally elevated, particularly with chronic disease. Pancreatic somatostatin concentrations are significantly decreased in diabetic Chinese hamsters (Petersson et al., 1977).

PATHOLOGY

A number of studies using the Chinese hamster have focused on structural changes in the islets of Langerhans. Primary morphological alterations are observed in the β cells, which degranulate as the disease progresses (Boquist, 1969; Like et al., 1974a, 1974b; Malaisse et al., 1967). The defective β cells in the Chinese hamster are characterized by proliferation of rough endoplasmic reticulum, expansion of the Golgi apparatus, and glycogen deposition (Boquist, 1969; Luse et al., 1967; Soret et al., 1974). The islets are reduced in size due to β -cell death (Carpenter et al., 1967, 1970). Some peripheral tissues and organs have been studied by light and/or electron microscopy, to better characterize the diabetic Chinese hamster. Glycogen deposition in the renal tubules is the most consistent change in the kidney (Soret et al., 1973, 1974). Examination of the eyes of diabetic Chinese hamsters documents the presence of retinal lesions. The most consistent finding involves glycogen deposition within Müller cells of the outer nuclear layer (Soret et al., 1973, 1974). Structural changes have been identified in the central, peripheral, and autonomic nervous systems of diabetic Chinese hamsters. Alterations of the hypothalamic-hypophyseal axis may provide the basis for the development of diabetic syndrome in this animal model (Bestetti and Rossi, 1982; Deslex and Rossi, 1976; Luse et al., 1970). Extensive studies of the autonomic nervous

system, including both qualitative and morphometric, have been conducted in the diabetic Chinese hamster. Aberrant myelination and axonal degeneration of pelvic viscera may contribute to urinary bladder distension in the diabetic Chinese hamster (Dail et al., 1977). Reduction of myelinated and unmyelinated fiber size in the abdominal sympathetic trunk and ventral abdominal vagus nerve might be a causative factor underlying gastrointestinal malfunction in the diabetic Chinese hamster (Diani et al., 1981, 1984).

GENETICS

The original hypothesis for diabetes in the Chinese hamster proposed that a single recessive gene and certain modifier genes are responsible for the syndrome (Meier and Yerganian, 1959, 1961a, 1961b; Yerganian, 1964). However, based upon epidemiologic examination of diabetic animals from other colonies, a polygenic hypothesis has been proposed for the disease. This hypothesis states that the Chinese hamster possesses a minimum of four recessive diabetogenic genes. In the homozygous state, any two of the genes produce a glycosuric hamster. Extensive genetic studies involving backcrosses have now shown that certain sublines of hamsters possess a single recessive gene for diabetes, whereas other sublines possess two recessive genes (Gerritsen et al., 1984). The onset of glycosuria in the diabetic hamsters is regulated by a multi-gene system. Inbred non-diabetic Chinese hamsters appear to lack any recessive genes for diabetes.

EFFECT OF DIET

Dietary modification has been shown to have a pronounced effect on diabetes development in this animal model. In prediabetic hamsters, hyperphagia is often observed. By limiting feed consumption to that of a non-diabetic hamster, a delayed onset of glycosuria and prevention of urinary ketones is achieved (Gerritsen, 1974, 1975; Gerritsen and Dulin, 1972). In another study, dietary restriction was shown to delay the development of glycosuria and hyperglycemia. Hyperinsulinemia is typically not observed in non-hyperphagic prediabetic animals (Gerritsen et al., 1981). Metabolic complications associated with diabetes are reduced or prevented by changes in dietary composition. A reduction in dietary fat and replacement of animal fat with vegetable fat in the diet ameliorates or prevents ketonuria (Gerritsen et al., 1981; Grodsky et al., 1974).

THERAPY

The diabetic Chinese hamster is an insulin-deficient model of diabetes mellitus. Treatment with exogenous insulin has a beneficial effect on the metabolic alterations. Severely diabetic animals treated with Neutral Protamine Hagedorn (NPH) insulin exhibit

a substantial reduction in glycosuria (Gerritsen and Dulin, 1966). In other investigations, continuous insulin infusion for 7 or 14 days was demonstrated to elicit improvements in blood glucose levels and endogenous insulin release from diabetic animals (Bringer et al., 1981; Frankel et al., 1979). However, insulin treatment of prediabetic Chinese hamsters does not delay the onset or the severity of glycosuria or hyperglycemia (Frankel and Grodsky, 1979).

Chemically Induced Diabetes Mellitus

Several chemical agents have been used to induce the metabolic and pathologic complications of diabetes mellitus in hamsters which do not possess the genes for diabetes. Experimentally induced clinical abnormalities include hyperglycemia, glucosuria, abnormal plasma insulin levels, and β cell abnormalities. These abnormalities are similar to those observed in chemically induced diabetic rats and mice. Diabetogenic substances include: streptozotocin, N-nitrosomethylurea, alloxan, monosodium glutamate, cortisone, and zinc deficiency.

STREPTOZOTOCIN

Streptozotocin, an antibiotic derived from *Streptomyces achromogenes*, has a direct cytotoxic effect on pancreatic islet β cells of the Chinese hamster. A single intravenous streptozotocin injection (75 mg/kg) into fasted animals causes a decrease in insulin levels, hyperglycemia, an increase in serum free fatty acids, and an elevation in hepatic glucose-6-phosphatase activity. There is no observed concurrent degranulation and degeneration of islet β cells (Losert et al., 1971; Richter et al., 1971). A transient glycosuria is produced with low-dose streptozotocin (25 mg/kg) administered in concert with NPH insulin therapy (Berman et al., 1973). In several studies, streptozotocin was administered to adult animals as a single dose (175 or 200 mg/kg). Changes indicative of diabetogenesis included hyperglycemia, glycosuria, and pancreatic islet changes affecting both α and β cells. Further, pancreatic insulin and glucagon levels were significantly reduced (Dulin and Soret, 1977). Others have shown that, following treatment with streptozotocin, the β cells undergo severe degeneration characterized by cytoplasmic vacuolization, nuclear pyknosis, and cell necrosis (Wilander and Boquist, 1972).

ALLOXAN

Alloxan, a pyrimidine nucleic acid, has a cytotoxic effect on cells and has been used to induce diabetes in the Chinese hamster. Intramuscular or intraperitoneal injection of a single alloxan dose (300 mg/kg) is shown to cause a temporary, modest rise in blood glucose levels in the Chinese hamster (Boquist, 1967). Alloxan is considered a transitory diabetogenic agent in the

Chinese hamster (Boquist, 1968; Boquist and Falkmer, 1970). Normalization of blood glucose and regeneration of islet β cells are shown to occur within several weeks of alloxan administration.

MONOSODIUM GLUTAMATE

A single subcutaneous injection of monosodium glutamate (4mg/kg) into newborn Chinese hamsters causes a marked change in the hypothalamus including severe necrosis and loss of nerve cell bodies in the arcuate and ventromedial hypothalamic nuclei (Komeda et al., 1980). These pathological changes are suggestive that the hypothalamus plays a central role in the development of monosodium glutamate-induced diabetes.

General Toxicology

The non-diabetic Chinese hamster has received considerable attention as a rodent model for the toxicological evaluation of a wide range of agents. Its usefulness is attributable to its small size, relatively long lifespan, and ease of care and handling. The Chinese hamster has been utilized to evaluate a wide range of chemicals and physical agents including radioisotopes, X-irradiation, microwaves, environmental pollutants, therapeutic drugs, pesticides, and potent tumorigenic agents. Table 35.7 provides an overview of some select general toxicology studies conducted in the Chinese hamster.

Genetic Toxicology

The Chinese hamster has been widely used for cytogenetic testing. It is an ideal candidate for genetic toxicology studies, due to its well-defined, stable karyotype, short gestation period, large chromosomes, and low chromosome number. Cytogenetic testing requires careful attention to a number of factors including: growth conditions, controls, doses, treatment conditions, and the time interval between treatment and sample analysis. In vivo assays for chromosomal aberrations involve treatment of intact animals and later collection of intact cells for cytogenetic analysis (Kirkland et al., 1990; Tice et al., 1994). The main advantage of an in vivo cytogenetic assay is that mammalian metabolism, DNA repair, and pharmacodynamics can be evaluated. The target is tissue from which large dividing cells can be easily prepared. Bone marrow from rats, mice, or Chinese hamsters has been most commonly used.

Cytogenetic assays have been developed utilizing mammalian cell lines to identify cells that have experienced genetic damage (e.g., chromosomal aberrations, micronuclei, sister chromatid exchanges, or changes in chromosome number). A large body of literature

TABLE 35.7 Chemical Agents (Environmental Pollutants and Therapeutic Drugs) Which have been Tested for their Ability to Produce Genetic Aberrations in Chinese Hamsters

Agent	Genetic Aberration	Reference
3,4-benzopyrene	Sister chromatid exchange in bone marrow cells	Bayer et al. (1981)
7,12-dimethylbenzanthracene	Chromosome breakage in bone marrow cells	Kato et al. (1969)
Formaldehyde	Chromatid gaps, breaks and exchanges of ovary cells	Natarajan et al. (1983)
Phenanthrene	Sister chromatid exchange in bone marrow cells	Bayer et al. (1978)
Styrene	None	Norppa et al. (1980)
Vinyl chloride	Chromatid breaks, fragments, gaps, deletions and sister chromatid exchange in bone marrow cells	Basler and Rhorborn (1980)
Acyclovir	Chromosome breakage in bone marrow cells	Clive et al. (1983)
Benzodiazepine	None	Schmid and Staiger (1969)
Cyclophosphamide	Chromosome gaps and exchanges in bone marrow cells and spermatogonia	Miltnerburger et al. (1981)
Phenylbutazone	None	Muller and Strasser (1971)
Praziquantel	None	Machemer and Lorke (1978)
Quinine	None	Munzer and Renner (1983)
Sulfonylureas	Sister chromatid exchange in bone marrow cells	Renner and Munzer (1980)

exists on the effects of a variety of chemical insults on chromosome composition in Chinese hamster cells. These insults include radiation, environmental pollutants, therapeutic drugs, pesticides, mycotoxins, and other agents. Metaphase analysis is used in conventional cytogenetics to detect chromosomal anomalies, especially unstable chromosomes or chromosomal aberrations.

Two in vitro assays for chromosomal damage have been developed in cloned Chinese hamster ovary cells to identify chemicals capable of inducing chromosomal aberrations or sister chromatid exchanges in mammalian cells. Both assays are described in detail by Galloway et al. (1985, 1987).

Chromosomal Aberration (CA) Test

Since many birth defects and most cancers are associated with abnormal chromosome complements, it is important to identify chemicals that can induce chromosome damage. To assess induction of chromosomal aberrations, cells are harvested during the first mitotic division after initiation of chemical exposure. The CA assay only detects structural chromosomal damage; it does not detect changes in chromosome number (aneuploidy) induced by chemical exposure.

Sister Chromatid Exchange (SCE) Test

Sister chromatid exchanges are a measure of DNA damage. Increased levels of DNA damage are associated with mutation induction and cancer. Sister chromatid exchange assays require examination of cells that have entered their second mitotic division following the initiation of chemical exposure, and assessment of the number of chromatid exchanges that have occurred.

Cytogenetics of Germ and Somatic Cells

A large body of cytogenetic literature exists for the Chinese hamster due to the small number of chromosomes present in this rodent species as well as a low rate of spontaneous chromosomal anomalies. The Chinese hamster has a small number of chromosomes compared with humans and other vertebrates, which has permitted in-depth analysis of banding patterns and systematic analysis of mitosis, meiosis, spermatogenesis, and oogenesis. [Tables 35.8 and 35.9](#) summarize select cytogenetic studies performed on germ and somatic cells of the Chinese hamster.

Cell Lines Derived from Chinese Hamster Tissues and Organs

Puck et al. (1958) obtained a female Chinese hamster from Yerganian's laboratory and used it to derive the original Chinese hamster ovary (CHO) cell line. Cell lines derived from Chinese hamster tissues and organs have been utilized for in vitro experimentation in a wide range of scientific disciplines including cytogenetics, toxicology, immunology, and cell physiology. The Chinese hamster ovary (CHO) and V-79 lung fibroblast are two cell lines derived from the Chinese hamster that have been used extensively in biomedical research (Bradley et al., 1981).

The adaptive ability of CHO cells and their ease of maintenance have been utilized in many basic biomedical research disciplines. G-coupled protein receptors and their signaling pathways have been studied by stable expression in CHO cells, and have contributed to advances in the fields of anesthesiology and

TABLE 35.8 Cytogenetic Studies of Male and Female Germ Cells in Chinese Hamsters^a

Study	Reference(s)
Chromosome activities during meiosis of male germ cells	Jhanwar et al. (1981)
Chromosome activities during meiosis of female germ cells	Mikamo and Sugawara (1980)
Chromosome anomalies of oocytes	Mikamo and Kamiguchi (1983)
Chromosome activities during spermatogenesis	Murkherjee and Ghosal (1969); Barcellona and Brinkley (1973)
Chromosome banding patterns of sperm	Utakoji (1966); Pathak et al. (1976); Vistorin et al. (1982)
Cytochemistry and behavior of dense bodies in sperm	Takanari et al. (1982)
Karyotyping of synaptonemal complex in sperm	Dresser and Moses (1980)

^aFrom Diani and Gerritsen(1987); reprinted with permission of Elsevier.

TABLE 35.9 Cytogenetic Studies of Somatic Cells in the Chinese Hamster^a

Study	Reference(s)
Banding pattern of chromosomes	Raicu (1970); Kakati and Sinha (1972); Ray and Mohandis (1976); Gamperl et al. (1978)
Chromosome activities during mitosis	Bregman (1972); Vig and Miltenburger (1976); Jhanwar and Chaganti (1981)
Chromosome analysis of Rous sarcoma virus	Kato (1968)
Cytotaxonomy	Yerganian (1972)
DNA replication	Utakoji and Hsu (1965)
Effect of bromodeoxyuridine on chromosomes	Lin et al. (1984)

^aFrom Diani and Gerritsen (1987); reprinted with permission of Elsevier.

pharmacology, and ultimately to patient therapy (Figler et al., 2003; Hornigold et al., 2003; Schulte and Fredholm, 2003). CHO cells serve as a tool for understanding cytoskeletal structure, adhesion, and motility, and have provided a better understanding of cancer biology and treatment (Hari et al., 2003; Zeng et al., 2003). CHO cells are relatively easy to synchronize, which has facilitated their use in cell cycle studies (Fiore et al., 2002). In addition, CHO cells are used for studies to assess the effects of various agents on mammalian cells (Cai et al., 2001; Fernandez et al., 2003; Soloneski et al., 2002). CHO cell mutants have been utilized in DNA repair and radiation research studies (Batista et al., 2006; Dunkern and Kaina, 2002).

Some early cell biology studies conducted in CHO cells involved mutagenesis and isolation of certain auxotrophs (Puck and Kao, 1967) that facilitate cell growth and viability in cell culture. Cell mutants with deficiencies in metabolic enzymes such as adenosine

phosphoribosyl transferase (APRT) and dihydrofolate reductase (DHFR) have been isolated and utilized as research tools (Taylor et al., 1977; Urlaub and Chasin, 1980). In addition, mutants defective in transcription, translation, and amino acid/polyamine biosynthesis have been isolated. The isolation of cells deficient in DHFR enzyme has led to an effective means for selection of stable clones and amplification of genes (Puck, 1985). DHFR is an enzyme that catalyzes the conversion of folic acid to tetrahydrofolate (THF). THF is a carrier for one-carbon molecules required in the biosynthesis of glycine, purine, and thymidine. In the early 1980s, Chasin et al. isolated two DHFR-deficient mutants (DXB11 and DG44), that are common parent cell lines used today (Graf and Chasin, 1982; Urlaub and Chasin, 1980; Urlaub et al., 1983). Both DHFR-deficient mutants are derivatives of a proline-requiring auxotroph established by Kao and Puck (1968).

SUMMARY

The Chinese hamster has played an invaluable role in biomedical research from rigorous in vivo scientific investigations of its unique biological features to cell-culture-based experimentation. With the advent of cell culture techniques developed within the past 50 years, Chinese hamster-derived cells have played a major role in cytogenetic toxicity assays and the production of glycosylated therapeutic proteins. Numerous recombinant cell lines have been produced using Chinese hamster cells transfected with genes derived from human or other mammalian cells. Advances in cell culture techniques have obviated the need for performing many in vivo studies, and Chinese hamster cell culture research has led to the prolific production of recombinant proteins through bioengineering. During the past 20 years, CHO cells have been utilized to produce numerous

TABLE 35.10 Selected List of Biologics Produced in Chinese Hamster Ovary (CHO) Cell Lines^a

Product	Type	Therapeutic Use	Manufacturer	FDA Approval
Vectibix	Anti-EGFR mAb	Metastatic colorectal cancer	Amgen	2006
Myozyme	α -Glucosidase	Pompe disease	Genzyme	2006
Aldurazyme	Laronidase	Mucopolysaccharidosis I	Genzyme	2006
Orencia	Ig-CTLA4 fusion	Rheumatoid arthritis	Bristol-Myers Squibb	2005
Naglazyme	N-AGA-4-sulfatase	Mucopolysaccharidosis VI	Biomarin Pharm	2005
Luveris	Luteinizing hormone	Infertility	Serono	2004
Avastin	Anti-VEGF mAb	Metastatic colorectal & lung cancer	Genentech	2004
Advate	Factor VIII	Hemophilia A	Baxter	2003
Xolair	Anti-IgE mAb	Moderate/severe asthma	Genentech	2003
Raptiva	Anti-CD11a mAb	Chronic psoriasis	Genentech	2003
Fabrazyme	α -galactosidase	Fabray disease	Genzyme	2003
Rebif	Interferon- β	Relapsing multiple sclerosis	Serono	2002
Humira	Anti-TNF α mAb	Rheumatoid arthritis	Abbott	2002
Aranesp	Erythropoietin	Anemia	Amgen	2001
Campath	Anti-CD52 mAb	Chronic lymphocytic leukemia	Genzyme, Bayer	2001
ReFacto	Factor VIII	Hemophilia A	Wyeth	2000
Tenectaplast	Tissue plasminogen activator	Myocardial infarction	Genentech	2000
Herceptin	Anti-HER2 mAb	Metastatic breast cancer	Genentech	1998
Enbrel	TNF α receptor fusion	Rheumatoid arthritis	Amgen, Wyeth	1998
Benefix	Factor IX	Hemophilia B	Wyeth	1997
Follistim/Gonal-F	Follicle stimulating hormone	Infertility	Serono/NV Organon	1997
Rituxan	Anti-CD20 mAb	Non-Hodgkin's lymphoma	Genentech, Biogen Idec	1997
Avonex	Interferon- β	Relapsing multiple sclerosis	Biogen Idec	1996
Cerezyme	β -glucocerebrosidase	Gaucher's disease	Genzyme	1994
Pulmozyme	Deoxyribonuclease I	Cystic fibrosis	Genentech	1993
Epogen/Procrit	Erythropoietin	Anemia	Amgen/Ortho	1989
Activase	Tissue plasminogen activator	Acute myocardial infarction	Genentech	1987

^aFrom Jayapal et al. (2007). Reprinted with permission from the American Institute of Chemical Engineers.

recombinant therapeutic proteins to treat a wide array of human diseases. A list is provided in Table 35.10 of some select therapeutic proteins derived from Chinese hamster cell cultures. Currently, greater than 50% of all recombinant therapeutic proteins are derived from cells of Chinese hamster origin, with annual world-wide revenues exceeding 30 billion dollars (Jayapal et al., 2007). The current focus on the use of Chinese hamster cells in cell culture research exemplifies animal welfare initiatives to refine, reduce, and replace the use of animals in research. The history of the Chinese hamster as a laboratory animal is replete with studies clearly demonstrating its value as a unique animal model in biomedical research.

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The European Hamster

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OUTLINE

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TAXONOMY

Cricetus cricetus is officially known as either the European or Common hamster, though it may be less commonly referred to as the European field hamster, black-bellied hamster, or the Alsace hamster. It belongs to the family Cricetidae and subfamily Cricetinae of the order Rodentia (Mohr and Ernst, 1987). Like the Chinese hamster, the European hamster has a chromosome diploid number 22 (Hankenson and Van Hoosier, 2002).

HISTORY

The wild European hamster was originally found in large numbers across the lowlands in much of Eurasia from Belgium to eastern Russia. They favored rural areas

bordering industrialized regions, especially in the grain and root crop areas (Mohr and Ernst, 1987; Neumann et al., 2004; Reznik et al., 1979). As the species thrived and populations expanded, it was considered a pest by farmers, as they caused extensive damage and hoarded the farmers' crops. Hamster trappers and rodenticides were used to control the population (Nechay et al., 1977; Ziomek and Banaszek, 2007). In some years, the numbers of hamsters caught were extremely high, from 1.5 million in 1973 and 2.4 million in 1974 in Hungary, or 1.3 million annually between 1953 and 1966 in Germany (Nechay et al., 1977). In the 1970s, these mass outbreaks quickly turned to the other end of the spectrum – dramatic population decline that has continued since. Since the 1979 Bern Convention, this species is listed in Appendix II as a strictly protected species (Figure 36.1). It later became listed in Appendix IV of the Habitats Directive,

Bern Convention.

At the 1979 Bern (Bern Switzerland) Convention, which was sponsored by the Council of Europe, a set of procedures were established to protect Europe's wild flora and fauna and their habitat. The European hamster is one species covered. Each country that signed the agreement agreed to submit a report every six years on how the country is doing following the Convention guidelines. According to the Convention and procedures established, man was prohibited from collection, killing or deliberate disturbance or destruction of resting or breeding sites- especially during breeding, rearing and hibernation seasons. No possession or internal trade of live or dead animals is allowed.

and is therefore provided with strict legal protection in all European countries (Ziomek and Banaszek, 2007). In some countries of Western Europe, such as France, Belgium, and the Netherlands, this hamster is now threatened with extinction (Nechay, 2000). Despite their protected status, the conservation efforts to include breeding and release programs, and improvements in their natural agricultural habitat, the wild European hamster population continues to decline, as does their distribution range across western and central Europe (Neumann et al., 2004).

The European hamster is not a widely used research laboratory animal. In the first half of the twentieth century, it was used in the study of thermoregulation and hibernation (Kayser, 1939). It was later developed as a research model when several wild-caught animals from a West German industrial area were found to have bronchogenic squamous cell carcinoma (Reznik et al., 1979). A closed institutional breeding colony was established in the early 1970s from 50 wild-caught hamsters in Hannover, Germany (Mohr and Ernst, 1987). Method sections from the total published literature cited herein indicate that prior to the early 1990s, either wild-caught or institutionally bred hamsters were used in research; however, since the mid-1990s, the source of the European hamster for research is only from European institutional research breeding colonies. As of 2010, there is a single institution with an active breeding colony – the University of Strasbourg in France (Monecke, 2010). There is no breeding colony or active research using this species in the United States. The current research efforts, based on topics of recent publications, are on circadian/circannual biology, hibernation, and species conservation.

BIOLOGY

Appearance

The European hamster is the largest of all hamsters, bearing a size resemblance to the guinea pig (three

FIGURE 36.1 Convention on the Conservation of European Wildlife and Natural Habitats, 1979.



FIGURE 36.2 The adult European hamster (rear) is approximately three times the size of the Syrian hamster (fore).



FIGURE 36.3 Coloration of the ventral surface of an adult European hamster.

times the size of the Syrian hamster) (Reznik et al., 1979) (Figure 36.2). Its coloration is similar to the Syrian hamster. Their bodies are yellow-red brown to grayish brown on the dorsal and lateral surfaces, and the cranioventral areas are completely black, except for a small white patch on the chest (Figure 36.3) (Monecke, 2010).

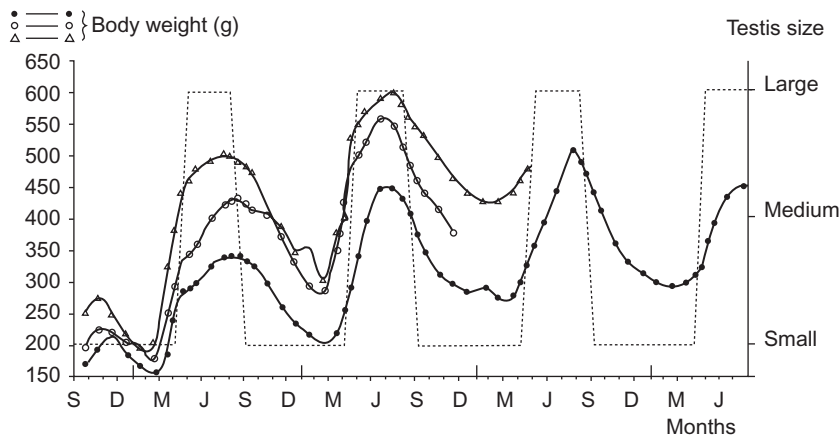


FIGURE 36.4 Changes in body weight and testis size over time in European hamsters. Parameters increased during the reproductive season and decreased during hibernation. (from Masson-Pévet *et al.*, 1994.)

TABLE 36.1 Body Weight of European Hamsters in the Laboratory Setting (Reznik *et al.*, 1979)

	Males	Females
Summer	451 ± 49 g	359 ± 63 g
Winter (4°C, 90% RH)	245 ± 92 g	174 ± 49 g

In the summer, the fur is lighter than in the winter. The adult body conformation is stocky with a short tail. Its eyes are an intense deep, black color, its nose is non-haired, and the brown or white facial whiskers extend laterally. Their prominent relatively avascular external ears are directed dorsomedially.

The males are larger than the females. The body weight of both the male and female is heaviest in the reproductive season (spring and summer) and lightest during hibernation (fall and winter) (Figure 36.4 and Table 36.1). In addition, the maximum body weight of an individual from year to year continues to increase over time (Masson-Pévet *et al.*, 1994). It is important to note, there are dramatic body weight changes in individuals between the reproductive and non-reproductive seasons (e.g., individual body weight with losses of ~200g in a 500-g animal). The weight gain after food deprivation/hibernation is faster in the spring and summer than the decrease in the autumn and winter (Canguilhem and Marx, 1973). They maintain large subcutaneous fat pad depots throughout the year. The more impressive fat pads are seen on the abdomen (Figure 36.5) (Reznik *et al.*, 1979). The average body length of the adult male is between 27–32cm and adult female is 22–25cm, excluding the tail (Mohr and Ernst, 1987).

Males and females have a sac-like cutaneous organ in the umbilical region, called the umbilical glandular organ. At the level of the anterior process of the ilium, there are bilateral flank organs that appear as dark cutaneous stripes, 2cm long by 2mm wide. The flank organs are sebaceous glands and active in sexually mature males. These glands are used to mark the male's territory.

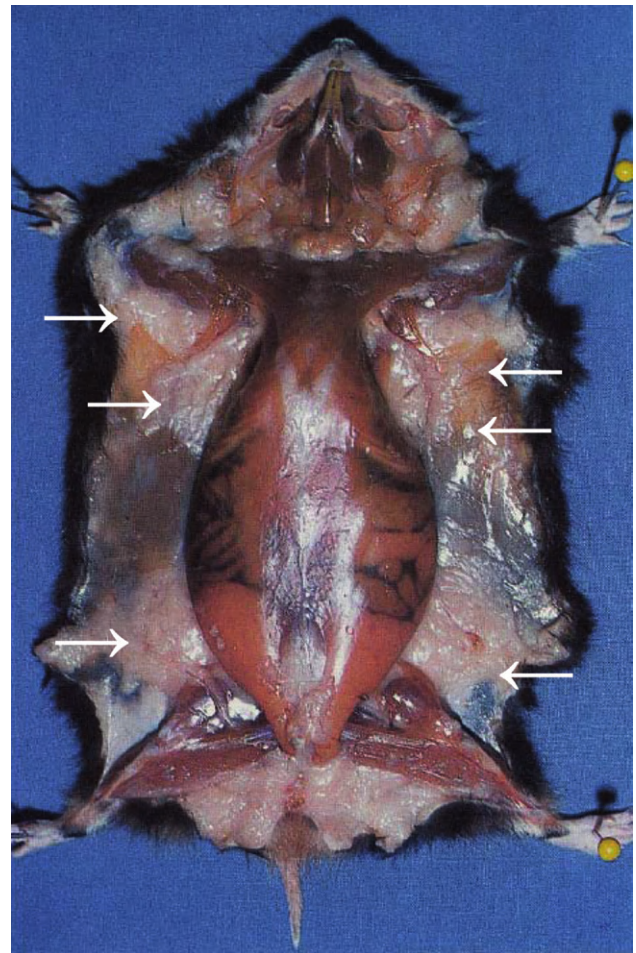


FIGURE 36.5 Subcutaneous fat pads (indicated by arrows) in an adult European hamster.

Life Cycle

Animals in the wild are currently reported to live up to 4 years of age, although there are older published references (e.g., Reznik *et al.*, 1979) indicating a life span

TABLE 36.2 Reproductive Data for the European Hamster

Sexual maturity	Females, 80–90 days
	Males, 60 days
Estrus cycle	4–6 days
Gestation	18–21 days (wild caught) 15–17 days (laboratory-born) 17–18 days (Monecke and Wollnik, 2008; Vohralik, 1974)
Litter size	7–9 7–8 (Vohralik, 1974; Monecke and Wollnik, 2008)
Weaning	25–28 days

up to 6–8 years in the wild. It is not known if the lifespan has actually significantly shortened or if the older information was incorrect.

The average lifespan of the research-bred European hamster is reported to be 31 months for females and 28 months for males (Ernst et al., 1989), attributing changes from the wild due to loss in hibernation pattern (Mohr and Ernst, 1987), dental lesions (Althoff et al., 1986), and higher incidence of neoplasia (Brandes et al., 2004; Ernst et al., 1987). The University of Strasbourg reports the average lifespan in captivity is slightly shorter, 2.5 years for females and 2.0 years for males (Monecke 2010).

Similar to other rodents, the male has a large, round prepuce and the space between the anus and prepuce is larger than in the female (2 and 1 cm, respectively) (Mohr and Ernst, 1987). The female clitoris has a pointed shape with a very small urinary opening. Females have eight teats, obvious by 10–14 days of age (Mohr and Ernst, 1987). Only during the reproductive season are the testes located within the scrotum (Reznik et al., 1979). During hibernation, the testes are smaller and situated in the abdominal cavity (Reznik et al., 1979). Before sexual maturity and during hibernation, the vagina is closed by a layer of squamous epithelium (Mohr and Ernst, 1987). The females reach sexual maturity around 200 g (Reznik et al., 1979). During the reproductive season, the vaginal orifice opens and the epithelial layer disappears (Reznik et al., 1979).

The reproductive indices are noted in Table 36.2. In the wild and in natural conditions simulated in the laboratory setting, they are reproductively active from spring to early summer (Masson-Pévet et al., 1994; Monecke et al., 2009; Nechay, 2000; Reznik et al., 1979; Vohralik, 1974). European hamsters become reproductively quiescent in the non-reproductive season when gonadal regression occurs (Canguilhem et al., 1988). Even if maintained under constant photoperiods in a laboratory setting, there are annual rhythms in body weight and the reproductive state due to the circannual endogenous reproductive cycle (Monecke and Wollnik, 2004; Masson-Pévet et al., 1994). Some animals may remain reproductively active for a longer period, depending upon the

individual period length of its circannual clock, which drives the reproductive cycle (Monecke and Wollnik, 2004).

Laboratory studies of breeding females show that they have a regular four-stage estrus cycle of 4–6 days (Reznik et al., 1979) during the ~4-month reproductive season. The female is responsive to the male for a short period during estrus (Reznik-Schüller et al., 1974). Females are markedly aggressive toward the male in the other three stages of the cycle. The four-stage estrus cycle is characterized by a proestrus stage lasting a few hours, an estrus of 1–2 days, a metestrus of 6 hours, and a diestrus stage lasting 2–4 days (Reznik-Schüller et al., 1974). Monecke and Wollnik (2004) reported a regular estrous cycle of 4–5 days at the University of Stuttgart. Male acceptance is indicated by reduced aggression of the female toward the male and interest in each other. When receptive, the female is followed closely behind by the male uttering a mating call, which is a loud “ffff” sound (Monecke, 2010) that increases in volume when the female is ready to mate (Mohr and Ernst, 1987). Copulation occurs several times during mating. Pregnancy lasts 17–18 days in the laboratory setting (Vohralik, 1974).

The average litter size is 7–8 (range 1–13) young, with 1–3 litters being produced per year (Monecke and Wollnik, 2008; Vohralik, 1974), with the smaller number of litters (two/year) occurring in a semi-natural laboratory setting or natural (wild) environment, and the larger annual litter number occurring under a constant photoperiod cycle in the laboratory setting throughout the year. Fecundity is reduced after 14 weeks under a long photoperiod (Monecke, 2010).

Incisors are the first teeth to erupt on day 4–5 post-partum. The young remain sucklings for about 28–30 days post-partum. Dental eruption is complete at 35 days of age, with only one set of permanent teeth (Reznik et al., 1979).

Anatomical and Histological Characteristics

Reznik et al. (1979) provide a very comprehensive description of pathology of the European hamster and include gross anatomy and histopathology illustrations and descriptions. Table 36.3 is intended to be a brief anatomic description of major organ systems in the European hamster.

Hematology and Clinical Chemistry

The normal blood values of the laboratory-bred European hamster show seasonal and lifecycle changes (Mohr and Ernst, 1987). There are no significant differences between the sexes for most hematology parameters. Normal hematology and clinico-chemical parameters for the non-hibernating hamster are briefly

TABLE 36.3 Summary of Anatomic Information for Adult European Hamsters (Reznik et al., 1979)

Organ	Characteristics
Salivary glands	Parotid, mandibular, sublingual, and zygomatic glands
Brain	2.85 g; well-developed cerebral hemispheres without sulci; large olfactory bulbs; cerebellum about half size of cerebrum
Cheeks	Cheek pouches – nonkeratinizing epithelium
Teeth	Continuously growing incisors (4) and molars (12); canines and premolars are lacking; one set of permanent teeth
Lungs	Right lung divided into five lobes (cranial, middle, diaphragmatic, accessory, and intermediate accessory); left lung has only one lobe
Heart	Four chambers with two atria and ventricles; average heart rate 175 beats/minute
Stomach	Divided by a distinct constriction into the larger forestomach lined with squamous cell epithelium and a smaller glandular stomach lined with typical gastric mucosa
Duodenum	120 mm long and 3.5 mm wide; common duct, a combination of bile and major pancreatic ducts, enter into the duodenum about 30 mm from pylorus
Jejunum	510 mm long and 5 mm wide; similar histology to duodenum, with fewer and smaller villi
Ileum	25–35 mm long and 3 mm wide; goblet cells found frequently and at the base of the broad based villi
Cecum	120 mm long and 15 mm wide; distinct apex, body and freely mobile base
Colon	370 mm long and 4 mm wide; crypts are deeper than small intestine; epithelium consists of mostly goblet cells; lymphatic tissue diffuse; folds of colon occur at 1–2 mm intervals
Rectum	Longer in male (40–60 mm) than female (35–45 mm); many lymph nodes along the lamina propria and submucosa; increased thickening of tunica muscularis; fewer goblet cells than colon
Liver	15 g; usually brown; divided into six parts (bipartite left portion, left lateral lobe, caudate and papillary processes of the caudal lobe, quadrate lobe, and right medial lobe)
Pancreas	Three well-defined, elongated lobes (gastric, duodenal, and splenic); light in color similar to fat; main pancreatic duct forms a common duct with the bile duct 15 mm before it reaches the duodenum
Kidneys	930 mg each; smooth surface, reddish brown; adrenal gland at cranial pole; cortex and medulla have no distinct border; glomerula and tubules not visible macroscopically
Urinary bladder	4–20 mm in diameter; transitional epithelium; urethra discharges urine separately from the vagina and anus in females and via penis in the males
Male genital organs	Testes external during breeding season; all organs involute during hibernation; epididymis firmly attached to testes; paired vesicular glands; prostate gland
Female genital organs	Ovaries – 5 mm, 26 mg each; bipartite uterus – undivided body and paired horns ~59 mm long; firm cervix ~10 mm long; epithelium closing the vagina disappears in the spring

summarized in [Tables 36.4 and 36.5](#), respectively. During hibernation, the entire metabolism of the European hamster is reduced to a minimum. The most notable changes occur in leucocyte and thrombocyte indices. Leukopenia occurs during hibernation with a decrease of leucocytes from an average of 5350 to 740 cells/mm³ ([Mohr and Ernst, 1987](#)). The lowest counts appear around 16 weeks into the hibernation cycle. Thrombocytes decrease from 226000 to 24170 cells/mm³. Within 1 day of awakening from hibernation, leucocyte and thrombocyte levels rebound to average levels recorded in the summer. Hamsters kept in a non-hibernating state during the winter show no seasonal changes in hematology parameters ([Mohr and Ernst, 1987](#)).

Hibernation Cycle

In the wild and in natural to semi-natural environmental conditions in the laboratory setting, the European hamster is a hibernator during the cooler, shorter months of the year. Less than 15.5 hours of light will induce gonadal regression for animals previously

exposed to long photoperiods ([Canguilhem et al., 1988; Saboureau et al., 1999](#)). The short days induce testicular regressions; a reduction in food intake and body weight; and hibernation when the ambient temperature is lowered ([Canguilhem, 1989](#)). On average, hibernation lasts 104.2 ± 23.8 hours ([Wollnik and Schmidt, 1995](#)), and the animals arouse at 5–7-day intervals to feed on feed stored in the burrow. The entry into hibernation typically occurs around midnight, reported between 00:00–08:00 hours ([Canguilhem et al., 1994](#)) or a mean time of day 01:48 hours ± 3.4 h ([Wollnik and Schmidt, 1995](#)). Spontaneous arousal is less predictable, scattered throughout the day and night ([Canguilhem et al., 1994; Wollnik and Schmidt, 1995](#)).

Body temperature of the European hamster during the active, reproductive period is approximately 36.1–37.9°C ([Wollnik and Schmidt, 1995](#)). During hibernation, body temperature and metabolism are reduced to save energy. Like all other hibernators, they run through rhythmical succession of torpor episodes characterized by low body temperature alternating with

TABLE 36.4 Normal Hemogram of the European Hamster

Hemogram	Silverman and Chavannes (1977)		Emminger et al. (1975)	
	Value	Percentage	Value	Percentage
Leucocytes ($10^3/\mu\text{l}$)	7.4 ± 2.6		8.3 ± 2.2	
Neutrophils	1.71 ± 0.06	23.2 ± 2.5	2.87 ± 3.74	34.6 ± 17
Lymphocytes	5.47 ± 0.06	74.0 ± 2.3	5.02 ± 0.39	60.0 ± 17.6
Monocytes	0.192 ± 0.015	2.6 ± 0.6	0.083 ± 0.022	1.00 ± 1.00
Eosinophils	0.005 ± 0.002	0.07 ± 0.10	0.093 ± 0.018	1.13 ± 0.83
Basophils	0.001 ± 0.002	0.02 ± 0.07	0	0
Thrombocytes ($10^3/\mu\text{l}$)			210 ± 32	
Erythrocytes ($10^6/\mu\text{l}$)	7.64 ± 0.42		7.45 ± 0.49	
PCV (%)	49.2 ± 1.6			
Hemoglobin (g/dl)	18.0 ± 0.7			

TABLE 36.5 Clinical Chemistry Parameters

Parameter	Mean $\pm$ SD	Range	Ref.
Urea nitrogen (mg/dl)	29.0 ± 4.3	19–44	Silverman and Chavannes, 1977; Mohr and Ernst, 1987
Alanine aminotransferase (SGPT) (Reitman-Frankel units)	27.7 ± 5.2	20–56	
Cholesterol (mg/dl)	110.9 ± 17.4	80–140	
Lipase (Cherry-Crandall units)	0.4 ± 0.3	0–1	
Total bilirubin (mg/dl)	0.4 ± 0.2	0.2–0.8	Malan and Arens, 1973
Total protein (mg/dl)	6.5 ± 0.3	6.0–7.3	
Thyroxin ($\mu\text{g/dl}$)	2.4 ± 0.7	1.6–3.3	
Glucose (mg/dl) (non-fasted)	57.8 ± 22	15–115	
Blood pH	7.4 ± 0.02	–	

short periods of eutheria in which the body temperature returns to 37°C (Waßmer and Wollnik, 1997). The lower body temperature is correlated with ambient temperature, following a lag time of 3–5 hours. Body temperature in the European hamster as low as 4.0°C has been recorded during hibernation (Wollnik and Schmidt, 1995).

A photoperiod of approximately 13 hours or more is required to stimulate gonadal regrowth in animals previously exposed to short photoperiods (Monecke and Wollnik, 2004; Monecke et al., 2009). As hibernation ends, there are spontaneous, light-independent increases in body weight and food intake; and gonadal regrowth occurs (Canguilhem, 1989).

BEHAVIOR

The European hamster is well acclimated to the laboratory setting. Though historically reported to be very aggressive, those in captivity have shown slightly reduced aggression to their kind and to man (Mohr

and Ernst, 1987); however, they continue to be aggressive toward each other and are typically singly housed (Canguilhem et al., 1988; Monecke et al., 2009; Tournier et al., 2007; Waßmer and Wollnik, 1997).

European hamsters are not a typical nocturnal rodent, as they are active only during the dark cycle with limited or no seasonal differences in activity. In 2 months of reproductive season (May to July), they are most active from the few hours before sunset to the few hours before dawn. They have an arrhythmic cycle, with no clear rest or activity phase, during the other 10 months of the year (Monecke and Wollnik, 2005; Wollnik and Schmidt, 1995; Wollnik et al., 1991).

In the wild, the solitary male travels over a large territory and does not occupy a single burrow (Bartlett, 2003). They are prodigious collectors of food, with as much as 180 lb of food reported to be stored in a burrow. The male mates with many females and his territory may cover the ranges of three or more females. Females have a smaller territory and spend less time out of the burrow than males and more time with her young (Bartlett, 2003). The species prefers to burrow in dry clay or loess soil. They

inhabit grassy steppes, plowed land, cultivated fields, and areas along riverbanks, ranging from lowlands to 2000-foot elevation (Bartlett 2003; Mohr and Ernst, 1987). The burrows are shallow (30–60 cm) in summer and deeper (up to 2 meters) in the winter; and each burrow has several exits in winter (Mohr and Ernst, 1987; Reznik et al., 1979). The European hamster maintains the same burrow for winter and summer. The burrow becomes more complex as the animal grows older (Eisentraut, 1928; Kayser, 1961). Within the adult burrows, there are separate areas for food, sleeping, and excreta (Bartlett, 2003; Eisentraut, 1928; Mohr and Ernst, 1987). The diet of the European hamster includes cereal seeds, cabbage, peas, potatoes, and roots and green parts of plants, insect larvae, small mice, and frogs (Bartlett, 2003).

DISEASES

Viruses

Little is reported in the literature with respect to the health profile of wild or laboratory-reared European hamsters. According to Svrcek et al. (1980), rabies virus was identified in five out of 266 free-living hamsters in Eastern Slovakia in 1972 and 1973. Hannoun et al. (1974) described a spontaneous and subclinical infection with papovavirus in European hamsters captured in France.

Bacteria

Secondary bacteria infections with *Corynebacteria*, *Staphylococcus*, *Pasteurella pneumotropica*, and *Pasteurella multocida* have been associated with pathological processes of the jaw and head (Kunstyr et al., 1976). These occasionally appeared as fistulating abscesses in both captured and laboratory-bred European hamsters.

Protozoan and Metazoan Parasites

Matthiesen et al. (1976) reported an outbreak of chronic enteritis associated with *Spironucleus muris* in seven newly captured European hamsters. The main pathological findings were hepatic swelling and edema of the intestinal wall, pancreas, and mesenteric lymph nodes. There was histological evidence of hepatic necrosis, cysts, and bile duct proliferation. In the intestine, the villi were shortened and the dilated Lieberkühn's crypts contained necrotic debris and inflammatory cells. There were large numbers of *Spironucleus muris* seen intra- and extracellularly in the small intestine. Co-infections with *Hymenolepis diminuta* and trichomonads were also seen in some of the hamsters.

Spontaneous, clinically inapparent infections with *Sarcocystis*, *Trypanosoma*, *Taenia*, *Cysticercus*, *Metastrongyles*, and *Capillaria* have also been described (Kunstyr et al., 1976; Meszaros, 1977; Murai, 1970; Tenora and Murai, 1970).

Spontaneous Tumors and Other Pathologies

Spontaneous benign and malignant tumors in older European hamsters are very common. The tumor incidence is up to 70% in those reaching 2 years of age. The most common tumors found in European hamsters at the Hannover Medical School were lymphatic in origin (Mohr and Ernst, 1987). Another report indicated that spontaneous neoplasia occurs more frequently in males than in females (overall frequency of 57.9% and 47.2%, respectively). Malignant tumors were more frequent than benign neoplasias. The most frequent tumors in descending order are leukemias and lymphomas, adrenal pheochromocytomas, and granulosa cell tumors in females (Ernst et al., 1989). Bronchogenic squamous cell carcinomas were found in the wild-caught hamsters from an industrialized area of West Germany, though it could not be determined whether these were spontaneous or due to environmental pollution (Mohr et al., 1973).

Thymomas have been reported in wild European hamsters (Ghadially and Illman, 1965). Brandes et al. (2004) reported a large number of spontaneously occurring thymomas in a laboratory colony of European hamsters. Of the 40 hamsters evaluated, 22 (55%) presented clinically with masses of variable diameter in the cranial mediastinum. The thymomas were characterized according to the World Health Organization (WHO) human classification to be type B1 thymomas, predominantly cortical or organoid low-grade malignant tumors. Typically, thymomas are rare neoplasms compared to other spontaneously occurring tumors in the European hamster. These tumors were evaluated for viral particles by electron microscopy and polymerase chain reaction, but none were identified.

The European hamster is prone to development of peritoneal cysts, particularly in the liver, with a higher incidence in females than males. Peritoneal cysts may also be found in the cecum, ovaries, spleen, kidneys, and colon (Kaup et al., 1990).

Spontaneous pathological processes may occur in the head, particularly the jaw. These include malocclusion, osteomyelitis, and dysplasia. There is evidence to suggest that the European hamster may be useful in dental research, as the lesions increase with age (Althoff et al., 1986; Kunstyr et al., 1987). These spontaneous pathologies may become complicated by secondary bacterial infections that may be fatal (Ernst et al., 1989).

MANAGEMENT, HUSBANDRY, AND COLONY HEALTH

The housing conditions for the European hamster vary depending upon the type of research being conducted and the location in which it is conducted. Conditions vary and range from natural conditions to semi-natural conditions (Canguilhem et al., 1994), or constant photoperiod (Wollnik and Schmidt, 1995) laboratory conditions. In the summer, animals have been maintained in light: dark (LD) 16: 8 photoperiod, temperature $20 \pm 2^\circ\text{C}$, and $55 \pm 5\%$ relative humidity. In winter, some investigators have moved them into a cold room with LD 8: 16 photoperiod and decreasing temperature gradually lowered to $8 \pm 1^\circ\text{C}$. They are typically housed individually (Canguilhem et al., 1988; Monecke et al., 2009; Tournier et al., 2007; Waßmer and Wollnik, 1997). The cages used for non-hibernating European hamster containers have included Makrolon cages (type III, 800cm^2) (Mohr and Ernst, 1987; Monecke et al., 2009) and Macrolon type IV cages (Monecke and Wollnik, 2004, 2005; Monecke et al., 2006; Wollnik et al., 1991). These same cages can be used during the hibernation season (Revel et al., 2007). However, a larger cage may also be used for winter housing (reported as 1m^3). The cages may contain soil and/or hay to be used as burrowing material and various nests boxes. During the hibernation period caging changes may be suspended to prevent interruption to the normal cycle (Wollnik and Schmidt, 1995).

Standard rodent chow may be fed to European hamsters. Food pellets and water are given *ad libitum*, supplemented with fruit (apples, bananas) and nuts (Wollnik and Schmidt, 1995). For gestating or lactating females, additional protein-rich food (white cheese, milk pudding, baby nutrition) may be used (Monecke, 2010). The average water consumption is $5\text{ml}/100\text{g BW}$, and food consumption is $2.9\text{g}/100\text{g BW}$ in summer and $1.8\text{g}/100\text{g BW}$ in winter (Silverman and Chavannes, 1977).

EXPERIMENTAL METHODOLOGY

Reproductive assessment is an important data point used in hibernation or circadian/circannual research. Under anesthesia, testis size is measured externally with a caliper square to measure the reproductive status of male hamsters. When the length of the testis is $\geq 1.8\text{cm}$, they are considered fully developed and reproductive, and the levels of testosterone are maximal (Monecke and Wollnik, 2004; Monecke et al., 2006). Gonadal regression begins when the testis length falls below 1.8cm . Gonadal regression is completed when the testes are no longer palpable, as they have ascended

into the peritoneal cavity (Monecke et al., 2006). The female reproductive state is determined by the opening of the vagina which correlates well with ovarian cyclicity (Monecke and Wollnik, 2004). Vaginal smears are no longer considered appropriate for determining estrus due to significant stress effect on the animal (Monecke et al., 2006). Rather than rely on smears, estrus is determined by animal behavior and male acceptance.

Parameters typically evaluated in hibernation or circadian/circannual research include body temperature; body weight; activity; cardiac and respiratory function; oxygen consumption; and blood parameters such as clinical chemistry, complete blood count, and measurement of testosterone, melatonin, and neuropeptides (Canguilhem and Masson-Pévet, 1988; Kayser, 1961). Light: dark cycle and room temperature modifications to emulate or control the natural seasonal changes are often manipulated as variables. Body temperature is typically monitored using surgically implanted temperature-sensitive radio transponders (Wollnik and Schmidt, 1995). Surgical procedures commonly employed in studies related to hibernation/circadian rhythm include pinealectomy (Garidou et al., 2003; Masson-Pévet et al., 1994); castration (Canguilhem et al., 1988); cerebral ventricular (lateral septal) cannulation (Hermes et al., 1989); telemetry or transponder implantation; and silastic capsule or osmotic pump implantation for infusion (Hermes et al., 1989). Inhalant gases, such as ether, halothane, enflurane, and isoflurane, have been used for anesthesia (Canguilhem et al., 1988; Monecke and Wollnik, 2004). Injectable anesthetics have included ketamine/xylazine ($50/10\text{mg}/\text{kg}$ body weight (bw)) (Waßmer and Wollnik, 1997; Wollnik and Schmidt, 1995); pentobarbital $1\text{mg}/\text{kg}$ bw IP (Hermes et al., 1989); and zoletil $20/\text{xylazine}$ ($80/10\text{mg}/\text{kg}$ bw) (Monecke, 2010).

USE IN BIOMEDICAL RESEARCH

Carcinogenicity

There are several publications evaluating the organ distribution of experimentally induced tumors in the European hamster following administration with nitroso-compounds (e.g., Althoff et al., 1974; Mohr et al., 1972, 1974; Reznik-Schüller, 1978; Reznik-Schüller and Lijinsky, 1979), as well as tumors from concentrated smoke inhalation (Reznik et al., 1975). Reznik et al. (1979) stated that their goal was to find a sensitive experimental animal that would fulfill the following criteria for carcinogenicity in vivo studies: (1) the lesions are induced in a reasonable time period after administration of known carcinogens; (2) development of well-differentiated tumors (adenoma and squamous cell carcinomas) originating from the respiratory tract; and (3) lack of infectious respiratory

tract diseases that might interfere with the experimental results. Mohr et al. (1972) found European hamsters to be susceptible to development of pulmonary tumors following administration of N-diethyl nitrosamine. Reznik-Schüller and Lijinski (1979) determined that respiratory tract tumors would develop within 13 weeks following exposure to nitroso-compounds. The incidence of pulmonary tumors in this study approached 100% with no tumor induction in the liver. The tumors were found to be well-differentiated and included adenomas, adenocarcinomas, squamous carcinomas, and mixed tumors. The authors further advocated for the use of European hamsters over other hamsters due to their size which (1) facilitated clinical evaluations such as radiology, bronchography, cytology; (2) facilitated administration of compounds by the intratracheal and intrabronchial routes; and (3) a larger respiratory tidal volume than the Syrian hamster. Lastly, the laboratory-bred European hamsters examined were determined to be free of infectious diseases of the respiratory tract. Thus, the European hamster met key criteria and the species was determined to be a better animal model for carcinogenicity than other hamsters.

Hibernation, Circadian and Circannual Rhythm

The majority of biomedical research using the European hamster is in the field of chronobiology and involves study of hibernation, circadian rhythm and circannual cycles.

Hibernation is considered to be a form of survival adaptation for animals that live in areas with striking seasonal changes in their environment. Offspring are born in the most favorable season for survival, while the harsh conditions of winter are survived by hibernation (Garidou et al., 2003; Kayser, 1961; Monecke et al., 2006, 2009; Waßmer and Wollnik, 1997). The European hamster is a well-characterized hibernator and especially interesting due to its large seasonal variations in physiological processes relative to other rodents (Kayser, 1961; Monecke et al., 2004). There are seasonal variations in body weight, food intake, gonadal activity, gonadal hormone levels, body temperature, and locomotion for the hibernating animal. Physiological processes such as heart and respiratory rates, immune and renal functions, and neural activity all run at greatly reduced levels during hibernation (Revel et al., 2007).

Circadian rhythms are endogenously driven patterns that have a period length of 1 day and are synchronized to the environmental cycle by the light: dark cycles. The suprachiasmatic nucleus (SCN) of the hypothalamus controls the master circadian clock. During hibernation, the SCN is metabolically very active (Monecke and Wollnik, 2004). The entry into, but not the arousal from, a torpor bout is coupled to a certain time of day (Waßmer

and Wollnik, 1997) indicating that the circadian clock which drives the daily activity and rest cycle is involved only in the former. During hibernation, the circadian clock seems also to be inactive (Revel et al., 2007).

Circannual rhythms are endogenously driven patterns that have a period length of approximately 1 year. The seasonal timing of the hibernation phase is driven by a circannual clock (Masson-Pévet et al., 1994) which is synchronized by changes in photoperiod (Monecke and Wollnik, 2004; Monecke et al., 2009; Saboureau et al., 1999). With the help of the circannual clock, the upcoming seasons can be predicted such that all the necessary changes in physiology can be initiated before they are needed. If perfectly timed, the animals will survive the harsh conditions of the winter through hibernation (Monecke et al., 2009). Rhythms that are under the control of an endogenous circannual clock include reproduction (Masson-Pévet et al., 1994); hibernation and body mass (Canguilhem et al., 1973); seasonal activity pattern changes (Tournier et al., 2007; Wollnik et al., 1991); serotonin and noradrenaline levels and turnover rates (Kempf et al., 1978); urinary sodium excretion (Haberey et al., 1967); and aldosterone excretion (Bloch and Canguilhem, 1966). Additional seasonal physiological changes occur in the European hamster that affect the melatonin binding sites of the pars tuberalis (Skene et al., 1993); pineal melatonin secretion (Garidou et al., 2003; Revel et al., 2007; Tournier et al., 2007; Vivien-Roels et al., 1992, 1997, 1998); daily body temperature patterns (Wollnik and Schmidt, 1995); vasopressin release (Buijs et al., 1986); and cortisol and progesterone secretions (Franceschini et al., 2007).

Gonadal regrowth in the spring is controlled by an endogenous timer rather than through a direct photoperiod effect. The gonadal regrowth begins prior to leaving the hibernation burrow and at a time when the animal has no information about the photoperiod from the outside world. There also appears to be a period of sensitivity to long photoperiods, which is unique to the European hamster compared to other hamster species. Sensitivity to long photoperiods following a shortening photoperiod does not induce gonadal regrowth until mid-November to March/April, as it does in other hamster species (Monecke and Wollnik, 2004).

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Other Hamsters

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INTRODUCTION

The hamsters described in this chapter have served over the last 40 years as important animals models in biological and biomedical research, but they are used less often than *Mesocricetus auratus*. Not all of these hamsters are commercially available from North American research rodent vendors, so research programs that use these hamsters generally maintain their own breeding colonies, acquire these animals from other research laboratories with breeding colonies, or trap hamsters from their native habitat to establish intramural colonies. As interest in use of these hamsters in research increased, the sharing of hamsters between laboratories and use of common names to describe the species has caused confusion as to which species of hamster was actually used in the published research. More recently, information about the taxonomy or genetics of these species is included in

descriptions of the hamster models to accurately reflect the species.

PHODOPUS SUNGORUS (DJUNGARIAN HAMSTER)

Taxonomy, History, and Genetics

There are three species in the genus *Phodopus*: *Phodopus sungorus*, *Phodopus campbelli*, and *Phodopus roborovski*. The most studied species, *Phodopus sungorus*, is called by many common names, including the hairy-footed hamster, Siberian hamster, and the Djungarian (or Dzungarian) hamster. *Phodopus campbelli*, once considered a subspecies of *P. sungorus*, was recognized as a separate species in 1993 (Carelton and Musser, 1993). *Phodopus campbelli*, often referred to as Campbell's hamster, shares many of the same common names as *P. sungorus*, which has caused

confusion in the life sciences literature. The most obvious phenotypic feature that distinguishes *P. campbelli* from *P. sungorus* is the lack of dramatic coat color change in response to a short photoperiod. *P. sungorus* molts to pure white haircoat, while *P. campbelli* retains a gray haircoat. In reviewing literature about dwarf hamsters, it might be advised to critically evaluate the species under discussion by evaluating the supplier, the geographic region of origin and whether the haircoat is pure white in short photoperiods (Ross, 1998). Information in this section will be focused on *P. sungorus*, with relevant comparisons to *P. campbelli*.

Phodopus sungorus is found in dry mountain steppes and semi-deserts of southwest Siberia, and eastern Kazakhstan, and in one isolated area in Khakassia, near the Yenisey River in Russia (IUCN, 2008; Ross, 1998). The first colony was established at the Max Planck Institute for Behavioral Physiology in Andechs, Germany from four hamsters captured in western Siberia (Figala et al., 1973; Hoffman, 1973). Colonies of *P. sungorus* have been established worldwide, and there are experimental data that suggest there are genetic differences between colonies from Germany and the United States with respect to photoresponsiveness (Lynch et al., 1989). In the United States, colonies of these hamsters molt later, have more photo-unresponsive animals and lose weight more slowly than those in German colonies. It was unclear whether these differences were due to genetic drift, or from inadvertent selection for photoresponsiveness.

P. sungorus has a diploid number (2N) of 28 chromosomes, all of which are metacentric except for chromosomes 12, 14, 15, 16, and Y, which are acrocentric (Schmid et al., 1986). Karyotype analysis using G-banding and chromosomal painting with probes from the golden hamster *Mesocricetus auratus* and the Chinese hamster *Cricetulus griseus* has confirmed close phylogenetic relationship between *P. sungorus* and *P. campbelli* (Romanenko et al., 2007). Analysis of partial DNA sequences from mitochondrial genes cytochrome b and either the 12S rRNA gene (Neumann 2006) or the tRNA-Thr gene (Meshchersky et al. 2009) characterize *Phodopus* as a distinct clade, and the oldest in the subfamily Cricetinae. The data suggest *P. sungorus* and *P. campbelli* separated about 0.8–1.0 million years ago, and are well separated from the other dwarf hamster, *P. roborovski*. Unique C-banding patterns in *P. sungorus* chromosomes also distinguish the karyotype from that of *P. campbelli* (as described in Ross, 1998). Interspecies mating produced females with reduced fertility and subfertile to sterile males (as described in Ross, 1998; Safronova et al., 1999).

Anatomy, Physiology, Husbandry, and Breeding

Phodopus sungorus is a small hamster with a unique characteristic of dense fur on the footpads, which

accounts for the common name of “hairy-footed hamster”. Adult males are about 11 cm long while mature females are about 9 cm long. Body weights vary with length of photoperiod, with the heaviest weights noted during the longest daylight hours that correspond to summer months. At peak body mass, males weigh from 40–50 grams while females weigh about 30 grams. In winter months, hamsters can lose from 40–50% of their body weight (Figala et al., 1973; Hoffmann, 1973). Hamsters have short tails, 5–15 mm in length, which are partially covered with white hair (Ross, 1998). The dorsal pelage is dense and gray with a wide, black-brown mid-dorsal stripe that extends from a dark black-brown patch on the crown of the head to the base of the tail (Figure 37.1). Black patches on the shoulders and flanks are connected with a line of black hairs that demarcate the dorsal from the ventral fur. The ventral pelage is white with a few black hairs. In contrast, *P. campbelli* has a yellow-brown tinge to gray haircoat with a gray underside; blackish fur patches on the shoulder only; narrow mid-dorsal stripe; and lack of dramatic coat color change in response to a short photoperiod. Winter molt under natural conditions begins in September and is complete in 2 months. The summer molt begins in late January and is complete by March (Figala et al., 1973). For either seasonal change, the molt begins on the head and the rump, and proceeds to the sides and ventrum. *P. sungorus* has lateral and midventral sebaceous marking glands. The glands are responsive to testosterone, vitamin D, and possibly estradiol and so are larger in males, especially in the breeding season (Stumpf et al., 1993). Like other hamsters, *Phodopus* has cheek pouches that are extensive for storing food. In contrast to other hamsters, *Phodopus* has an epidermal pocket between the skin of the cheek and the opening of the cheek pouch. This pouch is lined by stratified squamous epithelium that exfoliates into the center of the pouch, forming a whitish material with a pungent odor (Sokolov et al., 1994). The amount of secretion is greatest in females during the breeding season, and may be important in communicating age and sex, and for marking pelage and food stores (Sokolov et al., 1994). Females have four pair of mammary glands. The dental formula is: incisor 1/1 and molar 3/3, with continuously growing incisors as in other rodents. Detailed anatomical descriptions of the gastrointestinal tract (Vorontsov, 1979) and microscopic descriptions of the salivary glands (Suziki et al., 1983, 1984) and cecum (Snipes, 1979) have been published. Light and electron microscopic evaluations of the Harderian gland documented the similarities in glands between male and female hamsters collected during long and short photoperiods (Schreckenberger and Reuss, 1993). The body temperature ranges from 36.1°C to 37°C (Heldmaier and Ruf, 1992).



FIGURE 37.1 *Phodopus sungorus* (Djungarian hamster).

Phodopus are reported to be more docile and less aggressive than Syrian hamsters (Hoffmann, 1981). These hamsters can be housed in same-sex groups, and breeding pairs can stay together for the duration of their breeding life (Lerchl, 1995). Solid-floored cages with various kinds of bedding have been used for *Phodopus*. Material for nest building can be provided as enrichment, and is important in cages of breeding hamsters. Room temperature (20–25°C) and humidity levels (>50% RH) typical for rodent housing are acceptable conditions for colonies of *Phodopus* (Lerchl, 1995).

P. sungorus is nocturnal, crepuscular, and active year round in the wild (Ross, 1995). In captivity, the daily light cycles will influence the reproductive performance and body weight of *P. sungorus*. Puberty in male is influenced by the photoperiod exposure of the mother during gestation (Gündüz and Stetson, 2003; Stetson et al., 1986) and the postnatal photoperiod exposure (Gorman and Zucker, 1997; Shaw and Goldman, 1995). Male hamsters born and raised in 16h light and 8h dark photoperiodic cycles initiate spermatogenesis on postnatal day 11. Elongated spermatids are in seminiferous tubules by day 31 (Bergmann et al., 1991). Under these conditions, male hamsters reach puberty by 35–40 days of age. Puberty is delayed to around 150 days of age in male hamsters raised under short day conditions (8h light and 18h dark) (Hoffmann, 1979, 1981). Females kept in 15–16h daylight photoperiod have a 4-day estrous cycle (Schlatt et al., 1993; Scribner and Wynne-Edwards, 1994), and can be paired with males as adults.

P. sungorus are seasonal breeders, with the breeding season in the wild beginning in April to May and extending through September (Ross, 1998). Breeding colonies use stable photoperiods of 15–16h light with 8–9h darkness to match the photoperiod of the hamster's breeding season. Hamsters can breed year round with a constant long-day photoperiod, but may become obese (Jordan, 1971). Gestation length for the first litter is around 18–19 days (Figala et al., 1973; Scribner and Wynne-Edwards,

1994). Litters are typically born about 12 weeks after pairing, so females generally produce their first litter at about 4 or 5 months of age (Lerchl, 1995). Mean litter size is from 5–6, with litter size peaking at the third litter. Females have a fertile post-partum estrus, and the interbirth interval is from 18–25 days (Lerchl, 1995). The interbirth interval delay of over 19 days may be the result of post-implantation embryonic diapause (Newkirk and McMillan, 1997). Male *P. sungorus* do not participate in the rearing of offspring. In contrast, male *P. campbelli* are integral for the successful raising of litters (Wynne-Edwards, 1987). The breeding life of a pair of hamsters is about 1 year, and up to 13 litters can be produced (Lerchl, 1995). A dip in reproductive rates has been described in summer and winter in captive hamsters maintained under long day photoperiod (Jordan, 1971). However this seasonal variation was not observed in a 5-year study of breeding records from a *P. sungorus* colony that had been in captivity for 30 years, suggesting that selection over time may have influenced the loss of seasonal variation. While reproductive abnormalities are infrequent in *Phodopus*, there is one report of abdominal pregnancies in a breeding colony of hamsters used for mutagenesis experiments (Buckley and Caine, 1979). Affected hamsters were multiparous dams who all had previously delivered normal litters. Macerated fetuses were found in the abdomen, some attached by placentas to the abdominal wall, but most were free in the abdominal cavity which was often inflamed. There was no macroscopic or microscopic evidence of rupture of the uterus or oviduct, and the majority of affected dams were either stock or control animals, so the experimental procedures were not considered causative.

Young are born hairless except for vibrissae and weigh 1–2 grams. Incisors and claws are present at birth. The dorsal stripe is first recognized by day 3 and separation of the pinna from the head occurs by day 4. Pups are fully haired by day 8. When the eyes open around day 10, pups leave the nest and chew on food. Young can be weaned at 16–18 days, but commonly are weaned at 21–23 days of age (Lerchl, 1995). Weaned young can be housed with same-sex littermates. Weaned weight at 20 days averages 17.4 grams (range of 11.7–21.5 grams) (Jordan, 1971), and adult weight is achieved by 4 weeks of age.

Diseases and Veterinary Care

P. sungorus is a robust animal with few reports of serious contagious diseases. A report of *Trichophyton mentagrophytes* causing alopecia and hyperkeratosis of the extremities and ventrum was reported from a captive colony (Young, 1973). Other infectious diseases noted in this colony included fur mite infestation with *Myocoptes musculinus* and infected wounds. Bacterial pseudomycetomas

or cutaneous botryomycotic lesions were described in the forelimb of a solitary pet *P. sungorus*. The etiology of the pseudomycetomas was interpreted to be *Staphylococcus aureus* based on the Gram staining and histologic appearance of the bacteria in the lesion (Martorell et al., 2006). The majority of reports of diseases in *Phodopus* describe spontaneous neoplasia, and the mean age at diagnosis ranged from 12–28 months (Cooper et al., 1991; Kondo et al., 2008; Pogosianz and Sokova, 1996). The incidence of spontaneous neoplasia in captive research and domestic colonies ranged from 1–10%, and cancers included mammary adenomas and adenocarcinomas, cutaneous papillomas and squamous cell carcinoma and basal cell tumors, integumentary sarcomas, pulmonary adenomas and carcinomas, renal cell carcinoma, various mesenchymal tumors and endocrine neoplasia, including thyroid parafollicular or C-cell adenocarcinomas and adrenal pheochromocytoma (Cooper et al., 1991; Kondo et al., 2008; Li et al., 1995; Pogosianz and Sokova, 1996; Quimby et al., 1982).

Experimental Models

Seasonal-induced changes in morphology, physiology, and behavior in *P. sungorus* have made the hamster a popular model for the study of circadian rhythm and pineal gland activity. These changes include molting to the pure white winter pelage, loss of gonadal function, and weight loss. The neuroendocrine response to shorter days when seasons shift from summer to winter is an increase in nocturnal pineal secretion of melatonin (Illnerova et al., 1984). Melatonin secretion is proportional to the length of dark phase, and is extended in the winter months with longer dark cycles and compressed in summer months with shorter dark periods (Illnerova and Sumova, 1997). The melatonin signal is further enhanced by exposure to cold and reduced food (Stieglitz et al., 1991). The physiologic response to decreasing day length and increasing melatonin secretion is a molt to the pure white winter pelage. A decrease in serum prolactin is thought to initiate the growth of the unpigmented winter coat (Duncan and Goldman, 1984). In male hamsters, testicular weight drops to 10% of adult testicular weight. Histologic changes include reduction in seminiferous tubular diameter, loss of spermatids with arrest of spermatogenesis, and retention of primary spermatogonia. Decreases in circulating follicle-stimulating hormone (FSH) occur within 5 days of short day cycles, and at this time, testicular apoptosis is increased almost five-fold (Furuta et al., 1994). Serum and testicular testosterone levels subsequently drop and the rate of apoptosis is correlative with rate of hormone decline. In females, drops in FSH levels lead to ovarian involution with no progression in oogenesis past the secondary follicular stage (Schlatt et al., 1993). Hamsters lose up to 30% of body weight (Figala et al., 1973; Hoffmann, 1973, 1979). The loss of

body mass is due to a dramatic reduction in white fat from melatonin-stimulated, norepinephrine-induced lipolysis of white adipose (Bowers, 2005). Brown fat stores remain relatively constant (Hoffmann, 1981). In short days and temperatures <20°C, hamsters do not hibernate, but rather enter torpor. Their metabolic rate decreases and the body temperature drops to 17–23°C depending on the ambient temperature (Figala et al., 1973). Hamsters will hunch over, and hide their heads and forepaws in the fur of the ventral abdomen, fluff up their fur and will stay in this position with sporadic feeding activity (Heldmaier, 1975). The ability of *Phodopus* to withstand cold temperatures in torpor is from norepinephrine-induced non-shivering thermogenesis (Heldmaier and Buchberger, 1985; Heldmaier and Ruf, 1992). In short photoperiod and cold temperatures, brown fat respiration increases, as measured by increases in mitochondrial protein and GDP-binding of mitochondria. Additionally, there is activation of brown fat lipoprotein lipase and an increase in brown fat lipolysis providing the substrate for non-shivering thermogenesis (Klingenspor et al., 1989).

Change from short (8-h day) to long photoperiod (16-h day) results in recrudescence or stimulation of weight gain, gonadal function, and molt to the summer pelage (Hoffmann, 1981). A short burst of light for 1 or 5 minutes was shown to have the same effect on reinitiating spermatogenesis as did a 16-h daily exposure to light (Hoffmann, 1982). Response to photostimulation is the release of FSH, with increased levels measured by the third day in intact male hamsters (Furuta et al., 1994). By day 5 of long days, there was a 65% reduction in testicular apoptosis, increase in testicular weight and increases in serum testosterone levels (Futura et al., 1994; Wolfe et al., 1995). Female hamsters also show a rise in FSH that results in reinitiation of oogenesis (Schlatt et al., 1993). Interestingly, FSH administered exogenously to photo-inhibited male hamsters can reinitiate spermatogenesis when testicular testosterone levels are still low (Schlatt et al., 1995), suggesting this hormone, and not testosterone, was responsible for initiation of spermatogenesis.

Phodopus that remain in prolonged short photoperiods will undergo spontaneous recrudescence (Hoffmann, 1981). Nocturnal melatonin synthesis is disrupted (Lerchl 1992) and hamsters gain weight, regain normal gonadal function, and the molt to the summer pelage (Hoffmann, 1975). In spontaneous recrudescence, serum FSH levels return to normal levels 6 weeks before a rise in serum testosterone levels was detected (Schlatt et al., 1995), confirming the role of FSH in initiating gonadal function in spontaneous recrudescence.

Photoperiod also influences puberty in the male and female *P. sungorus*. Young males born in the long day length (16-h daylight) will develop active spermatogenesis and show increased testicular weight by 35 days

of age. However, *Phodopus* males and females raised in short day length (8-h daylight) will have delayed puberty. Testicular growth is sensitive to photoperiod stimulation at around 15 days of age and will stop developing in short daylengths. Testicular growth can be delayed for months (up to 150 days) and then proceed to full development by 5–7 months of age (Hoffmann, 1981, Stetson et al., 1986). Female hamsters raised in short days will also experience delayed puberty and gonadal development (Hoffman, 1981). Juvenile males raised in short day exposures have lower serum FSH, luteinizing hormone, and prolactin levels as compared to cohorts raised in 16-h daylight (Yellon and Goldman, 1984).

Seasonal changes in the immune system also occur in *Phodopus* as well as other mammals, and are linked to changes in day length (Yellon et al., 1999). Immunologic changes in immune cells from hamsters exposed to short day length include decreased circulating granulocytes and increases in circulating B-lymphocytes (Prendergast et al., 2002), reduced phagocytosis, and oxidative burst activities of macrophages and enhanced natural killer cell activity (Yellon et al., 1999), and diminished IgM production in T-cell-dependent adaptive immune responsiveness (Yellon, 2007). The mechanisms by which immune competence is diminished in response to short day photoperiod are not well understood, however, melatonin and sex hormones have been shown to have some influence on immune function in these hamsters. Pinealectomized male hamsters exposed to short days had serum IgM responses comparable to those of intact hamsters exposed to long days, suggesting that melatonin plays an inhibitory role in adaptive immunity (Yellon, 2007). The low granulocyte, high B-lymphocyte leukograms in *Phodopus* exposed to short day length were not observed in leukograms from castrated hamsters that were exposed to short days (Prendergast et al., 2002), and that sex hormones may also be involved. *Phodopus* offers a unique model system to continue to evaluate the responsiveness of immune cells to hormonal changes associated with photoperiod.

Phodopus are used in behavioral studies on separation anxiety and aggression. When a mated pair of *Phodopus* is separated, male hamsters display increase in body mass and altered behaviors, including increased food consumption, inactivity, decreased testicular and seminal vesicular weights, and increased serum cortisol levels. These responses to separation mimic some of the patterns observed in atypical human depression, and allow opportunities to evaluate the neuroendocrine influence on this induced behavior (Castro and Matt, 1997). *P. sungorus* are also considered ideal animal models for studies on aggression. Male hamsters display increased territorial aggression during the non-breeding season as compared to hamsters in the breeding season even though non-breeding hamsters have lower levels of testosterone (Jasnow

et al., 2000). Interestingly, aggressive encounters at night did result in increased serum testosterone levels, suggesting a possible role of melatonin (Scotti et al., 2009). Female *P. sungorus* display more maternal aggression when males are removed after birth of the litter than when the male stays postpartum (Gammie and Nelson, 2005). Female *P. sungorus* are more aggressive compared to female *P. campbelli* in maternal aggression experiments, and may prove useful in future studies needed to dissect the neurobiological correlates of maternal aggression.

P. sungorus is not often used in infectious disease studies. However, the recognition of the susceptibility of the Syrian hamster (*Mesocricetus auratus*) to prion disease prompted the investigation of the susceptibility of other rodents, including *P. sungorus* and *P. campbelli*, to the prion responsible for cervid chronic wasting disease or CWD (Raymond et al., 2007). Of the nine *P. sungorus* inoculated with various CWD inocula, two hamsters developed disease after prolonged incubations, and were considered to have limited susceptibility. In contrast, of the 15 *P. campbelli* inoculated, none developed disease and were considered resistant. The authors concluded that transmissibility of CWD to rodents might be useful for further comparison of strains of prion agents or for testing potential therapies.

MESOCRICETUS BRANDTI (TURKISH HAMSTER)

Taxonomy, History, and Genetics

Mesocricetus brandti is commonly referred to as the Turkish hamster or Brandt's hamster and is closely related to *Mesocricetus newtoni* (Romanian hamster) and *Mesocricetus auratus* (Syrian hamster). In the wild, the Turkish hamster is found in dry open steppe habitat at 1000–2200 meters above sea level over a large range including Turkey, Georgia, Armenia, and Iran (IUCN, 2008).

Research colonies have been established in the United States from animals trapped in Turkey and Iran. Chromosomal number varies among *M. brandti* populations with most populations having a diploid number (2N) of 42 chromosomes. However, one population trapped near Ankara Turkey has a diploid number of 44 chromosomes. Hybrid animals, produced from mating these two populations, are viable and fertile and contain a diploid number of 43 chromosomes. Chromosomal analysis suggests that chromosome pair #19 in the Ankara population is absent in Mayla populations (Lyman and O'Brien, 1977; Shimada, 2007). Breeding experiments crossing *M. auratus*, which also has a diploid number of 44, with *M. brandti* has not resulted in offspring (Lyman and O'Brien, 1977) indicating that *M. brandti* is a separate



FIGURE 37.2 *Mesocricetus brandti* (Turkish hamster).

species from the Syrian hamster. Reproductive crosses between *M. brandti* and *M. newtoni* have resulted in viable offspring but they are sterile with underdeveloped reproductive organs also indicating that *M. brandti* is a separate species from the Romanian hamster (Lyman and O'Brien, 1977). Recent molecular characterization of subfamily *Cricetinae* shows that hamsters from the genus *Mesocricetus* are a distinct phylogenetic group of hamster species. Within this group *M. brandti* is most closely related to *M. newtoni* and these two species comprise a subclade which is well separated from the other subclade comprised of *M. auratus* and *M. raddei* (Neumann et al., 2006).

Anatomy, Physiology, Husbandry, and Breeding

M. brandti has a slightly rougher hair coat and a dark subaruricular band, otherwise it is very similar in appearance to *M. auratus* (Figure 37.2). *M. brandti* is larger than *M. auratus* and averages 163 grams (range 137–258 grams). Median lifespan is 670 days and animals have a tendency to become obese after the first year of life (Lyman and O'Brien, 1977). *M. brandti* hibernates when exposed to cold and is considered a more consistent hibernating species when compared to *M. auratus*. Longevity in *M. brandti* has been linked to hibernation (Lyman et al., 1981). Animals categorized as good or moderate hibernators showed a statistically significant increase in longevity compared to animals not exposed to cold. However, poor hibernators had a decreased longevity compared to animals not exposed to cold and aged animals, which were apparently healthy when exposed to cold for a third season, died within a short time frame after cold exposure. This study shows that many animals within a colony have an increased longevity when exposed to hibernation temperatures for part of the year, but this benefit may not occur in all individuals. In particular, aged animals may die within a short time period when exposed to hibernation conditions.

Husbandry and management of *M. brandti* is the same as that of *M. auratus*. They can be raised in shoebox-type cages with wood shavings. Young are born blind and hairless, but hair coat quickly comes in and eyes are open around 12–13 days. Young can be weaned around 20 days and can be group housed until about 6 weeks of age. After 6–7 weeks of age the animals become aggressive and must be singly housed (Lyman and O'Brien, 1977). Puberty occurs around 7–8 weeks of age but can be delayed particularly if animals are born in late summer and exposed to a natural light cycle. Peak fecundity occurs around 1 year of age (Lyman and O'Brien, 1977). Female estrous cycle is 4 days in duration. Males are placed with females during estrus and can be left for 3–4 days after which time the incidence of fighting increases and males should be removed (Lyman and O'Brien, 1977). Gestation is 15 days and litter size averages six pups. Cannibalism is common especially if females are disturbed. Ample bedding and darkness may help prevent cannibalism of newborn litters. *M. brandti* are photoperiodic seasonal breeders. Light cycle is critical for testicular and ovarian function. Light cycles of 15–17 hours of light are associated with normal reproduction. Light cycles less than 15 hours light or greater than 17 hours light are associated with testicular regression in males and anestrus cycles in females (Hong and Stetson, 1986; Ogilvie et al., 1992).

Diseases and Veterinary Care

Very little has been published regarding naturally occurring diseases of *M. brandti*. Fighting and traumatic injury are common in group housed hamsters older than 7 weeks of age and in males co-housed with females for longer than 4 days (Lyman and O'Brien, 1977). Other reported diseases included “wet tail” diarrhea which was controlled with tetracycline added to the drinking water and resolved over time with diet modification and dental malocclusion with upper and lower incisor overgrowth (Lyman and O'Brien, 1977).

Experimental Models

M. brandti is used as a model animal for hibernation studies (Bartness, 1990; Bartness et al., 1991; Darrow et al., 1987; Giedke and Pohl, 1985; Goldman and Darrow, 1987; Hall and Goldman, 1982; Hall et al., 1982; Okulicz et al., 1988; Papafrangos and Lyman, 1982; Spangenberg et al., 1995). *M. brandti* is easily bred and reared in research facilities and is a more reliable hibernator than *M. auratus* and it hibernates readily when exposed to cold temperatures (Lyman and O'Brien, 1977). Hibernation is characterized by 2–7 day bouts of torpor followed by 1–2 days of activity. Unlike marmot species which acquire large fat stores prior to hibernation, *M. brandti* stores food which

is consumed during periods of activity. *M. brandti* is a seasonally breeding mammal that is a photoperiodic long-day breeder so many research studies focus on the photoperiodic control of reproduction and the interplay of the photoperiodic control, hibernation, and gonadal regression (Badura and Goldman, 1992; Butler et al., 2008; Carter et al., 1982; Darrow et al., 1986; Fang and Jagiello, 1991; Gunduz and Stetson, 1998; Hong and Stetson, 1987, 1988; Hong et al., 1993; Jagiello et al., 1992; Myoga et al., 1991; Ogilvie and Stetson, 1990; Pevet, 1988; Stetson and Watson-Whitmyer, 1986). Other photoperiodic research study focus areas include circadian rhythm studies and retinal studies (Albers et al., 1983; Pohl 1985, 1987; Williams and Jacobs, 2008; Youngstrom and Nunez, 1986).

Another major research focus for *M. brandti* relates to behavioral studies (delBarco-Trillo et al., 2009a, 2009b; Heth et al., 1999; O'Connell, 1983). *M. brandti* in the wild are solitary nocturnal animals and in captivity fighting is common in adult animals requiring single-animal housing. Behavior studies tend to focus on odor cues that allow animals to distinguish kin, species, and even familiar animal exposure. Other areas of use include infectious disease studies in which it was found that *M. brandti* can harbor *Yersinia pestis* (Karimi, 1980). *M. brandti* has also been used as a model for transmissible spongiform encephalopathy when inoculated intracranially with 263K scrapie agent (Meade-White et al., 2009). Other research studies with *M. brandti* examine estrogen-induced hepatic injury, estrogen-initiated renal carcinogenesis, and amyloidosis (Coe and Ross, 1988; Coe et al., 1997).

CRICETULUS MIGRATORIUS (ARMENIAN HAMSTER)

Taxonomy, History, and Genetics

Cricetulus migratorius is also known as the Armenian hamster, gray hamster or gray dwarf hamster. The Armenian hamster can be found in regions that overlap with the habitat of the Chinese hamster (*Cricetulus griseus*), but its geographic distribution is quite broad, extending from Balkan regions in western Europe through the middle of Asia, including southern Russia, Turkey, Iraq, Iran, and Afghanistan to western China (IUCN, 2008). This hamster prefers a semidesert environment, inhabiting the steppes, dry grasslands, and desert regions with limited vegetation. However, it is also been found in the foothills of south-east Bulgaria where the habitat includes field crops and pastures (Milchev, 2009).

This species was brought to the United States from the former Soviet Union and first used in cytogenetic studies in the mid 1960s as a complement to ongoing studies in the Chinese hamster, *C. griseus* (Yerganian and Papoyan,



FIGURE 37.3 *Cricetulus migratorius* (Armenian hamster).

1964). The Armenian hamster has a diploid chromosome number of $2N = 22$, and cytogenetic analysis revealed the X and Y chromosomes are isomorphic, sub-telocentric, and practically indistinguishable from each other (Gharkheloo, 2006; Yerganian and Papoyan, 1964). Even though the karyological data of the Armenian hamster are similar to those of the Chinese hamster, recent DNA-based phylogeny of the subfamily Cricitinae revealed a lack of monophyly within the genus *Cricetulus* (Neumann et al., 2006). Analysis of DNA sequences from two mitochondrial genes and one nuclear gene places *C. migratorius* outside of the genus *Cricetulus* and within a separate but related group that includes *Tscherskia*, *Cricetus*, and *Allocricetulus*. Multiple inbred strains of the Armenian hamster had been developed for genetic and genotoxic investigations. A characteristic of some of the inbred strains was a late-onset, progressive autoimmune disease without neoplasia (Yerganian et al., 1978). Outbred stocks and some inbred strains are currently available commercially.

Anatomy, Physiology, Husbandry, and Breeding

C. migratorius weighs from 33–80g (average 45 grams), has gray dense fur with a cream belly and a short tail (Figure 37.3). The husbandry for this species is similar to that used to care for Syrian and Chinese hamsters. Armenian hamsters reach sexual maturity at about 50 days of age. The estrous cycle, like that of other hamsters, is from 4–4.5 days. Males and females are paired prior to weaning, and males are removed from the cage prior to parturition. Gestation averages 19 days (range from 16–21 days) and litter size ranges from 5–7 pups. The reproductive life of females is about 1½ years and that of the male is about 2 years.

Diseases and Veterinary Care

Armenian hamsters are hardy and spontaneous diseases are uncommon. *Demodex cricetuli* is a mite found in the hair follicles that can induce clinical signs of alopecia and dermatitis (Hurley, and Desch, 1994). Armenian hamsters carry a myriad of intestinal protozoa, including trichomonads, which are likely commensals for the hamster, but have been documented to be transmissible to sentinel mice that were housed on soiled bedding from hamster cages (Lipman et al., 1999).

Experimental Models

Use of the Armenian hamster in biomedical research is primarily in studies of cytogenetics and infectious disease. This hamster is susceptible to many mutagens, carcinogens, and oncogenic viruses. Cells from *C. migratorius*, when recovered for primary in vitro cultures, frequently remain in euploidy, a feature that enhances use for mutagenic studies (Yerganian, 1972). The low diploid number ($2N = 22$) and large chromosome size facilitated analysis of insults from genotoxic agents. Much of the toxicological literature compares the relative susceptibility and resistance of the Chinese as well as the Armenian hamster to mutagens (Gad, 2007). Limited studies have demonstrated that Armenian hamsters develop hepatocellular injury and carcinoma from chronic exogenous estrogen exposure (Coe et al., 1990, 1998).

With the recognition of the susceptibility of the Syrian hamster to experimental infection with prion agents from ruminant diseases (scrapie and cervid wasting disease), there are several studies that compare the susceptibility of *C. migratorius* to wild-type and hamster-passaged prion strains. These studies were performed to not only develop new rodent models of disease (Raymond et al., 2007), but also to investigate the molecular events associated with cross-species transmission of the prion protein, that is transmission of the prion protein from the natural ruminant host to another mammalian species. In the latter study, six hamster species, including the Armenian hamster and the Chinese hamster were infected with a Syrian-hamster-passaged scrapie prion (Meade-White et al., 2009). The authors used these two *Cricetulus* species because of apparent taxonomic relatedness, but many characteristics of the induced disease and protein profiles of the prions recovered from infected hamsters were quite different.

Interspecies mouse:Armenian hamster hybridoma clones that produced hamster monoclonal immunoglobulins were first described by Sanchez-Madrid et al. (1983). Armenian hamster monoclonal antibodies are now common reagents for immunologic assays.

MESOCRICETUS NEWTONI (ROMANIAN HAMSTER)

Taxonomy, Physiology, and Genetics

Mesocricetus newtoni is known by its common name, the Romanian hamster. This hamster has a small range in the lowlands of northern Bulgaria and Romania where scrubby, rocky steppes and dry grassland make up the habitat. Recent surveys conclude that the wild populations are vulnerable and likely declining (IUCN, 2008). This hamster has been highlighted as a small European mammal that should be targeted for conservation efforts such as habitat improvement and local captive breeding for re-introduction (Gippoliti and Amoru, 2007).

Morphologic and physiologic similarities between *M. newtoni* and *M. auratus* resulted in early genetic studies, including karyotypic analysis as well as intercross breeding experiments. *M. newtoni* has a diploid number of chromosomes $2N = 38$, while *M. auratus* has $2N = 42$ (Yigit et al., 2000). Breeding *M. newtoni* males with *M. auratus* females resulted in hybrids with a $2N$ of 41 (Raicu et al., 1969; Todd et al., 1972). Hybrid males were infertile, but hybrid females ranged from expressing behavioral estrus with no conception to fertile matings with *M. auratus* male hamsters. Successful production of hybrids was also accomplished with mating of male *M. brandti* (the Turkish hamster) with *M. newtoni* females. When given choices of male hamsters of different species, female *M. newtoni* preferred *M. brandti* males over *M. auratus* males (Murphy, 1977). Not only did these studies help evaluate behavior related to sympatric evolution, but also outcomes of mixed mitochondrial populations in cells from hybrid hamsters.

More recent studies verify the genetic separation demonstrated by behavioral preferences between *M. newtoni* and *M. auratus*. DNA sequence data from two mitochondrial genes and one nuclear gene reveal greater genetic separation from *Mesocricetus auratus* than anticipated by morphologic data. *M. newtoni* and *M. brandti* are in a subclade separate from that of *M. auratus* and *M. raddei*. Their habitat is geographically separated as well, and the split from *M. auratus* likely happened in the upper Pliocene epoch (Neumann et al., 2006).

Anatomy, Husbandry, and Breeding

The Romanian hamster has brown fur on the dorsal side and a white belly. There is a dark stripe that extends from the top of the head to the neck. Dark stripes also start along each cheek and above the forearms and extend to the shoulder. The head has a pointier nose than the closely related *M. auratus* even though the shape of the nasal portion of the skull is not narrower. The length

from nose to rump is 180 mm and its weight ranges from 80–115 grams (Musser and Carleton, 2005). Like other members of the *Mesocricetus* genera, *M. newtoni* has cheek pouches for carriage of food, bedding, and offspring. Hamsters reach puberty when about 56–70 days of age. Management of breeding colonies is similar to the husbandry practices for *M. auratus*. The Romanian hamster has a 15–16-day gestation and gives birth to litters that range from one to 11 pups.

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Taxonomy and History

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OUTLINE

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INTRODUCTION

Chinchillas are a recent and relatively specialized addition to the biomedical research armamentarium. Considering use of the species in Pubmed-published articles as a reasonable reflection of all biomedical research use, their representation can be followed through the years.

Articles from the 1940s through the early 1960s focused on basic descriptive biology and clinical medical conditions, especially spontaneous infectious diseases. One of the earliest listed prospective studies using chinchillas appeared in 1952. That study assessed vitamin requirements and referenced only four pertinent articles involving chinchillas (King and Orcutt, 1952). Of those articles, two related to breeding and the others reflected captive management and anatomy. The King and Orcutt article also provided situational perspective on chinchilla research by explaining that breeding pairs cost over \$1000 and thus limited their use. Adjusted for inflation, this equates to over \$8000 a pair in 2009 prices.

Chinchillas appear in a variety of studies in the later 1960s and early 1970s with research related to the auditory system appearing among vaccine, radiologic, reproductive, and other studies. Late in the 1970s, chinchillas appear to have become established as an important model in acoustic research based on the rapid increase in frequency and numbers of published papers involving their use. Research related to the auditory system remains as the primary field utilizing chinchillas as experimental models.

TAXONOMY

Evolutionary History

As members of the Order Rodentia, the family Chinchillidae is represented in the fossil record from early in the Oligocene epoch, nearly 34 million years ago (Hartenberger, 1985; Patterson and Pascual, 1968). The group arose in the Patagonian region of the South American continent and while having once expanded

in species diversity and geographic distribution, the evolutionary record suggests that both decreased by the Pliocene epoch approximately 5 million years ago (Patterson and Pascual, 1968).

Classification

The Chinchillidae are hystricomorphous rodents. Anatomical adaptations within Rodentia accommodating bite force and jaw mobility have followed several paths of masseter muscle modification. The hystricomorphous condition is characterized by a portion of the masseter muscle passing through greatly enlarged infraorbital foramina. Other well-known hystricognath rodents include the guinea pig (*Cavia porcellus*) and the Old World and New World porcupines (e.g. *Erethizon dorsatum*, the North American porcupine) (Animal Diversity, 2009).

Modern examples of Chinchillidae were first scientifically described as *Mus laniger* by Molina in 1782 (Osgood, 1941; Sanborn, 1926; Woods and Kilpatrick, 2005). Osgood's review suggested that Molina based this speciation on a composite of non-living material from several species (Osgood, 1941). The genus was changed to *Chinchilla* by Bennett following study of skins and living specimens in London Zoo. Bennett also adapted Molina's species terminology as *C. lanigera* and further recognized that the species group belonged in a unique Family and coined the name Chinchillidae in 1833 (Osgood, 1941).

The species representatives within this Family and Genus have a long history of uncertainty (Bennett, 1835; Bidlingmaier, 1937; Bowen and Jenkins, 1988; Partington, 1836; Spotorno et al., 2004b). In 1937, Bidlingmaier condensed what had previously been described as four chinchilla species into three (Bidlingmaier, 1937). Less than 10 years later, Osgood supported a single species (Woods and Kilpatrick, 2005); yet, some authors were describing three species as late as 1969 (Bowen and Jenkins, 1988). A recent effort to resolve the chinchilla species number examined mitochondrial DNA of both wild and domestic representatives. While the number of individuals sampled was limited, there was clear evidence for there being two rather distantly related chinchilla species. (Spotorno et al., 2004b). The two species of chinchilla are the long-tailed chinchilla, *Chinchilla lanigera*, and the short-tailed chinchilla, *Chinchilla chinchilla* (Woods and Kilpatrick, 2005) (Figure 38.1). Unfortunately these are still frequently designated as *C. laniger* and *C. brevicaudata*, respectively.

It is currently believed that the Family Chinchillidae consists of six species in three genera. In addition to *Chinchilla* with two representatives, Chinchillidae has living specimens of *Lagidium* and *Lagostomus* which are the mountain vaschacha with three species and the

Kingdom:	Animalia
Phylum:	Chordata
Subphylum:	Vertebrata
Class:	Mammalia
Subclass:	Theria
Infraclass:	Eutheria
Order:	Rodentia
Suborder:	Hystricognatha
Infraorder:	Hystricognathi
Family:	Chinchillidae
Genus:	<i>Chinchilla</i>
Species:	
	<i>Chinchilla lanigera</i>
	Long-tailed Chinchilla
	<i>Chinchilla chinchilla</i>
	Short-tailed Chinchilla

FIGURE 38.1 Chinchilla taxonomy (adapted from Woods, 2005).

plains vaschacha with a single species, respectively (Animal Diversity, 2009; ITIS, 2009; Vanderlip, 2006). This is surprisingly consistent with the earliest descriptions of the group (Bennett, 1835; Partington, 1836).

Geographical Distribution

The Chinchillidae developed in and are currently limited to the western reaches of South America. The historical range of chinchilla was arid, rocky, and sandy areas of the Andean mountains of modern countries Chile, Bolivia, and Peru. The evidence indicates that the short-tailed chinchilla occupied the more northerly areas from central Chile to Peru, the long-tailed chinchilla occupied central Chile, with some overlap of the two ranges near present-day Potrerillos, Chile. The precise ranges are not well characterized as the populations were severely decimated prior to ecological study and persistent disagreement about how many different species existed (Jiménez, 1996).

Both chinchilla species are "critically endangered" in the wild, the last step before extinction (IUCN, 2009). Both species are listed in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) Appendix 1 (CITES, 2009). The short-tailed chinchilla was recently believed to be extinct having not been seen in the wild after 1953 (Jiménez, 1996). However, tissue samples collected from wild representatives for a recent genetic study indicate that some still survive (Spotorno et al., 2004b). A survey of wild long-tailed chinchillas in 1996 located 42 colonies in two areas of Chile (IUCN, 2009). Despite legal protection since 1929, wild populations continue to decline even in a Chilean national park created specifically for their protection (Spotorno et al., 2004a). While the pressures on chinchilla populations are not fully understood, continued trafficking in pelts is reportedly involved (Spotorno et al., 2004b; Vanderlip, 2006).

HISTORY

Origin

Early Spanish explorers noted that the Chincha Indians used chinchilla meat and fur (Vanderlip, 2006). This association is believed to have been the origin of the Genus name. In the Chincha Quechaun language, the words for silent, *chin*, and courageous or strong, *sinchi*, formed the basis for the name. The Spanish diminutive word suffix, *lla*, was added to complete it (Bowen and Jenkins, 1988; Vanderlip, 2006). The genus name may have actually originated as a derivative of the country of origin as that is the explanation given just 3 years after the fact (Partington, 1836).

The fur industry has been both bane and salvation for the chinchilla. The Spaniards took furs to Spain in the 18th century and commercial harvest began in earnest in 1828 (Jiménez, 1996). Early in the 19th century, chinchilla were described as “very abundant” (Partington, 1836); however, further expansion of the fur trade late in that century decimated the chinchilla populations. Fur exports decreased from about 700 000 in 1900 to 24 600 in 1910 despite rapidly increasing fur prices, demonstrating the disappearance of the chinchilla (Bowen and Jenkins, 1988; Jiménez, 1996). A regional agreement in 1910 was enacted to protect the chinchilla. Further legislation followed in 1929 but there was no practical enforcement of these protections prior to the 1970s (Jiménez, 1996).

Domestication

Practical continuation of a fur trade required domestic cultivation. A popular-press natural history article written by a curator of the Field Museum of Natural History indicated that Chilean efforts to raise them in captivity were not going well. Chinchillas fought and killed each other during breeding, litters were killed by parents, and young were attacked by unrelated adults (Sanborn, 1926). Mathias F. Chapman, a US mining engineer working in Chile, contracted for the collection of a few animals from the wild. Chinchilla were so scarce in the early 1920s that it required several years to acquire a small group of individuals (Bowen and Jenkins, 1988). Chapman imported 12 chinchillas (11 wild-caught and one born in transit) into southern California in 1923. Chinchillas were protected in Chile at that time and an exportation permit does appear to have been secured (Bowen and Jenkins, 1988; Parker, 1975).

While not a furrier himself, Chapman’s nascent chinchilla colony was supported by the US fur industry from its origin. However, domestic maintenance was again confronted with extensive challenges. Basic feeding and housing methods had to be developed

and no offspring were produced for several years. Domestic cultivation was further hindered by the Great Depression and Chapman’s death in 1934 (Bowen and Jenkins, 1988). Continued production by Chapman’s family was extremely poor with colony size increases of only about 37% per year in the late 1920s, 65% per year in the early 1930s and only doubling by the late 1930s in the absence of any production for furs (Parker, 1975).

Through the 1940s and early 1950s domestic colonies could not support a chinchilla fur industry. That period represented a speculative market of breeding stock; breeding pairs were so expensive they were often purchased by owner conglomerates. The first organized sale of chinchilla pelts occurred on June 21, 1954. Prices were far below expectation and the ensuing collapse of the breeder market led to dispersion of chinchillas to other parts of the world (Bowen and Jenkins, 1988).

Following the shake up, chinchilla farming entered into a period of slow development of fur quality and colony management for productive cultivation. There is no recorded history describing the process of chinchillas becoming available for biomedical research. One prominent vendor’s experience is probably typical (Moulton, 2010). In that case, after approximately 10 years as a fur farm, the facility was contacted in 1975 by a research organization seeking chinchillas. Over time, the business slowly converted from fur farming to that of a research vendor, based on demand. That vendor development process seems to reflect the current state of affairs, as well.

Genetics

Genetic information commonly associated with research rodents does not exist for chinchillas. No genetic information related to chinchillas could be found in the biomedical research literature in preparation of this chapter. There are no chinchilla “breeds” as the term is usually applied but color varieties are now common. Sporadic color mutations were reported in the 1940s but none were commercially developed. The first persistent color variety was with white fur in the mid-1950s, followed by black and brown fur types in the late 1950s. There was a wide expansion of color varieties in the 1960s but none were commercially viable by late in that decade (Bowen and Jenkins, 1988). Coat color genetics are not well-described in the scientific literature. Popular press sources indicate that the basis of color is in five dominant and four recessive mutations each with various phenotypic and genotypic designations (Trochinchillas, 2009).

While no specific genetic features of chinchillas are noted in biomedical research, there are issues related to domestication that could impact this species’ application to some types of research. Although these issues

are not scientifically well-documented, biomedical researchers should not discount them without due consideration. These issues are: first, the readily available and widely reported story about the domestic chinchilla industry says that it was founded on only 12 animals (three female and nine male) and this tightly restricted gene pool could have importance; and second, the domestic chinchilla may be an interspecies hybrid.

Clearly the original introduction of chinchillas into the US was of 12 individuals (Bowen and Jenkins, 1988; Parker, 1975; Spotorno et al., 2004a, 2004b; Vanderlip, 2006). Sources routinely state that these were the only introductions and that all current chinchillas are descendants of those 12 (e.g., Spotorno et al., 2004a; Vanderlip, 2006; and routinely in pet chinchilla popular press). However, two books describing the early domestication, by those close to the events, fail to note this limited source (Bowen and Jenkins, 1988; Parker, 1975). While such an omission is weak evidence, Parker further indicates his own association with efforts to capture wild chinchillas near Lampa, Chile in the 1960s (Parker, 1975). Although not indicating any collection success, the author does not indicate that the effort was out of the ordinary. One source that says all chinchillas are derived from the Chapman group also indicates that 10–50 chinchillas were imported per year up until the 1950s, although without attribution (Vanderlip, 2006). In all likelihood, domestic chinchillas were derived from a reasonably large pool of individuals captured and imported over many years and the story about them all being derived from the Chapman group is a widely reported quaint story perpetuated due to a lack of readily available contradicting evidence.

The second problem of domesticated chinchillas potentially being an interspecies hybrid may have validity. Statements to that effect appear in both popular press and scientific literature (Chincare, 2009; Jiménez, 1996), while other sources express doubts (Spotorno et al., 2004a). It is generally believed that the Chapman animals were all long-tailed chinchillas (Vanderlip, 2006); however, they were captured in the region of Chile where the species may have had overlapping ranges (Spotorno et al., 2004a). Indeed, early breeders believed they were dealing with multiple species (Bowen and Jenkins, 1988) and a recent book indicates that the US population currently includes both species (Vanderlip, 2006). On the basis of the short-tailed chinchilla having a number of advantageous characteristics over the predominant long-tailed chinchilla, there are clear descriptions of cross-breeding efforts. This was an arduous process complicated by male F1 sterility and 25% sterility of male offspring when F1 females were bred to males of either species. Bowen indicates that while body size, fur quality, and disposition were desirable, smaller litter sizes and general difficulty in

generating the hybrid rendered the idea unsustainable (Bowen and Jenkins, 1988). Hybridization was not commercially successful; however, it was biologically successful. Given the high economic value of chinchillas, the positive attributes of the hybrids, and the general confusion about speciation, if the hybrids were capable of contributing to the growth of the industry, they certainly did so. Both early and recent authors indicate phenotypic differences between domestic and wild chinchillas (Bowen and Jenkins, 1988; Spotorno et al., 2004a). While active selection for production would likely account for differences, Bowen attributes some of the difference to residual effects of the active hybridization programs (Bowen and Jenkins, 1988). A recent genetic study was conducted to clarify chinchilla speciation and the authors concluded that there was no evidence for either domestic long-tailed or short-tailed chinchillas being hybrids (Spotorno et al., 2004b). However, the study had several limitations to an authoritative conclusion to that question. All chinchillas sampled were from Chile and may not have been representative of stocks typically used in biomedical research. Also, the study sampled two short-tailed and five long-tailed chinchillas from single breeders and the limited sample size may have been insufficient to detect genetic contamination. Further, the study analyzed mitochondrial *Cytochrome b* sequences as the basis for comparison and the maternal lineage of mitochondrial DNA would support a further need for larger sample sizes in such a study. While evidence of interspecies hybridization remains sparse, biomedical researchers should remain cognizant that residue genetic contamination may persist and maintain awareness of this potential complication.

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Anatomy, Physiology, and Behavior

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INTRODUCTION

Chinchillas possess several key anatomic, physiologic, and behavioral characteristics which in many ways are adaptations derived from their evolution in the high Andean mountains of South America. As a consequence, chinchillas are useful animal models for a number of human diseases. This chapter discusses certain features in detail, while treating other attributes only briefly, so that key differences can be explored in sufficient detail while leaving other sources to discuss systems in common with other mammals.

EXTERNAL FEATURES

Chinchillas weigh an average of 400–600 grams (Hillyer and Quesenberry, 1997; Hoefer 1994), although they can weigh up to 800 grams (Riggs and Mitchell, 2009). Females generally weigh more than males of the same age (Hillyer and Quesenberry, 1997; Johnson-Delaney, 1998; Strake et al., 1996; Williams, 1976). Their bodies, about 12–14 inches long (Medow, 1969), are short, stocky, and compact (Hillyer and Quesenberry, 1997; Riggs and Mitchell, 2009), with comparatively long, delicate limbs (Hillyer and Quesenberry, 1997;

Riggs and Mitchell, 2009). The front limbs are shorter than the longer, more muscular, powerful back limbs (Donnelly and Quimby, 2002; Lyon, 2003; Nowak, 1999). The front feet possess four digits (digits 2, 3, 4, and 5) as digit 1 is not present. The hind feet possess three normal digits (digits 2, 3, and 4), with digits 1 and 5 being small and rudimentary (Kraft, 1987; Lyon, 2003). Each toe has a small, short claw (Hillyer and Quesenberry, 1997; Hrapkiewicz and Medina, 2007; Williams, 1976). There are stiff bristles on the digits of the rear feet, which may be used for grooming (Nowak, 1999). The palmar and plantar surfaces of their feet are furless (Hillyer and Quesenberry, 1997; Hoefer 1994; Hrapkiewicz and Medina, 2007; Nowak, 1999). The toe pads are irregularly shaped, prominent, and extend to the hock on the rear feet (Williams, 1976). The tail is about 4–5 inches long (Medow, 1969), and bushy, with coarse fur (Hillyer and Quesenberry, 1997; Hrapkiewicz and Medina, 2007; Riggs and Mitchell, 2009; Williams, 1976). It curls towards the body, and is normally carried high (Hillyer and Quesenberry, 1997) and erect (Lightfoot, 1999).

Chinchilla fur is soft and thick (Hillyer and Quesenberry, 1997; Hrapkiewicz and Medina, 2007). The density of the body fur is due to the large number of hairs which emerge from one opening. There are an average of 50–60 hairs, with a maximum of 75 (Hoefer and Crossley, 2002; Wilcox, 1950), emerging as a cluster from each opening. In each cluster there is one central “guard hair” approximately 12–15 μm in diameter. Surrounding the “guard hair” are two lateral bunches of “wool hairs”, each approximately 5–11 μm in diameter. The “guard” and “wool” hairs have no bulb at their base. There is a single erector pili muscle attached to each cluster. For each cluster, 3–4 sebaceous glands serve the “wool hairs” and one sebaceous gland serves the “guard hair” (Wilcox, 1950). On the tail, hairs all emerge separately, although they are arranged in groups of 3–8 hairs. On the pinna, hairs also emerge separately, and are not grouped (Wilcox, 1950). Each hair is approximately 2–4 cm long (Bennet, 1835), and has three color areas. The tip is black, and the longer the tip, the darker the animal’s appearance. Proximal to the tip is a clear/unpigmented bar, about 1/8–3/8 inch long. From the bar to the root is the rest of the hair, which has the animal’s undercoloring (Medow, 1969). In the wild, the original coloring was mottled yellow-gray. Through selective breeding, the natural color is now blue-gray (Donnelly and Brown, 2004) or silver gray with black ticking (Hillyer and Quesenberry, 1997; Riggs and Mitchell, 2009) and yellow under parts (Hillyer and Quesenberry, 1997). There are color mutations of white, silver, black, and beige, and the fur and pet trades have led to the development of more colors and variations (Hillyer and Quesenberry, 1997). Beige, white, and ebony are inherited via dominant genes, and sapphire,

violet, and velvet are inherited via recessive genes (Donnelly and Brown, 2004). The fur colors are beige (blond, pearl, pastel); white (Wilson white, white mosaic, silver, platinum, pink white, apricot); violet (Afro violet, Rhodesian violet, German violet); sapphire; charcoal (pastel charcoal, sapphire charcoal, violet charcoal); ebony (brown ebony, chocolate ebony, pastel ebony); and velvet (black velvet, brown velvet, pastel velvet, blond velvet) (Donnelly and Brown, 2004). Coulton (1989) provides a detailed explanation of chinchilla coat color heredity. The fur coat grows in waves from the head caudally, and a pale bar line in the fur indicates its stage of growth (Hrapkiewicz and Medina, 2007; Williams, 1976).

The head of the chinchilla is large relative to its body (Donnelly and Quimby, 2002; Hrapkiewicz and Medina, 2007; Riggs and Mitchell, 2009). Interestingly, one study found that the average skull size of captive-bred chinchillas was significantly larger (16% longer and 6% wider) than the average skull size of wild-caught chinchillas (Crossley and del Mar, 2001). The eyes are large (Donnelly and Quimby, 2002; Hillyer and Quesenberry, 1997; Hrapkiewicz and Medina, 2007), within a shallow bony orbit (Hillyer and Quesenberry, 1997; Hoefer and Crossley, 2002; Williams and Kern, 2008). The cornea is also large relative to the size of the globe and palpebral fissure (Peiffer and Johnson, 1980), which is characteristic of a nocturnal animal (Donnelly and Brown, 2004). There are fine cilia on the upper and lower lids, and the medial canthus has a fleshy caruncle (Peiffer and Johnson, 1980). The eye also has a poorly developed nictitating membrane (Peiffer and Johnson, 1980; Williams and Kern, 2008). The pupil is a vertical slit (Hoefer and Crossley, 2002; Peiffer and Johnson, 1980; Williams and Kern, 2008). When relaxed, the pupil is a vertical ellipse, which contracts to a fine slit with a teardrop end, and then finally to complete closure (Donnelly and Brown, 2004). The iris is heavily pigmented (Donnelly and Brown, 2004; Hoefer and Crossley, 2002; Williams and Kern, 2008), appearing dark brown (Peiffer and Johnson, 1980). The dark iris and slit-like pupil help the animal cope with their behavior of basking in bright sunlight at their natural high altitudes (Donnelly and Brown, 2004). The chinchilla also has large ears (Donnelly and Quimby, 2002; Hoefer and Crossley, 2002; Hrapkiewicz and Medina, 2007; Riggs and Mitchell, 2009; Strake et al., 1996; Williams, 1976), which have been described as “bat-like” (Hoefer 1994; Strake et al., 1996; Williams, 1976). The pinnae are thin (Hoefer 1994; Hoefer and Crossley, 2002; Strake et al., 1996; Williams, 1976) and pigmented (Williams, 1976), with sparse fur. The chinchilla has long vibrissae (Hillyer and Quesenberry, 1997; Hrapkiewicz and Medina, 2007; Williams, 1976) on either side of the upper lip (Hoefer and Crossley, 2002). The vibrissae are independent sensory hairs, with a

bulb and papilla, which resemble a typical hair follicle. They are approximately 100–130 mm long, strong, and abundant (Wilcox, 1950). The chinchilla, being nocturnal, uses these to find its way in the dark (Hoefer and Crossley, 2002). The chinchilla mouth is small (Riggs and Mitchell, 2009).

Only the female chinchilla has mammary glands and teats, with three pairs (Hoefer and Crossley, 2002; Hrapkiewicz and Medina, 2007). There are two thoracic pairs and one inguinal pair (Donnelly and Brown, 2004; Johnson, 2006; Nowak, 1999; Weir, 1974), although the caudal thoracic pair has been referred to as an abdominal pair (Yu and Anderson, 1975). The number of galactophores per teat varies. The cranial thoracic pair has two per teat, the inguinal pair has one per teat, and the caudal thoracic (or abdominal) pair can have one or two per teat (Yu and Anderson, 1975). During lactation, the area around the teats loses fur (Spotorno et al., 2004).

The female chinchilla has a large, prominent cone-shaped urogenital papilla (clitoris/urethral cone/urinary papilla) (Hillyer and Quesenberry, 1997; Hoefer 1994; Hrapkiewicz and Medina, 2007; Strake et al., 1996; Williams, 1976). It can be manually extruded through the urethral orifice (Donnelly and Brown, 2004), and, due to its size, may be mistaken for the prepuce/penis (Donnelly and Brown, 2004; Hillyer and Quesenberry, 1997; Hoefer 1994; Hoefer and Crossley, 2002; Hrapkiewicz and Medina, 2007). The urethral orifice sits within the urogenital papilla (Hoefer and Crossley, 2002), but the chinchilla female has a separate vaginal opening between the urethra and the anus (Hrapkiewicz and Medina, 2007; Kraft, 1987; Strake et al., 1996). The vulva is immediately caudal to the urogenital papilla (Hillyer and Quesenberry, 1997; Hoefer 1994; Strake et al., 1996; Williams, 1976). It is slit-like, but oriented transversely/horizontally to the body's craniocaudal axis (Hillyer and Quesenberry, 1997; Kraft, 1987). The vulva has no labial folds (Kraft, 1987; Lyon, 2003). There is a vaginal closure membrane present except during estrus and parturition (Hoefer 1994; Hoefer and Crossley, 2002; Hrapkiewicz and Medina, 2007; Johnson-Delaney, 1998; Strake et al., 1996; Williams, 1976). The vaginal closure membrane is sturdy, and the opening and the perforation and sealing are distinct. The perforation and sealing usually take 2–4 days, but can occur over 12 hours (Weir, 1974). When the vaginal closure membrane is present, the urethral orifice can be mistaken for the vulva. The membrane-covered vaginal orifice can be distinguished by a slightly raised semicircular area (Hillyer and Quesenberry, 1997). When the vaginal closure membrane is absent close to parturition, the vagina takes on a bluish hue (Strake et al., 1996).

Because the external genitalia of females and males appears similar (Hrapkiewicz and Medina, 2007),

the anogenital distance is the best criterion for sexing chinchillas (Brower, 2006; Hillyer and Quesenberry, 1997; Strake et al., 1996). The male's anogenital distance, between the penis and the anus, is approximately 1–1.5 cm (Hillyer and Quesenberry, 1997), which is about twice the anogenital distance in females (Hrapkiewicz and Medina, 2007; Johnson-Delaney, 1998; Strake et al., 1996; Williams, 1976). An additional distinguishing feature is that the penis is larger than the clitoris. Also, the extruded penis can be separated and distinguished from the prepuce, while the extruded clitoris tends to evaginate and the prepuce is not apparent (Donnelly and Brown, 2004). Also, compared to the female, the male chinchilla has a larger genital papilla (Hrapkiewicz and Medina, 2007; Merry, 1990).

The penis normally sits in an s-shape when in the retracted position (Kraft, 1987; Lyon, 2003; Weir, 1974). It originates with two supports in the pelvic region which fuse together to point caudally; and it then bends in a cranial and ventral direction, then turns again to point posteriorly (Kraft, 1987). The glans penis terminates just below the anus, and the prepuce/foreskin extends nearly to the anus (Kraft, 1987; Lyon, 2003). The glans is partially covered with small horny spines, which point backwards, towards the body (Kraft, 1987; Spotorno, 1979). There is also a supporting bone/baculum (Strake et al., 1996), approximately 1 cm long within the penis (Kraft, 1987; Lyon, 2003). The penis may be manually extruded (Strake et al., 1996), and measures 1–2 cm (Hillyer and Quesenberry, 1997). There is no true scrotum (Brower, 2006; Donnelly and Brown, 2004; Hoefer 1994; Hoefer and Crossley, 2002; Hrapkiewicz and Medina, 2007; Johnson-Delaney, 1998; Merry, 1990; Strake et al., 1996; Weir, 1974; Williams, 1976), and the genital papilla is separated from the anus by bare skin (Hillyer and Quesenberry, 1997; Weir, 1974). The inguinal canal is open, and the testes remain in the abdomen or are subcutaneous in the anal region (Brower, 2006; Donnelly and Brown, 2004; Hoefer 1994; Hrapkiewicz and Medina, 2007; Johnson-Delaney, 1998; Strake et al., 1996; Williams, 1976). The caudal epididymus can drop into two small external sacs (postanal sacs) (Donnelly and Brown, 2004; Weir, 1974). However, if the male is close to a female in estrus, his testes swell and become easier to observe (Strake et al., 1996).

UNIQUE BIOLOGICAL CHARACTERISTICS

The organ systems of the chinchilla are essentially similar to those of most other mammals. They do, however, have unique biological attributes which may either influence approaches to their medical care or demonstrate their value as research animals.

Reproductive System

Anatomy

The chinchilla's ovaries are 6–8 mm long (Riggs and Mitchell, 2009), approximately 0.3 g each (Kraft, 1987), and located lateral and caudal to the kidneys (Bennett and Mullen, 2003). The ovaries are smooth in the immature chinchilla, but as she reaches sexual maturity, small lumps form on the ovary surface, due to eggs migrating to the surface (Kraft, 1987; Lyon, 2003). The oviduct has an ampule-like enlargement with a strongly developed longitudinal fold where the eggs are fertilized after ovulation (Kraft, 1987). The chinchilla has two uterine horns, each with a separate internal os (Hoefer 1994; Hoefer and Crossley, 2002; Hrapkiewicz and Medina, 2007; Strake et al., 1996; Williams, 1976), and two cervixes (Spotorno, 2004). Each uterine horn is approximately 6 cm long (Kraft, 1987), and the vagina is approximately 1.5 cm long (Kraft, 1987).

The chinchilla's testes are approximately 2.0–2.5 cm long (Kraft, 1987; Strake et al., 1996) $\times$ 1 cm, and approximately 2.6 g each (Kraft, 1987). The epididymus is lateral to the testis (Kraft, 1987). The testes are relatively large (Weir, 1974), and the gonadosomatic index (testes mass/body weight) in the adult chinchilla is relatively high compared to most mammals, but is similar to rats and mice (Leal and França, 2008). The chinchilla testis has a high seminiferous tubule volume and density (Leal and França, 2008). There is also a seasonal variation in testis volume, with a reduced volume in the summer (Busso et al., 2005). The male accessory sex glands include seminal vesicles (Williams, 1976), prostate gland, and bulbourethral glands (Kraft, 1987). The seminal vesicles/spermatocystic glands/deferentio-vesical glands are long (Kraft, 1987; Weir, 1974), tubular (Williams, 1976), and branched (Kraft, 1987). The ducts from the seminal vesicles enter the urethra along with the vas deferens from the testes/epididymus (Kraft, 1987; Weir, 1974). The prostate's presence is variable (Adaro et al., 1999). When present, it consists of two triangular reddish brown lobes approximately 1–2 cm $\times$ 0.5 cm. The lobes are lateral and dorsal to the urinary bladder and proximal urethra. The ducts open into the urethra close to the vas deferens (Kraft, 1987). The bulbourethral glands/Cowper's glands/bulb of urethra glands are reddish, round (Kraft, 1987), and approximately 5–6 mm diameter (Cepeda et al., 1999; Kraft, 1987). They are covered by a capsule consisting of dense connective tissue and striated muscle (Cepeda et al., 1999). They are located below the root of the tail, lateral to the rectum and dorsal to the urethra (Kraft, 1987).

Physiology

Females are seasonally polyestrous (Donnelly and Quimby, 2002; Hrapkiewicz and Medina, 2007; Webb,

1991), even in captivity (Weir, 1974), but males produce spermatozoa of similar quality continuously (Busso et al., 2005) and are fertile throughout the year (Weir, 1967). The breeding season in the southern hemisphere is May to November (Nowak, 1999), and in the northern hemisphere, it is November to May (Donnelly and Quimby, 2002; Webb, 1991). Estrous cycles may be irregular (Williams, 1976), with a range of 30–50 days (Donnelly and Quimby, 2002; Hoefer 1994; Hrapkiewicz and Medina, 2007; Kuroiwa and Imamichi, 1977; Webb, 1991; Weir 1967; Williams, 1976). Estrus is 2–3 days long (Weir, 1967), and ovulation is usually spontaneous (Hrapkiewicz and Medina, 2007). Several eggs mature and are ovulated simultaneously (Kraft, 1987; Lyon, 2003). The age at which chinchillas reach sexual maturity varies, depending on when they are born (Weir, 1974). Chinchillas born in spring breed in the fall of the same year. Those born in the fall first breed a year or more later (Williams, 1976). Thus, they reach puberty at 4–12 (Hoefer, 1994), or 18 months (Williams, 1976). Hence, the age of vaginal opening in the female ranges from 71–308 days (Kuroiwa and Imamichi, 1977). The placentation type is hemoendothelial (Williams, 1976).

Urinary System

Anatomy

The kidneys are approximately 18 $\times$ 13 $\times$ 5 mm, and 1 g (Kraft, 1987). They have a relatively thick medulla compared to the cortex (Prong et al., 1969), and the ratio has been measured as 5.6: 1 (Díaz and Cortés, 2003). The kidneys have elongated renal papillae (Díaz and Cortés, 2003; Prong et al., 1969), which protrudes into the ureter (Gutman and Beyeth, 1970; Prong et al., 1969). This thick medulla, with its long renal papilla, resembles that of some desert rodents (Prong et al., 1969). The ureters empty into the urinary bladder near its neck (Kraft, 1987).

Physiology

Chinchillas produce a wide (100-fold) range of urine concentrations, from diuresis at 60 mOsm/kg to a highly concentrated 6000 mOsm/kg (Prong et al., 1969). They are able to excrete a concentrated urine (Weisser et al., 1970), and can survive without drinking water from 8 days (Weisser et al., 1970) to 2 weeks (Prong et al., 1969). In general, mammals from arid and semiarid habitats tend to have a thick medulla relative to their kidney size, and long loops of Henle when compared to mammals from mesic habitats (Sperber, 1944). The chinchilla's urine-concentrating ability has been an object of study, as the kidneys have a thick medulla (Díaz and Cortés, 2003; Prong et al., 1969), thus the species can withstand long periods of water deprivation (Ness,

1999). Several studies have demonstrated that chinchillas can indeed efficiently concentrate urine. Weissner et al. (1970) observed that chinchillas were able to maintain a positive water balance (water loss did not exceed intake) when drinking a salt solution up to 0.6 Molarity NaCl. Further, chinchillas deprived of water up to 8 days had urine osmolality up to 7599 mOsm/kg with an average of 3505 mOsm/kg (Weisser et al., 1970), and chinchillas deprived of water for 2 weeks had urine concentrations of up to 6000 mOsm/kg (Prong et al., 1969). These high concentrations are comparable to those of desert rodents (Schmidt-Nielson and O'Dell, 1961). Chinchilla urine often has a specific gravity greater than 1.045 (Hrapkiewicz and Medina, 2007). Chinchillas also have high serum osmolality (Weisser et al., 1970), which is similar to other rodents in arid climates (Sakai et al., 1965).

Cardiovascular and Pulmonary Systems

Anatomy

Although there is a relative dearth of information on the cardiovascular and pulmonary systems in this species, recent studies on the cardiovascular system of chinchillas have revealed several features worth mentioning. Two arterial branches, the brachiocephalic trunk and left subclavian artery, originate from the aortic arch in a manner similar to rabbits and guinea pigs. The left common carotid branches from the brachiocephalic trunk; this trunk then divides into the right subclavian artery and the right common carotid artery (Ozdemir et al., 2008a). Internal carotid arteries are absent, as the blood to the brain is supplied solely by a vertebral-basilar artery system (Sportorno et al., 2004). Further, the chinchilla has only a left coronary artery. The left coronary artery arises from the aorta at the sinus of the aorta and courses towards the coronary groove, eventually dividing into paraconal, interventricular, and circumflex branches (Ozdemir et al., 2008b). Cardiac musculature in the chinchilla is distributed in both large-sized and medium-sized intrapulmonary venous walls (Endo et al., 1992). Eken and coworkers (2005) determined that the course of the chinchilla femoral artery and its branches are similar to the rat. The left lung has three lobes, whereas the right has four (Sportorno et al., 2004).

The cerebral arterial system is described in conjunction with the nervous system later in this chapter.

Musculoskeletal System (Figures 39.1–39.3)

Anatomy

The dental formula of the chinchilla is $2(I \ 1/1 \ C \ 0/0 \ P \ 1/1 \ M \ 3/3)$, and all teeth are open-rooted and grow continuously throughout life. Various authors cite different

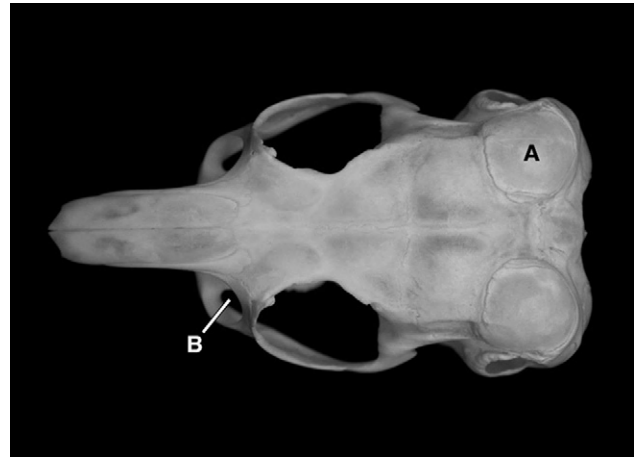


FIGURE 39.1 Dorsal view of the chinchilla skull (mandible not included). (A) Tympanic bulla; (B) infraorbital foramen.

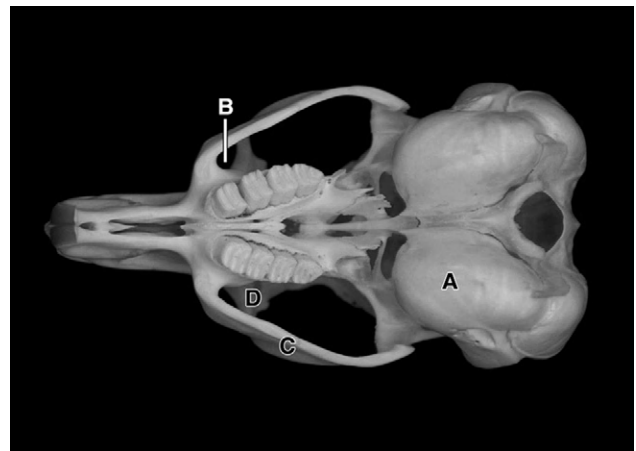


FIGURE 39.2 Ventral view of the chinchilla skull (mandible not included). (A) Tympanic bulla; (B) infraorbital foramen; (C) zygomatic bone; (D) lacrimal bone.

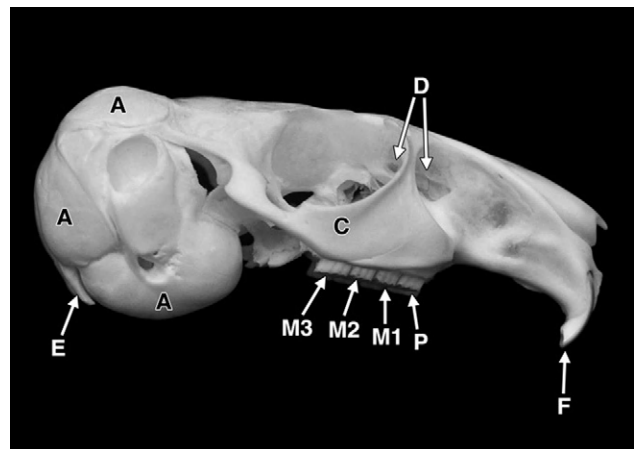


FIGURE 39.3 Right lateral view of the chinchilla skull (mandible not included). (A) Tympanic bulla; (C) zygomatic bone; (D) lacrimal bone; (E) paraoccipital process; (F) incisors; (M1) first molar; (M2) second molar; (M3) third molar; (P) premolar.

growth patterns for the yellow incisors of this species. Strake et al. (1996) and Hoefer (1994) indicate that incisors grow 4–6 cm per year; Sportorno et al. (2004) report that the incisors grow 5–7.5 cm per year. In contrast, Sulik et al. (2007) cite that incisors grow only 1.5–3 cm per year. The teeth are considered “aradicular hypsodont” (Crossley et al., 1998) and have long crowns with no anatomic roots (Brenner et al., 2005). The occlusal planes of premolar and molar teeth are slightly angled from buccal to lingual (Brenner et al., 2005). As chinchilla teeth do not form a true anatomic root, some authors refer to the portion of the root below the alveolar margin as the “reserve crown” (Brenner et al., 2005; Crossley et al., 2001). Chinchillas demonstrate an anisognathism whereby the mandible is wider than the maxilla (Brenner et al., 2005).

The skeletal system of chinchillas has several features worth mentioning. The skulls of male and female chinchillas are visually indistinguishable, and there is minimal skull growth beyond maturity (Crossley et al., 2001). The tympanic bullae are large, with three vesicular protuberances, and the mean total volume of the middle ear is $1.52 \text{ ml} \pm 0.26 \text{ SD}$ (Sportorno et al., 2004; Vrettakos et al., 1988). Further distinctive features of the chinchilla skull include large infraorbital foraminae, reduced zygomatic arches, an absence of a masseteric crest of the mandibles, large lacrimal bones, and lacrimal canals that open in the side of the rostrum (Brenner et al., 2005). The vertebral formula is C7, T13, L6, S2, Ca23, for a total of 51 vertebrae, with 13 pairs of ribs.

Studies of the anatomy of the chinchilla nasal cavity revealed that the naso- and maxilloturbinates of the chinchilla nasal cavity more closely resemble the simple turbinates found in other rodents, rather than the branched or complex turbinates found in canids and felids. The nasal mucosa of the chinchilla also has numerous lymphoid aggregates (nasal-associated lymphoid tissue, or NALT) similar to other rodents. The NALT is responsible for induction of both secretory and systemic immune responses in the upper respiratory tract (Jurcisek et al., 2003).

Digestive System

The digestive system of chinchillas presents a number of interesting characteristics which might influence a clinician's approach to their veterinary medical care.

Anatomy

Chinchillas possess a palatal ostium, an opening in the soft palate through which the oropharynx communicates with the rest of the pharynx (Hillyer et al., 1997; Sportorno et al., 2004). Martinez et al. (2000) report that the posterior third of the tongue's dorsal surface has a

pair of vallate papillae, which is similar to that seen in insectivores and armadillos. The gallbladder is located between the right and median sides of the four-lobed liver. In the chinchilla liver, there is little lobulation which is grossly visible at the surface (Lyon et al., 2003; Sportorno et al., 2004). Hopkins and Whidden (1959) reported observing “sex chromatin” bodies in hepatocytes of most female chinchillas, a finding which has not been reported elsewhere.

The stomach is pyriform-shaped (Sportorno et al., 2004), similar to that of a horse or pig (Lyon et al., 2003). The gastrointestinal tract itself is very long, and the length of the small and large intestine together often exceeds 3 m in adult animals (Hoefer, 1994; Johnson-Delaney, 2006; Sportorno et al., 2004). The large intestine is 1.5 times longer than the small intestine (Chen-Pan and Fujiwara, 1989), although the jejunum nearly fills the entire abdominal cavity (Hoefer, 1994; Hrapkiewitz and Medina, 2007). The volume of the cecum is relatively small compared to the volume of the colon. The cecum holds 23% of the dry matter content of the large intestine compared to the rabbit (57%) and guinea pig (44%). The thin-walled, sacculated, and coiled cecum is comparable to that of the pig, horse, and rabbit (Chen-Pan and Fujiwara, 1989).

Physiology

Similar to other rodents, chinchillas do not vomit, and are hindgut fermenters (Jenkins, 1992). Chinchillas produce two types of fecal pellets; a nitrogen-rich pellet for cecotrophy, and a nitrogen-poor pellet as a typical fecal pellet (Donnelly and Brown, 2004) at a 3:1 ratio (Holtenius and Bjornhag, 1985). Ebino (1993) reports that chinchillas, guinea pigs, and rabbits all exhibit “vigorous coprophagy” as compared to mice, rats, hamsters, and voles. Holtenius and Bjornhag (1985) characterized the “colonic separation mechanism” in chinchillas, a concept which is further explored by Sakaguchi (2003) and Wolf et al. (2003). These authors found that chinchillas exhibited a diurnal activity rhythm, in that they eat and deliver all of their fecal pellets at night, and are inactive during the day except for periods of cecotrophy. This “mechanism”, found in both chinchillas and guinea pigs, is characterized by a longitudinal furrow in the proximal part of the colon which facilitates production of caecotrophs by which bacteria are moved towards the cecum via an antiperistaltic movement. This capability permits rapid passage of less-digestible food particles while retaining microorganisms and more digestible food particles in the cecum to allow adequate opportunity for fermentation (Bjornhag and Snipes, 1999). Worthington and Fulghum (1988) explored the cecal and fecal bacterial flora in healthy chinchillas, isolating species of *Bifidobacterium*, *Bacteroides*, *Eubacterium*, and anaerobic *Lactobacillus*.

Nervous and Endocrine Systems

Endocrine System

ANATOMY

The endocrine glands of the chinchilla have not been shown to be significantly different in structure or location from those of other mammals. The adrenal glands are elongated and large (Williams, 1976), dirty white to yellow, and dimorphic in size, shape, weight (Kraft, 1987). The location of the thymus is entirely thoracic (Cartee, 1979; Hrapkiewicz and Medina, 2007). It is located at the base of the heart and extends along the heart's ventral surface approximately half of the distance between the base and the apex (Cartee, 1979). Histologically, the cortical and medullary areas appear similar to other species (Cartee, 1979). Studies have documented age-related changes in the thymus. Chinchillas aged 2–8 weeks were compared with chinchillas aged 4–8 years. In general, there was involution with age. The thymus of older chinchillas was lighter in color and more lobulated. There was decreased parenchymal tissue, a lower concentration of lymphocytes, and increased adipose tissue (Cartee, 1979). The pituitary gland has a ventrocaudally oriented infundibulum (pituitary stalk), and the glandular anterior pituitary is positioned nearly horizontal (Kraft, 1987). The thyroid gland's isthmus is only sometimes present (Kraft, 1987).

Nervous System

GENERAL ANATOMY

In chinchillas, blood to the brain is supplied only through the vertebral arteries, with no internal carotid blood supply (Gielecki et al., 1996; Jablonski and Brudnicki, 1984). The vertebral arteries anastomose to form the ventral basilar artery, which then divides cranially to form the cerebral arterial circle. The cerebral cortex is supplied by three cerebral arteries branching from this cerebral arterial circle: caudal, medial, and rostral (Panesar et al., 2001). The cerebral arterial circle resembles that of other rodents (Panesar et al., 2001), but it is open rostrally, because the rostral cerebral arteries do not anastomose to complete the circle of Willis (Gielecki et al., 1996). The rostral arterial circle is also asymmetric, due to the unusually strongly developed right anterior cerebral artery (Kuchinka et al., 2005). The middle cerebral artery supplies the ventral surface of the cerebral hemisphere via three branches – the rostral, caudal, and central (striated) branches (De Araujo and Campos, 2009).

There is little published describing the chinchilla nervous system in general, although the auditory cortex has been described. The primary auditory cortex is 2–3 mm caudal to the medial cerebral artery (Panesar et al., 2001). The auditory cortex is made of distinct

regions in the temporal cerebral cortex. The structure and number of regions varies with species. In chinchillas, there are regions dorsal and caudal to the primary auditory cortex in which the neurons are less responsive to tone stimuli but more broadly tuned and complex than neurons in the primary auditory cortex (Pienkowski and Harrison, 2005). In chinchillas, three separate auditory areas have been revealed in the temporal cortex by optical imaging of intrinsic signals, a method of indirectly monitoring neural activity in the cortex. These areas correlate with the primary auditory cortex, and two secondary auditory areas. The primary auditory cortex is the most dorsocaudal field, with the secondary areas ventral and rostral to it. Neuron response latencies in the primary auditory cortex are shorter than those in the secondary auditory areas (Harel et al., 2000). The cochlear nucleus is large and well developed, but its organization is generally similar to that described in other mammals (Morest et al., 1990).

The epicardial ganglia have been examined. The highest density of epicardial ganglia is on the ventral surface of the right atrium. There is another, less dense plexus on the left atrium, and a few small ganglia on the ventricles' dorsal surfaces (Kuder et al., 2003).

The trigeminal ganglion has also been described. It is closely connected to the trigeminal nerve where it divides into the ophthalmic and maxillary nerves (Szczerkowski, 2005).

Special Senses (Vision, Olfactory, Somatosensation, Taste, Auditory)

Auditory System

ANATOMY

The auditory system of the chinchilla has been closely examined. Chinchilla ears have features characteristic of species adapted to open, arid areas. These include a large middle-ear space divided into compartments; a large tympanic membrane and ossicular dimensions; and increased mechanical sensitivity to low-frequency acoustic stimuli relative to other animals with a similar hearing range (Rosowski et al., 2006). The tympanic membrane is difficult to see via the external ear canal because the canal runs dorsal–ventral (Browning and Granich, 1978). The tympanic membrane diameter is an average of 8.3–8.5 mm, and the circumference is an average of 26.5 mm (Vrettakos et al., 1988).

Beyond the tympanic membrane is the middle ear, with the auditory bullae and conductive ossicles. The chinchilla is known for its large auditory bullae, which are thin-walled (Hillyer and Quesenberry, 1997), well-developed, and extend upwards towards the top of the skull (Williams, 1976). Because the bone of the bulla is

very thin, its strength relies on a large number of internal septae (Browning and Granich, 1978). The dorsal part of the bulla is subcutaneous, and mostly palpable on the top of the head, except for the anterior and posterior rims which are covered, respectively, by the temporal and platysma muscles (Browning and Granich, 1978). The labyrinthine part is at the bottom of the bulla, and is palpable in the neck. The digastric muscle originates from its surface. The eustachian tube enters into the labyrinthine part, but aerates the entire bulla (Browning and Granich, 1978). The eustachian tube has a vertical orientation (Hoa et al., 2009). The mastoid part of the bulla sits in the middle, but is not palpable because it is covered by the occipital muscles (Browning and Granich, 1978). The total middle ear air volume is large (Rosowski et al., 2006; Vrettakos et al., 1988), and has been measured from 1.5–2.8 cc (Drescher and Eldredge, 1974; Rosowski et al., 2006; Teas and Nielson, 1975; Vrettakos et al., 1988). The middle ear's multiple compartments are joined by narrow passages (Browning and Granich, 1978), so the air space is segmented into multiple subcavities (Browning and Granich, 1978; Vrettakos et al., 1988; Williams, 1976). Some details about the ossicular chain (malleus, incus, stapes) have been published. The structure of the chinchilla's conductive apparatus, similar to the human's, is described as "freely mobile", in which the malleus and incus are attached by suspensory ligaments and are able to move independently. This contrasts with the less highly evolved "micro-type" apparatus of other species such as the mouse, gerbil, and hamster, in which there is a bony fusion between the malleus and the tympanic ring (Fleisher, 1978).

The handle of the malleus and posterior margin of the long process of the incus are almost perpendicular to the rotational axis (Vrettakos et al., 1988). The joint between the malleus and the incus is fused, and these two ossicles form a curved bar which fits snugly into the epitympanic recess (Vrettakos et al., 1988). The long process of the incus articulates with the stapes (Browning and Granich, 1978) at a right angle (Vrettakos et al., 1988). The stapes has been measured at 1.78 mm high (Vrettakos et al., 1988). Its footplate is convex on the labyrinthine (medial) side and concave on the tympanic (lateral) side (Vrettakos et al., 1988). It has a toe oriented posteriorly, and its crura are more central than in mice (Vrettakos et al., 1988) or humans (Browning and Granich, 1978; Vrettakos et al., 1988). The crura provide the surface onto which the stapedius muscle inserts (Vrettakos et al., 1988). There is no stapedal artery (Vrettakos et al., 1988). The tensor tympani and stapedius muscles are well developed (Vrettakos et al., 1988).

The chinchilla's inner ear is easily accessible, via the large middle ear bulla (Panesar et al., 2001). The cochlea makes $3\frac{1}{2}$ turns, and projects into the labyrinthine part of the tympanic bulla (Browning and Granich, 1978).

The organ of Corti averages 18.4 mm in length and has 102 inner and 405 outer hair cells per mm (Browning and Granich, 1978). The oval window's diameter has been measured at 2.19×0.78 – 0.93 mm, with an average area of 1.55 mm^2 , and the round window's diameter has been measured at 1.4×0.95 mm (it is oval in shape) with an average area of 1.06 mm^2 (Vrettakos et al., 1988). The round window membrane is thin, one-sixth of the thickness of that of the human (Chen et al., 1999).

PHYSIOLOGY

Chinchillas are frequently used in hearing research due to the easy access to the inner ear via the large auditory bullae. The chinchilla's auditory sensitivity is similar to that of humans (Henderson and Hamernik, 1973) and thus has been extensively investigated. Chinchillas are more sensitive than humans at lower frequency ranges (<1100 Hz) and higher frequency ranges (>6500 Hz). In the middle range of 1100–6500 Hz, chinchillas are less sensitive than humans (Miller, 1970). Chinchillas also appear to be more sensitive than guinea pigs, except at very high frequencies (Miller, 1970). In general, chinchillas have increased mechanical sensitivity to low-frequency acoustic stimuli relative to other animals with similar hearing ranges (Fay, 1988; Miller 1970).

Chinchillas show presbycusis. McFadden et al. found a small but significant decline of auditory sensitivity and outer hair cell function in 44 chinchillas examined between 3–15 years of age (McFadden et al., 1997). This age-related hearing loss is similar to age-related hearing loss in humans and more similar to humans than that of rodents with shorter life spans. As in humans, the high-frequency losses exceed low-frequency losses, and the rate of loss in chinchillas approximates that of the human male (McFadden et al., 1997).

Vestibular System

ANATOMY

The semicircular canals sense angular acceleration. The semicircular canals' orientation is relatively planar, and all of them have a similar deviation from that plane (approximately 10°) (Hullar and Williams, 2006).

PHYSIOLOGY

Because their semicircular canals are all relatively planar with similar deviations from planarity, chinchillas are relatively equally sensitive to rotations in all directions (Hullar and Williams, 2006).

Olfactory System

ANATOMY

The chinchilla's bilateral vomeronasal organs are about 6 mm long, lie along the base of the nasal septum, and are encircled rostrally by cartilage, and caudally by

bone (Oikawa et al., 1994). It communicates with the nasal cavity via a pore at the rostral end (Oikawa et al., 1994).

PHYSIOLOGY

Histologically, the vomeronasal organ appears functional (Oikawa et al., 1994).

Vision

ANATOMY

The internal anatomy of the eye includes an anterior chamber of moderate depth and a large, spherical lens (Peiffer and Johnson, 1980). The large and spherical lens is characteristic of a nocturnal animal (Donnelly and Brown, 2004). Another nocturnal characteristic is the sensitive retina (Donnelly and Brown, 2004), which is anangiotic, without visible vessels (Peiffer and Johnson, 1980; Williams and Kern, 2008). The optic disc is gray, with a variable shape, being round to oval, in either a horizontal or vertical plane (Peiffer and Johnson, 1980). The vascularization of the optic disc varies (Williams and Kern, 2008). For example, small blood vessels were visible on the optic disc of approximately half of the chinchillas examined by Peiffer and Johnson (1980). The size of the physiological (i. e., optic) cup varies, from a small central depression on the optic disc to almost total involvement of the nerve head (Peiffer and Johnson, 1980). The average intraocular pressure is 18.5 mmHg (Peiffer and Johnson, 1980).

PHYSIOLOGY

There are no reports of the chinchilla's vision being different from that of other rodents.

BEHAVIOR

Chinchillas are described as gentle, docile, quiet, timid (Hillyer and Quesenberry, 1997; Lyon, 2003; Riggs and Mitchell, 2009), lively, and curious (Hillyer and Quesenberry, 1997; Hoefer and Crossley, 2002; Johnson, 2006; Lightfoot, 1999; Lyon, 2003; Merry, 1990). They use their mouths to explore, and may perform trial bites (Johnson, 2006) when exploring an object or person. They also gnaw regularly (Hoefer, 1994; Hoefer and Crossley, 2002), similar to other rodents. Chinchillas are naturally nocturnal, being active at dusk and at night in the wild, but in captivity they can adapt to a diurnal lifestyle (Hoefer 1994; Johnson-Delaney, 1998; Hillyer and Quesenberry, 1997; Lyon, 2003; Riggs and Mitchell, 2009; Webb, 1991). They are active (Riggs and Mitchell, 2009) and agile, and enjoy climbing and jumping (Hillyer and Quesenberry, 1997; Hoefer 1994; Hoefer and Crossley, 2002; Johnson, 2006; Johnson-Delaney, 1998). They are "excellent leapers" (Honeycutt, 2003),

and can jump over 6 feet high, with no apparent fear of heights (Johnson, 2006). They will run on an exercise wheel (Johnson, 2006).

Chinchillas are mainly herbivorous, eating grasses and seeds. However, they will also eat insects (Honeycutt, 2003). Chinchillas show biphasic ingestion behavior. An examination of chinchilla ingestion indicated that their highest level of ingestion (70% of their daily intake) is from 9pm–7am, with peak ingestion from 9pm–11pm. No ingestion was seen from 7am–3pm (Wolf, 2003). Their daily food intake was approximately 4–5% of their body weight (Wolf, 2003). Daily water intake was low, at 20–40 ml/chinchilla (Wolf, 2003). Chinchillas eat by sitting upright (Nowak, 1999) and holding food in their front paws (Honeycutt, 2003; Johnson, 2006; Williams, 1976). They sometimes throw pellets out of their cages (Williams, 1976). They are also coprophagic (Johnson, 2006; Lightfoot, 1999).

Generally, chinchillas will urinate in a specific location (Coulton, 1989; Johnson, 2006), and will use a litter box (Johnson-Delaney, 1998). However, they do not always defecate in the same specific place (Coulton, 1989; Johnson, 2006), and are harder to train to a litter box for defecation (Johnson-Delaney, 1998).

Chinchillas sit upright when grooming and sunbathing (Nowak, 1999). When they sleep, their most common posture is to be upright, but other normal postures include lateral or dorsal recumbency, or even wedged upside down in tight spaces (Johnson, 2006).

Chinchillas are fastidious groomers (Johnson, 2006). Part of their normal grooming behavior is dust-bathing (Donnelly and Quimby, 2002; Johnson-Delaney, 1998; Webb, 1991). They dust-bathe in the wild (Merry, 1990), and in captivity a dust bath must be provided to remove lipids from the fur (Barber and Thompson, 1990) and maintain their coat health (Hrapkiewicz and Medina, 2007). Some chinchillas chew on their own fur, or that of other chinchillas, a behavior known as "fur-chewing" (Kitchen and Kitchen, 1967). Because self-chewers can only chew off the fur they can reach on their lower, caudal body, they can look like a lion, with motley fur (Hrapkiewicz and Medina, 2007). Trichobezoars are found in the stomachs of fur-chewers at necropsy (Hrapkiewicz and Medina, 2007) and are associated with fur-chewing (Donnelly and Quimby, 2002). The definitive cause is unknown (Hrapkiewicz and Medina, 2007). Some causes postulated have been maladaptive displacement behavior (Donnelly and Quimby, 2002), boredom, housing stress, malnutrition (Eidmann, 1992), poor diet (low fiber), or hereditary factors (Merry, 1990; Webb, 1991). Fur-chewing does occur more in certain families (Hrapkiewicz and Medina, 2007). Hormonal imbalances have also been suspected. Thyroid hyperplasia has been correlated with the size of the area of the body with fur removed, and the hyperplasia was interpreted

as a reaction to loss of insulation with respect to body heat (Eidmann, 1992). In fur-chewing chinchillas, there is increased hormone activity of the thyroid and adrenal glands consistent with stress-induced changes. Varonjack and Johnson (1973) compared thyroid activity and corticosterone levels in fur-chewers to those of non-fur-chewers. Both were higher in the fur-chewers. Additionally, changes in the adrenal glands and skin consistent with hyperadrenocorticism have been described in fur-chewing chinchillas, suggesting that stress and genetic factors are involved (Tisljar et al., 2002).

Chinchillas are creatures of habit, do not adjust well to change (Johnson, 2006), and are easily frightened (Webb, 1991). They are sensitive to noise, startle easily, and are disturbed by unfamiliar sounds (Johnson, 2006). When alerted to potential danger, they will stand on their hind limbs (Johnson, 2006). In general, their defense mechanism is flight rather than fight (Hillyer and Quesenberry, 1997; Johnson, 2006), and when they are fearful, they run for cover or cower in corners or small spaces (Johnson, 2006). As a prey species, they also hide illness (Riggs and Mitchell, 2009).

Chinchillas are social, and live in colonies of multiple smaller family groups in the wild (Hoefer, 1994; Honeycutt, 2003). These colonies can be composed of several hundred individual (Donnelly and Brown, 2004; Donnelly and Quimby, 2002; Nowak, 1999). Due to their social nature, they can generally be housed as pairs, single-sex groups, or polygynous (harem) groups in captivity (Hoefer and Crossley, 2002; Johnson-Delaney, 1998), although they have been reported to be monogamous in the wild (Brower, 2006; Honeycutt, 2003).

Intraspecies behaviors include physical contact. Chinchillas participate in allogrooming, and family groups huddle when at rest (Kleiman, 1974). Another interactive behavior is the "frisky-hop", an active performance of vertical leaps, body twists, head tosses, racing, pivoting, and prancing, which involves little physical contact (Kleiman, 1974).

They rarely fight (Hoefer, 1994; Hoefer and Crossley, 2002), but they can be aggressive (Weir, 1967). When fighting, they will sometimes kill each other. They kill by climbing onto the opponent's back and biting the back and neck (Johnson, 2006). Generally, aggression occurs during breeding or introduction (Johnson, 2006). Chinchillas can be territorial and will defend against intruders (Johnson, 2006). Female chinchillas are dominant, with a larger body size and higher levels of aggression than males (Donnelly and Quimby, 2002; Johnson, 2006). Anecdotally, females are more territorial (Johnson, 2006). A female in estrus can be aggressive (Johnson, 2006), especially towards males (Brower, 2006; Williams, 1976), and this must be taken into consideration when breeding chinchillas. Females can be aggressive if approached by a male if she is not ready to copulate. She may jump

vertically, and then kick him or urinate on him (Bignami and Beach, 1968). Near parturition, females can also show increased aggression towards previously compatible males (Strake et al., 1996). After parturition, females with pups may show aggression towards males, even the father (Brower, 2006). Females are also aggressive towards other females' young. If one female's offspring get into the cage of another female, she may kill the offspring (Weir, 1967). However, orphans can be adopted by another lactating chinchilla (Strake et al., 1996; Webb, 1991; Williams, 1976) or a guinea pig (Strake et al., 1996; Webb, 1991). Females which were raised together, or mother and daughter pairs are compatible (Johnson, 2006). However, newborn pups may be aggressive with each other, especially if there are more than two of them (Johnson-Delaney, 1998). Males may also show aggressive behavior, and intact males housed in a group may fight (Johnson, 2006). Chinchillas are known to use an unusual form of defense or protest: spraying urine at the offending party. Chinchillas urinate as a defensive or perhaps aggressive behavior. Chinchillas may project urine 2–3 feet with accurate aim in defense (Weir, 1967) or if annoyed (Donnelly and Brown, 2004; Williams, 1976). Both females and males defend their territory with this method, but females reportedly have better aim in general (Johnson, 2006).

Chinchilla are highly vocal (Honeycutt, 2003), and their vocalizations have been studied to produce guidelines as to their likely meanings. Donnelly and Brown (2004) described many vocalizations. The "click" sound has a similar function to the "chutt/cluck" of guinea pigs. A female will click at her nursing pups if they nip her. A young chinchilla uses a "squawk" to elicit a grooming response by its mother. A chinchilla that is injured or handled awkwardly may "squeal". Other chinchillas react to this sound by leaping around in their cages. They will "tooth chatter" when they are confronted with an unknown chinchilla or are being aggressive for another reason. During fighting, they may make a "rasp-like snarl". Males make a "purr" sound after mating, which is described as sounding like hiccups. Others have categorized some vocalizations. Chinchillas "bark" when they are defensive or irritated, "honk" for attention, (Johnson, 2006); and "chattering teeth" or "growling" sounds are used as threat noises (Nowak, 1999). "Soft cooing" indicates contentment; a "spitting growl" is made when upset; and chinchillas "cry" when injured or frightened (Merry, 1990).

Chinchillas can habituate to humans when handled frequently at a young age (Hillyer and Quesenberry, 1997). They respond best to gentle handling and a light touch (Hrapkiewicz and Medina, 2007), and are generally easy to handle (Donnelly and Brown, 2004; Donnelly and Quimby, 2002). They are not usually aggressive towards people (Hrapkiewicz and Medina, 2007; Johnson, 2006; Weir, 1967; Williams, 1976) and rarely bite

(Hillyer and Quesenberry, 1997; Hoefer 1994; Johnson-Delaney, 1998; Lyon, 2003; Webb, 1991). However, they may bite if agitated (Donnelly and Brown, 2004; Hrapkiewicz and Medina, 2007; Weir, 1967; Williams, 1976), although the bite may be preceded by a warning nip (Donnelly and Brown, 2004).

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Management, Husbandry, and Colony Health

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INTRODUCTION

Chinchillas (*Chinchilla laniger*) have proven to be a practical animal model because of their small size, ease of handling, long life span (12–20 years), and relative freedom from diseases that interfere with research. Maintaining chinchillas in a laboratory setting is easily accomplished because their care and husbandry are similar to that of rabbits and other hystricomorph rodents. Facilities already housing rabbits and other rodents can readily adapt to housing chinchillas with few problems.

HOUSING SYSTEMS

Caging Systems

Chinchillas are housed in either solid-bottom or suspended (metal or plastic) cages. Solid-bottom caging is preferred for pregnant females that are nearing parturition. Standard rabbit cages are typically used for housing chinchillas in the laboratory setting because they are readily available and because cages designed specifically for housing chinchillas in a research setting are not commercially produced. Care should be taken to



FIGURE 40.1 Chinchilla caging. (A) Standard plastic rabbit cages can be modified for housing chinchillas. White arrow indicates potential escape point. (B) Close-up of unmodified rabbit cage. White arrows indicate potential escape points. Note that when dropping pan from the above cage is removed, chinchillas can escape between individual cage units. (C) Close-up of rabbit cage modified for housing chinchillas. Black arrows indicate where additional enclosures have been added at potential escape points. Arrow with asterisk points to stainless steel panel added horizontally between individual cages to prevent escape when dropping pans are removed.

evaluate the spacing of metal bars or perforated flooring when using rabbit cages for housing chinchillas. A minimum of 15×15 mm has been recommended to prevent injuries to limbs (Hoefer, 1994) and to prevent escape of neonates in breeding situations. Animals housed on suspended metal floors should also be provided with a solid resting board or other enrichment devices on which to rest. A further consideration when using suspended plastic cages is to ensure that the chinchillas cannot escape since their bodies fit in any opening through which their skull fits. Many plastic rabbit cage systems currently produced allow the escape of chinchillas unless modifications are made to close potential points of egress (Figure 40.1).

There are no published floor space or height requirements for chinchilla housing but chinchilla fancier and breed organizations recommend approximately 1–2 ft² per animal (Donnelly and Brown, 2004) and 12 inches of cage height. Since most commercially available rabbit cages exceed these dimensions, pair and group housing of chinchillas is easily accomplished. Chinchillas are very active and will climb and jump when provided

with enrichment devices and sufficient space to exhibit these behaviors.

Bedding

Hardwood shavings, heat-treated softwood shavings, or paper-based (cellulose) animal bedding are used in solid-bottom cages for chinchillas. Pine shavings and untreated softwood products should be avoided because these products can contain aromatic hydrocarbons that may alter drug and anesthetic metabolism, and induce respiratory problems. For animals housed in suspended cages, dropping pans are handled like those of rabbits. The pans can be lined with a variety of absorbent materials including plastic-backed paper products, cardboard, pelleted paper, or alfalfa pellets.

Chinchillas need regular access to a dust bath because they produce a significant amount of lipid from their sebaceous glands like other rodents adapted to arid environments (Barber and Thompson, 1990). Without access to a suitable substrate, their fur becomes matted and greasy. Chinchilla dust is generally a mixture of

silver sand and Fuller's earth (a type of clay) but a variety of dusting materials including diatomaceous earth, talc, cement byproducts, and volcanic ash are available (Donnelly and Brown, 2004). It should be noted that talcum has been shown experimentally to induce pulmonary granulomas and epithelial hyperplasia (Trautwein and Helmboldt, 1967). Avoiding dust containing this material may be warranted.

Recommendations for the frequency of offering bathing substrate range from daily to monthly. In our experience, a 3-day-a-week schedule (Monday, Wednesday, and Friday) in the laboratory setting is sufficient. The dust bath should be removed from the primary enclosure daily so that it can be cleaned of urine and feces. Dust can also be an irritant to the eyes and daily removal will prevent overuse. Dust baths should not be shared between cages of animals since chinchillas can be inapparent carriers of ringworm dermatophytes and the dust and bath can serve as a fomite for these agents. Marking the individual baths with the cage identifier allows for storage and reuse. Otherwise, empty the bath and replace with fresh dust in a clean, sanitized bath or disposed of the bath daily. In a research setting, standard mouse shoebox-style cages work well as dust baths.

ENVIRONMENTAL CONDITIONS

Ventilation

The recommended ventilation rate of 10–15 air changes per hour (NRC, 1996) is usually appropriate for rooms housing chinchillas. Actual ventilation rates should take into account the number of animals housed in the room, room dimensions, frequency of cage/dropping pan changing, and the temperature and humidity requirements of the animals (see Temperature and Humidity below). Caging should be situated such that animals are not subject to drafts from the ventilation system.

Illumination

A survey of the literature indicates that chinchillas in research institutions are typically housed in conditions of 12 hours of light and 12 hours of darkness or 14 hours of light and 10 hours of darkness. Some articles describe the use of natural photoperiod similar to what the animals would be exposed to in the wild (Busso et al., 2005, 2007). A 14 light: 10 dark photoperiod approximates the conditions at maximum day length (i.e. summer solstice) in the chinchillas' natural habitat. This photoperiod may be more desirable when breeding is occurring.

One report in the literature indicates that the spectrum of artificial (incandescent) light used to maintain

chinchillas can influence the sex ratio of offspring (Ott, 1964). When housed outdoors under natural light, chinchillas produced an equal distribution of male and female offspring but when moved indoors under daylight incandescent bulbs, litters were predominantly male. When the bulbs were changed to an alternate spectrum, sex ratios returned to normal.

Temperature and Humidity

Chinchillas are very resistant to hypothermia and are known to withstand sub-freezing temperatures in their natural habitat while showing a significant sensitivity to heat. At temperatures above 35°C, chinchillas appear stressed and mean body temperatures rise significantly (Orsetti and McManus, 1971). The thermoneutral zone of chinchillas has been established to be between 20 and 35°C (Arends and McNab, 2001; Gorecki, 1967). A stable temperature of 18–22°C is therefore recommended as optimal for chinchilla housing (Donnelly and Brown, 2004).

Chinchillas are also relatively sensitive to elevated humidity. Their natural habit in northern Chile has an average humidity below 60%. Prolonged elevations in humidity above 60% can result in adverse effects on hair coat quality and contribute to the risk of heatstroke with elevated temperatures (Hoefer, 1994). The recommended humidity range for chinchillas is 40–60%.

Enrichment

Environmental enrichment for chinchillas can be similar to that provided for rabbits. Toys that can be gnawed to assist in wearing down teeth should be provided. Wood blocks (e.g., birch, oak, maple), young tree branches (e.g., elm, grapevines, birch), bark (e.g., apple, pear, peach, ash trees) or porous stones (e.g., pumice) can be given for gnawing (Donnelly and Brown, 2004). Appropriately sized plastic or hard rubber toys (e.g., dumbbells, balls or drums with bells, KONG®, Nylabone®) are readily accepted and chinchillas will orally manipulate stainless steel chains and other items hung on the front of the cage. Because chinchillas are adept and relentless chewers, it is critical that the condition of enrichment items be assessed daily and damaged toys removed. In our experience, gastrointestinal foreign bodies have not been a clinical issue; however daily observation for missing pieces of enrichment devices is advisable. For animals housed in suspended caging, checking the cage pan may reveal bells and other small, missing pieces of concern.

Chinchillas are a shy, prey species and in the wild they conceal themselves in rock crevices (Donnelly and Brown, 2004). This habitat can be mimicked in captivity by providing them with a structure in which to hide (e.g., a plastic igloo, T- or L-shaped sections of

polyvinyl chloride plumbing pipes). When structures with flat tops are provided, the animals will also use these as a perch.

Food treats can be provided in moderation, either in the feeder, on the cage floor or on top of structures. Acceptable treats include hay (loose or cubed), fresh or dried produce (e.g., apples, carrots, figs, pears, raisins, sweet potatoes, and snow peas), nuts and seeds (e.g., hazelnuts, sunflower seeds), and grain or cereal mixes (Hoefler, 1994; Lightfoot, 1999; Quesenberry et al., 2004; Ryerson, 2009). Fresh produce should be well washed to minimize the risk of introducing pathogens. Perishable items should be removed daily to prevent consumption of spoiled produce. Any changes in types of foods provided, including treats, should be done slowly to avoid the development of enteric problems (Hoefler, 1994).

Sanitation

The sanitation schedule for the chinchillas' primary enclosures is dependent on the type of housing system used. Solid-bottom caging is changed as often as necessary to keep the animals clean and dry; and is dependent on the size of the cages, the bedding material and the animal housing density.

Dropping pans from suspended cages are changed at a minimum once a week but more typically 2–3 times a week to remove accumulated urine and feces. Primary enclosures should be cleaned minimally once a month, but more commonly this is necessary every 2 weeks because of accumulation of urine scale in the enclosure. Individual facilities should establish standard operating procedures for cage cleaning based on housing density and experience with particular cage types. However, primary enclosures should be cleaned whenever they become visibly soiled.

The room sanitization schedule should take into account the fur and dander generated by these animals as well as the use of the dust bath. Chinchilla rooms may need to be swept and mopped daily after the use of the dust bath because dust may be thrown from the primary enclosures during use. The walls of the room can also become coated with the dust and should be monitored for such accumulation and wiped down as appropriate. If filters are present on the HVAC exhaust, these should be monitored regularly for accumulation of dust and fur.

NUTRITION

Feed Type

Ideally a palatable pelleted formulation should constitute the bulk of the chinchillas' diet along with daily

high-fiber supplementation (grasses or hays) since most pelleted diets alone have insufficient fiber to maintain the health of the lower intestinal tract. Several commercially produced chinchilla diets that are modeled after guinea pig and rabbit formulations (based on the anatomic similarities of the lower gastrointestinal tract) are available (e.g. Mazuri® and Oxbow Animal Health). Unlike other hystricomorph rodents, chinchillas manipulate food items with their forepaws. The pellets should therefore be long enough to handle. For this reason, guinea pig and rabbit diets should not be substituted when a specific chinchilla formulation is available. Loose, mixed-feed diets should be avoided, since chinchillas tend to select out the most palatable components and leave the less desirable ones. Over-supplementing chinchillas with dietary treats can also alter intestinal microflora and motility. Supplemental grains, fruits, and seeds should be provided, but cautiously rationed either daily or weekly.

Nutritional Requirements

Current chinchilla-specific formulations provide crude protein 16–21.6%, crude fat 2.5–4.0%, and crude fiber 15–23% (Wolf et al., 2003). Chinchillas will consume between 4–5% of their body weight daily when offered a complete pelleted diet or hay (Wolf et al., 2003). If feed is provided *ad libitum*, most chinchillas will self-regulate feed intake. Chinchillas are nocturnal and consume more than 70% of their daily feed ration during the dark phase (Wolf et al., 2003), therefore a single daily feeding in late afternoon is acceptable.

Few studies on chinchilla nutrition have been performed and the early nutritional studies on daily ascorbic acid and thiamine requirements (King and Orcutt, 1952) lacked sufficient numbers of animals or were prematurely terminated due to disease onset (Larrivee and Elvehjem, 1954). These early studies therefore drew only tentative conclusions on nutritional needs of this species. While chinchilla-specific diets are available, their exact dietary needs, reflective of their natural intake, are still unknown.

Hydration

Chinchillas are native to arid habitats at high elevations and are physiologically adapted to low daily water intake. Similar to desert-dwelling rodents, the renal medulla of the chinchilla is significantly larger than the cortex, allowing for significant urine concentration and water retention (McManus, 1972). Water consumption correlates directly with the moisture content of the food provided, but on average is 20–40ml/day (Wolf et al., 2003). Given enough succulent feed, chinchillas can cope without drinking water.

To assure sufficient hydration, clean drinking water should be available each day (Donnelly and Brown, 2004). Most laboratory animal facilities provide water *ad libitum* either from bottles or an automatic watering system.

Nutrition-related Diseases

Fiber is critical to the chinchillas' diet and deficiencies in the amount and type of daily fiber can contribute to health problems. Chinchillas, like guinea pigs, have an open-rooted system for all teeth. Malocclusion is more prevalent in species with full open-rooted dentition (Legendre, 2002). It is most frequently associated with the uneven wear of the molars or "cheek" teeth. However, incisor abnormalities can also occur and if caught early these animals may have absent or limited clinical signs (Crossley, 2001). In moderate cases the animal can present with reduced appetite and hypersalivation due to oral pain. Bone spurs on the buccal or lingual aspect of the molars can cause trauma to the oral mucosa. Severe cases may also present with dental abscessation or fistulation into the palate and nasal cavity. Clinical evaluation of the oral cavity may prove to be very difficult unless animals are chemically immobilized. The oral cavity is long and narrow, making visualization of the molars challenging even with the use of a speculum. Examination of the oral cavity may require anesthesia. Radiograph and CT imaging can be used to assist in the examination and diagnosis. Although malocclusion is considered a polygenic trait, inadequate nutrition also contributes to the development and progression of this condition. Providing long-stranded coarse hays and grasses allows for appropriate mechanical wear on the teeth compared to consumption of fiber through highly processed pelleted diets alone. Proper dental trimming should occur as often as necessary to maintain healthy weights and minimize oral pain in animals with malocclusion.

Fiber deficiencies can also contribute to reduced intestinal motility (constipation). A diet predominated by highly digestible energy and proteins and limited in fiber will significantly reduce gastrointestinal motility. To prevent constipation, provide chinchillas with grass hay or high-fiber leafy greens on a regular basis. If chinchillas become constipated, supplementation with fresh apple, pear, or leafy greens is therapeutic (Quesenberry et al., 2004).

Chinchillas are highly sensitive to mycotoxins and can die from acute aflatoxicosis. Aflatoxins are a group of naturally occurring mycotoxins that can grow on a wide variety of improperly stored feed, particularly when stored under conditions of high humidity and high temperatures. Gonzalez Pereyra et al. (2008) described an outbreak of acute aflatoxicosis that resulted in the death of 200 animals on an Argentine

chinchilla farm. Feed analysis identified the presence of aflatoxins B₁, B₂, G₁, and G₂ in the pelleted oat-based commercial feed. Diagnosis of aflatoxicosis is difficult because clinical signs are non-specific, but may include inappetence, weight loss, diarrhea, and discoloration of the fur and skin. Grossly, livers were friable and were characterized by generalized hepatomegaly with diffusely pale areas throughout the parenchyma. Histologic changes were characterized by moderate to severe intrahepatic vacuolation and lipid deposition. Diagnosis requires feed analysis and corresponding histopathologic lesions.

BREEDING

Reproductive Biology

Chinchillas are sexually dimorphic, with adult females typically larger than males (Table 40.1). Sexing chinchillas through examination of the genitalia can be challenging since the urethral papilla (urethral cone) in females strongly resembles the penis in its location and appearance. It is prominent, cone-shaped, and situated ventral to the vaginal opening (Strak et al., 1996). However, chinchilla sex is best determined by measurement of the anogenital distance and may be performed at a very young age. Like most rodents, this distance is significantly greater in males than in females. Sexing females during estrus can be easier since the vaginal closure membrane is perforated at this time and the perineum intensely deep red in color (Brookhyser and Aulerich, 1980). The testicles are not typically well visualized because they are situated just beneath the skin overlying the inguinal canal or retracted into the abdomen and a true scrotum is not present (Quesenberry et al., 2004). The testicles become more prominent when females are present. Gentle pressure along the prepuce will expose the penis.

Puberty in females occurs between 4–5 months of age; however, they are typically 8–10 months old before the first breeding estrus occurs. Females are seasonally polyestrous, with a 7-month breeding period (November to May) and a 5-month anestrus period in their natural habitat. In captivity, under a constant light-cycle, chinchillas breed year round with a mild breeding depression in the winter months. Chinchillas are spontaneous ovulators with cycling occurring every 30–50 days throughout the breeding season. Females can demonstrate a post-partum estrus; however, breeding during this period is typically not productive. Chinchillas raised in captivity typically only breed until 10 years of age despite their 20-year lifespan.

Chinchillas, similar to other species in the suborder Hystricomorpha, have a relatively long estrus cycle compared with other rodents. Vaginal cytology

TABLE 40.1 Reproductive Physiology

	Females	Males
Adult weight (g)	400–600	400–500
Puberty (months)	4–5	3–4
Mating maturity (months)	8–9	17
Reproductive duration (years)	1–10	1–10
Estrus (days)	28–35	
Implantation (days)	5.25	
Gestation (days)	105–115	
Litter size	1–6	
Weaning (weeks)	6–8	

is a reliable method for determining the sexual cycle in chinchillas (Bekyrek et al., 2001). The cycle is divided into four periods (proestrus, estrus, metestrus, and anestrus) with the start of estrus correlating clinically with evidence of vaginal closure membrane perforation. As with most mammalian species, the cytologic profile of the vaginal epithelium exhibits a continuum of cellular change that correlates with the hormone profile of the estrous cycle. Each cycle period is defined by the predominating cell types. The proestral period is typified by a mixed cellularity that undergoes a shift from moderate numbers of parabasal and small intermediate cells predominating early in the period to large numbers of superficial cells typifying the late period. A high neutrophil density and the presence of goblet cells are also characteristic of proestrus. Non-cornified and cornified superficial squamous epithelial cells predominate estrus and neutrophils, small intermediate cells and parabasal cells re-emerge during metestrus. Neutrophils and large intermediate cell densities are greatest during metestrus. Only during this period may foam cells (non-cornified cells containing vacuoles) and metestrum cells (non-cornified cells containing leukocytes) be present. Anestrus is characterized by the presence of superficial and parabasal epithelial cells with small numbers of intermediate cells and neutrophils.

Large, waxy vaginal plugs are produced to signify the beginning of estrus. Behavioral displays of female receptivity involve lordosis, hindlimb extension, and lateral deviation of the tail allowing for exposure of the perineum. Male mounting behavior involves clasping his forelimbs just cranial to the pelvic region of the female and the initiation of a short series of preliminary thrusts that increase with depth of insertion until ejaculation has occurred. Ejaculation terminates with the deposition of a seminal (copulatory) plug in the vagina that can remain in place up to several hours after mating (Bignami and Beach, 1968).

Females are not consistently receptive to males throughout estrus. Despite receptive posturing, females may not consent to male mounting, insertion, or ejaculation; or mounting may be allowed but insertion may be refused. The likelihood of receptivity is significantly reduced after a female has already engaged in one successful mating (Bignami and Beach, 1968). Following copulation, females may display intense aggression towards males, which can result in severe trauma to the male or even death. For this reason, breeding cages are designed to allow for male escape. The classic cage design involves small exit tunnels for males that preclude neck-collared females from entering.

Common Breeding Systems

Although chinchillas typically breed in monogamous pairs in the wild, most commercial breeding systems utilize a harem scheme with one male for every 4–6 females. With the breeding tunnel cages, one male can visit multiple females during periods of receptivity.

Since female-to-male interactions can be very aggressive, some breeding facilities utilize artificial insemination in addition to natural breeding techniques. Testicular volumes are typically smaller during the summer months compared with the fall and winter breeding season; however, total ejaculate volumes, spermatozoa numbers, and functional activity demonstrate minimal monthly variability (Busso et al., 2005; Leal and Franca, 2009). Puberty, defined by the presence of sperm within the epididymis, occurs by 3 months of age. However, full sexual maturity does not occur until about 17 months. By this time the testicular weight and tubular diameter have stabilized (Leal and Franca, 2008). Unanesthetized electroejaculation using a rectal probe set at 220 V (sinusoidal wave), 50 cycles/s repeated at 5–10-second intervals can produce good ejaculate volumes and high spermatozoa concentrations during early stimulation (Ponce et al., 1998). Alternatively, electroejaculation performed under heavy sedation or anesthesia requires higher voltages and more stimuli to achieve ejaculation (Busso et al., 2005). Chinchilla spermatozoa have been successfully cryopreserved, however viability was significantly lower compared to fresh samples. Freezing and thawing compromises membrane integrity that can lead to a reduction in viable acrosome-intact gametes and functional motility (Ponce et al., 1998).

Care of Young

Dams display minimal nesting behaviors in preparation for parturition and deliver in the early morning similar to other rodents. Kits are precocious, born

fully haired with their ears and eyes open and weighing between 30–50 g. Unlike altricial rodents, at parturition, chinchilla kits have a low tolerance for CO₂ and a high demand for oxygen (Brower, 2006). Once placental detachment occurs, any delay in delivery can quickly lead to compromise of the kits. Chinchillas rarely experience dystocia but are prone to retained feti due to late-term fetal death (Brower, 2006). Dams should always be palpated after parturition to evaluate the uterus for fetal retention and any retained, decomposing feti should be surgically removed. Failure to do so can result in compromise of the dam and litter. Dams may display a prolonged post-partum agalactia (12–72 h) with a gradual increase in milk production (Brower, 2006); during this period kits may require hand feeding. Consumption of solid food typically occurs within a week of birth, with weaning at 6–8 weeks.

COLONY HEALTH

Preventive Medical Strategies

Husbandry is often the most significant factor in medical problems of chinchillas (Lightfoot, 1999). Problems with husbandry are less common in a well-designed research program. A periodic review of procedures and expectations with animal care and research staff can assist in detecting any deviations that might eventually negatively impact animal health. Like other species, a daily observational health check should be performed. The daily check should include assessment of the volume and character of feces and urine, and the amount of food and water consumed (if on water bottle). Healthy animals are active, hold their tail erect, and their fur will be thick and smooth (Lightfoot, 1999). Healthy animals that are comfortable in their surroundings are curious and investigate activities in their housing room. Typically, they come to the front of the cage when a familiar caregiver enters the room, particularly if accustomed to receiving a food treat at this time. If time permits, it is beneficial to gradually accustom each animal to a gentle handling each day. Most chinchillas will soon seek both treats and attention, which will make handling for physical examinations and other procedures less stressful. Some animals may not come all the way to the front of the cage, but should still appear alert and responsive to their surroundings. Animals that appear disengaged or depressed (tail down, or a ruffled, moth-eaten, or barbered appearance to their fur) could be ill or may simply be experiencing excessive dominance from a cage mate. If dominance issues are suspected, standing well back from the cage for a longer observation period can be helpful in determining whether animals should be separated.

Colony Health Monitoring

Health monitoring is generally limited to physical examinations at acquisition and clinical observations made during daily visual examinations. Areas of particular concern are anorexia/weight loss, dental abnormalities, dermatologic conditions (barbering, dermatophytosis, environmental issues such as excessive temperature or lack of dust bath), diarrhea or constipation, or straining to urinate. Physical exams and specific diagnostic tests are typically only performed if clinical abnormalities are noted. However, some conditions that develop gradually are difficult to detect visually. For example, the chinchillas' thick fur can disguise weight loss (Lightfoot, 1999). For animals housed long term, handle periodically (e.g., monthly) to assess for weight loss (by simple palpation or actual weight), dental abnormalities, length of nails and lesions on feet, fur ring on the penis of males, and any conditions commonly seen in the colony. In large breeding colonies, individual health exams of animals that appear healthy at cage side during daily checks may be limited to veterinary assessment immediately prior to shipment.

RECORD KEEPING

Use of Records

Records should function to meet regulatory requirements, aid in providing adequate husbandry and veterinary care, and facilitate research objectives. Each facility should review the activities and needs of their program when determining number and type of records to be used to meet professional standards. To assure accurate communication between all people involved in animal care, the number and complexity of records should be minimized.

Breeding Records

Record systems developed for laboratory mouse colonies can be adapted to chinchillas. These include breeder cards at the cage level and pedigree and production logs to evaluate long-term colony performance (Fox et al., 2007). Various styles of cage cards, production logs, and pedigree charts are also available from chinchilla breeder organizations (e.g., www.chinchillabreeders.org). As with other species, production planning and management of large colonies is best accomplished with a computer database.

Husbandry Records

A single check sheet for recording daily care and environmental parameters is sufficient for documentation of

CHINCHILLA ENRICHMENT LOG

Bldg/Room: _____

Month/Year: _____

X=Present	Activities / Toys									Food Treats								
DAY	Dust Bath	House	Kong®	Lattice Ball	Nylabone®	Ringer Ball	Washer/bell Chain	Wood	Other (Write in)	Apple	Carrots	Dried Fruit	Forage Mix	Hay	Pear	Sweet Potato	Other (Write in)	Initial(s)
1																		
2																		
3																		
4																		
5																		
6																		
7																		
29																		
30																		
31																		
Notes:																		

FIGURE 40.2 Chinchilla enrichment log. Example of a monthly log sheet for documenting provision of dust bath and environmental and nutritional enrichment.

husbandry activities. However, to adequately record the variety in an extensive enrichment program a separate enrichment log should be used (Figure 40.2). Regardless of style or number of forms, there should be a place to document provision of dust baths and information regarding enrichment.

Health Records

The Animal Welfare Act and Animal Welfare Regulations do not specifically require or provide guidance regarding documentation of medical care for this species. Similarly, the *Guide for the Care and Use of Laboratory Animals* discusses the value of medical records in general and individual medical records specifically for some species (dogs, cats, non-human primates, farm animals, “large long-lived animals”), but does not specifically comment on chinchillas or other larger rodents (NRC, 1996). However, health records

for regulated rodents are included in the USDA Animal Care Policy 3 – Veterinary Care (USDA-APHIS, 2007), which stipulates that at minimum records should include identity, description and resolution of clinical abnormalities, dates and details regarding medically related activities (observations, diagnostics, treatments). In regards to the style of the records, it states, “As long as all required information is readily available, records may be kept in any format convenient to the licensee/registrant (e.g., on cage cards for rodents)” (USDA-APHIS, 2007). A cage card-style record will be sufficient for many colonies. These can either be created for each animal when it is received into the facility (or weaned) or issued only if a health concern is reported (Figure 40.3). A larger, standard medical record (hard copy or electronic system) may be preferred when managing colony-wide outbreaks or if frequent clinical interventions are required to support a particular research model.

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Basic Experimental Methods

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INTRODUCTION

Performing basic experimental procedures on chinchillas can be a challenge due to their small size, their unique characteristics, and the limited amount of literature available describing these techniques. However, in general, many of the methods and concepts used in other rodent species may be applicable when performing basic procedures in chinchillas. Procedures such as handling, restraint, collection of samples, or administration of compounds can all be done relatively stress-free with minimal complications if performed

appropriately by skilled personnel. A thorough understanding and knowledge of the experimental procedure should be obtained prior to initiation to ensure the task is carried out properly. Oftentimes, first practicing the procedure on animals that have not yet begun an experimental study may be warranted. This allows the person who is performing the experimental technique to establish a level of comfort with the method to assure optimal execution when performed on experimental animals. The following text and figures highlight basic experimental methods in chinchillas based upon the literature and personal experiences of the authors.

HANDLING AND RESTRAINT

General Precautions

Chinchillas are typically quiet and shy rodents that can be easily handled if done so in a calm, quiet, and gentle manner. However, as a prey species, chinchillas may exhibit a flight response when presented with an uncomfortable situation. They may thus move about in short darting bursts or quick forceful lunges to attempt escape. Chinchillas may vocalize, lunge, box, and even urinate on people when attempts are made to pick them up, and so the handler should be quick and confident as well. Although chinchillas rarely bite, biting or scratching can occur if they become agitated, frightened, or stressed (Donnelly and Brown, 2004; Hrapkiewicz and Medina, 2007). Leather or Kevlar[®] gloves may be worn to add a layer of protection for the handler (Strake et al., 1996); however, gloves may be bulky and limit the handler's ability for delicate feel which could lead to inadvertent application of excessive pressure during manual restraint. Care should be exercised when attempting to handle and restrain chinchillas so as to avoid "fur slip," an escape mechanism in which a patch of hair is abruptly shed if enough tension is applied to the fur while the chinchilla is struggling to escape (Hoefer and Crossley, 2002; Riggs and Mitchell, 2009). Fur slip is not a degloving injury since typically the skin is not damaged but rather remains smooth and intact, though hairless. The fur may take months to regrow, and may grow in thin, sparse, and discolored in comparison to the original fur coat (Strake et al., 1996). Although mostly cosmetic in nature, fur slip may be alarming to the handler or restrainer if it occurs unexpectedly. On the other hand, attempting to catch a moving chinchilla by the tail may result in a degloving injury of the tail and is thus not recommended (Quesenberry et al., 2004). Other complications that may occur if chinchillas are handled improperly or restrained inadequately include bone fractures (e.g. tibia) from falls or trauma, restriction of respiration from excessive compression around the thorax, or loss of fetuses in pregnant chinchillas from rough handling or poor support of the dam's larger body size (Quesenberry et al., 2004; Strake et al., 1996). As is the case with other species, proper acclimation of the chinchilla to the handling or restraint method, whether physical or mechanical, should be done to minimize stress.

Physical Methods

Chinchillas should be handled in a manner that uses the least amount of restraint but is still firm enough so that the handler has good control of the animal. Excessive force or aggressive restraint beyond what is needed may promote additional stress to, and cause



FIGURE 41.1 Handling of a chinchilla with one hand supporting the hind legs and tail, and the other hand supporting the ventral thorax.

further struggling by, the chinchilla. Procedures should be performed quickly since the chinchilla, as a high-stress animal, may not tolerate handling, restraint, or procedures very well (Briscoe and Syring, 2004). Having additional hands available to help restrain the chinchilla may be of use in some circumstances (Donnelly and Brown, 2004), but may be limited by the body surface area available to hold the animal.

Chinchillas should not be scruffed (Donnelly and Brown, 2004; Riggs and Mitchell, 2009), but rather should be picked up by grasping the base of the tail with one hand while supporting the front end with the opposite hand. For additional control, it is often best to incorporate the hind limbs of the chinchilla in the same hand used to grasp the base of the tail. There are a variety of options for supporting the front end of the chinchilla depending on what is being performed. If minimal restraint is needed, such as during transport of a docile chinchilla for a short distance, the animal may be secured by the base of the tail with one hand while the opposite hand is placed gently under the chinchilla's ventral thorax for support (Figure 41.1). Alternatively, the chinchilla may be secured and carried in a "football" hold. This is done by allowing the chinchilla to rest on the handler's forearm with the base of the tail secured and the chinchilla's head and face tucked against the handler's body and arm (Figure 41.2). Nervous chinchillas often prefer to be held close to, and with their heads tucked into, the handler's body (Lightfoot, 1997, 1999).

For procedures that may require more control of the chinchilla or which have the need to orient the animal in a particular position (e.g. venipuncture), the animal may be secured by the base of the tail with one hand



FIGURE 41.2 The "football" hold. The chinchilla is placed on the handler's forearm with the base of the tail secured and the head tucked against the handler's body.



FIGURE 41.4 Restraint of a chinchilla in lateral recumbency.



FIGURE 41.3 Restraint of a chinchilla with one hand grasping the base of the tail and hind legs, and the other hand securing the back of the skull and dorsal neck.

and the head with the opposite hand (Figure 41.3). In this method, the restrainer's thumb is placed on one side of the chinchilla's head below the ear, the index and middle fingers placed over the top of the cranium, and the remaining fingers placed near the base of the skull, opposite the thumb. Depending on the size of the handler's hands or the size of the chinchilla, this hold may be modified by changing the position of the fingers on top of, or on the side of, the chinchilla's cranium to suit



FIGURE 41.5 Restraint of a chinchilla with one hand grasping the base of the tail and hind legs, while the other hand is placed around the dorsal thorax. Photo shows ventral aspect of the chinchilla.

the handler's preference. The chinchilla may then be securely presented in a variety of positions, such as in lateral recumbency (Figure 41.4), to facilitate whatever procedure is being conducted. Alternatively, support of the front end of a chinchilla may be accomplished by placing the hand over the animal's dorsal thorax with the handler's thumb cranial to the chinchilla's forelimb and the index and middle fingers encompassing the animal's opposite forelimb (Figure 41.5). Variations of this restraint technique may be performed by placing the thumb caudal to the animal's forelimb rather than cranial to the forelimb (often referred to as the "three-finger hold" in other species), or by encompassing the animal's forelimb with the thumb and index finger and the opposite forelimb with the middle and ring finger (often referred to as the "four-finger hold"). It should be noted that when performing restraint techniques that require the hand to be placed around the



FIGURE 41.6 Wrapping a chinchilla in a towel (A to D) as a method of restraint.

chinchilla's thorax, care should be exercised so that respiration is not inadvertently limited. Careful attention should always be paid to the respiratory pattern and other physical signs to assess for hypoxia due to excessive pressure around the thorax.

Towels or drapes may be of great use for handling and restraint of chinchillas. A towel should be laid out on a flat surface, the chinchilla placed on top, and then the towel is wrapped snugly around the chinchilla's body with only the head or necessary limb exposed (Figure 41.6). This method may be of use when performing oral exams, syringe feedings, or oral gavage to prevent chinchillas from using their front limbs to knock syringes or otoscopes away, or from using their powerful hind limbs to kick or lunge. Towels or drapes may also be useful for aggressive chinchillas prone to biting or scratching.

MECHANICAL METHODS

There are only a few reports in the literature describing restraint devices for chinchillas. Snyder and Salvi

(1994) describe a novel, custom-made restraint device to support auditory research constructed from inexpensive and readily available hardware supplies such as polyvinyl chloride (PVC) piping. Another custom-made restraint device described by Strout (1976) was designed to assist with restraint and oral dosing of chinchillas. It was also reported that this restraint device is suitable for performing clinical procedures such as taking rectal temperatures, clipping teeth, or obtaining blood samples. Although both of these devices permit restraint without anesthesia, their utility may be limited to procedures in which the required body part is accessible. To the authors' knowledge, no commercially available mechanical restraint device is available for chinchillas.

Chemical Methods

Chemical restraint of chinchillas may be performed by either using injectable or gas anesthetics. Many of the same principles, drugs, and dosages utilized in chinchillas for complete anesthesia may be suitable for restraint and will depend on the procedure being performed and the desired recovery time. Both gas and injectable



FIGURE 41.7 Delivering gas anesthetics to a chinchilla in an induction chamber.

anesthetics may be titrated to provide complete immobilization and restraint, or to calm and sedate an otherwise active chinchilla. Typically, for short-term restraint, gas anesthesia can be used since recovery is often rapid after cessation of anesthetic administration. Injectable anesthetics or sedatives may take longer for the chinchilla to metabolize and recover, however many of these drugs are now available with reversing agents; therefore, when selecting injectable agents, those with corresponding reversing agents are preferred. Specific anesthetic drugs and regimens for chinchillas have been reported in the literature (Heard, 2004; Hoefer and Crossley, 2002; Hrapkiewicz and Medina, 2007; Johnson-Delaney and Harrison, 1996; Riggs and Mitchell, 2009).

Gas anesthetics may first be delivered to the chinchilla via an induction chamber (Figure 41.7) and then, once immobilized, the chinchilla is transferred over to a nose cone on a non-rebreathing circuit (Figure 41.8). Alternatively, the chinchilla may be exposed to gas anesthesia solely via a nose cone; however, the chinchilla's protest to the gas anesthetic odor or the initial anesthetic excitement phase may necessitate additional physical restraint during these periods. Wrapping a chinchilla in a towel or drape as described in the section on physical methods of restraint is a suitable solution for these scenarios. Intubation can be difficult in chinchillas and so maintenance via a nose-cone may be sufficient for quick procedures which only require a short period of immobilization or restraint. However, if intubation is desired, direct visualization of the glottis using a 1.9- or 2.7-mm rigid endoscope to assist with the placement of an endotracheal tube in chinchillas has been described (Riggs and Mitchell, 2009). Both isoflurane and sevoflurane are commonly used gas anesthetics for chinchillas and can easily be titrated to the desired effect and duration of restraint. Sevoflurane may have a less objectionable odor than isoflurane (Riggs and Mitchell, 2009) and in one author's experience, chinchillas recover more rapidly with sevoflurane than with isoflurane. Halothane



FIGURE 41.8 Delivering gas anesthetics to a chinchilla using a nose cone and non-rebreathing circuit.

and methoxyflurane use in chinchillas has also been reported (Hoefer, 1994). Atropine (0.01–0.2mg/kg) given subcutaneously (SQ) or intramuscularly (IM), or glycopyrrolate (0.01–0.02mg/kg) given SQ or IM may be administered 10–20 minutes prior to induction if needed.

Use of injectable drugs and drug combinations for chemical restraint may induce prolonged recovery times when compared to the use of gas anesthetics. Therefore, deciding on which chemical restraint method to use and the amount or dose should be given careful consideration. Protocols used by the authors to provide mild sedation or short-term restraint include: (a) midazolam (0.2mg/kg, IM) to make chinchillas calm and “malleable” without significant sedation to perform short procedures such as radiographs; (b) midazolam (0.2mg/kg) + butorphanol (0.2mg/kg, IM) for short procedures of less than 20 minutes; this is reversible with flumazenil (0.02mg/kg, with half the dose given IM and half the dose given SC) and naloxone (0.02mg/kg, IM); (c) ketamine (5mg/kg, IM) + dexmedetomidine (0.03mg/kg, IM). Note that ketamine and dexmedetomidine can be mixed in the same syringe; and the combination may be used for longer procedures lasting about 45 minutes. This combination is reversible with atipamezole. These protocols may be supplemented with isoflurane and oxygen as needed.

Although some withhold food from chinchillas for periods ranging from 1–6 hours prior to an anesthetic event (Hrapkiewicz and Medina, 2007; Riggs and Mitchell, 2009), fasting may not be necessary in chinchillas since they cannot vomit or regurgitate (Boschert, 2005; Donnelly, 2004; Riggs and Mitchell, 2009; Strake et al., 1996; Zeinert, 1983), making the risk of aspiration low. Since fasting of many rodents may cause ileus and exhaustion of glycogen stores leading to hypoglycemia

(Heard, 2004), the same may be true of chinchillas, and the risks of fasting as opposed to the benefits should be evaluated for each animal.

SAMPLING TECHNIQUES

Blood

Collection of blood from chinchillas can be performed from a variety of anatomic locations. The volume of blood needed, the accessibility of the vessel, the temperament of the chinchilla, and the phlebotomist's skill are all factors to consider when selecting a site for blood collection. Larger vessels are better for obtaining samples in which it is critical that clotting does not occur since a larger and faster flow of blood can be obtained than from vessels of smaller size. Smaller vessels may be suitable for samples requiring less volume or samples in which clotting is acceptable (e.g. serum) since the slow blood flow and prolonged collection time may increase the likelihood of clotting. Regardless of which vessel is selected for blood collection in the chinchilla, clipping the hair and/or applying sufficient amounts of alcohol to wet down the fur may assist with visualization of the vessel. Sedation or anesthesia may be used to reduce the amount of stress a chinchilla may undergo during the procedure, as long as the potential effects of the anesthetics on experimental data obtained from the blood sample are taken into consideration (Briscoe and Syring, 2004). Because blood collection in anesthetized chinchillas may in fact be more challenging due to the drop in blood pressure caused by the anesthetic agent, it is recommended that blood collection first be attempted without chemical restraint.

As with all rodent species, the amount of blood removed from a chinchilla should be closely monitored to avoid any side effects related to iatrogenic blood loss. In general, the total circulating blood volume of most animal species can be estimated as 7% of their body weight and approximately 10% of the total blood volume may be safely removed (Table 41.1) (Diehl et al., 2001; Hrapkiewicz and Medina, 2007; Morton et al., 1993; Quesenberry et al., 2004). Alternatively, the amount of blood in ml equal to 1% of the body weight in grams may be safely removed from a healthy animal (Table 41.1) (Joslin, 2009; McClure, 1999; Pilny, 2008). Based upon recommendations in other rodent species (Diehl et al., 2001; Hoff, 2000), removal of 10% of the circulating blood volume should be limited to once every 2 weeks in healthy animals. Fluid supplementation should be provided for single blood collections of volumes greater than 10% of the circulating blood volume. However, even with fluid administration, blood collection volumes in healthy chinchillas should still

TABLE 41.1 Estimates of Blood Total Circulating and Withdraw Volumes in Adult Chinchillas

Chinchilla Weight (g)	Total Blood Volume (ml) ^a	Withdraw Volume: 10% of Total Blood Volume (ml)	Withdraw Volume: 1% of Body Weight (ml)
400	28	2.8	4.0
500	35	3.5	5.0
600	42	4.2	6.0

^aEstimated based upon 7% of body weight.

TABLE 41.2 Needle Sizes, Average Volumes Collected, and Anesthesia Requirements for Various Blood Collection Methods in Chinchillas

Method	Needle Size (gauge)	Average Volume Collected (ml)	Anesthesia Required
Jugular	22–25	1–3	No
Femoral	25–27 ^a	0.5–1	No
Cephalic	25–27 ^a	0.3–1	No
Lateral saphenous	25–27 ^a	0.3–1	No
Cranial vena cava	22–23	1–3	Yes
Retro-orbital	Not applicable	1–3	Yes
Transverse sinus	25	1–3	Yes
Cardiac	20–23	>5	Yes ^b

^aAn insulin syringe may also be used.

^bTerminal procedure, euthanize while under anesthesia after blood collection.

be limited to 15–20% of the animal's circulating blood volume.

As with other species, removing the needle prior to transfer of the blood sample from the syringe to a tube is recommended in order to limit the amount of hemolysis that may result from attempts to force red blood cells through a narrow needle. For insulin syringes, a hemostat can be used to grasp the base of the needle to detach it from the syringe prior to transferring the sample to the collecting tube.

Table 41.2 summarizes the needle sizes, average volumes collected, and anesthesia requirements for the various blood collection techniques described in the following sections.

Jugular Vein

Collection of blood from the jugular vein of chinchillas is generally well tolerated by the awake, unsedated, or unanesthetized animal. The jugular veins, located in the ventral cervical neck, may be the best option when large samples of blood are required that need to



FIGURE 41.9 Jugular vein blood collection from a chinchilla in dorsal recumbency.

be collected in a timely fashion (e.g., for anticoagulated blood samples). One method to access the jugular vein in chinchillas is with the animal on its back. As with some rabbits, chinchillas may become relaxed when placed on their backs, thus aiding in the procedure. This technique is performed by having an assistant restrain the hind end of the chinchilla with one hand while the other hand supports and extends the neck by grasping the loose skin around the ear and neck (Figure 41.9). After a sufficient amount of alcohol is placed over the area of the jugular vein to help with visualization (clipping the fur may also be used), the phlebotomist uses the thumb or index finger of one hand to apply gentle pressure on the vessel at the level of the thoracic inlet, and the other hand to position the syringe and insert the needle into the jugular vein at an approximately 20° angle. Since chinchillas may attempt to use their forelimbs to bat at the syringe in the phlebotomist's hand, it may be necessary to provide additional restraint of the chinchilla's front legs.

Alternatively, jugular vein blood collection in chinchillas may be performed similar to the method used in cats in which their bodies are in sternal recumbency, their head and neck extended, and their forelimbs extended forward and ventrally (Figure 41.10) (Briscoe and Syring, 2004; Ness, 1999; Pilny, 2008). The hind legs should be securely supported and controlled to prevent the chinchilla from kicking during the blood draw. A 22–25-gauge needle should be used attached to a 1–3-ml syringe. The needle can be bent slightly at a 30–45° angle to facilitate the angle of approach into the vessel if necessary (Joslin, 2009).

Femoral Vein

The femoral vein in chinchillas can be used to collect blood in a way similar to the method used to sample the medial saphenous vein in cats (Joslin, 2009). This vein is located on medial aspect of the hind limb providing easy



FIGURE 41.10 Restraint of a chinchilla in a sternal position for jugular vein blood collection, similar to the method used in cats.

access with a 25–27-gauge needle and a 1-ml syringe (Ness, 1999). The chinchilla should be placed in lateral recumbency with the non-recumbent hind limb held in a flexed position against the chinchilla's body. The medial aspect of the recumbent hind limb is now accessible and slight pressure can be applied proximally on the medial thigh over the vein to assist with venipuncture.

Cephalic Vein

The cephalic vein runs along the cranial aspects of the lower forelimbs and can be sampled using a 25- or 27-gauge needle and a 1-ml syringe (Briscoe and Syring, 2004; Lightfoot, 1997; Riggs and Mitchell, 2009). The chinchilla should be restrained on a table and the hair clipped along the cranial aspect of the forelimb. The forelimb can then be extended by the restrainer while simultaneously holding off the cephalic vein (Figure 41.11). The phlebotomist can then hold and secure the paw with the non-dominant hand and manipulate the syringe and needle with the dominant hand. Paolini et al. (1993) reported that they rarely collected greater than 0.5 ml of blood when using the cephalic vein. Although typically only small amounts of blood may be obtained due to the small size of the cephalic vein, this vessel, along with the jugular and femoral veins, are preferred venipuncture sites in the chinchilla since most phlebotomists are familiar and have experience with these vessels in other species.

Lateral Saphenous Vein

Similar to the cephalic vein, the lateral saphenous vein in the chinchilla may be used to collect small samples of blood. The vein is located on the lateral aspect of the

lower hind legs (Figure 41.12) and can be sampled using a 25- or 27-gauge needle on a 1-ml syringe or using an insulin syringe (Briscoe and Syring, 2004; Riggs and Mitchell, 2009).

Cranial Vena Cava

Although there have been reports describing the use of the cranial vena cava for blood sample collection in the chinchilla (Briscoe and Syring, 2004; Hrapkiewicz and Medina, 2007; Joslin, 2009; Riggs and Mitchell, 2009), this procedure, as in other species, is more invasive and may have a higher incidence of complications (e.g. bleeding into the thoracic cavity) when compared to blood collection from other anatomic sites. However, large volumes of blood can be obtained from the cranial vena cava and Joslin (2009) categorizes this anatomic

location as a recommended site for chinchilla venipuncture in the private practice setting as opposed to a venipuncture site of last resort. To perform this procedure, the anesthetized chinchilla is placed in dorsal recumbency with the forelimbs pulled downwards and caudally. The phlebotomist, standing at the head of the animal, secures the head and inserts a 22–23-gauge needle connected to a 3–5-ml syringe at a 45° angle into the notch between the manubrium and the first rib slightly lateral to midline, directing the needle toward the opposite hind leg (Briscoe and Syring, 2004). The plunger on the needle is then pulled back to create a slight vacuum while the needle is slowly withdrawn until a flash of blood is seen in the hub. If unsuccessful, probing for the vessel is not recommended due to the risk of laceration and damage to surrounding structures. After the sample is collected, direct pressure should be placed on the sampling site, and the chinchilla should be watched closely to ensure adequate recovery.

Orbital Sinus

Collection of blood from the orbital sinus has been described for use in the laboratory setting (Brookhyser et al., 1977; Strake et al., 1996), however it is generally recommended only as a last resort (Joslin, 2009) or not recommended at all for use in pet chinchillas (Hoefer, 1994; Ness, 1999). In the laboratory setting, as is the case with this technique in other rodents, minimal complications may be observed if performed properly by skilled personnel. Complications reported in the chinchilla include hemorrhage, conjunctivitis, and corneal opacities (Brookhyser et al., 1977), although no differences in food consumption or body weights were noted in chinchillas that were bled twice weekly for approximately 2 months vs. those that were not bled (Brookhyser et al., 1977).



FIGURE 41.11 The cephalic vein (arrow) in the forelimb of a chinchilla.

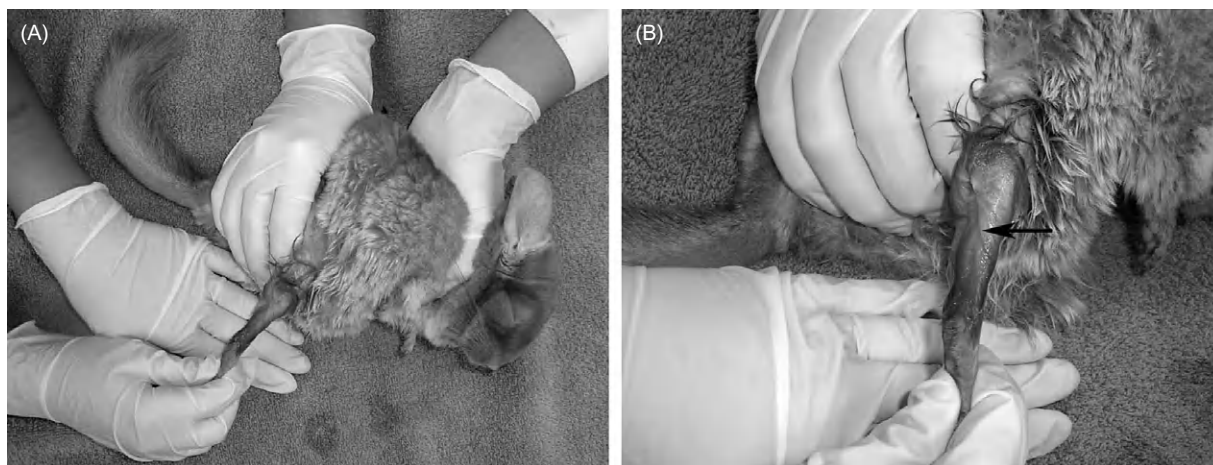


FIGURE 41.12 (A) Restraint of a chinchilla for blood collection from the lateral saphenous vein. (B) The lateral saphenous vein (arrow) in the hind limb of a chinchilla.

The technique is similar to that described for mice and rats and can be used to obtain 1–3 ml of venous blood (Brookhyser et al., 1977). The chinchilla should be anesthetized, the head held firmly with its nose pointing downward, and the eyelids held wide open with the thumb and forefinger. A microhematocrit tube is placed at the medial canthus of the eye between the globe and the eyelid, and directed medially and caudally. The microhematocrit tube should be gently advanced while rotating it along the long axis of the tube until blood is seen. Blood can be collected in the microhematocrit tube itself, or allowed to drip from the end of the microhematocrit tube into a larger collection tube. After removal of the microhematocrit tube, hemostasis should be ensured by using a piece of gauze to hold the eyelids closed and applying gentle pressure over the eye.

Transverse Sinus

The transverse sinus is a venous system that encircles the large auditory bullae in chinchillas and is connected to the zygomatic venous system and the superior sagittal sinus (Figure 41.13A) (Browning and Granich, 1978; Paolini et al., 1993). The transverse venous system may be used to reliably obtain 1–3 ml of blood in an anesthetized chinchilla and may even be used to obtain 10 ml of blood or more (Boettcher et al., 1990; Paolini et al., 1993). The fur over the auditory bullae, located on the dorsal aspect of the head and between the two ears, should be clipped and the skin prepared aseptically. A 25-gauge, 3/8-inch butterfly needle attached to a 1-ml syringe should then be inserted perpendicular to the skull approximately 1 mm medial from the edge of the auditory bulla, just slightly lateral to the midline of the skull (Figure 41.13B). The needle should be gently advanced approximately 1–2 mm with a slight twisting motion to get through the skin and then through the thin layer of bone overlying the sinus. Once through the roof of the sinus, the bony floor of the sinus can be found only 1 mm from the roof and so care should be taken to avoid advancing the needle too far into the auditory bulla or cranium. With the needle seated in the sinus, negative pressure can be applied to the 1-ml syringe to begin blood collection. Once proper placement has been verified, the 1-ml syringe can be changed to a larger 5- or 10-ml syringe. Paolini et al. (1993) suggested starting with a 1-ml syringe to aspirate as opposed to a larger syringe to avoid producing excessive negative pressure and potentially rupturing the tympanic membrane should the auditory bulla be inadvertently accessed. Unintentional entry into the auditory bulla was the most common complication reported, however no adverse sequelae were noted (Paolini et al., 1993). Boettcher et al. (1990) described damage to the central nervous system and partial paralysis in one of 15 chinchillas when the subject was not completely anesthetized and

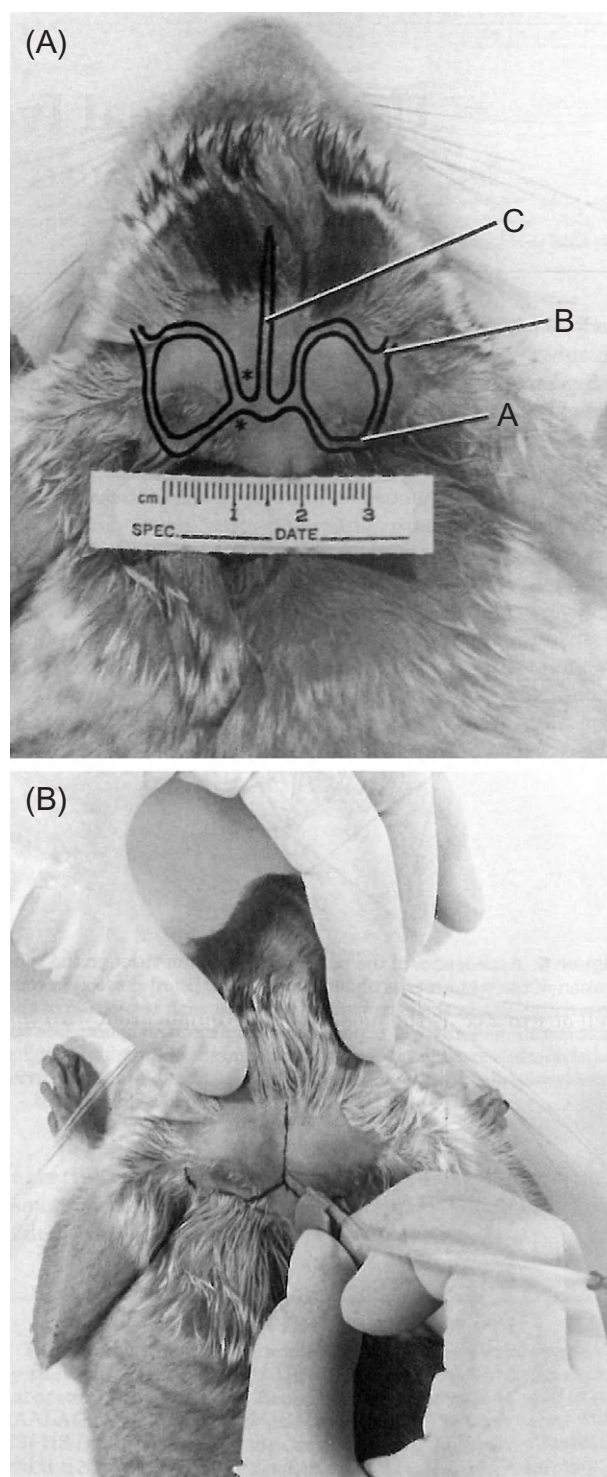


FIGURE 41.13 (A) Dorsal aspect of the head of a chinchilla with the fur removed and the venous system highlighted (A: transverse venous sinus; B: zygomatic venous system; C: superior sagittal sinus). The needle is inserted approximately 1 mm lateral to the asterisks into the transverse venous sinus. (B) Blood collection from the transverse venous sinus of the chinchilla. Figures published in and obtained, with permissions, from Paolini et al., 1993.

subsequently moved during the procedure. However, they described use of a 25-gauge, 5/8-inch needle, thus use of a shorter needle, such as the 3/8-inch butterfly needle, is recommended.

Cardiac Puncture

Cardiac puncture should only be performed with the animal under complete anesthesia or immediately after euthanasia has been confirmed. Large volumes of blood may be obtained via this method, however it should only be utilized as a terminal procedure (Hrapkiewicz and Medina, 2007). The anesthetized chinchilla may be placed in lateral recumbency and a 20- or 23-gauge, 1-inch needle on a 5- or 10-ml syringe used to access the heart through the ribs. Alternatively, the chinchilla may be placed in dorsal recumbency with the needle entered just below the xiphoid process, directed cranially and at a 30° angle toward the heart.

Other Peripheral Veins

Collection of blood from other peripheral veins such as the auricular, tail, dorsalis penis, and lateral abdominal vein have been described in the chinchilla (McClure, 1999; Ness, 1999; Tappa et al., 1989). Sampling from these sites is typically performed by laceration of the vein and drip collection of the blood into a tube since these vessels will collapse due to their small size if aspiration is attempted with a needle and syringe. However, these sites typically only yield very small amounts of blood, are not easily accessible, and may carry a greater risk of post-venipuncture complications. As a result, these veins are not commonly used and may be considered unsuitable for blood sampling in some applications. For example, the tail vein, lateral abdominal vein, and dorsal vein of the penis are all very difficult to identify and locate and so are generally not practical for routine blood collection (Ness, 1999; Tappa et al., 1989). Boettcher et al. (1990) and Paolini et al. (1993) state that the thick cartilaginous sheath surrounding the tail vessels in the chinchilla makes venipuncture difficult and results in variable yields.

As with pet rabbits, the use of the auricular vein in chinchillas is generally frowned upon and not recommended when treating the pet population due to risk of thrombosis and trauma of the pinna (Hoefer, 1994; Joslin, 2009). In contrast, the auricular vessels are often used in rabbits in the research setting with few complications. Nonetheless, since such small volumes are obtained from the auricular vessel in chinchillas and other more convenient sites are available, the ear may not be the optimal choice for blood collection unless only very small amounts of blood are needed and other collection sites are not practical. The vessel may be pricked with a needle, or a 27-gauge needle may be inserted into the vein, and then the blood collected with

a microhematocrit tube (Ness, 1999). However, repetitive sampling should be avoided to minimize the risk of complications with the ear (Hoefer, 1994; Hrapkiewicz and Medina, 2007).

Urine

Collection of urine from chinchillas may be performed similar to other species. The simplest method of urine collection is to place them in a metabolic cage. Similarly, performing a free catch or collection from a table or floor may be sufficient as chinchillas will often urinate when agitated or annoyed (Hrapkiewicz and Medina, 2007). Manual expression may also be performed by applying gentle pressure to the urinary bladder through the abdominal wall (Hrapkiewicz and Medina, 2007; Ness, 1999). However, if urine is needed for microbiologic culture, these methods may not be suitable due to contamination with environmental bacteria. To obtain a sample for urine culture, cystocentesis may be performed. Sedation or anesthesia may be used, although some chinchillas may tolerate this procedure awake. The chinchilla should be placed in dorsal recumbency and the urinary bladder located through the abdominal wall (Figure 41.14A). While holding the urinary bladder securely with the non-dominant hand, a 25-gauge, 5/8-inch needle with a 1- or 3-ml syringe should be inserted cranial to the external genitalia and at an approximately 30–45° angle to the table (Figure 41.14B). Ultrasound may also be used to assist with cystocentesis if available (Riggs and Mitchell, 2009). Catheterization of the urinary bladder to obtain urine may be difficult due to the chinchilla's small size, however it may be possible when necessary to relieve an obstructed urethra (Ness, 1999). An open-ended, 3½-French, 5½-inch-long tom cat catheter or a 3½-French red rubber catheter is recommended for use in chinchillas.

Cerebrospinal Fluid

Cerebrospinal fluid collection in the chinchilla can be performed in the subarachnoid space beneath the atlantooccipital membrane (Juhn et al., 1974). Although a detailed description of the procedure has not been described for chinchillas, the cisterna cerebellomedullaris, also known as the cisterna magna, is the most common site for obtaining cerebrospinal fluid in other species and is located where the caudal surface of the cerebellum meets the dorsal surface of the medulla oblongata (Fenner, 2000; Fletcher, 1993). To perform the procedure, the chinchilla is placed under anesthesia and the cranial portion of the dorsal neck and occipital area of the skull are then clipped to remove the fur. The chinchilla is then placed in lateral recumbency and with the neck in slight flexion. The clipped area is

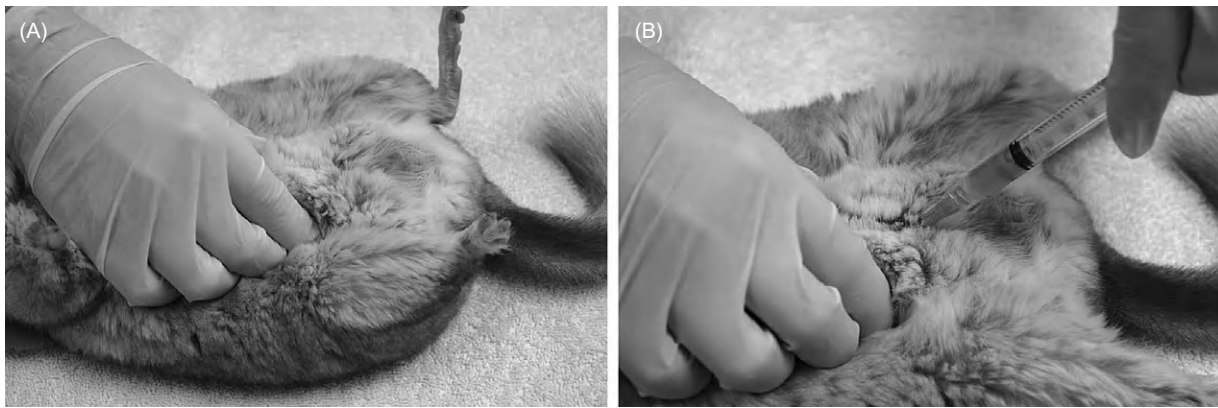


FIGURE 41.14 (A) Palpation of the urinary bladder through the abdominal wall. (B) Cystocentesis in a chinchilla using a needle and syringe.

aseptically prepared. Sterile technique should be followed for the procedure. A spinal needle is then inserted on the midline caudal to the occipital protuberance in the space between the base of the skull and the atlas bone. The needle should be inserted approximately perpendicular to the long axis of the spine and parallel to the table surface. A loss of resistance can be felt when the needle enters the subarachnoid space. The stylet is then removed and cerebrospinal fluid is collected. A 25-gauge, 1.6-cm-long hypodermic needle is described for use in cerebrospinal fluid collection in ferrets (Platt et al., 2004) and so needles of similar size may be suitable for cerebrospinal fluid collection in chinchillas.

Semen

Collection of semen and spermatozoa by electroejaculation has been described in the chinchilla (Busso et al., 2005; Ponce et al., 1998). Electroejaculation provides the advantage of being able to collect multiple samples from one individual, as well as avoiding surgery or euthanasia for sample collection. The authors describe using a bronze bipolar electrode with a length of 40 mm and diameter of 4.2 mm. The chinchilla was restrained, the genital area cleaned, the prepuce retracted, and the penis introduced into a 2-ml plastic tube. The electrode was lubricated and inserted in the rectum approximately 20–30 mm and an alternating current (sinusoidal wave, 50 cycles/s) was applied for 5 of every 10 seconds. A rheostat was used to control the electrical potential across the poles of the electrode and measured with a voltmeter. A series of one to six pulses at 6–6.8 volts was used (Ponce et al., 1998), or four series of up to five pulses were applied at 6–6.5, 7.5–8, 9.5–10, and 11.5–12 volts (Busso et al., 2005). Busso et al. (2005) found that ketamine (40 mg/kg, intramuscular) anesthesia significantly reduced the success rate of electroejaculation from 100% to 60% in unanesthetized

vs. anesthetized chinchillas, respectively. Also the volume of semen obtained in anesthetized chinchillas was greatly reduced.

Bone Marrow

Bone marrow collection in chinchillas may be performed as a single procedure, or at the same time as placing an intraosseous catheter to administer fluids or compounds. The proximal femur (via the trochanteric fossa) or the proximal tibia (via the tibial crest) are the most common sites to access the medullary cavity (Briscoe and Syring, 2004; Riggs and Mitchell, 2009), although the ileum or the proximal humerus may be utilized in some small exotic species and may be useful for chinchillas (Pilny, 2008). To perform the procedure, the chinchilla should be put under general anesthesia, the hair over the insertion site clipped, and the site aseptically prepared. All subsequent steps should be performed using sterile technique. Injection of 2% lidocaine in the sampling site should be performed to locally anesthetize the surrounding structures and periosteum. A small stab incision can then be made through the skin using a scalpel or needle. The bone is then firmly grasped with the forefinger placed along the long axis of the bone to assist with orientation and needle placement. A 20-, 22-, or 25-gauge, 1-1/2-inch spinal needle with an internal stylet is then inserted through the stab incision and seated into the medullary canal by rotating the needle on its long axis while concurrently applying gentle pressure to advance the needle. The stylet prevents a core of bone from plugging the tip of the needle; however standard hypodermic needles may be used if spinal needles are unavailable. Once the needle is advanced through the cortex, a sudden loss of resistance will be noted as the medullary space is entered. The stylet is then removed and a syringe attached to apply negative pressure until bone marrow is aspirated into the hub of the needle.

COMPOUND ADMINISTRATION

Administration of compounds or fluids in chinchillas may be done by a variety of routes including oral, intravenous, intramuscular, subcutaneous, intraperitoneal, or intraosseous. Selecting the optimal route of delivery for compound administration is dependent on multiple factors. Absorption of the compound into the bloodstream and subsequently into the target organ will vary depending on the route, thus contributing to the length of the time before the desired effect occurs. In general, intravenously administered compounds have a very rapid effect since the compound is delivered directly into the bloodstream and thus immediately distributed systemically after administration. On the other hand, intramuscularly or subcutaneously delivered compounds must first be absorbed from the muscle or subcutaneous tissues, respectively, into the bloodstream, thus prolonging the time for distribution. Furthermore, selection of a delivery route may be dependent on the compound itself since some compounds may cause tissue irritation or necrosis via one route but not the other. The volume to be administered also plays a role in selecting a route of administration since some locations accommodate larger volumes than others (Table 41.3). Considering these factors, careful thought and consideration should be made prior to selecting a method of delivery.

Oral

Delivering compounds to chinchillas orally may be done by administering the compound in food, in the drinking water, via a syringe, or by gavage. Although putting the compound in the chinchilla's daily food or water source may be the simplest method, it is often difficult to control the amount of compound that is ingested. Also, if the compound is not palatable, it

may decrease the chinchilla's intake of food and water. Therefore, it is recommended that compounds in tablet form be administered by crushing them into a powder and placing them in treats such as raisins (Donnelly and Brown, 2004; Hrapkiewicz and Medina, 2007; Quesenberry et al., 2004). Alternatively, the powder may be mixed with water in a syringe and manually fed into the side of the chinchilla's mouth. Small syringes or eyedroppers may be used to administer small volumes (Strake et al., 1996), while large volumes may be dispensed with a 35-ml catheter tipped syringe. Mixing the compound with fruit-flavored electrolyte solution (Riggs and Mitchell, 2009) or with sweetened water (5ml of sugar or syrup per liter of water) (Hrapkiewicz and Medina, 2007) may increase palatability and success. A feeding tube may also be used to deliver compounds directly into the stomach. It is recommended that a speculum or other device be used to prevent the chinchilla from biting through the tube during the procedure (Strake et al., 1996). However, passing a feeding tube in an awake and excited chinchilla may be difficult and so should only be attempted in animals with a calm, quiet demeanor. Additionally, their long and narrow oral cavity may add to the level of complexity of this procedure (Riggs and Mitchell, 2009).

Intravenous

Generally, the same methods and sites described to access a vessel for blood collection may also be used for intravenous administration. The more common sites for intravenous compound administration include the jugular, cephalic, femoral, and lateral saphenous vein. Other veins which can be used or that have been described include the auricular, lateral abdominal, tail, or dorsalis penis vein. For most of these vessels, a 22–25-gauge needle connected to a 1- or 3-ml syringe, or an insulin syringe is suitable. Alternatively, butterfly needles of the same gauge can be used.

Catheters may be used to facilitate intravenous compound administration as well as blood sampling. Although catheter placement in chinchillas may be a challenge due to their small size and fragile veins (Quesenberry et al., 2004), 24-gauge or smaller catheters may be placed in the cephalic, femoral, or lateral saphenous veins (Briscoe and Syring, 2004; Riggs and Mitchell, 2009). It may be helpful to first puncture the skin with a 22-gauge needle and then place the catheter through the hole in the skin to avoid burring and bending the catheter (Briscoe and Syring, 2004). Alternatively, a jugular cut down may also be performed if the other peripheral vein catheterization attempts are unsuccessful. As with other species, an Elizabethan collar may be necessary to prevent the animal from chewing and removing the catheter (Riggs and Mitchell, 2009).

TABLE 41.3 Single Bolus Injection Volume Limits in Adult Chinchillas

Route of Administration	Volume (ml)
Subcutaneous ^{a,g,h}	25–50
Intraperitoneal ^{a,c,d}	10
Intravenous ^a	1
Intramuscular ^{a,b,c,d,e,f}	0.3–0.5
Per os ^a	5
Intraosseous ^h	0.1–0.5 (given slowly)

^aHrapkiewicz and Medina, 2007.

^bStrake et al., 1996.

^cHoefer, 1994.

^dJohnson-Delaney and Harrison, 1996.

^eDonnelly and Brown, 2004.

^fHoefer and Crossley, 2002.

^gQuesenberry et al., 2004.

^hAuthors' experience.

Vascular access ports, or VAPs, are commonly used in laboratory animals to provide long-term intravenous access. Typically, the titanium or plastic ports are surgically implanted under the skin. The ports are connected to catheters that are tunneled through the tissues into the lumen of a blood vessel. The vessel can then be accessed through the silicone septum for either administration of compounds or withdrawal of blood. Drawbacks of using VAPs include the need for surgery to implant the ports, continuous maintenance and flushing of the ports to ensure patency, and risk of infection if a break in aseptic technique occurs during access. However, when properly implanted and cared for, VAPs can be used in a variety of vessels and maintained for long periods.

Intramuscular

Intramuscular injection in the chinchilla may be performed in the epaxial muscles (typically lumbar region) or the muscles of the hind leg (Hrapkiewicz and Medina, 2007; Quesenberry et al., 2004). To locate the lumbar muscles, the chinchilla should be securely restrained in the “football” hold or on a flat surface. The spine should be located at the level of the lumbar vertebrae. A 23- or 25-gauge needle should be used to inject just laterally to the spine where the lumbar muscles can be palpated. Injection into the muscles of the upper hind leg or thigh of the chinchilla is similar to that in other species, and the cranial or caudal portion of the leg may be used. As in other species, avoidance of the sciatic nerve is recommended.

Subcutaneous

Subcutaneous injection is commonly performed on the dorsum of the chinchilla anywhere from the neck/interscapular region down to the lumbar region. Injections can also be performed more laterally over the thorax or in the flank region as well. Care should be taken to avoid fur slip when attempting to administer a subcutaneous injection (Johnson-Delaney and Harrison, 1996; Riggs and Mitchell, 2009). When compared to other administration routes, large volumes may be injected subcutaneously due to the elasticity of the skin and the ability to use multiple injection sites at one time. However, the amount injected per site should still be limited as the stretching of the skin may become painful with high volumes. Although some report a maximum of 8–35 ml per site (Hrapkiewicz and Medina, 2007; Quesenberry et al., 2004), the authors have routinely administered up to 50 ml of sterile saline per site. Of course, the maximum amount will depend on the chinchilla’s size and demeanor, elasticity of the skin,

characteristics of the injected material, and frequency of injection. A 22-gauge needle attached to a syringe may be used, or alternatively butterfly needles and fluid bags may be utilized.

Intraperitoneal

The intraperitoneal cavity is a common route of compound and fluid administration in small animals such as the chinchilla. Absorption is presumed to occur through the serosal surfaces of the abdominal organs and the peritoneal membrane (Riggs and Mitchell, 2009). The procedure is best performed with two people. The restrainer should place the chinchilla in dorsal recumbency with the head tilted slightly down so that the visceral organs will presumably rest cranially due to gravity. Administration into the right lower quadrant of the abdomen is preferred when performing intraperitoneal injections in other rodent species in order to avoid the spleen and cecum (Bihun and Bauck, 2004). The person performing the injection should use one hand to extend the chinchilla’s right hind leg caudally while performing the injection with the other hand. A 22-gauge or smaller needle is then inserted in the posterior right quadrant of the abdomen at an approximately 30° angle in relation to the chinchilla’s ventrum. Negative pressure should be applied to the syringe to see if any material is aspirated. If any fluid or material is seen in the syringe, then the gastrointestinal tract may have been inadvertently entered. In this case, the needle and syringe should be removed and discarded as the compound is now contaminated with gastrointestinal bacteria and should not be injected into the sterile abdominal cavity. A new needle, syringe, and fresh compound should be obtained and the procedure attempted again. If no fluid or material is seen upon aspiration, then the compound may be injected.

Intraosseous

Intraosseous catheters provide access to the medullary canal in which fluids or compounds may be infused and are rapidly absorbed (Briscoe and Syring, 2004). Since vascular access may be challenging in smaller species like chinchillas, intraosseous catheters may be a reliable and useful alternative technique for compound administration. These catheters may remain in place for up to 72–96 hours if necessary (Briscoe and Syring, 2004; Riggs and Mitchell, 2009). Placement of an intraosseous catheter is the same as already described in the section on bone marrow collection in the chinchilla. Once the spinal needle is seated in the medullary canal of the proximal femur or tibia, placement can be verified by moving the limb since the

needle should move in the opposite direction to the limb when the limb is moved. Antimicrobial ointment should be applied to the insertion site and the catheter secured with a thick non-compressing bandage encompassing the hub of the catheter and the limb distally. Alternatively, the catheter may be secured by applying an adhesive tape butterfly around the hub of the needle and suturing the tape to the skin. An extension set or intravenous fluid line can be attached to the end of the intraosseous catheter for compound and fluid administration.

SPECIALIZED RESEARCH TECHNIQUES

Auditory Research

The chinchilla is well known for its utility as an animal model for auditory research and diseases of the ear (Amoils et al., 1992; Bakaletz, 2009; Henderson et al., 1994; McFeely et al., 2000; Tang et al., 2009; Tanaka et al., 2009). Chinchillas have large, thin-walled tympanic bullae that measure approximately 25 mm long craniocaudally, 22 mm high, and 12 mm wide (Brown, 2007; Daniel et al., 1982; Hanamure and Lim, 1987). These large bullae lie subcutaneously and can be palpated on the dorsal skull of the chinchilla between and slightly rostral to the base of the pinnae (Brown, 2007; Daniel et al., 1982; Browning and Granich, 1978), thereby providing easy access to the structures of the middle and inner ear. Brown (2007) describes the surgical approach to access the middle ear of chinchillas when used as a model for otitis media. This approach is used to collect fluid or inflammatory material from the normally air-filled tympanic cavity, or to inoculate bacteria to induce the middle ear infection. Briefly, the chinchilla is anesthetized and placed in sternal recumbency. The area over the tympanic bullae on the dorsal part of the skull is clipped, prepared aseptically and draped. A 14–16-gauge needle is placed perpendicular to the bone and gently advanced while rotating the needle on its long axis into the tympanic cavity through the bone. The needle is then removed and the cut end of tubing from a butterfly infusion set can be placed through the hole left by the needle. A syringe attached to the butterfly tubing can then be used for aspiration or infusion. Alternatively, the needle used to enter the tympanic bulla may be left in place and a polyethylene catheter passed through the needle for sample collection (Antonelli et al., 2006). Antonelli et al. (2006) also describe the use of a second needle (23-gauge) concomitantly inserted into the tympanic bulla to vent the middle ear and prevent rupture of the tympanic membrane. After the procedure, the tube is removed, the skin is

closed with tissue adhesive, and the bone left to heal by second intention (Brown, 2007).

Access of the large tympanic cavity of chinchillas also allows access to the structures of the inner ear. Perilymph, a fluid contained in the inner ear, may be collected with small glass capillary micropipettes by exposing the round window, a membrane separating the middle and inner ear (Juhn et al., 1974; Rybak et al., 1979).

The tympanic membrane, or ear drum, is a thin membrane that separates the outer ear canal from the middle ear. The chinchilla is used as a model to induce and study diseases of the tympanic membrane (Amoils et al., 1992; McFeely et al., 2000). Otoscopy, otomicroscopy, myringotomy/ectomy, and tympanometry are just some examples of specialized research techniques that have been used in chinchillas to evaluate the tympanic membrane (Antonelli et al., 2006; Valtonen et al., 2001).

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Diseases and Veterinary Care

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INTRODUCTION

Almost all publications describing disease prevalence for chinchillas have been related to the pet trade or raised for fur rather than for chinchillas that are housed within a limited number of well-managed

research facilities. For pet chinchillas, most abnormal health conditions are a result of husbandry or diet deficiencies (Donnelly, 2004). Diseases resulting from bacteria in chinchillas usually relate to opportunistic agents by commensal bacterial residents that will cause overt disease, either localized to one organ (e.g., *Streptococcus*,

colibacillosis) or as septicemia. Organisms, such as *Listeria* may lead to disease through contamination of feed. Disease by opportunistic agents or parasites usually occurs in animals that are immunocompromised whether through age, nutritional status (improper diet), or environmental stress. Other conditions in chinchillas that require veterinary care are typically secondary to metabolic, nutritional, traumatic, reproductive disorders, and a limited number of age-related and miscellaneous conditions. Additional comprehensive reviews of diseases observed in chinchillas are found in Donnelly (2004), Donnelly and Quimby (2002), Hayes (2000), and Pridham et al. (1996). A comprehensive review of normative clinical data and supportive therapy for chinchillas is described by Quesenberry et al. (2004).

INFECTIOUS DISEASES

Bacterial Diseases

The indigenous bacterial flora of the lower intestinal tract was evaluated in two chinchillas by Worthington and Fulghum (1988) from the Virginia Polytechnic Institute Anaerobe Laboratory. Using anaerobic techniques, the predominant bacterial flora of the colon, cecum, and fecal pellets were evaluated. Species of *Bacteroides* and *Lactobacillus* were commonly identified, and *Bifidobacterium* was cultured once. The recovered flora did not differ significantly from other species of rodents (Worthington and Fulghum, 1988) and their findings are similar to earlier work by Mathieu et al. (1982). Bacterial infections and inadequate husbandry were the most common causes of death in chinchillas at one veterinary college in Canada (Pridham et al., 1966). To alleviate some bacterial infections, chinchillas on fur farms in Germany are vaccinated to prevent disease from *Pseudomonas*, *Escherichia coli*, and *Yersinia* (Matthes, 1985) and chinchillas in Canada have shown immunity to *Pseudomonas* for 36 weeks following vaccination with two doses of a *P. aeruginosa* bacterin (Lusis and Soltys, 1971a).

Listeriosis

BACKGROUND

Listeriosis in chinchillas is common in fur-ranched chinchillas but is not frequently observed in chinchillas within a research environment (Finley and Long, 1977; MacDonald et al., 1972; Nilsson and Soderlind, 1974; Wilkerson et al., 1997). MacKay et al. (1949) provided the first report of listeriosis in chinchillas. Several authors suggested that chinchillas appear to be the most susceptible animal to infection with *Listeria monocytogenes* (Gray and Killinger, 1966; MacDonald et al., 1972), but the prevalence is probably attributed to case reports of listeriosis in chinchillas located on ranches in

cold climates where the animals are fed silage or substitute roughage in pellets, such as beet pulp (Cavill, 1967; Finley and Long, 1977; Wilkerson et al., 1997). Similarly, listeriosis is reported in group-housed animals such as cattle, sheep, goats, deer, swine, rabbits, rodents, birds, and turtles maintained with similar environmental and dietary circumstances (MacDonald et al., 1972; Nilsson and Soderlind, 1974). Advances in taxonomy, isolation methods, bacterial typing, molecular biology, and cell biology of *L. monocytogenes* have contributed to understanding of the agent and associated disease (Low and Donachie, 1997; Rouquette and Berch, 1996; Schlech, 1996).

ETIOLOGY

Listeria monocytogenes is a pleomorphic, motile, non-spore-forming, β -hemolytic, Gram-positive bacillus that inhabits the soil for long periods of time. The organism is a highly adaptable environmental bacterium that can exist both as an animal pathogen and plant saprophyte with a powerful array of regulated virulence factors.

EPIZOOTIOLOGY

Most animal and human cases of listeriosis arise from the ingestion of contaminated food and water, and the disease is particularly common in animals fed silage (Low and Donachie, 1997). The organism is a resident of normal microbial flora in healthy ruminants and is found in environmental sources such as decaying vegetation. The visceric form of listeriosis is more common in chinchillas, chickens, and monogastric species, while the encephalic form is more common in agricultural ruminant species (Gorham and Farrell, 1955; Gray and Killinger, 1966; MacDonald et al., 1972; Shalkop, 1950; Wilkerson et al., 1997).

CLINICAL MANIFESTATIONS

Listeriosis may cause an acute, sporadic disease associated with visceral and neurological signs or result in a chronic infection that may be subclinical. Although most food-borne pathogens primarily cause gastrointestinal disease, *L. monocytogenes* causes a number of additional invasive syndromes, such as encephalitis, abortion, and septicemia. The disease may present as an isolated case or with multiple animals affected. In chinchillas, clinical signs may include sudden death, anorexia, listlessness, diarrhea, weight loss, and ataxia.

PATHOLOGY

In chinchillas, listeriosis is a cecal disease with blood-borne dissemination to other tissues. Grossly, miliary abscesses (approximately 1-mm white foci) are commonly observed in affected organs, and microabscesses may be observed in the liver (Figure 42.1A,B), spleen, cecum (Figure 42.2), and colon. The main target organ is the liver

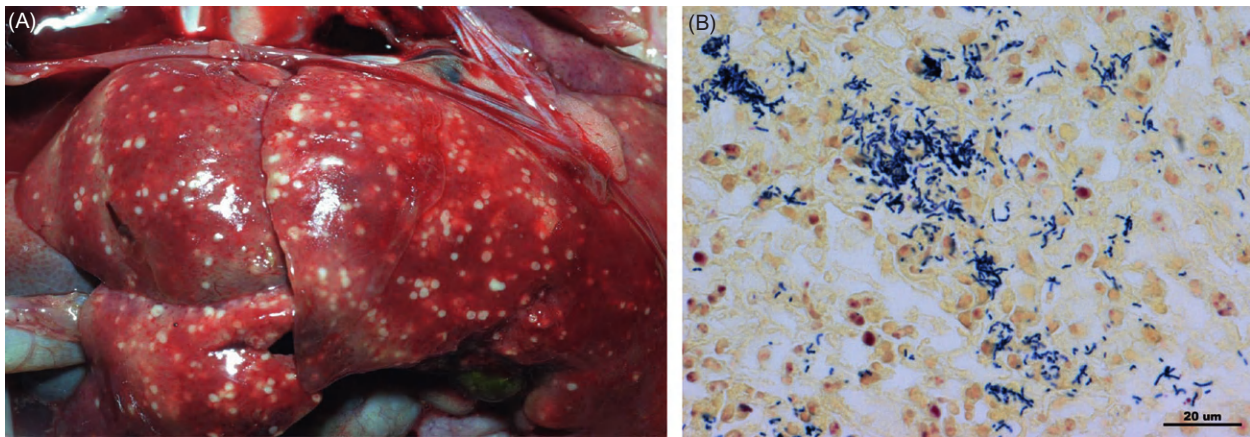


FIGURE 42.1 Listeriosis. (A) Classic lesions of multiple random white foci on the capsular surface of a chinchilla liver. (B) Necrotic areas of chinchilla liver containing numerous intrahistiocytic and extracellular Gram-positive bacilli. Photograph courtesy of Animal Health Laboratory, University of Guelph and Armed Forces Institute of Pathology.



FIGURE 42.2 Listeriosis. Scattered white foci visible on the serosal surface of the cecum in a chinchilla. Photograph courtesy of Animal Health Laboratory, University of Guelph and Armed Forces Institute of Pathology.

as bacteria multiply inside hepatocytes. Early recruitment of polymorphonuclear cells leads to hepatocyte lysis, bacterial release, septicemia, and in surviving hosts, abscesses may develop within the lung, brain, spleen, lymph node, and liver (Donnelly, 2004; Donnelly and Quimby, 2002). Necrotizing enteritis of the small intestine, particularly within the duodenum, may be observed (Loellinger, 1961). The invasion of peripheral nerve cells and rapid entry into the brain, giving the classic histopathological lesion of monocytic perivascular cuffing, is believed to be a unique characteristic of its virulence.

DIAGNOSIS

Initial diagnosis is made from clinical signs, and diagnosis is confirmed by culture and immunohistochemistry. The organism can be isolated from the liver, intestine, colon, and spleen, but other locations include

the lung, stomach, and heart. Impression smears will demonstrate the pleomorphic Gram-positive characteristics of *Listeria*. Of the 16 known serotypes of *L. monocytogenes*, 30% of human cases are caused by three serotypes (Low and Donachie, 1997; Rouquette and Berche, 1996). Although somatic (O) and flagellar antigens (H) may be used to discriminate among strains of *Listeria* (Low and Donachie, 1997), serotyping is not used commonly in infectious outbreaks of listeriosis in animals.

PREVENTION AND THERAPY

Prevention and control involve general precautions to break the cycle of oral transmission, such as disinfection of cages, water and feed containers, and dust baths. Removal of contaminated feed or hay to preclude the development of new infections is also recommended. Treatment, particularly of moribund animals, is not recommended because of the zoonotic potential of the organism.

Yersiniosis

BACKGROUND

Although 11 species of *Yersinia* are recognized, only *Y. pestis*, *Y. pseudotuberculosis*, and specific serovars of *Y. enterocolitica* are important infectious organisms for humans and warm-blooded animals. Yersiniosis is associated with *Y. pseudotuberculosis* and *Y. enterocolitica*. The disease entity occurs worldwide in areas of moderate and subtropical climate, and outbreaks in chinchillas have been described (Gueraud, 1988; Raevuori et al., 1979; Wuthe and Aleksic, 1992). Environmental contamination from asymptotically infected warm-blooded animals is considered the most important factor for spread of the disease agents.

Molecular biological techniques have contributed extensively to the understanding of *Yersinia* pathogenicity. A 70-kilobase plasmid, which is essential for

virulence expression, is present in *Yersinia* associated with human disease and microbiologists have identified 13 plasmid-encoded polypeptides. Pathogenic factors, such as survival in serum, resistance to phagocytosis, cell adhesion, and cytotoxicity, are mediated through the plasmid-encoded polypeptides (Donnelly and Quimby, 2002; Heesemann, 1990). Although virulence of *Y. enterocolitica* depends on a chromosomal gene, endocytosis in intestinal epithelial cells does not appear to be encoded by a plasmid. For *Y. pseudotuberculosis*, virulence depends on a single chromosomal locus (Cornelis et al., 1987). Serologic diagnosis of yersiniosis using plasmid-encoded proteins in immunoblotting and indirect immunofluorescence techniques has resulted in more reliable diagnostics relative to using whole bacterial antigens (Heesemann, 1990). A "chinchilla-type" strain of *Y. enterocolitica* (biovar 3, antigens, or serovar 1, 2a, 3) that appears to persist enzootically among chinchilla stock worldwide was described by Wuthe and Aleksic (1992). Cases of yersiniosis have been reported in mammals (including chinchillas) and birds in both the United States and Canada (Hubbert, 1972; Langford, 1972), and the incidence of yersiniosis in mammals and birds in Antwerp Zoo was reported by Kageruka (1970).

ETIOLOGY

Yersinia enterocolitica is a Gram-negative, aerobic, and facultative anaerobe of the family Enterobacteriaceae. *Yersinia pseudotuberculosis* is a Gram-negative, non-hemolytic, exotoxin- and enzyme-producing, pleomorphic rod. Enteric infections predominate in the diseases caused by these bacteria. *Y. pseudotuberculosis* causes classical pseudotuberculosis or mesenteric adenitis in guinea pigs and has been isolated from many rodents including rabbits. However, *Y. enterocolitica* is currently the species of *Yersinia* most frequently isolated from human and animal infections. *Yersinia* includes both pathogenic and non-pathogenic strains. Among the human pathogens, those isolated in America are more virulent than those isolated elsewhere, especially in Europe and Japan, and these isolates differ biochemically and serologically (Cornelis et al., 1987).

EPIZOOTIOLOGY

Most animal cases of yersiniosis arise from the ingestion of contaminated food, by inhalation, or through dermal lacerations. Offspring may be infected from mothers. A chinchilla epizootic of *Y. enterocolitica* in France was reported by Guerard (1988) and the pathological effects, distribution of the organism within chinchillas, and microbiological isolation methods were described. Raevuori et al. (1979) investigated the virulence and experimental pathogenicity of *Y. enterocolitica* in chinchillas.

CLINICAL MANIFESTATIONS

Yersiniosis is typically an enteric disease in chinchillas and signs may include diarrhea, weight loss, anorexia, severe abdominal pain, dehydration, and bloody feces. Endometritis and septicemia also have been reported in chinchillas (Furowicz et al., 1996). In acute cases, animals may present with sudden death.

PATHOLOGY

Yersiniosis results in epithelial lesions of the ileum, cecum, and colon, which results in mucosal hemorrhage and ulceration. Lymphoid infiltration of the Peyer's patches and mesenteric lymph nodes results in hypertrophy with necrotizing granulomas. Septicemia results in granulomatous lesions in the lungs, spleen, and liver (Donnelly, 2004; Donnelly and Quimby, 2002; Langford, 1972; Wuthe and Aleksic, 1992).

DIAGNOSIS

Palpation of enlarged mesenteric lymph nodes are suggestive of Yersiniosis. Organisms can be observed and cultured from lesions.

PREVENTION AND THERAPY

Yersiniosis can be controlled by obtaining disease-free animals and then preventing contact with wild rodents and birds. Immunization of chinchillas against *Yersinia pseudotuberculosis* has been reported (Furowicz et al., 1996). Tissues from affected chinchillas should be handled and disposed of properly, and housing areas of infected animals should be adequately sanitized.

Pseudomoniasis

BACKGROUND

Opportunistic *Pseudomonas aeruginosa* systemic infections have been described in chinchillas (Lusis and Soltys, 1971b; Menchaca et al., 1980). Pseudomoniasis may result in a rapidly progressing epizootic characterized by significant mortality (Keagy and Keagy, 1951) or sporadic deaths of individual animals (Gorham and Farrell, 1955). Epizootic outbreaks are usually associated with contamination of the water supply to animals (Keagy and Keagy, 1951).

ETIOLOGY

Pseudomonas aeruginosa is a motile Gram-negative rod.

EPIZOOTIOLOGY

Pseudomonas aeruginosa is not a normal inhabitant of the enteric flora of the chinchilla (Mathieu et al., 1982; Worthington and Fulghum, 1988), but is an opportunist agent that inhabits moist environments. Following inhabitation of a host, the agent may be found in the nasopharynx, oropharynx, and gastrointestinal tract. *Pseudomonas*



FIGURE 42.3 Pseudomoniasis. Inguinal dermal pustules and swollen genital region in a chinchilla with *Pseudomonas aeruginosa* infection. Photograph from Doerning et al. (1993) with permission from Laboratory Animals.

aeruginosa has been cultured in a limited number of clinically healthy chinchillas (Mathieu et al., 1982).

CLINICAL MANIFESTATIONS

Pseudomoniasis may be asymptomatic, unless chinchillas are stressed by experimental procedures or an underlying disease (Graham, 1980). Alternatively, affected chinchillas may present with a variety of rapidly progressing clinical signs prior to death (Doerning et al., 1993), or animals may be found dead without any clinical evidence of disease (Gorham and Farrell, 1955). A variety of clinical signs may be observed prior to death, including conjunctivitis, otitis (Wideman, 2006), anorexia, weight loss, diarrhea, corneal and oral ulcerations, scrotal swelling, and inguinal pustules (Figure 42.3).

PATHOLOGY

Septicemia results in necrosis or abscess formation in the liver and other affected organs. Histological findings may include centrilobular vacuolization of hepatocytes as well as hemorrhage, inflammation, and necrosis of affected tissues, such as skin, muscle, and mesenteric lymph nodes.

DIAGNOSIS

Pseudomoniasis is diagnosed on the basis of history, clinical signs, lesions, and isolation of the organism.

PREVENTION AND THERAPY

Control of *P. aeruginosa* often focuses on water quality and reducing colonization of animals through chlorination (10–13 ppm) or acidification (pH 2.5–3.0). Treatment with an antimicrobial may eliminate the infection in chinchillas but may not be practical for large groups of animals. A vaccine against *P. aeruginosa*

has been developed for immunization and is used in fur-ranched chinchillas, but the vaccine may have limited duration of immunity (Lusis and Soltys, 1971a).

Miscellaneous Diseases

ENTEROTOXICOSIS

Enterotoxemia was described in chinchillas attributed to *Clostridium* spp. (Moore and Greenlee, 1975) and *C. perfringens* (Nowakowska et al., 1991). Similarly, Bartoszcze et al. (1990a) described acute deaths in a chinchilla flock due to *C. perfringens* A enterotoxin. Clinical signs included rectal prolapse, flatulence, and diarrhea. Acute deaths, particularly in young chinchillas of 2–4 months of age, are common. Gross findings include hepatic centrilobular necrosis, congestion and necrosis of intestinal mucosa, and proliferation of splenic white pulp. Enterotoxemia is diagnosed on the basis of history, clinical signs, lesions, and identification of the enterotoxin by immunological assay. Treatment for enterotoxemia is primarily through supportive fluid therapy.

SALMONELLOSIS

Several case reports of *Salmonella* infection in chinchillas were available. *Salmonella arizona* (Mountain, 1989), *S. enteritidis* (Yamagishi et al., 1997), and *S. pullorum* (Jones and Henderson, 1953) have been isolated from individual chinchillas. Outbreaks of salmonellosis in multiple animals have been associated with undefined *Salmonella* sp. (Holm and Graves, 1940), *S. sofia* (Naglić et al., 2003), *S. dublin* (Watson and Watson, 1966) as well as *S. enteritidis* and *S. typhimurium* (Misirlioğlu et al., 2002).

KLEBSIELLOSIS

Klebsiella pneumoniae infection was reported in breeding chinchillas (Bartoszcze et al., 1990b). Affected animals had clinical signs of depression, respiratory distress, inappetence, diarrhea, and death. Gross lesions included congestion and consolidation of the lungs, hydrothorax, cardiomegaly, gastroenteritis, and subcapsular renal petechiation. Histological findings were consistent with gross lesions where lungs demonstrated congestion of alveolar capillaries and suppurative exudate of the alveoli. Treatment of the affected chinchillas with gentamicin resulted in recovery of about 50% of treated animals (Bartoszcze et al., 1990b).

Viral and Chlamydial Infections

Two reports were available of herpes virus infection in chinchillas. Goudas and Giltroy (1970) described a spontaneous herpes-like viral infection in an adult female chinchilla. Following acute death of the chinchilla, mild discoloration of the perineal fur was observed. Gross lesions included a pale, friable liver and 2–5 mm white foci dispersed through the adrenal

glands. Wohlsein et al. (2002) described a spontaneous human herpes virus type 1 in a 1-year-old male chinchilla. The animal exhibited conjunctivitis followed by neurological signs of seizures, disorientation, recumbency, and lethargy. Gross and microscopic lesions of non-suppurative meningitis and polioencephalitis with neuronal necrosis and intranuclear inclusion bodies were observed in the chinchilla on postmortem examination. Other findings included bilateral ulcerative keratitis, uveitis, retinitis, retinal degeneration, optical neuritis, and a purulent rhinitis. No other viral or chlamydial diseases have been described for chinchillas.

Parasitic Diseases

Protozoa

TOXOPLASMOSIS

BACKGROUND Although once commonly found in fur-ranched chinchillas (Keagy, 1949), toxoplasmosis has not been reported for chinchillas maintained in laboratory environments as laboratory chinchillas do not have access to sporulated cysts shed by infected cats.

ETIOLOGY *Toxoplasma gondii* is an obligate intracellular coccidial parasite of the family Eimeridae. Cats are the only definitive host, but numerous warm-blooded animals may serve as intermediate hosts.

EPIZOOTIOLOGY Transmission of the protozoon by the definitive host is through ingestion of tissue cysts.

CLINICAL MANIFESTATIONS Clinical signs depend on the location of the parasite within organ tissues, but may include vulvar bleeding, abortion, stillbirths (Green and Morgan, 1991; Vetterling, 1976) or encephalitis (Markham, 1937).

PATHOLOGY Asexual stages of *T. gondii* may be distributed in most organ tissues as tachyzoites or bradyzoites in brain, heart, and skeletal muscle. Necropsy lesions include hemorrhagic lungs, splenomegaly, and enlarged mesenteric lymph nodes. The wide pathological manifestations of toxoplasmosis, with or without necrosis, and tissue cyst formation as it occurs in sheep, cattle, pigs, horses, dogs, cats, chinchillas, and humans was reviewed by Turner (1978). Chinchillas with focal necrotic meningoencephalitis due to *Toxoplasma gondii* have been reported by Meingassner and Burtcher (1977). Several lobulated *Frenkelia* cysts up to 0.6 mm diameter in the brain of two chinchillas were present independent of and remote from the *Toxoplasma* inflammatory reaction. The cysts resembled both morphologically and in staining *Frenkelia* in various *Microtus* species and *Ondatra zibethica*.

DIAGNOSIS Diagnosis is based on serological methods and histopathology.

PREVENTION AND THERAPY Control and prevention depend on limiting access of chinchillas to feces or to items contaminated with feces from cats. As the oocytes are resistant to deactivation by dessication, temperature extremes, and disinfectants, extensive cleaning of infected environments is required.

GIARDIASIS

Giardia duodenalis (synonymous with *G. lamblia*) is a flagellated protozoan that colonizes and reproduces in the small intestine of animals, including the chinchilla.

Giardia sp. are considered to be normal inhabitants of the chinchilla gastrointestinal tract with 66% of fecal samples from chinchillas showing the presence of *Giardia* sp. (Pantchev et al., 2005). Although an examination of feces from chinchillas found a number of *Giardia* organisms (Figure 42.4), a histological examination of the intestines was not conducted (Eidmann, 1992). Historically, group-housed chinchillas, such as in fur ranches and research colonies, often had a high prevalence of *Giardia* infection (Newberne, 1953; Shelton, 1954a, 1954b). A case report of *Giardia* infection in chinchillas located in Taiwan showed a large number of trophozoites within the intestinal lumen and mucosal crypts of the jejunum and ileum (Chang et al., 1986). Stress and poor husbandry can result in an increased number of *Giardia* organisms and predispose animals to opportunistic gastrointestinal bacterial infections, resulting in severe diarrhea and death. While *Giardia* sp. may be present in healthy animals, an increased number of organisms on a fresh fecal smear along with diarrhea may be suggestive of giardiasis. If giardiasis is suspected, treatment may be

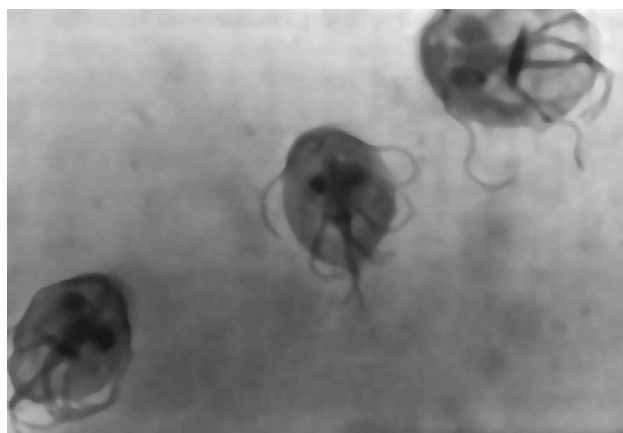


FIGURE 42.4 Giardiasis. *Giardia* trophozoites in a wet mount of a fresh fecal sample. Structural characteristics include the prominent pair of nuclei, longitudinally oriented flagella, and curved median bodies. Photograph from Donnelly (2004) with permission from Saunders.

initiated with fenbendazole, albendazole, or metronidazole (Alves et al., 2008; Donnelly, 2004).

MISCELLANEOUS PROTOZOA DISEASES

EIMERIA The etiology, pathology, and host range of *Eimeria chinchillae* in chinchillas, guinea pigs, hamsters, mice, rats, and rabbits in South Africa have been reported (Vos, 1970; Vos and Dobson, 1970). Infected chinchillas showed clinical signs of depression, anorexia, and diarrhea. Necropsy revealed congestion and edema of the cecum and colon, and a large number of oocysts were present within the large intestine. Sporulated oocysts from infected chinchillas resulted in transmission of *Eimeria* infection to other species.

CRYPTOSPORIDIUM Gastroenteritis associated with a *Cryptosporidium* sp. in a pet chinchilla (Yamini and Raju, 1986) has been described. An 8-month-old chinchilla had severe diarrhea unresponsive to antimicrobials for 8 days prior to death. Grossly, the stomach, small intestine, and colon were bloated and contained yellowish fluid. Histologically, numerous spherical bodies (2–5 µm diameter) characteristic of *Cryptosporidium* were noted on the luminal surface of the epithelial cells of the stomach, small intestine, and colon. Electron microscopy of the small intestine confirmed the presence of *Cryptosporidium*.

SARCOCYSTOSIS Acute hepatic sarcocystosis in a pet female chinchilla has been described (Rakich et al., 1992) in an animal found dead. Hepatic architecture was disrupted by numerous randomly distributed coalescing areas of inflammation and necrosis (Figure 42.5A) and the lung was congested and atelectatic. Various stages of *Sarcocystis* sp. (Figure 42.5B) were dispersed within the areas of hepatic necrosis and inflammation.

Nematodes

Cerebral nematodiasis caused by the raccoon ascarid (*Baylisascaris procyonis*) in chinchillas in western Canada has been described (Sanford, 1989, 1991). Chinchillas from multiple ranches presented with central nervous system signs of ataxia, tumbling, torticollis, paralysis, incoordination, and death. Grossly, no lesions were evident. Histologically, malacic tracts or areas of necrosis, swollen axons, and infiltrates of inflammatory cells in perivascular sites and necrotic areas were noted in the brainstem and cerebellum. The usual source of infection is contamination of feed stored in barns by raccoon feces. Treatment is supportive care.

Although uncommon, other nematodes have been reported in chinchillas. *Ostertagia* sp. females and a *Trichostrongylus colubriformis* male were found in one animal from a group of emaciated chinchillas (Stampa and Hobson, 1966), and chinchillas infected with *Haemonchus contortus* have been reported (Kraft, 1960). In both reports, chinchillas had been fed grass from pasture housing ruminants. Susceptibility for chinchillas to be infected with *H. contortus* was reported by Boisvenue and Hendrix (1968).

Cestodes

Chinchillas may be infected with a variety of cestodes. Gorham and Farrell (1955) reported that the chinchilla could serve as an intermediate host for *Taenia serialis*, *T. pisiformis*, *Echinococcus granulosus*, and *Hymenolepis nana*. Stampa and Hobson (1966) reported infection of chinchillas with *H. nana*; animals in the postweaning period exhibited listlessness and poor body conditioning, while adult and unweaned animals appeared normal. *H. nana* may have both a direct and intermediate life cycle in the chinchillas and can cause

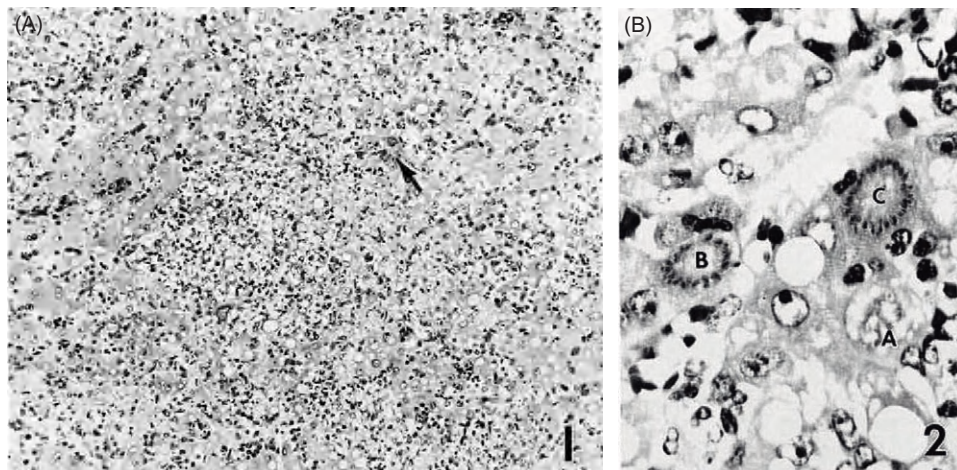


FIGURE 42.5 Sarcocystosis. (A) Necrosis of hepatocytes in liver from a chinchilla infected with *Sarcocystis* sp. Arrow points to schizonts (150x). (B) Higher magnification of the schizonts in (A). Three schizonts (A, B, C) in various stages of division and arranged in presumed order of development in hepatocytes (1050x). Photograph from Rakich et al. (1992) with permission from the Journal of Veterinary Diagnostic Investigation.

heavy intestinal infestations (Olsen, 1950). Several reports describe infections of chinchillas with *Taenia* sp. A *Taenia* coenurus located in the ventral ocular orbit was reported in a single chinchilla (Holmberg et al., 2007), and *Taenia* coenurus was reported in seven chinchillas by Bracken and Olsen (1950). Several hundred cysts of *Taenia crassiceps* were found in the abdominal cavity of a pet chinchilla (Kugi et al., 1999). Staebler et al. (2007) reported the presence of *Echinococcus multilocularis* in a single chinchilla exhibiting painful abdominal distension. Postmortem examination revealed numerous hepatic vesicles and cysts.

Other

Subcutaneous myiasis by *Cuterebra* larvae in Argentina was reported by Led and Brandetti (1974).

Mycoses

Dermatophytosis

BACKGROUND

Ringworm has been reported primarily in chinchillas maintained under poor husbandry practices. These reports of ringworm are in regard to chinchillas raised in the fur industry, but cases for pet or research animals are rare (Hayes, 2000).

ETIOLOGY

Trichophyton mentagrophytes is the dermatophyte most commonly isolated from fur-bearing animals and infected chinchillas. However, *Microsporum canis* and *M. gypseum* also have been incriminated in spontaneously occurring outbreaks in chinchillas (Bohm and Loliger, 1969; Gimesi, 1980; Graham, 1961; Hagen and Gorham, 1972; Horvath and Keri, 1980; Janovitz and Long, 1984; Male and Fritsch, 1966; Morganti and Gomez Portugal, 1970; Torres-Rodriguez et al., 1992).

EPIZOOTIOLOGY

Lesions are seen most often in young or malnourished animals, or animals that are housed in an unclean or stressful environment.

CLINICAL MANIFESTATIONS

Dermatophytosis of chinchillas results in small scaly patches of alopecia on the nose, eyes, behind the ears, anus, or on the forefeet. Lesions may appear on any part of the body, and in advanced cases a large circumscribed area of inflammation with scab formation occurs.

PATHOLOGY

Skin lesions consist of exfoliated debris, exudate, mycelia, and arthrospores associated with hyperemic dermatitis.

DIAGNOSIS

Diagnosis is based on clinical presentation, skin scrape, and fungal culture. Fungal cultures of chinchillas on ranches showed a 5% incidence of *T. mentagrophytes* in animals with normal skin; in contrast, a 30% incidence of *T. mentagrophytes* was observed in animals with fur damage (Hagen and Gorham, 1972; Male and Fritsch, 1966).

PREVENTION AND THERAPY

Prevention is through providing a clean living environment and minimizing stress to animals. Oral or topical antifungals can be used to treat chinchillas with dermatophytosis, and an antifungal powder may be mixed with the dust bath to assist in minimizing transmission to group-housed chinchillas. A live attenuated vaccine, Trichopelen (Bioveta, Inc.), containing *T. mentagrophytes* has been used to prevent dermatophytosis in fur-bearing animals, including chinchillas (Lund and DeBoer, 2008; Sarkisov and Nikiforov, 1981). The vaccination schedule includes two doses by intramuscular injection at an interval of 8–12 days with a 1-year immunity established 1 month after the last dose.

Histoplasmosis

Two reports of spontaneous histoplasmosis due to *Histoplasma capsulatum* have been described in chinchillas. Burtscher and Otte (1962) described histoplasmosis in a chinchilla exported from the United States to Switzerland. Owens et al. (1975) reported histoplasmosis in a female chinchilla originating from a commercial ranch in central Missouri. Clinical signs in the affected chinchilla included anorexia, weight loss, constipation, and tachypnea. Necropsy revealed pulmonary lesions of multiple hemorrhagic foci, consolidation, bronchopneumonia, and epithelioid cells containing the organism. In addition, the spleen and liver contained multifocal pyogranulomatous lesions, and focal glomerular nephritis characterized by accumulation of polymorphonuclear cells in the proximal tubules. Epithelioid cells containing the organism were present in the liver, spleen, and kidneys. Culture of the timothy hay used for food demonstrated the presence of *H. capsulatum*.

GASTROINTESTINAL AND METABOLIC DISEASES

Esophageal Disease

Chinchillas cannot vomit, and esophageal choke can result after the ingestion of large objects. Although choke is not commonly observed, it has been described in chinchillas of all ages (Cousens, 1962). When observed, choke is more common in postparturient

females that eat their placentas or animals that consume their bedding. Affected animals show drooling, retching, dyspnea, and anorexia. Diagnosis is by palpation, endoscopic examination of the oropharynx and esophagus, and radiography. Treatment is through removal of the lodged foreign material from the esophagus.

Megaesophagus has been reported in a female chinchilla that presented with severe, recurrent pneumonia. The recurrent pneumonia was attributed to aspiration of regurgitated content secondary to megaesophagus (Jopp et al., 2004).

Bloat

Gastric tympany or bloat has been reported to be a problem of lactating female chinchillas resulting from overeating of clover, gastroenteritis, or a dietary change such as an addition of fresh green grasses or fruits (Donnelly, 2004). Tympany also may result in chinchillas with gastrointestinal disease. Clinical signs in affected animals are distended and painful abdomens, lateral recumbancy, lethargy, dyspnea, and in severe cases, death. Treatment is through alleviation of gastric distension by decompression with a gastric tube or use of a transabdominal trocar in sedated or anesthetized animals.

Gastric Trichobezoars

Although uncommon, gastric trichobezoars may result from excessive fur chewing. Affected chinchillas typically present with anorexia and lethargy. Diagnosis is by palpation and radiography. Treatment is through administration of fluids and laxatives. Proteolytic enzymes also may be useful to degrade the trichobezoars and animals may be fed a high-fiber diet (Donnelly, 2004).

Constipation

In chinchillas, constipation can be associated with many infectious and non-infectious gastrointestinal disorders and may occur more often than diarrhea (Hartmann, 1993). Constipation is associated with chinchillas fed a diet that is high in calories and protein but contains insufficient roughage or fiber (Cousens, 1962). Other causes of constipation may include obesity, lack of exercise, intestinal obstruction, and intestinal compression due to large fetuses during pregnancy (Hartmann, 1993). Clinical signs of constipation may include straining to defecate, decreased fecal pellets, and any pellets passed are thin, short, hard, and occasionally bloodstained. In chronic cases of constipation, ileus, intestinal torsion, intussusception, and impaction of the cecum or colonic flexure may occur (Bowden, 1959; McGreevy and Carn, 1988). Rectal prolapse may

also occur in severe cases of constipation or diarrhea. From 91 chinchillas necropsied during a 4-year period in a Japanese laboratory, prolapse of the rectum was noted in 6% of the animals (Kuroiwa and Imamichi, 1977). Treatment of constipation is dependent upon the underlying basis, but increasing dietary fiber through gradual provision of hay or fresh vegetables and providing a bulk laxative may be beneficial.

Intestinal Torsion

A single case report of intestinal torsion in a 4-year-old male chinchilla was reported by McGreevy and Carn (1988). Anorexia and listlessness were described for the animal. Necropsy revealed a 360° torsion of the distal small intestine through the mesocolon.

Diabetes Mellitus

Diabetes mellitus in a 5-year-old overweight female chinchilla has been described (Marlow, 1995). This chinchilla had a 3-week history of poor appetite, lethargy, weight loss, and polydipsia/polyuria. Bilateral cataracts were identified in the chinchilla during its evaluation. Blood and urine analysis demonstrated a hyperglycemia greater than 400mg/dl, glucosuria, and ketonuria. Following euthanasia, microscopic examination of pancreatic islets revealed prominent vacuolation consistent with diabetes mellitus. Ewringmann and Göbel (1998) also reported cases of diabetes mellitus in chinchillas.

Hyperthyroidism

Hyperthyroidism was described in a 6-year-old female chinchilla that exhibited progressive weight loss, elevated glucose levels, glucosuria, and elevated T4 levels (Fritsche et al., 2008).

TRAUMATIC LESIONS

Fur Slip

Fur slip in chinchillas is defined by a release of a patch of fur, and it has been attributed to a predator avoidance syndrome, a behavioral disorder, nutritional imbalance, and/or endocrine imbalance. Chinchillas which fight, are handled roughly by humans, or during scruffing may release quantities of fur. The lost fur may take several months to regrow and cover the smooth area of skin (Donnelly, 2004). Prevention of fur slip can be aided through wearing of cotton or latex gloves to prevent hand oils from damaging fur, handling of chinchillas by gently cupping the animal in both hands, and provision of a good-quality diet.

Bite Wounds

Although chinchillas housed in groups rarely fight, frequent bite wounds that abscess occur during breeding. Female chinchillas are larger than males and highly selective in their choice of males for mating. Males not selected for breeding are disenchanted by the female through urination, kicking, and biting (Bignami and Beach, 1968; Weir, 1973). On occasion, a young male housed in the same cage as older females may be killed (Weir, 1970). Culture of the abscesses often yields *Staphylococcus* species (Jenkins, 1992), but collecting samples for culture and sensitivity is recommended. Bite wounds may result in the loss of a portion of the ears and toes. Provision of hiding areas for males in group housing can minimize aggressive behavior by female chinchillas during breeding.

Limb Injuries

Limb injuries usually result from an inadequate housing environment. Wire-bottom caging can result in foot pad injuries to chinchillas, particularly if overweight (Hayes, 2000). Provision of a resting board or other solid surface can prevent foot pad injuries. Traumatic fractures of the tibia are commonly seen and are associated with the animal catching its hind limb in a cage bar (Hoefer, 1994). The tibia is a straight bone longer than the femur and minimal muscle is located over the bone. Tibial fractures are either transverse or short spiral and may be associated with bony fragments. Treatment of tibial fractures commonly requires surgical repair, but malunion may result unless physical activity is restricted during convalescence. Amputation of the hindlimb may be considered if surgical repair is unsuccessful.

MANAGEMENT-RELATED DISORDERS

Fur Chewing

The loss of hair, alopecia, in chinchillas may result from fur chewing in group-housed animals. One theory for fur chewing is based upon a stereotypy transferred from dams to their offspring. Similar to maladapted behavior in other species, a higher incidence of fur chewing is observed in chinchillas maintained in the captivity of commercial herds and not being able to engage in more normal behaviors of free-ranging animals (Donnelly, 2004). Animals also may suffer from malnutrition and chew their fur for dietary requirements (Eidmann, 1992); however, the etiology of the malnutrition has not been defined. Historically, fur chewing in chinchillas has been attributed to

an increased thyroidal and adrenocortical activity (Vanjonack and Johnson, 1973) and a fungus (Shaull, 1988). Thyroid hyperplasia was correlated to the size of chewed fur over the body and was interpreted as a reactive response of the thyroids due to the inability of the body to maintain warmth following fur removal (Eidmann, 1992). An infectious basis of fur chewing was dispelled following review of mycological and bacterial cultures of skin and fur from 39 fur chewers relative to that of 19 healthy chinchillas (Eidmann, 1992).

Matted Fur

Chinchillas are fastidious groomers and dust-bathing is common. Frequent availability of dust baths is recommended for at least 30 minutes per day (Donnelly, 2004). Chinchillas denied dust-bathing may develop matted fur from oily secretions on the back (Hillyer et al., 1997). Schaeffer and Donnelly (1997) reported that dust-bathing can lead to irritative conjunctivitis without clinical signs of upper respiratory infection.

NEOPLASTIC DISEASES

Reports for neoplasia in chinchillas are limited despite a life span up to 20 years of age (Schaeffer and Donnelly, 1997). Postmortem examinations by Brenon (1953, 1955) on 1005 chinchillas (527 males and 478 females) before mid-1949 and another 1000 chinchillas over the subsequent 3 years did not list neoplasia as a cause of death. Although the postmortem examinations were conducted in chinchillas ranging between less than 6 months and 11 years in age, the majority of the animals were less than 3 years of age. Newberne and Robinson (1957) described a malignant lymphoma in a 3-year-old chinchilla that was found dead without any prior untoward clinical signs. A uterine leiomyosarcoma was reported in a 1-year-old female chinchilla as an incidental finding at necropsy (Schaeffer and Donnelly, 1997). Other reported types of neoplasia in chinchillas include malignant lymphoma (Newberne and Seibold, 1953), hepatic carcinoma (Nobel and Neumann, 1963), lumbar osteosarcoma (Simova-Curd et al., 2008), and carcinoma of salivary gland (Smith et al., 2010).

MISCELLANEOUS CONDITIONS

Dental Disease

BACKGROUND

Malocclusion is a common problem in chinchillas, whereby approximately half of all deaths in adult chinchillas in the 1960s were due to digestive tract disorders

and malocclusion accounted for one-quarter of those deaths (Cousens, 1962; Dall, 1963). Abnormalities of cheek tooth overgrowth remain common in chinchillas (Crossley, 1997; Crossley et al., 1997), and chinchilla breeders commonly refer to the condition as “slobbers” based upon the clinical signs of hypersalivation. However, other conditions of the oral cavity may result in hypersalivation, which is not specific to malocclusion (Crossley, 2001).

ETIOLOGY

The dental formula of chinchillas is 1/1 incisors, 0/0 canines, 1/1 premolars, and 3/3 molars. Incisors and cheek teeth of chinchillas grow continuously throughout life at a rate of 2.5–3 inches per year (Hayes, 2000).

EPIZOOTIOLOGY

Genetic predisposition and husbandry practices are considered contributory in malocclusion of chinchillas. Crossley and Miguélez (2001) reported that cheek tooth lengths in captive-bred chinchillas were significantly elongated in comparison to wild-caught animals (Figure 42.6), suggesting that physical form or composition of the diet is a contributing factor in malocclusion and resulting dental disease. During examination of 123 chinchillas, dental disease was found in 32.5% of the animals and chinchillas had a higher incidence of gingival erosions and gingivitis in comparison to rabbits and guinea pigs. In chinchillas, significantly more pathology of teeth and associated soft tissues was noted that corresponded to the fourth premolar (bilateral) and first molar (right side) of the upper arcade relative to other teeth including incisors (Jekl et al., 2008). Similarly, Crossley (2001) reported dental abnormalities, such as tooth elongation, in 35% of apparently healthy chinchillas ($n = 651$) in the United Kingdom.

CLINICAL MANIFESTATIONS

The most common signs of dental disease include hypersalivation and drooling, while other signs may include ventral mandibular swelling, pawing at the mouth, anorexia, dysphagia, weight loss, nasal discharge, and selective choice of finer relative to coarser food particles (Crossley, 2001; Donnelly, 2004). Progressive growth of the cheek teeth (premolars and molars) results in ulceration of the buccal mucosa and tongue. Although uncommon, advanced stages of malocclusion may lead to periodontitis and alveolar abscessation of maxillary and mandibular cheek teeth (Emily, 1991; Griner, 1983). In a pet chinchilla exhibiting anorexia and malocclusion, caries and odontoclastic resorptive lesions were reported by Crossley et al. (1997). During postmortem examination of 104 chinchillas in the United Kingdom, 37% of the animals had caries and resorptive lesions (Crossley, 2001).

PATHOLOGY

Lesions may include root elongation, ulceration of buccal mucosa and tongue with periodontitis, alveolar abscesses, caries and resorptive lesions, and perostitis in advanced stages.

DIAGNOSIS

Clinical signs are pathognomonic for malocclusion in chinchillas. Examination of the narrow oral cavity using an otoscope can assist in visualizing the cheek teeth. Rigid endoscopy provides excellent visualization of the oral cavity to assess dental disease in chinchillas (Jekl and Knotek, 2007). Although manual restraint of chinchillas for the examination of the oral cavity is possible, anesthesia of the animal contributes to a more thorough examination. Radiography of the skull (Crossley, 2001) and computerized tomographic (CT) scanning

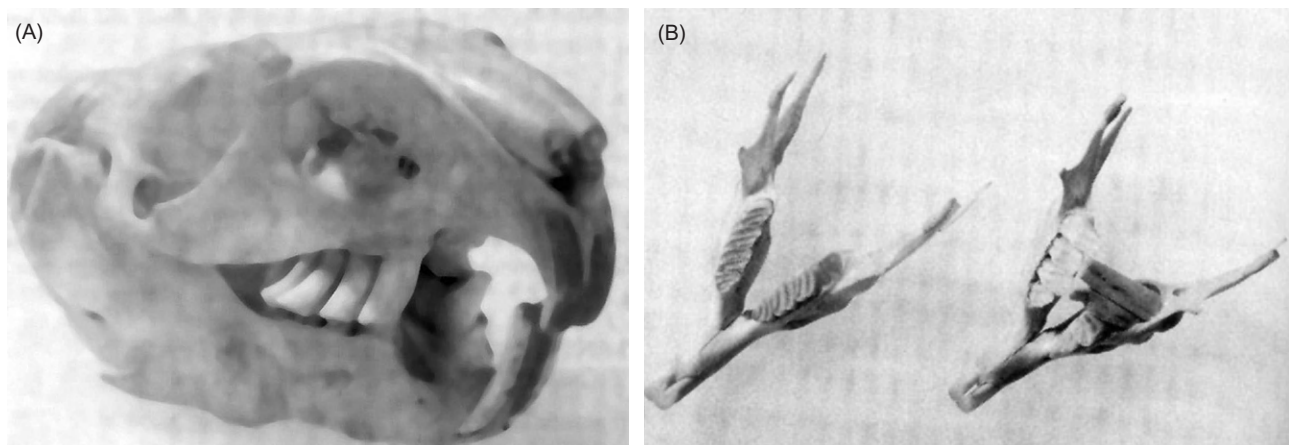


FIGURE 42.6 Malocclusion. Malocclusion of the dental arcade in the chinchilla. (A) Lateral view of the chinchilla skull showing outward curvature of the upper molars. (B) Normal mandible of a chinchilla (left) in comparison to a mandible with malocclusion (right). Photograph from Donnelly (2004) with permission from Saunders.

(Crossley et al., 1998) to investigate tooth structure in chinchillas has been shown to be a useful tool in the early diagnosis of malocclusion.

PREVENTION AND THERAPY

Treatment consists of repeated trimming and adjustment of the occlusal surface of overgrown teeth in affected chinchillas. Malocclusion is influenced by husbandry practices and provision of a high-fiber diet may be beneficial. Although hard objects, such as wood blocks and pumice stones, may be provided to chinchillas to minimize malocclusion, wearing is limited to incisors and does not affect cheek teeth. Continued malocclusion of the cheek teeth may result in progressive dental disease. As malocclusion is caused by hereditary elements, affected animals should be eliminated from breeding stock.

Penile Fur Ring

BACKGROUND AND EPIZOOTIOLOGY

Adult male chinchillas commonly develop accumulation of fur surrounding the penis. Fur may come from other males or the same animal as the condition is also seen in group-housed and single-housed males not exposed to females. In some male chinchillas, the penis will extend from the prepuce all the time even when not engorged. In these males, the cause of this condition is not associated with fur ring but is due to overexcitement brought on by separation from their mates or overexhaustion due to too many females in the same cage.

CLINICAL MANIFESTATIONS AND PATHOLOGY

Affected animals may show signs of excessive grooming, frequent production of small quantities of urine, straining to urinate, and repeatedly cleaning of the penis (Schaeffer and Donnelly, 1997). Presumably, the ring of hair accumulates around the penis within the prepuce, which eventually prevents the penis from retracting back into the prepuce leading to paraphimosis. In severe cases, an engorged penis may protrude 4–5 cm from the prepuce. The condition may progress to cause severe pain in the affected animal, and result in urethral constriction and acute urinary retention. Chronic paraphimosis may culminate in infection and severe damage to the penis, which may ultimately impact the breeding ability of the animal. Congestion of penile tissues is associated with fur ring.

DIAGNOSIS

Clinical signs are pathognomonic for penile fur ring in chinchillas.

PREVENTION AND THERAPY

Many male chinchillas have fur accumulation around the penile base without any clinical signs, and

visual examination of penile base in male chinchillas should be routine during any physical examination. Any visible fur ring should be cut or gently rolled off the penis after applying a sterile lubricant. Occasionally, sedation or anesthesia of the male may be required to remove the fur ring.

Ocular Disorders

Eyes of 14 chinchillas ranging from 6 to 17 years of age were examined by Peiffer and Johnson (1980). Mean intraocular pressures in the chinchillas were 18.5 ± 5.75 mm/Hg. Bilateral posterior cortical cataracts and asteroid hyalosis were observed in two animals.

Conjunctivitis from environmental factors may be observed in chinchillas. Precipitating environmental factors range from dirty or poor-quality bedding, inadequate cage ventilation, and excessive dust bathing (Donnelly, 2004; Schaeffer and Donnelly, 1997). Conjunctivitis also may result from infection with *Pseudomonas aeruginosa* (Doerning et al., 1993; Lusi and Soltys, 1971b). Treatment should focus on minimizing exposure to any irritating factor (e.g., limiting duration of access to dust baths, providing fresh bedding) and providing a protective ophthalmic solution or ointment.

Reproductive Disorders

Chinchillas usually give birth early in the morning and only rarely before midnight (Weir, 1966). Cousens (1962) reported that dystocia is usually associated with the presentation of a single, oversized fetus or abnormal presentation of one or more kits, while uterine inertia has been reported as another cause of dystocia (Prior, 1986). Cesarean section is effective to resolve the dystocia (Caspari, 1990; Jones, 1990; Prior, 1986; Sims, 1990; Stephenson, 1990). Coexisting intrauterine and intraperitoneal pregnancy due to superfetation in a chinchilla has been reported (Gitlin and Adler, 1969). A retrospective review of birth-to-weaning mortality in a Japanese laboratory colony of chinchillas was described by Kuroiwa and Imamichi (1977). Among 91 animals that died, the cause of death in 23 (25.3%) was newborn-related. Thirty-seven chinchillas gave birth to 71 kits with an average litter size of 1.9, and 59 kits were successfully weaned (1.6 per litter). Of these kits, 50 (1.3 per litter) attained 240 days of age.

Oxalate Nephrosis

Six spontaneous cases of oxalate nephrosis were described in adult female chinchillas (Goudas and Lusi, 1970). Clinical signs include progressive weakness

followed by death. Histologically, deposition of refractive, translucent, yellowish crystals occurred within renal tubules. Oxalate nephrosis should be considered rare in chinchillas.

Urinary Calculi

Three cases of urinary calculi and urolithiasis in male chinchillas have been reported (Jones et al., 1995; Newberne, 1952; Spence and Skae, 1995) and should be considered uncommon in chinchillas. Animals had a history of hematuria or anuria. Clinical signs may include straining and erythematous preputial and perineal area. Ultrasound and radiography can be confirmatory as the bladder calculi are composed of calcium carbonate (Jones et al., 1995; Spence and Skae, 1995). Diet may have contributed to the cases.

Heat Stroke

Chinchillas are susceptible to stress from temperatures at 80°F or higher, particularly in humid conditions (Jenkins, 1992). Clinical signs include tachypnea, prostrate posture, salivation, hyperthermia, hemoptysis, cyanosis, and death. Treatment includes reducing body temperature through cool water baths and administration of intravenous fluids in severely affected animals.

Schistosomus Reflexus

A case of abnormal embryological development in a chinchilla, resulting in a schistosomus reflexus fetus, was reported by DeNooij (1985).

Diaphragmatic Hernia

A single case report of a diaphragmatic hernia in a female chinchilla was described by Dall (1967). The chinchilla displayed anorexia and depression for 2 days prior to examination. During a procedure, the chinchilla died. Necropsy revealed the rectum was empty, while the stomach, small intestine, and cecum were content-filled. Herniation of the large colon into the pleural cavity through the aortic hiatus was found upon further review, but no strangulation was evident.

Incidental Postmortem Findings

Trophoblastic Emboli

Hemochorial placentation is common to many species, such as humans, mice, guinea pigs, hamsters, porcupines, and chinchillas (Billington and Weir, 1967; Hamir and Rupprecht, 2008; Ilha et al., 2000; King and Tibbitts, 1976; Tvedten and Langham, 1974). As the interhemal membrane of the placental labyrinth is hemomonochorial in chinchillas, it consists of a single layer of syncytial trophoblasts. A trophoblastic pulmonary emboli (Figure 42.7) in a chinchilla was described by Tvedten and Langham (1974). Another report described trophoblastic pulmonary embolism in three chinchillas (Ilha et al. 2000), and one of these chinchillas had trophoblastic infiltration of the myometrium. Trophoblastic emboli in chinchillas are considered incidental findings on histopathological examination.

Fatty Livers

Fatty livers are commonly observed during routine necropsies without clinical signs or other pathological findings (Egri et al., 1994).

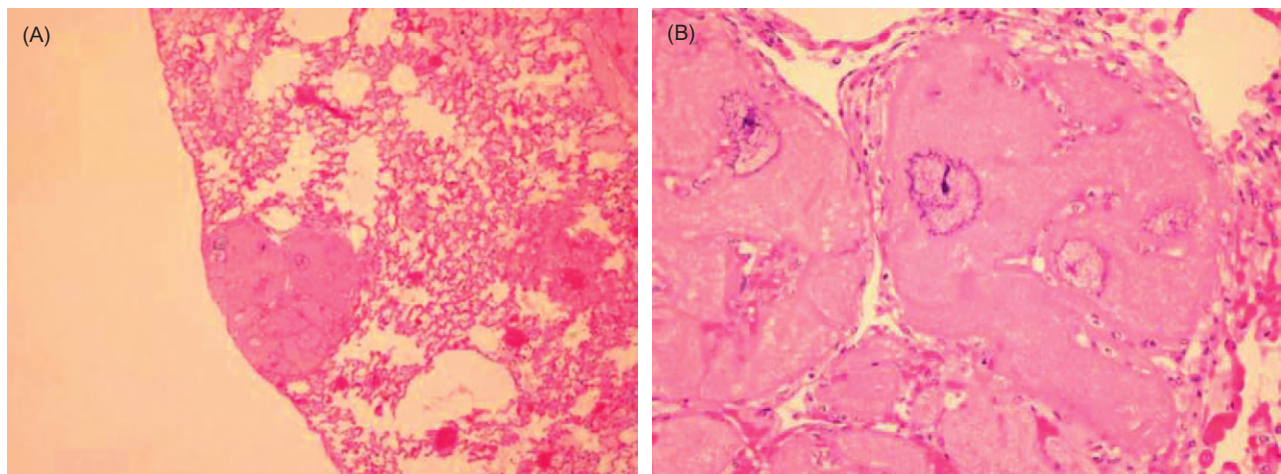


FIGURE 42.7 Trophoblast embolus. (A) Lung from a female chinchilla containing 100–200 μm trophoblast cells occurring singly or multiply. Trophoblast cells had abundant cytoplasm and 50–100-μm diameter nuclei (H&E stain). (B) Closer view of trophoblast embolus from (A) (H&E stain). Photograph courtesy of Brian Caserto, Kansas State University.

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Chinchillas as Experimental Models

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INTRODUCTION

Chinchilla is the traditional animal model for most types of research related to the ear (DeMaria, 1989), specifically, hearing loss and otitis media. According to the USDA, 2250 chinchillas were used in research in 2008

in the US, a decline over the last 20 years as chinchilla models are gradually replaced by mice and rats (Salvi and Boettcher, 2008). However, the chinchilla still has important characteristics that make it an ideal model for studying the ear. The chinchilla inner ear anatomy (including the cochlea) and ear physiology are similar

to humans. The chinchilla ear has three turns in the cochlea, a tubotympanum that shares similar anatomy with that of humans (Giebink, 1982; Giebink et al., 1976), a wide tympanic membrane, a cochlea readily accessible for microsurgical procedures, and a middle ear easily accessed through a large and very thin bulla; all attributes that enable researchers to conduct studies on the ear. Owing to the relatively longer lifespan of the chinchilla (15 years) versus a mouse (2 years), the auditory system does not undergo presbycusis (aging) in the chinchilla during the period of study (Giebink, 1982; Giebink et al., 1976), and thus allows a more consistent and less variable animal model (Bakaletz, 2009). The audiogram (range of hearing) of the chinchilla appears to resemble the human audiogram more closely than do other rodent's (Giebink, 1982; Heffner and Heffner, 1991) at 20Hz to 30KHz, making them popular for hearing research. The chinchilla's ear is more sensitive to toxicity because the round window is thinner (10–14 μ m) compared to that of humans (40–70 μ m), thereby enabling more drug penetration into the cochlea, and therefore serving as a sensitive indicator of ototoxic drugs. Low cost, ease of handling, and small size (Giebink et al., 1976) are other attributes that together with those already mentioned, make the chinchilla a reliable and precise model for research (Juhn et al., 1977).

Immunologically and genetically the chinchilla has many advantages for research. Chinchillas have well-defined nasal-associated lymphoid tissue as well as a simple naso- and maxilla-turbinate system that is more like that of other rodents and unlike the branched turbinate's of dogs, cats, and rabbits, which facilitates studies of intranasal delivery of vaccine candidates, adjuvants, and novel therapeutics for studying otitis media (Bakaletz, 2009). The immune response of the chinchilla can be studied with commercially available reagents due to the fact that chinchilla and human cytokines cross-react (Giebink, 1999; Sato et al., 1999). Human cytokines stimulate maturation of chinchilla bone-marrow-derived dendritic cells, and antibodies directed against human immunoglobulins cross-react with chinchilla immunoglobulins (Bakaletz, 2009). However, a lack of molecular and immunological reagents specific to chinchillas (anti-chinchilla antisera) is an obstacle to study at molecular levels that are routine in rats and mice (Bakaletz, 2009; Lim et al., 2005) including immune regulation, lymphocyte traffic, and genetic predisposition (DeMaria, 1989). At the genetic level there are now multiple examples of close homologies between the products of human genes and those of chinchillas including mucin genes, genes that encode effectors of innate immunity, and genes that encode for epithelial cell receptors (Bakaletz, 2009).

Chinchillas are not inbred or commercially available and are typically obtained from local chinchilla

ranchers whose coats are unsuitable for fur production (Green et al., 1994). SPF or transgenic lines are not yet available, so research results may be confounded by genetic and pathologic differences (Lim et al., 2005). However, the fact that preclinical trials conducted in this host are typically performed in mixed sex, outbred animals, chinchilla's likely better mirror the diversity of immunological responsiveness one would encounter in a human clinical trial (Bakaletz, 2009).

Chinchillas are used in multiple areas of research but mainly those involved with the ear. These include hearing loss, otitis media, tinnitus, tympanic membrane perforations, cochlear implants, perilymphatic fistulas, superior canal dehiscence, electrode implantation, cholesteatoma, otic drug testing, and pharmacokinetics of antibiotics for treatment of ear infections. They are also used on occasion to study kidney microanatomy, cardiology anatomy, arterial blood supply to the brain and ear, and ovarian endocrine activity. Other research fields in which chinchillas have been used include studies of Chagas disease, gastrointestinal disease, pneumonia, *Listeriosis*, as well as *Yersinia* and *Pseudomonas* infections. The more common and significant uses of chinchillas as models related to the ear will be covered in this chapter.

MODELS RELATED TO AURAL DISEASE

Models of Otitis Media

Several rodent hosts including the gerbil, rat, mouse, guinea pig, and chinchilla, have been used to develop models of single pathogen-induced otitis media, however the chinchilla is the predominant host and considered the gold standard (Bakaletz, 2009; McGillivray et al., 2007). The use of chinchilla in otitis media research is predated by its use in auditory and acoustic research owing to its anatomical and physiological similarities between the inner ear and that of man (Bakaletz, 2009; Giebink, 1999). Chinchillas make excellent models to study the pathogenesis of aural disease for several reasons. First, chinchillas do not naturally develop otitis media, as do some mice, rats, gerbils, guinea pigs, and rabbits (Bakaletz, 2009; Giebink, 1982), presumably because they are not coprophagic. Second, chinchillas can be colonized and experimentally infected with human pathogens (Harris et al., 2004; Lim et al., 2005; McGillivray et al., 2007) both active bacterial and viral infections in the uppermost airways whereas most rodents only support lower-airway disease. Third, chinchillas have a large and thin cephalid bulla, from which large quantities of middle ear fluids can be withdrawn easily and assessed (Bakaletz, 2009; Harris et al., 2004). This characteristic permits serial recovery of middle ear fluid without compromising the integrity of the

tympanic membrane, which is a distinct advantage to researchers (DeMaria, 1989; Giebink, 1999; Harris et al., 2004). Fourth, chinchilla have a permanently semipatulous Eustachian tube highly similar to the “floppy” Eustachian tube of children, making them a relevant model of pediatric otitis media (Bakaletz, 2009; Harris et al., 2004). Lastly, the juvenile chinchilla model mimics the immunological immaturity of the human child, the age when otitis media mostly occurs (Bakaletz, 2009; Lim et al., 2005).

The single pathogen chinchilla model of otitis media was first developed in 1975 by Dr. Giebink (Bakaletz, 2009), whereby the pathogen is directly instilled in the middle ear. This single-pathogen direct-challenge middle ear model is still used extensively to study therapeutics and surgical intervention strategies (Bakaletz, 2009). Single-pathogen models allow for a simple interpretation of experimental data over that of co-infection models described below (Yang et al., 1998). The drawback however to these models is that they bypass the early steps in the development of pathogenesis, i.e. colonization of the nasopharynx, ascension up the Eustachian tube, and initiation of middle ear infection typically seen in human otitis media cases. Single-pathogen intranasal inoculation models were developed later and became more popular because of their clinical relevance (Bakaletz, 2004).

The first dual-infection otitis media model in the chinchilla was developed later in 1980 (Bakaletz, 2009). These models attempt to replicate the synergistic relationships between upper respiratory tract viruses (influenza A, respiratory syncytial virus, adenovirus, rhinovirus), and one or more bacteria (*Streptococcus pneumoniae*, *Moraxella catarrhalis*, non-typeable *Haemophilus influenzae*) (Bakaletz et al., 1998; McGillivray et al., 2007). Partnering an upper respiratory tract virus with an otitis media bacteria and further timing the interval between inoculations of virus and bacterium, represents a specific interrelationship. Dual-infection models add significantly to the understanding of the mechanism by which upper respiratory infections predispose to bacterial otitis media. Chinchillas were the only viral–bacterial co-infection animal models for otitis media until a recent murine model was developed (Bakaletz, 2004). This chinchilla dual-challenge model has a sequence of events with all the hallmark signs of pediatric otitis media which significantly parallel the course of viral and bacterial disease seen in natural disease in humans (Gitiban et al., 2005). This model has evolved into a key system for use in studies of the molecular mechanisms of the pathogenesis of otitis media, the immune response to infection, and for preclinical evaluation of vaccine candidates for the prevention of otitis media (Bakaletz, 2009). This model also is used to identify and characterize antimicrobial peptides expressed by the epithelium lining the upper

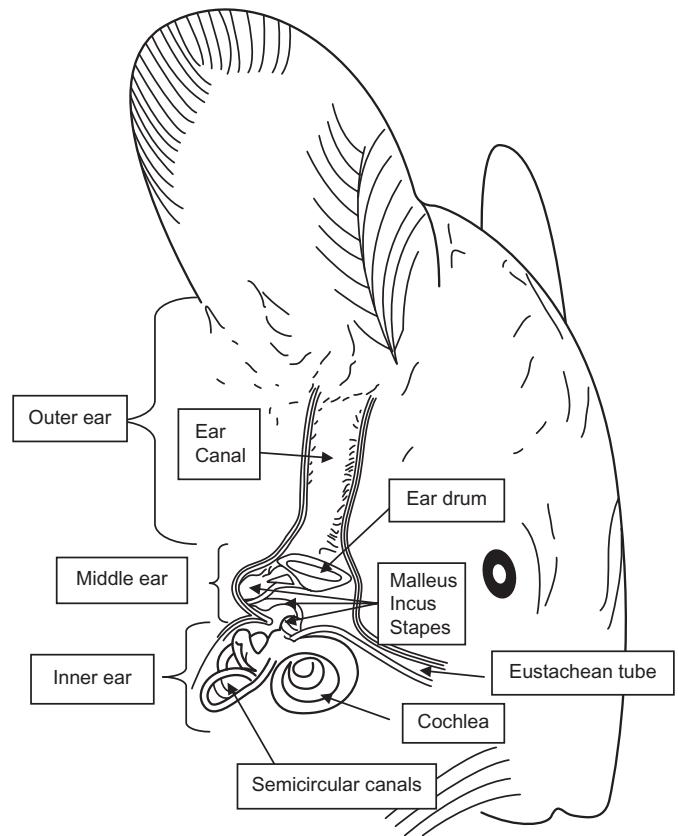


FIGURE 43.1 Schematic illustrating the three compartments of the ear; outer, middle, and inner. The round window is located between the stapes bone of the middle ear, and the fluid-filled cochlea in the inner ear. (Drawing courtesy of Brent de Riszner.)

airways, and help to determine their role in bacterial colonization and help to determine how many viruses might contribute to the pathogenesis of otitis media (Harris et al., 2004) (Figure 43.1). The following chapter describes specific-pathogen otitis media models.

Streptococcus pneumoniae

Streptococcus pneumoniae (SP) is one of three bacterial pathogens most frequently isolated by culture from middle ear effusions of children with both acute and chronic otitis media (others are *Haemophilus influenzae* and *Moraxella catarrhalis*) (Forbes et al., 2008). SP is the primary bacterial pathogen associated with 50% of all cases of otitis media in children (Carlsen et al., 1992; Linder et al., 1994). The first chinchilla model of otitis media was developed in 1975 using SP (Giebink, 1999). Until 1999 when a rat model was later developed (van der Ven et al., 1999), the chinchilla was the only non-bacteremic animal model of SP disease, whereby otitis media could be produced by very small amounts of inoculi and in which the disease remained localized (Giebink, 1999). The SP chinchilla model is still used to demonstrate hearing loss which has important

implications for transient sensorineural hearing loss seen in humans following otitis media (Giebink, 1999). In particular, the SP model is used most extensively to study local and systemic immune responses, pathogenesis, and efficacy of treatments (Alper et al., 1996) including the study of pharmacokinetics of antibiotics and passive and active immunoprophylaxis (vaccines) (Giebink, 1999). Chinchilla models of SP do not mimic human pathological processes on extension or severity (Giebink et al., 1976), but do provide a useful system to study the spectrum of disease induced by multiple clinical SP strains of unique genotype (Forbes et al., 2008).

Two methods to induce SP otitis media models are (1) direct transtympanic inoculation or inoculation of the middle ear via the cephalad bulla, and (2) intranasal inoculation (Giebink et al., 1979). Several intratympanic SP single-pathogen model variations have been developed. Strains used to induce models include serotypes 6A, 6B, 14, 19F, 23F which represent 65% of human clinical cases (Bakaletz et al., 1998; Linder et al., 1994). Other models use wild-type strain 3 (Sato et al., 1996), heat-killed encapsulated SP (Lowell et al., 1980), or non-encapsulated SP (Carlsen et al., 1992). To induce a transtympanic model for example, chinchillas are anesthetized and inoculated with 1.5×10^2 of SP in 0.3ml injected directly either through the transtympanic membrane or the epitympanic bulla, resulting in a greater than 75% success rate (Giebink et al., 1980). However, a model of acute otitis media that instills 10^2 – 10^8 bacteria/ml of type 23 SP, can result in 40% of chinchillas developing meningitis and/or bacteremia; conditions which are not desirable or comparable to the human clinical experience (Giebink et al., 1976).

Intranasal inoculation models are aided by applying mechanical or functional obstruction to the Eustachian tube, and then inoculating the nasal airway with SP (Carlsen et al., 1992). The addition of negative pressure (–25mmHg) to the intranasal otitis media model increases the success rate significantly by greater than 50% (Giebink et al., 1980). In humans, negative pressure is believed to be secondary to Eustachian tube dysfunction and a characteristic of otitis media with effusion (Giebink et al., 1979). When creating a model, negative pressure aids in the migration of bacteria along the eustachian tube from the nasopharynx to the middle ear. Negative pressure is applied on an anesthetized chinchilla by first applying saline to the nares (barotrauma), prepping the skin over the bulla (Green et al., 1994; Giebink et al., 1979), and inserting a needle into the bulla which is attached to a mercury manometer and firm plastic tubing. Negative pressure is applied while visualizing the tympanic membrane with an otoscope. When the membrane retracts the pressure is maintained for 5–20 minutes before the needle is removed. If negative pressure is applied 3 days after

nasal inoculation of SP, 59% of chinchillas will develop otitis media: 35% bilateral otitis media and 24% unilateral otitis media. For example, 0.5ml 10^6 type 7F SP instilled intranasally with negative pressure (–25), results in 98% incidence of otitis media within the first week and a purulent otitis media with middle ear effusion in 62% of chinchillas (Giebink et al., 1979).

Fluid samples (effusions) are typically collected from the middle ear for analysis following the establishment of the otitis media model by using either a capillary glass tube (0.7mm) (Giebink et al., 1980), aspiration with a 25-g needle, or lavage with 0.5ml of sterile saline. Disease progressions are assessed with tympanometry (an instrument that measures the eardrum responses to sound at different pressures) and otoscopy (Bakaletz, 2009). Otitis media is defined and identified as tympanic membrane changes with or without a visible fluid line (DeMaria, 1989), a red and inflamed tympanic membrane, detectable air–fluid levels, a bulging membrane with yellow fluid, or a tympanic membrane that is perforated with drainage to the external canal (Green et al., 1994; Tong et al., 2000).

Streptococcus pneumoniae and Influenza A

Chinchillas are significantly more prone to otitis media when *Streptococcus pneumoniae* (SP) is co-infected with influenza A, than when infected with either agent alone (Giebink et al., 1980). Only 4% of chinchillas develop otitis media with influenza alone and only 21% develop otitis media with SP alone ($17 \times$ increase in otitis media with both compared to influenza A alone, and $3 \times$ increase with both compared to SP alone) (Giebink et al., 1980). Overall, the incidence of otitis media increases to 67% in dually infected animal models (Bakaletz, 2009). In this dual-infection model, the virus compromises the eustachian tube mucosal integrity and results in impaired clearance function (Tong et al., 2002). This leads to development of negative middle ear pressure and viral suppression of polymorphonuclear white blood cell function which promotes a significant increase in nasopharyngeal colonization of Influenza A (Tong et al., 2002). Typically influenza A is inoculated intranasally, followed 3–4 days later by a SP intranasal inoculation (Giebink, 1999). The influenza strain most often used is A/Alaska/6/77 (H3N2) in a 0.2–0.5-ml suspension containing 6×10^6 CFU of virus/ml (Tong et al., 2002). The model is created by dripping viral solution into both nares (Abramson and Hudnor, 1994) followed by 0.5ml of SP type 6A containing 5×10^7 CFU/ml (Tong et al., 2002) or 1×10^5 SP (Abramson and Hudnor, 1994). This model causes a high incidence (67%) of otitis media with purulent effusions in middle ears but chinchillas survive without bacteremia. This model has been used extensively over the years for the preclinical assessment of various candidate antigens and adjuvants (Bakaletz, 2009).

Non-Typeable *Haemophilus influenzae*

Non-typeable *Haemophilus influenzae* (NTHi) is an opportunistic pathogen of the normal flora of the human nasopharynx and it accounts for 25–30% of all otitis media cases in humans (Tong et al., 2000). NTHi has mechanisms which enhance its infectivity, notably the ability to form biofilms which make it resistant to immune-mediated clearance (Jurcisek and Bakaletz, 2007). NTHi also does not require co-infection with virus to cause otitis media (Yang et al., 1998). Strains to induce animal models are selected from clinical isolates from patient specimens (Green et al., 1994). Non-typeable *Haemophilus* strains used as models include 1885, 1128, 1712, 86-028NP (Bakaletz et al., 1998; Hong et al., 2007). Because of virulence, each strain of NTHi must complete a dose-dependant curve prior to initiating an animal study to ensure otitis media will not result in complications (DeMaria, 1989). Possible complications are contralateral ear involvement, bacteremia, meningitis, and mortality (DeMaria, 1989). The NTHi chinchilla model is used to distinguish among multiple NTHi strains with respect to virulence parameters and varying ability to cause both local and systemic disease (Buchinsky et al., 2007), and are used for evaluation of antimicrobial agents and vaccine candidates.

NTHi is injected directly into the chinchilla middle ear to develop a transbullar challenge model (Yang et al., 1998). Chinchillas inoculated with 10^4 CFU of non-typeable NTHi result in 100% mortality from the development of meningitis by day 26 (Giebink, 1982). Likewise direct inoculation of 5×10^2 CFU, results in 40% of ears developing effusion by day 7, with only 25% mortality (Giebink, 1982). Severity of infection is therefore serotype- and dose-dependent (Giebink, 1982). Typically, NTHi transbullar models result in severe otitis media in virtually all ears of all animals within a very short period of time (24–48 hours). Direct middle ear inoculation of only a few bacteria produces a model without mortality and a 100% infection rate (Giebink, 1982). Elimination of negative pressure and co-infection reduces stress imposed on the chinchilla and uses fewer animals (Green et al., 1994; Yang et al., 1998). The transbullar model is useful for investigating the importance of host immunity in prevention of NTHi-related disease and for screening potential vaccines (Yang et al., 1998). This model is not useful to study NTHi adhesins which play a crucial role in the initial step of colonization (Yang et al., 1998). Other disadvantages are that the mechanism of this model is not analogous to the natural disease course of otitis media in children (Yang et al., 1998), which is typically subsequent to or coincident with a viral upper respiratory infection that then compromises host protective functions, predisposing the child to retrograde bacterial ascension up the eustachian tube (Bakaletz, 2009).

Intranasal inoculation of NTHi yields a low incidence of otitis media. NTHi must therefore be combined with adenovirus to produce a model with high incidence of otitis media by this route (Giebink, 1982). NTHi must also be followed by negative pressure to aspirate the bacteria into the middle ear via the eustachian tube which is usually done 18 hours after nasal inoculation (Yang et al., 1998). Typically a streptomycin-resistant strain of NTHi is used to create the animal model (Yang et al., 1998). When collecting middle ear fluid samples, streptomycin is added to the agar on which nasal lavage fluids are plated, to distinguish NTHi from other resident bacteria that colonize the chinchilla nasopharynx (Yang et al., 1998). This model is used as a tool to screen potential otitis media vaccine antigens, test colonization factors, and screen for NTHi adhesins (Yang et al., 1998). Disadvantages are that because chinchillas have lower infection rates, larger numbers of animals are required per experimental group. Moreover, additional manipulation and stress is experienced by the animals when applying negative pressure (Green et al., 1994).

Younger animals (2–3-month-old chinchillas) produce a higher incidence of NTHi otitis media compared to 4–5-month-olds or 1–2-year-olds (Yang et al., 1998), and thus serve as a model for pediatric NTHi otitis media. Age does not influence the degree of nasopharyngeal colonization with NTHi but has a marked effect on subsequent development of middle ear infection (Yang et al., 1998). Chinchillas of any age can be used to study colonization of the nasopharynx, but 2–3-month-olds are crucial to study middle ear infections, particularly with induction of 10 CFU with only a single nasal wash (Yang et al., 1998).

A significant improvement in the otitis media model of NTHi is the bioluminescence model (Novotny et al., 2005). A non-typeable *Haemophilus influenzae* has been developed with a plasmid-containing *luxCDABE* gene cluster of *Photobacterium luminescens* which emits light that penetrates through bony processes surrounding the middle ear space and that can be detected in a quantitative and qualitative manner. When chinchillas are inoculated intranasally or transbullarily with this strain, disease progression and colonization can be followed in the nasopharynx, eustachian tube, and middle ears. The advantage of this model is that it eliminates repeated nasal lavages, therefore significantly improving the ability to model otitis media in a non-invasive manner (Novotny et al., 2005).

Non-Typeable *Haemophilus influenzae* and Adenovirus

Synergistic models contribute to a better understanding of pathogenesis of virus-augmented NTHi disease (Yang et al., 1998). The development of the viral-bacterial super infection model in the chinchilla has been very

successfully used to study otitis media, highly reproducible (Bakaletz et al., 1993, 1995) and very similar to the disease process seen in children with otitis media. NTHi alone inoculated into the chinchilla nasal cavity will simply colonize the nasopharynx, however when a viral-bacterial dual challenge model is developed, NTHi ascends the eustachian tube to induce otitis media (Bakaletz, 2009). This model uses the juvenile chinchilla and challenges them first with an intranasal serotype of pediatric isolate of adenovirus. Once signs of viral upper respiratory tract infections develop, animals are subsequently challenged with an intranasal low-passage clinical isolate of NTHi, timed to coincide with maximal viral compromise of the eustachian tube (Bakaletz, 2009). For example, doses of 6×10^6 of 50% tissue culture of adenovirus are given intranasally with a pipette, followed 7 days later with an intranasal inoculation of 10^9 CFU of NTHi (Bakaletz, 2009; Suzuki and Bakaletz, 1994). NTHi usually reaches the tympanum at 7–10 days, with 77% of all ears showing disease by day 14 (Bakaletz, 2009). NTHi will multiply to high concentrations in the middle ear fluid, however as in children, these fluids are eventually cleared and the host re-establishes homeostatic conditions, however this process takes 5 weeks or more in chinchillas (Bakaletz, 2009).

When using preclinical vaccine efficacy studies, one additional step is added to this model and that is to passively transfer immune serum pools (derived from separate adult chinchillas directed against specific candidate vaccine antigen) to the juvenile chinchilla the day before they are challenged intranasally with NTHi. This protection maintains the immune status of the juvenile chinchilla, which helps support their permissivity to challenge with human wild-type viruses (Bakaletz, 2009). The advantage to this juvenile chinchilla model is that it seems to fit well with epidemiological data regarding the polymicrobial nature and natural disease course of otitis media in humans. The chinchilla passive transfer superinfection model is used to investigate the mechanism of pathogenesis and as well as its prevention, and has been used in several large cohort preclinical vaccine trials since its development in 1994 (Bakaletz, 2009).

Moraxella (Branhamella) catarrhalis

Moraxella catarrhalis (MC) is the third ranking commensal inhabitant of the upper respiratory tract (Chung et al., 1994) capable of inducing otitis media, sinusitis, conjunctivitis, and bronchitis in pediatric populations (Bakaletz et al., 1995; Luke et al., 2007). Traditionally, a MC otitis media animal model whereby viable organisms persist and multiply has been difficult to develop (Bakaletz et al., 1995; Chung et al., 1994). The only animal model that existed prior was the mouse pulmonary clearance model, but the organism was eradicated within 24 hours of inoculation (Luke et al., 2007). The

chinchilla model is therefore unique in that it serves as a self-limiting, less severe experimental nasopharyngeal MC model. Compared to other otitis media pathogens (Chung et al., 1994; Lim et al., 2002), this model only shows local infection with no bacterial multiplication, and eradication even after receiving large inocula (Bakaletz, 1995). MC will stably colonize the chinchilla nasopharynx for up to 2 weeks, and thus the chinchilla host can serve as a useful model of the earliest stage of otitis media development, namely colonization, inflammation, antibody development, and clearance (Bakaletz, 1995; Luke et al., 2007). This model is not used to evaluate effects of immunization or treatments due to the rapid elimination of bacteria from the ear (Chung, 1994).

Moraxella catarrhalis and Adenovirus

A dual model (pathogen synergy model) requires the addition of adenovirus to predispose the chinchilla to host *Moraxella catarrhalis*. To induce this model in the chinchilla, both organisms are introduced through intranasal inoculation. A 50% tissue culture of 6×10^6 pediatric adenovirus type 1 is inoculated in 0.5 ml divided between two nares via passive inhalation. This is followed 7 days later with inoculation of 10^8 CFU of *Moraxella* 1857 suspended in 0.5 ml. This model results in 100% pure culture of *Moraxella* from the nasopharyngeal lavages from days 1–4 post inoculation (Bakaletz et al., 1995). Despite colonization of MC in the chinchilla, no synergistic effect is found between these two organisms, as is with other combinations of bacteria and viruses. It is not possible therefore to use this model to evaluate effects of immunization or treatment modalities due to the rapid elimination of MC; however it does represent a model of colonization and clearance (Bakaletz, 1995; Chung et al., 1994).

Adenovirus

Chinchillas readily support infection, and respond serologically to adenovirus given either by intranasal or middle ear routes (Bakaletz et al., 1993). Adenovirus alone is used to study roles of upper respiratory viruses in the pathogenesis of bacterial otitis media, to assess the time course of disease and recovery, and to assess functional correlates of any induced histopathology. The model is induced with nasal inoculation of 0.6 ml of 6×10^6 TCID₅₀ divided between nares or transbulbarly with 0.3 ml per ear (Bakaletz et al., 1993). Intranasal inoculation results in mild-moderate tympanic membrane inflammation lasting 35 days, while transbulbar inoculation results in significant severe infection within 48 hours, lasting 10 days (Bakaletz et al., 1993). Chinchillas inoculated intranasally develop varying degrees of lethargy, malaise, wheezing, conjunctivitis, and labored breathing. These signs are not seen with

transbullar inoculation (Bakaletz et al., 1993). In comparison to influenza A virus, adenovirus time course is delayed both in disease onset and recovery in identical models (Bakaletz et al., 1993). The chinchilla is a valid model of adenovirus type 1 disease as it results in culture positives, seroconversion, cytopathologic changes, and distinct histopathology that correlate with functional assessments of the eustachian tube (Bakaletz et al., 1993). Adenovirus predisposes to NTHi and MC otitis media but does not predispose to *Streptococcus pneumoniae* otitis media (Bakaletz, 2004).

Influenza A

Influenza A induces acute otitis media (Lim et al., 1989) on its own and is used to develop a model of otitis media in the chinchilla. Eustachian tube and middle ear mucosa of chinchilla, similar to humans have glycoproteins with sialic acid which may have receptors for Influenza A (Lim et al., 1989). Influenza A acts to cause negative pressures in the eustachian tube which allow migration of bacterial pathogens to the middle ear (Lim et al., 1989). Influenza A attaches primarily to the ciliated cells of the tubotympanum, causing massive ciliated cell destruction (Lim et al., 1989) that does not restore for 3 weeks (Chung et al., 1993; Giebink, 1999). Influenza A/Alaska 16/77 (H3N2) virus strains are used to create the model (Chung et al., 1993). The virus is inoculated via bullar puncture with 7×10^6 PFU or 2.8×10^7 PFU (Chung et al., 1993; Lim et al., 1989). Ears show inflammation from 6–28 days (average infection lasts for 10 days), but few develop effusions (Chung et al., 1993; Giebink, 1999). The intranasal route results in a marked metaplasia of eustachian tube epithelium, increased secretions, and obstruction of the tube lumen (Giebink, 1999). Influenza A predisposes *Streptococcus pneumoniae* (Bakaletz, 1995, 2004) to develop otitis media.

Respiratory Syncytial Virus

Respiratory syncytial virus (RSV) is a key co-pathogen of otitis media (Bakaletz, 2004). In the past, a lack of relevant animal models prevented the study of mechanisms by which RSV predisposes to otitis media. Several animal models exist of RSV lower respiratory tract infections, but none of upper airway infections (Gitiban et al., 2005). Chinchillas are unique in that they are susceptible to RSV and show signs of illness, viral replication, and inflammation. The chinchilla model is useful to test the role of RSV in bacterial otitis media and efficacy of RSV vaccines, pathogenesis, and host immune function (Gitiban et al., 2005). The RSV model is created by inoculating a viral suspension of 10^6 or 10^7 PFU per animal intranasally via passive inhalation of droplets to juvenile chinchillas of ~3 months. At day 4, signs of ruffled fur, lethargy, and signs of upper respiratory tract infection develop in 33% of animals (Gitiban et al., 2005). Overall RSV shows

milder signs of illness than influenza A or adenovirus and clinical signs are dose-dependent. RSV's role in otitis media is to compromise the eustachian tube function by increasing mucous secretion which predisposes to bacterial superinfection (Gitiban et al., 2005). This chinchilla model is the first experimental system where the predisposition to bacterial otitis media by RSV was studied. This model however is still not practical for proof-of-concept studies and preclinical vaccine candidate trials (Gitiban et al., 2005).

Models of Biofilms

Biofilms are associated with the three primary causative agents of otitis media, non-typeable *Haemophilus influenzae* (NTHi), *Streptococcus pneumoniae*, and *Moraxella catarrhalis* (Bakaletz, 2009). Biofilms are a highly organized multicellular network of bacteria encased in a polymeric matrix and found in close association with a surface (Bakaletz, 2009). They grow into towers several hundreds of bacteria in height (Ehrlich et al., 2002). Biofilms promote bacterial persistence due to the matrix which protects against phagocytosis (Ehrlich et al., 2002) and increases resistance to clearance by host defenses or antibiotic therapy (Hong et al., 2007). The chinchilla biofilm model is developed by using *Haemophilus influenzae* strain 1128 bacteria, derived from a clinical isolate from a child with middle ear otitis media, injected into the bulla bilaterally (Ehrlich et al., 2002). After 72 hours the chinchilla is treated with ampicillin 150 mg/kg BID for 4 days to reduce the complications of systemic infection and to sterilize the middle ear effusion to culture negative (Ehrlich et al., 2002). In animals that develop middle ear effusion, ~50% develop biofilms within 5 days (Ehrlich et al., 2002). Multiple consecutive lavages provide a means to acquire biofilm samples without sacrificing the animal (Leroy et al., 2007). The chinchilla biofilm model of otitis media is consistent with the biofilm theory in many human infections (Ehrlich et al., 2002).

Models of Cholesteatoma

Cholesteatoma is associated with chronic ear disease. Cholesteatoma is defined as an accumulation of exfoliated keratin produced from stratified squamous epithelium which often overlays connective tissue. It develops within the middle ear and is an erosive space-occupying lesion associated with chronic otitis media (Masaki et al., 1989). It is difficult to develop a model with persistent tympanic membrane perforations seen with cholesteatoma, and since this syndrome is relevant to older patients, it requires a long-term animal model. The chinchilla serves as a model to evaluate factors involved in the development of otitis media that could play a role in the pathogenesis of acquired cholesteatoma (Lim

et al., 2002). This model is also used to explain the fate of prenatally acquired keratin and its relevance to post-natal ear disease (Camacho et al., 1997). To create a model of cholesteatoma in the chinchilla, non-viable keratin is introduced to the tympanic bulla. Tissue and hair are harvested from the abdomen of the same chinchilla (autologous) and minced, autoclaved, made into slurry, and instilled into one bulla. Chinchillas receive ampicillin for 3 weeks post inoculation, to sterilize the middle ear. Alternatively, a "viable" model is made by collecting autologous tissue excised from the conchal surface of the pinna of the chinchilla and inserted into the bulla. This viable model simulates the neonatal situation of amniotic fluid reflux into the eustachian tube. This model results in a robust inflammatory foreign body giant cell response from 1–9 months post inoculation (Camacho et al., 1997).

The cholesteatoma chinchilla also serves as a model to demonstrate the "migration theory" of how cholesteatoma develops. The chinchilla model demonstrates slow epithelial migration and true cholesteatoma formation in the middle ear with deliberate perforation of the tympanic membrane. No other animal model exists that can demonstrate the migration theory through a deliberate tympanic membrane perforation. This model is created by placing a chemically modified gelatin membrane from the external auditory canal through a tympanic membrane perforation, which can contact the epithelium of promontory (Hueb et al., 1993). This model simulates the migration of squamous epithelial cells and cholesteatoma, and mimics findings observed in humans. The advantage of the chinchilla model is that it can be easily and predictably repeated in over 50% of chinchillas (Hueb et al., 1993).

Another method to create a cholesteatoma model in the chinchilla involves the instillation of propylene glycol. Many commercially available otic preparations utilize propylene glycol as a solvent and it is thought to be the cause of this condition (Masaki et al., 1989). This model is created by instilling 0.2 ml of a solution of 50% of propylene glycol containing 5 mg/ml of neomycin and 10000 units/ml of polymyxin B into the middle ear by injection through a small hole in the superior aspect of the bulla, without damaging the tympanic membrane (Masaki et al., 1989). The result is hyperplasia of the tympanic membrane epidermis and epidermal invasion and penetration through an intact tympanic membrane (Masaki et al., 1989).

Models of Therapeutic Treatment of Aural Disease

Antibiotic Pharmacokinetics

Pharmacokinetic models are used to study the antibiotic drug distribution into the middle ear and evaluate

its relevance and significance to the clinical treatment of otitis media (Huang et al., 2001). Currently, there is little information on the middle ear distribution of antibiotics after systemic administration in clinical use, since reliable data from experimental animal models of otitis media treatment efficacy are rare. Inability to access and sample middle ear fluid frequently with traditional techniques is a major impediment in assessing pharmacokinetics and pharmacodynamics (Huang et al., 2001). Chinchilla otitis media models provide well-controlled conditions for measuring antibiotic pharmacokinetics. These models can be reliably produced with reasonable homogeneous pathology, enable repetitive sampling of middle ear fluid, and provide baseline pathology and fluid biochemistry that is well described as a means of reference (Canafax et al., 1994; Giebink, 1999).

The first type of model permits analysis of drug concentration on serial samples of very small volumes of ear fluid and blood samples (Canafax et al., 1989). Limitations are that only small volumes of middle ear fluid can be sampled (Jossart et al., 1990). This model is created by first surgically exposing the eustachian tube through the soft palate. Three silastic sponges (1–2 mm) are inserted to obstruct the eustachian tube. *Streptococcus pneumoniae* type 7F is inoculated in the lower portion of each bulla (10–50–400 CFU/ml). Antibiotics are given systemically, and a series of blood samples are collected. Middle ear fluid samples are collected on day 5 (purulent otitis media) or at 2–4 weeks (serous otitis media) using a 16-gauge needle inserted into the tympanic bulla. A catheter is passed through the needle into the bulla (Canafax et al., 1989). Most chinchillas survive 1 week, however by 3 weeks, 20% of chinchillas die due to sepsis, pulmonary edema, labyrinthitis, and/or pneumonia (Canafax et al., 1989). This model allows for the study of antibiotic pharmacokinetics and is used to determine how it relates to the biochemical, microbiological, and histopathological events that occur during treatment of acute otitis media (Canafax et al., 1994).

The Drug Diffusion Model measures the rate constant of antibiotic as it leaves the middle ear fluid (Canafax et al., 1994). This equates to the rate the drug penetrates the middle ear mucosa and is used to determine the concentration achieved in the middle ear (Jossart et al., 1990). This model assumes the exit rate is the same as the entry rate. This model is created 3 days after developing bacterial otitis media with SP type 7F and obstructing the eustachian tube as described above. Purulent material is aspirated out of the middle ear cavity before antibiotic solution is added. This model is created by placing a catheter in the central bulla (passing a 16-g needle then sliding a thin catheter through the needle), and inserting a 23-g needle to act as a pressure vent to prevent rupture of the tympanic membrane during injection of antibiotic. An antibiotic solution is infused

at 1ml/min to the middle ear through the catheter. Samples are aspirated using the same catheter at various time points. One disadvantage to this model is that only small effusion volumes can be obtained (Jossart et al., 1990).

A variation of this model is the drug systemic administration model which measures the constant rate K value calculated from the concentration of middle ear versus time after systemic drug administration. The K values from either a drug systemic administration model or a diffusion model are similar for assessing antibiotic pharmacokinetics (Canafax et al., 1994). After an otitis media model is established with *Streptococcus pneumoniae*, fluid is aspirated from the middle ear (3 days post inoculation). Following systemic antibiotic administration, fluid samples are collected from the middle ear (150µl) at 1, 2, 3, 4, and 6 hour time points. The rate constant K value is calculated from the middle ear concentration versus time. The advantage of this model is that middle ear fluid can be repetitively sampled. Limitations are the small middle ear fluid volumes, difficulty to assess the effects of various factors on antibiotic penetration, and difficulty to quantitate antibiotic appearance and disappearance from the middle ear (Canafax et al., 1994).

Chinchilla Microdialysis Model

The most advanced method to study antibiotic pharmacokinetics is the microdialysis model. This model is used to compare the antibiotic concentration in the middle ear fluid to serum levels (Cheung et al., 2006). It allows continuous measurements of plasma and middle ear fluid levels of an antibiotic in an awake, freely moving animal model which more closely resembles a pediatric human patient (Huang et al., 2001). This model is created by first obstructing the eustachian tubes, followed 24 hours later with instillation sterile PBS into the middle ear (artificial middle ear fluid), followed 24 hours later with the inoculation of 50CFU of *Streptococcus pneumoniae* into the middle ear. Catheters are placed 48 hours later in the jugular or femoral veins and femoral artery to administer antibiotics and collect blood samples. Microdialysis probes are implanted into both middle ears. Elizabethan collars are placed around the neck of the chinchilla to maintain the tether that contains the microdialysis tubing and catheters. Dosing of antibiotic occurs over the next 6–72 hours by IV bolus or infusion. Drug concentration in blood and middle ear fluid is monitored continuously with microdialysis and samples are analyzed by HPLC (Huang et al., 2001). A variation of this model is to use non-infected chinchilla ears prepared as described above but without the instillation of SP to determine unbound concentration of antibiotic drugs. Alternatively antibiotic can be delivered directly into the middle ears, and microdialysis

probe samples are collected over 17 hours to evaluate elimination kinetics from the ear (Cheung et al., 2006). The advantage of this chinchilla microdialysis model is that it allows continuous sampling and long-term monitoring of antibiotic concentration versus time in blood and middle ear fluid (Huang et al., 2001). It omits the potential side effects of anesthetics as a complicating factor. It overcomes the challenge of limited volumes of fluid because it does not remove fluid from the middle ear. Because of minimal restraint, normal physiological conditions are evaluated approximating those of human otitis media patients more closely. This model can be applied to a number of different antibiotics and is a powerful tool in the discovery and development of new anti-infective agents for treatment of otitis media (Cheung et al., 2006; Huang et al., 2001).

MODELS RELATED TO HEARING LOSS

Overview

Hearing loss is a major problem in industrialized societies (Henderson et al., 2006). Animal models play important roles in auditory research to study long-term well-controlled hearing loss over days, weeks, months, or years that is impossible to do in human populations (Salvi and Boettcher, 2008). Models with well-defined types and patterns of hair cell lesions (hearing loss) are aimed at improving cochlear prostheses, understanding central auditory plasticity, and developing strategies for inner ear rescue and repair. Most biological studies of sensorineural hearing loss have been conducted on cat, chinchilla, gerbil, guinea pig, mouse, and rat (Salvi and Boettcher, 2008). Chinchillas are an important animal model because their range of hearing is nearly identical to that of humans (Miller, 1970). They are an ideal animal model because of their ease of handling, excellent response to otoacoustic emissions testing (OAE), their contralateral ear can serve as its own control, and their thin round window membranes allow ototoxic drugs to cross easily (the membrane in the chinchilla is one-sixth as thick as that of a human) (Chen et al., 1999). Chinchillas are used by NIOSH (National Institute for Occupational Safety and Health), PHS (Public Health Service), and CDC (Centers for Disease Control) to determine guidelines for occupational exposure to noise in industry (Dunn et al., 1991). However, with rapid developments in molecular biology and genomics, research has shifted toward the use of rats and mice for hearing research, in particular, inbred strains in which the effects of genetic variability are greatly reduced (Salvi and Boettcher, 2008).

Animal models of hearing loss are developed using chemical agents or loud noise. Noise is thought to

create toxic free radicals and result in increases in reactive oxygen species (ROS). ROS damage is believed to be the common pathway of cell death leading to hearing loss resulting from loud noise and agents like aminoglycoside antibiotics, anticancer drugs, aging, industrial solvents (toluene) (Henderson et al., 2006), and quinolinic acid (Yellon et al., 1994). The administration of aminoglycoside antibiotics and antineoplastic drugs create hair cell lesions in a predictable pattern of damage in the mammalian inner ear.

The ear is divided into three compartments; outer, middle, and inner. Sounds are collected by the pinna and channeled toward the tympanic membrane (eardrum) which is coupled to the ear ossicles (malleus, incus, and stapes). Sound-induced vibrations transmitted through these three bones are transferred through the round window into the fluid-filled cochlea in the inner ear. The spiral tube of bone that forms the cochlea is subdivided along its entire length by three parallel, fluid-filled compartments; scala tympani, scala vestibule, and scala media. The cochlea spirals around the hollow bony core that contains the spiral ganglion neurons whose axons give rise to the auditory nerve. The outer hair cells (OHC) form three rows and the inner hair cells (IHC) form a single row that spirals along the length of the cochlea from base to apex. Outer hair cells help amplify sound vibrations entering the inner ear from the middle ear. When hearing is working normally, the inner hair cells convert these vibrations into electrical signals that travel as nerve impulses to the brain, where the impulses are interpreted as sound. The majority (95%) of auditory nerve fibers make synaptic connections with the IHC; only 5% connect with the OHC. The stereo cilia bundles protrude from the apical pole of the hair cells and are seen using a scanning electron microscope (Salvi and Boettcher, 2008). When typical cochlear damage occurs it is characterized by base to apex, outer hair cell to inner hair cell, and first row to third row gradients of hair cell loss (Figures 43.2–43.4).

To test the degree of hearing loss and assess methods of prevention or treatment, various dependent measures or tests are used. Typically the chinchilla is trained (operant conditioning) to distinguish characteristics of sound (via hearing), as a way for researchers to understand hearing loss and sound perception. This is accomplished by training the chinchilla to press keys through positive and negative reinforcement techniques to receive a food reward. Physiological measures of hearing loss used by researchers include measures of distortion product otoacoustic emissions (DPOAE), compound action potential (CAP), single auditory nerve fibers, and auditory brainstem response (ABR). DPOAE is a selective and sensitive tool for assessing the status of the OHC in noise-induced hearing loss. DPOAE amplitude is greatly reduced or absent with OHC loss. The CAP and single auditory

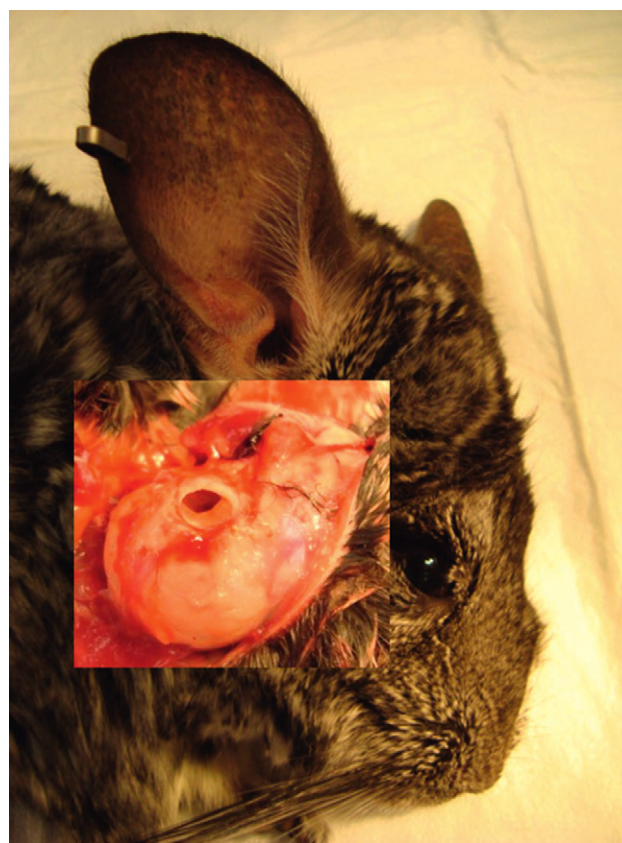


FIGURE 43.2 Dissection of the tympanic bulla of the chinchilla showing the relative location to the ear pinna. Bulla are extremely large in the chinchilla.

nerve fiber responses reflect the combined output from the OHC, IHC, and supporting cells of the cochlea. The ABR is a reflection of the neural activity that is transmitted from the inner ear to the auditory brainstem and used to measure the threshold of hearing to acoustic stimulation. These tests are all used in chinchilla models to assess hearing and quantify the degree of hearing loss (Salvi and Boettcher, 2008) (Figures 43.5, 43.6).

Models of Chemical-Induced Hearing Loss

Cisplatin

Cisplatin is a pharmaceutical chemotherapy drug that is used widely to treat different cancers including testicular, germ cell, head and neck, bladder, and lung cancer. The most common problems experienced during cisplatin therapy and overdosage are nephrotoxicity, electrolyte disturbances, myelosuppression, neurotoxicity, anaphylactic reactions, and ototoxicity. Hearing loss is thus a serious consequence for humans undergoing chemotherapy. Cisplatin can therefore be used to develop a model of hearing loss in chinchillas. Cisplatin primarily damages the outer hair cells more than the

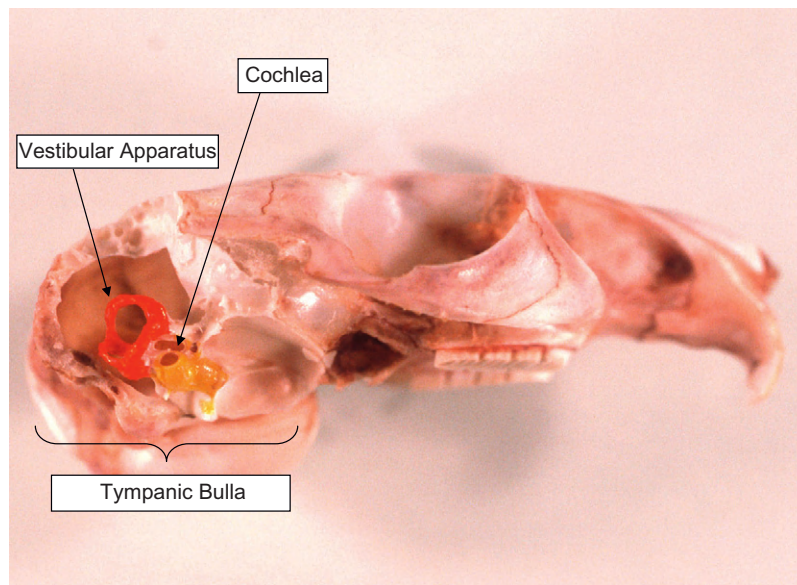


FIGURE 43.3 Chinchilla skull showing the bulla, cochlea, and vestibular apparatus. (Photo courtesy of Dalian Ding.)

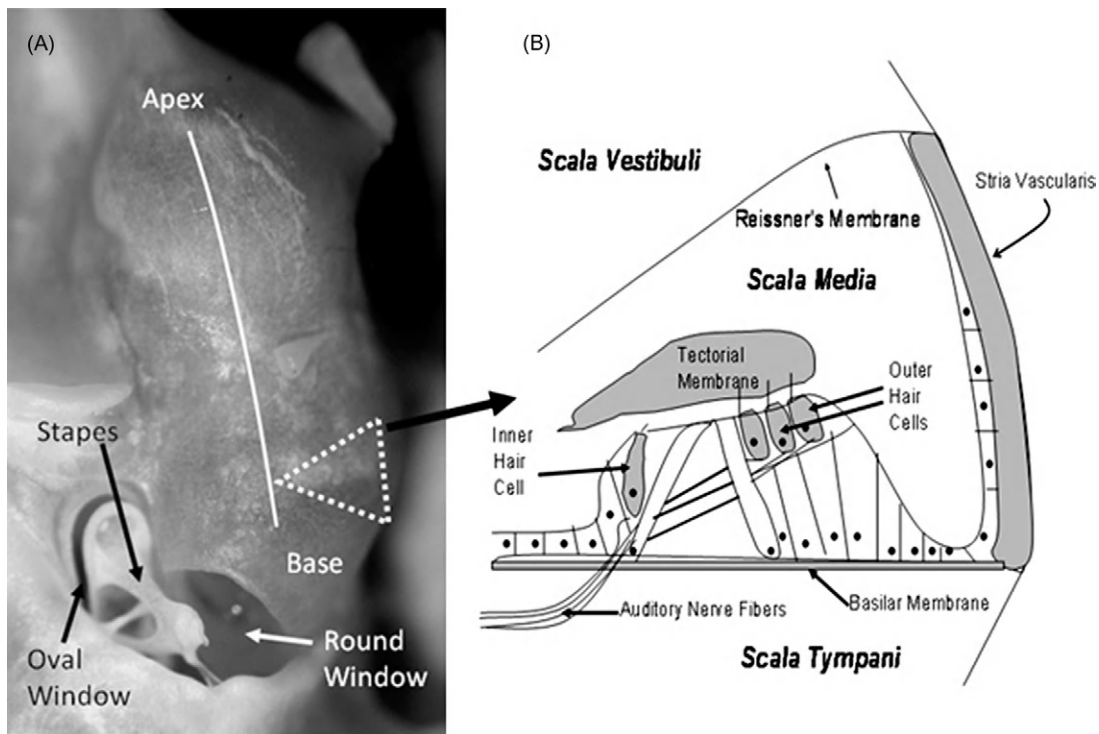


FIGURE 43.4 (A) Photomicrograph of the chinchilla cochlea showing the stapes plugged into the oval window in the base of the cochlea next to the round window. The cochlea spirals from base to apex around the modiolus, a central bony core containing the spiral ganglion neurons whose fibers give rise to the auditory nerve. The solid line depicts the axis of the modiolus within the cochlea; the dotted triangle depicts a cross-section through the basal turn of the cochlea. (B) Schematic cross-section of one turn of the cochlea showing the three fluid-filled compartment, scala media, scala vestibuli, and scala tympani. Scala media is separated from scala vestibuli by Reissner's membrane; the stria vascularis lies along the lateral wall of the cochlea. The organ of Corti containing the sensory hair cells and supporting cells rest on the basilar membrane. The tectorial membrane, an acellular structure, lies above the outer hair cells (OHC) and inner hair cells (IHC). Approximately 95% of the auditory nerve fibers contact the IHC; the remaining 5% contact the OHC. (Photo courtesy of Richard Salvi.)



FIGURE 43.5 Distortion product otoacoustic emissions (DPOAE) test on an anesthetized chinchilla. Probes/microphone insert is shown, that is placed in the ear. Microphone produces sound and the probe detects sound reflections. DPOAE is performed in a sound-proof chamber. (Photo obtained with assistance of Don Coling.)

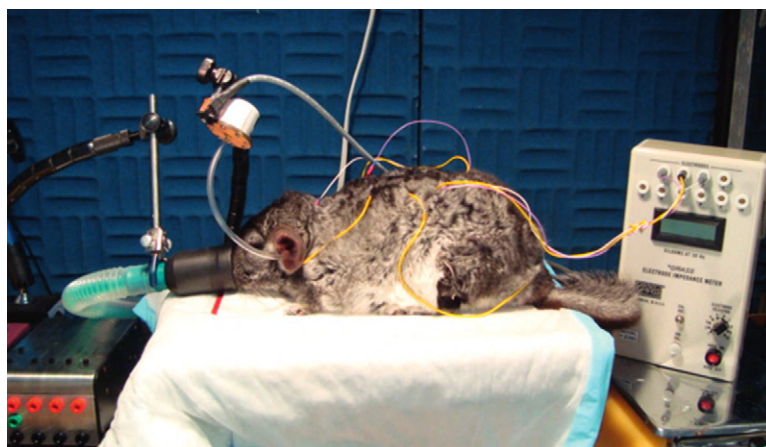


FIGURE 43.6 Auditory brainstem response test-pressure field (ABR) shown on an anesthetized chinchilla. Sound is directed into the ear. Electrodes to the head are used to record electrical activity from the auditory nerve which is converted to fiber optics by instrument on the left. Electrode impedance meter is shown on the right. ABR is performed in a sound-proof chamber. (Photo obtained with assistance of Don Coling.)

inner hair cells (Janning et al., 1998). It is applied topically directly to the round window membrane via the bulla with 25 μ l solution of 0.25 mg/ml in saline with a micropipette in an anesthetized chinchilla. One ear is used as the experimental model and the other ear serves as a control. Ototoxicity takes up to 7 days to develop with significant hearing loss as measured by increased ABR thresholds (Janning et al., 1998). Alternatively, cisplatin can be injected systemically at 2.75 mg/kg IP for 3 consecutive days (Ding et al., 2007). Subcutaneous fluids (saline) are administered during cisplatin administration to diurese the kidneys, but despite this precautionary effort, mortality can still be high due to kidney

damage. Direct application of cisplatin to the round window reduces the systemic side effects (nephrotoxicity) of the drug.

Cisplatin and Ethacrynic Acid

Ethacrynic acid diuretic alone causes temporary hearing loss that is reversible. By combining cisplatin with ethacrynic acid, the ototoxic effects are enhanced (Ding et al., 2007). This combination of drugs produces toxic hydroxyl radicals and decreases antioxidant levels; the result of which is 100% outer hair cell loss at the base of cochlea (and 10% at apex). Inner hair cell loss is uniform along the length of cochlea. This model is

created in chinchillas with 0.2 mg/kg IP of cisplatin and 40 mg/kg IV of ethacrynic acid (Ding et al., 2007). The benefit of this drug combination is that the toxic side effects of cisplatin can be reduced or avoided (nephrotoxicity) and a robust model of hearing loss can still be accomplished.

Carboplatin

Carboplatin is a chemotherapy drug used against some forms of cancer (mainly ovarian carcinoma, lung, head and neck cancers); however it can cause secondary auditory neuropathy. Auditory neuropathy is also associated with hereditary disorders such as Charcot-Marie-Tooth disease, Friedreich ataxia, kernicterus, and hypoxia theory due to history of high-risk birth (Harrison, 1998). Humans with auditory neuropathy have absent or severely abnormal ABRs that do not correspond to their audiometric thresholds or to relatively normal OAEs (Harrison, 1998). Carboplatin preferentially damages the inner hair cells, auditory nerve fibers, and type 1 spiral ganglion neurons, but spares the outer hair cells (El-Badry and McFadden, 2007; Wang et al., 2003). In contrast, most ototoxic drugs and trauma (noise) damage the outer hair cells. Carboplatin is therefore used to produce a chinchilla model of auditory neuropathy with a unique advantage to providing fundamental information about the function of the inner hair cells (Harrison, 1998; Wang et al., 2003). This model is developed by injecting 50 mg/kg IP of carboplatin (low dose) (Wang et al., 2003) or 75 mg/kg IP (high dose) (El-Badry and McFadden, 2007), or 400 mg/cm² given IV (Harrison, 1998) and results in death of 50% of the inner hair cells. The spiral ganglion neuron and afferent terminals are damaged a few hours after carboplatin application, and hair cell damage begins 1–2 days later (Wang et al., 2003). High doses of carboplatin in the chinchilla show IHC loss and partial OHC loss, whereby low to moderate doses show only IHC loss which is a unique feature in the chinchilla; the effects of which do not occur in other rodents or humans (McFadden et al., 2002; Wang et al., 2003).

Aminoglycosides

Aminoglycosides are bactericidal antibiotics that are active against aerobic Gram-negative bacilli and staphylococci. Although the ototoxic effects of aminoglycosides are well documented, this class of drugs is still widely used today. Aminoglycosides are cleared more slowly from inner ear fluids than from serum and therefore latency exists to the ototoxic effects resulting in a progression of hearing loss after cessation of treatment. Aminoglycoside toxicity primarily targets the renal and cochleovestibular systems. Kanamycin, amikacin, neomycin, and dihydrostreptomycin are preferentially cochleotoxic. Gentamicin affects both cochlear and

vestibular systems. Streptomycin and tobramycin are primarily vestibulotoxic (Mudd et al., 2008). Cochlear toxicity that results from aminoglycoside hearing loss usually begins in the high-frequency range and is secondary to irreversible destruction of outer hair cells in the organ of Corti, predominantly at the basal turn of the cochlea.

Aminoglycosides are used to induce models of hearing loss in chinchillas for two practical reasons; (1) to selectively destroy OHC (Ryan and Dallos, 1975), and (2) to mirror the clinical practice of chemical vestibular ablation used in the treatment of Ménière's disease (Chen et al., 1999; Hirvonen et al., 2005). The former is valuable to study IHC function, since aminoglycosides selectively destroy only the OHC. Unlike noise-induced hearing loss, which creates mixed hair cell death models, this model is selective and thus has a distinct advantage to allow for the study of IHC function (Ryan and Dallos, 1975).

Gentamicin is specifically used in chinchillas to create a model with damage to the vestibular apparatus of the inner ear (Hirvonen et al., 2005) and severe outer hair cell loss, primarily the inner two rows, while sparing the inner hair cells. The degree of neuroepithelial and vestibular damage is directly related to the dose; with higher dosages, the contralateral ear can have injury. To create this model an incision is made over the superior bulla to make a 3-mm opening to the middle ear cavity and 0.2–0.6 ml of gentamicin (26.7 mg/ml) is administered to fill the middle ear. The head of the chinchilla is turned toward the ceiling (ear up) to allow 30 minutes for the solution to bathe and diffuse across the round window (Lyford-Pike et al., 2007). Chinchillas will develop a static roll head tilt by approximately 2.5–3 weeks (anywhere from 4–22 days post injection) (Chen et al., 1999; Hirvonen et al., 2005). At 3 weeks, outer hair cells are eliminated from all the canals (Lyford-Pike et al., 2007). This animal model displays more cochlear than vestibular damage (McFadden et al., 2002) and will cause damage to the contralateral ear, both characteristics which do not reflect the human experience of hearing loss (Chen et al., 1999). Since this chinchilla model is unable to support vestibular selectivity it may not be an adequate model for Ménière's disease either, however it does replicate the histopathological changes seen in humans (Chen et al., 1999).

Streptomycin was the first clinically applied aminoglycoside antibiotic and was used successfully for treatment of tuberculosis. Streptomycin preferentially affects the vestibular system rather than the auditory system, with more severe damage in the basal turns and more outer hair cell involvement in a dose-dependent fashion (Chen et al., 1999; Hilton et al., 2002). Streptomycin has therefore become a popular form of vestibular treatment for Ménière's disease (Chen et al.,

1999). Streptomycin given in one instillation to the middle ear of chinchillas at full strength (26.7 mg) creates a model of severe OHC loss at basal and mid turns of the cochlea (Chen et al., 1999).

Kanamycin has a propensity to cause profound cochlear hair cell damage, marked high-frequency hearing loss, and complete deafness. The damaging effect is primarily to the cochlea, while the vestibular system is usually spared injury. Kanamycin (150 mg/kg SC) plus bumetanide (20 mg/kg IV) a diuretic, given 2 hours apart will cause primarily a basal inner hair cell loss model in the chinchilla (McFadden et al., 2002). Kanamycin given systemically at 200–300 mg/kg SC daily for 6–35 days results in OHC damage, threshold shifts of 42–52 dB, but maintains intact IHC (Ryan and Dallos, 1975).

Gentamicin and Ethacrynic Acid

Gentamicin can be combined with ethacrynic acid (EA) since ethacrynic acid greatly enhances ototoxicity. The combination decreases problems associated with long-term systemic effects associated with gentamicin. The two drugs can be administered together or given separately by 1–1.5 hours. By preserving normal vestibular end organ morphology while eliminating all cochlear hair cells, this model is useful to study vestibular function and responses of the vestibular system to sound. To create this model, 125 mg/kg of gentamicin is given IM and 40 mg/kg of ethacrynic acid given IV (McFadden et al., 2002). This model destroys all inner hair cells and outer hair cells; however up to 30% mortality can be seen with this regime. Dosages of 10–40 mg/kg of gentamicin and 40 mg/kg ethacrynic acid results in destruction of nearly all outer hair cells, with dose-dependent inner hair cell loss, normal vestibular function, and no mortality. Delaying the second administration of drug by 1 hour results in partial lesions of inner hair cells, and delaying second drug by 1.5 hours preserves most of the inner hair cells, with only less than 50% outer hair cell loss (McFadden et al., 2002). An animal model with normal vestibular end organs but no hair cells is useful to study vestibular function and responses of the vestibular system to sounds. This model does demonstrate a unique feature in that gentamicin on its own is vestibular toxic and shows base to apex IHC loss, but when combined with EA the vestibular system is spared and apical hair cell loss predominates. By inner hair cell lesions not following the base to apex gradient as seen in the carboplatin model, this model represents unique characteristics which are not hallmarks of human ototoxicity (McFadden et al., 2002).

Kainic Acid

Glutamate is an excitatory amino acid and neurotransmitter at the inner hair cell and auditory nerve

fiber synapse, in all mammalian cochlea. Excessive amounts of glutamate are excitotoxic to type 1 ganglion neurons in cochlea and are implicated in diseases such as sudden deafness, noise-induced hearing loss, and presbycusis (age-related hearing loss). Kainic acid is an analog of glutamate and establishes a model of hearing loss to study the recovery of function of the auditory nerve and inferior colliculus (Zheng et al., 1999). Glutamate agonists coupled with acoustic overstimulation causes early damage to type 1 afferent synapses (Wang et al., 2003). This model is created by first making the chinchilla monaural by surgical destruction of the left cochlea. Chronic recording electrodes are implanted in the left inferior colliculus and kainic acid is applied topically (60 mM) to the intact right round window membrane. Chronic electrodes are implanted directly on the trunk of the auditory nerve to record compound action potentials. Another electrode is implanted directly onto the auditory nerve within the internal auditory meatus after exposure of the cerebellum via posterior craniotomy. One end of the electrode is placed against the trunk of the nerve while the other is cemented to the top of the skull subcutaneously for EVP (evoked potentials) and DPOAE (distortion product otoacoustic emissions) measurements. Kainic acid induces cochlear excitotoxicity and immediately (3 hours post application) causes massive swelling of type 1 afferent endings under inner hair cells, but hair cells are spared. Ten days post inoculation the auditory afferent synapses rapidly regenerate and re-establish viable synapses with inner hair cells (fibers respond to sound). The purpose of this model is to understand the plasticity of the central auditory system to reorganize itself during restoration of input from the auditory periphery as might occur with cochlear prosthesis or other clinical interventions for hearing loss (Zheng et al., 1999).

Noise-Induced Hearing Loss Models

Loud noise exposure is a common health hazard in industrial, recreational, and military activities (Hu et al., 2008). Noise-induced hearing loss (NIHL) can be caused by a one-time exposure to an intense “impulse” sound, such as an explosion, or by continuous exposure to loud sounds over an extended period of time. Sound is measured in units called decibels (dB). On the decibel scale, an increase of 10 means that a sound is 10 times more intense. The humming of a refrigerator is 45 decibels, normal conversation is approximately 60 decibels, the noise from heavy city traffic can reach 85 decibels, and rock ‘n’ roll music can reach 120–125 dB SPL (Henderson et al., 2006). Long or repeated exposure to sounds at or above 85 decibels can cause hearing loss. Impulse (impact) sound can result in immediate hearing loss that may be permanent and

may be accompanied by tinnitus (phantom noise or ringing in the ears) (Sun et al., 2009). Continuous exposure to loud noise also can damage the structure of hair cells, resulting in hearing loss and tinnitus, although the process occurs more gradually than for impulse noise.

Noise can cause physical damage to the stereo cilia causing them to fuse, break, or lose structural integrity (Henderson et al., 2006). Dendritic terminals of the auditory nerve afferent fibers can swell and rupture (Henderson et al., 2006). Loud noise levels can decrease blood flow to the cochlea and damage the outer hair cells, particularly those at the basal end of the cochlea. The organ of Corti can be ripped from the basilar membrane from loud noise and hair cells can continue to die 30 days post exposure (Henderson et al., 2006). Early cell death and high levels of noise (>120 dB SPL) is attributed primarily to apoptosis, and later stages or lower levels of noise (<105 dB SPL) are the result of necrosis and to a lesser degree apoptosis (Hu et al., 2008; Yang et al., 2004).

Animal models have played an important role in determining how the intensity, duration, and spectrum of a noise affects the amount of hearing loss and how hearing perception changes when the OHC and IHC are damaged. High-level noise exposures tend to damage OHC before IHC (Salvi and Boettcher, 2008). Noise can be described as (1) impulse noise (impact) which is similar to the collision of masses or related to the release of compressed gases characterized by rapid fire and rapid decay (Dunn et al., 1991), or (2) steady state (continuous) which has no fluctuation (Dunn et al., 1991). Impulse noise is more damaging to hearing than continuous noise (Dunn et al., 1991).

Impulse and Impact Noise

Impact noise, which can be produced electronically, similar to the sound produced when striking a steel plate with a hammer (Henderson et al., 1991), can simulate the noise produced by the U.S. Army M-16A1 rifle with a time amplitude profile as would be seen utilizing a 5.56 caliber round (Henderson et al., 2006). Both impulse and impact noise can cause direct mechanical damage (Zheng et al., 2000). Hearing loss depends on the level, frequency, content, duration of exposure, and rate of repetition (Henderson et al., 1991). Outer hair cell loss is more likely a consequence of impulse/impact noise than inner hair cell loss, which is the contributing factor to hearing loss in humans (Zheng et al., 2000). Death of outer hair cells not only takes place during the noise exposure but continues for at least 30 days post exposure (Yang et al., 2004).

Chinchilla models can be used to study the applicability of equal energy hypothesis to impact noise in industrial settings (Henderson et al., 1991) and to determine a model of cell death and preventative measures of hearing

loss (Hu et al., 2008). Following ABR and DPOAE pre-testing, animals are placed in restraint tubes to ensure ear position remains constant, and then placed in a noise exposure chamber. Animals are awake during the exposure, and placed directly in front of the speaker at a distance 4 inches from the ear midline to speaker (Hight et al., 2003; Zheng et al., 2000). A microphone positioned within the cage at the level of the animal's head, measures the levels of the noise. Noise exposure can be presented at 145–150 dB SPL at 100 impulses delivered in 50 pairs, 50 ms apart with a 1000-ms pause between each pair (Hight et al., 2003; Zheng et al., 2000). Another model uses octave band noise of 4 KHz at a low level (85 dB SPL) for 10 days (6 hours/day) and then high levels (90 dB SPL) for 48 hours (Zheng et al., 2000). To assess noise-induced threshold shift and recovery in the animals, ABR testing is performed at several time points following the noise exposure period: for example 6h, 24h, 3 days, 7 days, and 21 days after the noise exposure (Bielefeld and Henderson, 2007).

Continuous Noise

To create hearing loss models, chinchillas can be exposed to continuous noise at >115 dB SPL (Pourbakht and Yamasoba, 2003), or 110 dB SPL for five consecutive days (Dunn et al., 1991). ABR is typically measured before and after exposure in a sound-proof test chamber. Continuous noise produces far less damage to outer hair cells than impact/impulse noise (Dunn et al., 1991). Levels of 110 dB SPL for 1 hour induce metabolic changes to the cochlea (reactive oxygen species formation, glutamate excitotoxicity), without generating significant mechanical damage (ripping of the OHC from their supporting cells, permanent fused stereocilia) (Bielefeld and Henderson, 2007).

Models of Aural Pathologic Conditions Associated with Hearing Loss

Treatment of Vestibular Symptoms Associated with Ménière's Disease

Vertigo (imbalance, dizziness) is one of the clinical signs of Ménière's disease, a disorder of the inner ear that can affect hearing and balance to a varying degree. Gentamicin is a popular method to treat this condition by chemical destruction of the vestibular labyrinth (Hoffer et al., 1999; Li et al., 2004). Gentamicin controls vertigo with a 90% success rate but causes 25% of patients to lose their hearing. The chinchilla is used as a model to study the treatment for Ménière's disease and is used to understand actions of gentamicin and facilitate safer use of this medicine. To create this model, gentamicin is placed in the middle ear in a sustained release vehicle, i.e. 0.5 ml of liquid fibrin tissue sealant containing 2.5 mg of gentamicin to completely fill the

middle ear. The sealant is allowed time to gel. Ears are then collected for histopathology (Hoffer et al., 1999). The advantage of this model is that cell damage is much faster than with systemic administration of gentamicin or when placed in the bulla in liquid form. This model can determine the most effective route of administration, total dose and frequency, end point of therapy, and correct dose to produce enough vestibular damage to cure vertigo with little or no cochlear damage. This model serves to study the regenerative properties of the inner ear following ablation and to examine medications that cure rather than just treat inner ear disorders (Hoffer et al., 1999).

The chinchilla is also a model for the cold-caloric nystagmus response used to study treatment for Ménière's disease. This model is used to assess the vestibular system, which is typically chemically destroyed for treatment of vertigo. This model is created by irrigating the ear canals with cold water (0.5 ml of 0°C water) and measuring time to return to normal nystagmus. Nystagmus results from the flow of endolymph through the semicircular canals induced by the temperature gradient between the external and inner ear. Since the semi-circular canal is closest to the cold water it experiences the largest temperature gradient and generates the largest response (Li et al., 2004). DPOAE measures the input and output function.

Tinnitus

Tinnitus is defined as the perception of sound without an external acoustic source commonly referred to as "ringing in the ears" (Bauer et al., 2008). Roughly 25 million Americans have experienced tinnitus. It is often triggered by cochlear hair cell damage due to hearing loss, or consuming high doses of salicylate (aspirin) (Sun et al., 2009). Tinnitus is assessed in animal models by using conditioned suppression of an operant behavior. This is performed by training chinchillas to press a lever for a food reward (operant training) and to refrain from lever pressing in the absence of acoustic stimulation (conditioned suppression). Animals are trained to discriminate between "sound on" and "sound off". Training occurs prior to cochlear trauma and tinnitus induction. After cochlear trauma, behavioral testing is repeated and tinnitus assessed. Tones of varying intensities are presented. Animals with tinnitus hear sounds differently and can hear sounds when the speaker is off. ABR thresholds are conducted to assess hearing loss, performed before and after acoustic exposure (Bauer et al., 2008).

Three models have been developed to study tinnitus; (1) acoustic exposure resulting in inner hair cell and outer hair cell loss, (2) round window cisplatin resulting in pronounced outer hair cell loss mixed with some inner hair loss, and (3) round window carboplatin with pronounced inner hair cell loss and no outer

hair cell loss. The acoustic exposure model of tinnitus is created by fitting a stainless steel speculum with an attached speaker driver into the left ear canal. A 4-kHz tone at 85 dB SPL sound pressure level is applied. This produces psychophysical tinnitus in chinchillas. The round window cisplatin model is created by applying 0.66 mg/ml to the round window in the left bulla through a 2-mm opening. The drug is applied to the tympanic membrane and left on contact for 15 minutes, then flushed away. This creates more high-frequency than low-frequency hearing damage. ABR tests will result in a 5–15 dB SPL increase in thresholds. The carboplatin model is produced with the application of 4 mg/ml of drug to the round window using the same techniques as the cisplatin model. This results in a 10–35-dB SPL increase in hearing threshold measured by ABR. All three models produce comparable tinnitus both qualitatively and quantitatively. Cisplatin results in slightly less levels of tinnitus than others but still gives significant results (Bauer et al., 2008).

Perilymphatic Fistulas

A perilymph fistula (PLF) is an abnormal opening in the fluid-filled inner ear or an abnormal connection between the air-filled middle ear and the fluid-filled inner ear. Perilymphatic fistulas may induce hearing loss, tinnitus, aural fullness, vertigo, disequilibrium, or a combination of these symptoms (Roland, 2010). Animal models of PLF that permit controlled experiments are sparse or non-existent, and currently no non-invasive method of detection is available to clinicians; only exploratory surgery. The chinchilla model provides a basis by which the sensitivity and specificity of potential perilymphatic fistula tests can be evaluated and compared. The chinchilla model simulates bony, round window, and oval window fistulas. The model is created with three sites of micropunctures made to recreate these three types of fistulas. Ice water caloric stimulation is used to assess semi-circular canal function before and after micropuncture surgery. Eye movements are recorded using electro-oculographic electrodes placed on the surface of the head. This model creates positive post-operative responses and has high sensitivity (78%) and specificity (100%) (Wall and Casselbrant, 1992).

Superior Canal Dehiscence

Superior canal dehiscence syndrome (SCDS) is a rare medical condition of the inner ear, leading to hearing and balance disorders in those affected. SCD is manifested by a hole in the bony wall between the superior semicircular canal and the brain case (Songer and Rosowski, 2007). The symptoms are caused by a thinning or complete absence of the part of the temporal bone overlying the superior semicircular canal of the

vestibular system. This may result from slow erosion of the bone or physical trauma to the skull and there is evidence that the defect or susceptibility is congenital. The surgical removal of the superior canal wall is used to treat severe cases of cholesteatoma in human patients but results in various degrees of conductive hearing loss (Siedentop et al., 2004) and vestibular symptoms (vertigo and nystagmus) (Songer and Rosowski, 2007). Loud sounds or changes in intracranial or middle ear pressures stimulate vestibular symptoms (Carey et al., 2004; Songer and Rosowski, 2007). Reconstruction of the wall is required to restore anatomic conditions in both these pathological and surgical treatment conditions of SCD (Siedentop et al., 2004). The gap in the temporal bone can be repaired by surgical resurfacing of the affected bone or plugging the superior semicircular canal. The chinchilla model is made by creating a fenestra 0.3 mm in size in the uppermost portion of the superior canal where it protrudes into the mastoid bulla (Hirvonen et al., 2001). This model is used to compare material to repair canal defects, and is used to evaluate effects of graft material on stability, healing processes, and integration into the canal wall (Siedentop et al., 2004). Various materials and combinations are tested in this model for repair including muscle, cyanoacrylate, cartilage, and fibrin tissue adhesives (Siedentop et al., 2004). This model is also used to predict the effect of dehiscence on auditory sensitivity and mechanics (Songer and Rosowski, 2007).

The superior canal dehiscence model is also used to investigate changes in firing rates of vestibular nerve afferents in response to changes in external auditory canal pressure before and after fenestration and after repair (Hirvonen et al., 2001; Siedentop et al., 2004). This model is created by exposing the bulla and internal canal on one side and making a small fenestra 0.25 mm at the top of the superior canal (Songer and Rosowski, 2007). The anesthetized chinchilla is mounted in a stereotactic frame mounted to a gimbaled structure for vestibular stimulation that allows roll and pitch tilts (Carey et al., 2004). Speakers deliver acoustic stimulation. This model is used to predict the effect of dehiscence location and size on auditory sensitivity and mechanics, and provides a framework for generating models on the effect of SCD in human patients. Limitations to this model are that the fenestra are open to the air in chinchillas rather than open to the dura and CSF as in humans (Carey et al., 2004). Differences in acute versus chronic and location and size of fenestra may also play important factors in this model that may not correspond well to human conditions of SCD (Songer and Rosowski, 2007).

Tympanic Membrane Perforation Repair

Tympanic membrane perforations are a common problem in humans secondary to infection, trauma,

or an extruded pressure-equalizing tube. Most membranes heal spontaneously but 10% will become chronic (Downey et al., 2003). The chinchilla is used as a model to test techniques of surgical repair and graft materials used to treat perforations to the tympanic membrane that do not heal. The chinchilla is an ideal model because of similarities between the anatomy of human and chinchilla tympanic membranes. To create this model, the anterior half of both ear tympanic membranes is perforated. A right-angle hook is used to fold the free edges of the drum medially (Spiegel and Kessler, 2005). Analgesics and antibiotics are administered post surgery (Downey et al., 2003). After 6–8 weeks post surgery, only chinchillas with mature perforations lacking signs of epithelial repair regeneration and with no signs of infection are selected as models (Downey et al., 2003). Grafts can then be tested in one ear and the other ear serves as a control. Repairs and grafts are evaluated after 6 weeks (Spiegel and Kessler, 2005). This animal model provides details of graft integration into the host tympanic membrane, of inflammatory responses, and is used to compare graft materials such as autografts, allografts, xenografts, and alloplasts (Downey et al., 2003).

Stem Cell Transplants

The regenerative activity of the inner mammalian ear is limited. Cell transplantation is pursued for cell therapy of degenerative inner ear diseases, of which most are incurable. Currently restoration of hearing with cochlear implants is the only therapy available for deafness and requires survival of a large number of spiral ganglion neurons. Stem cells have the potential to be utilized for regenerating inner ear cells and preventing further cell death. The chinchilla model is used to study if autologous bone marrow stromal cells have the potential to survive, migrate, and differentiate in damaged cochlea. The chinchilla model is based on a gentamicin-damaged inner ear model. This model is created by treating chinchilla with gentamicin (125 mg/kg IM) and ethacrynic acid (40 mg/kg IV). Bone marrow is harvested 2 weeks prior to drug treatment by exposing femur and aspirating 0.1 ml of the medullary cavity. Stem cells (10^5) are injected into the round window with a 30-g needle and into the bony axis of cochlea (where spiral ganglion and cochlear nerves are located). The fate of stem cells is monitored for migration and differentiation (Naito et al., 2004).

Otic Suspension Safety Testing

Chinchillas are used to assess safety of ototopical agents particularly in regards to the risk of ototoxicity, because they are a well-established animal model for hearing loss. Chinchillas are used because they have a wide tympanic membrane, a readily accessible cochlea,

and an audiogram more closely aligned with the human (Daniel and Munguia, 2008). Many ototoxic drugs are tested in the chinchilla including but not limited to aminoglycosides, otic preparations, Cerumenex®, and hydrogen peroxide (Daniel et al., 2008). (Hydrogen peroxide is used to flush ventilation tubes that are inserted to aerate the middle ear of children with recurrent ear infections (Nader et al., 2007).) These models are created by inserting ventilation tubes in both ears, and administering ear drops BID for 7 days. The chinchilla's head is positioned so the ear is facing upwards and four drops are instilled into the ventilation tube, followed by ten compressions. The head is held in place for 5 minutes before re-positioning. In this model, gentamicin serves as a positive control for its known ototoxic effects. The contralateral ear serves as a negative control by receiving 0.45% saline. ABR (auditory brain stem response) is measured at baseline and after tube placement at days 4, 8, 15, 22, and 60 to assess hearing loss (Daniel and Munguia, 2008; Nader et al., 2007). The disadvantages to this model are that ototoxicity varies between species and results may not correlate well to humans. Animals tend to be more sensitive to otic drug effects than humans because their round window is thinner and more permissive to agents, allowing more penetration to the inner ear than in humans. However if products are determined to be safe in chinchillas they are likely safe to use in humans (Daniel and Munguia, 2008).

FUTURE OF CHINCHILLA MODELS

Several new areas of research using chinchillas will be developed. To study pediatric otitis media, infant chinchilla models of pneumococcal otitis media will be developed to study relationships between maternal and infant antibodies. The young age will correlate better to human patients and may reveal determinants to the risk of otitis media at later stages in life (Giebink, 1999). Models of other disease pathogens such as *Neisseria*, *Pseudomonas*, and *Escherichia coli* will continue to be developed (Bakaletz, 2009). Ongoing efforts to obtain the chinchilla genome sequence are in progress (Bakaletz, 2009). Whereas inbred and knockout chinchillas are not likely to be available, knockdown chinchillas have already been achieved and can be expected to be more widely used for avenues of disease and prevention research (Bakaletz, 2009). Already several middle ear cell lines have been established from chinchilla tissues (Lim et al., 2002). Chinchillas will still continue to be important models for ear-related research and play critical roles due to their specific attributes, despite the trend toward mouse models.

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Degu

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INTRODUCTION

The degu, *Octodon degus* (Figure 44.1), a moderate-sized, precocious yet slow-maturing hystricomorph rodent from central Chile has increased in use and popularity as a laboratory animal species. Though most frequently associated with the study of circadian rhythms due to their diurnal activities in the wild, degus have also been established as valuable animal models in the study of a wide range of scientific areas including developmental biology, diabetes mellitus, cataracts, and Alzheimer’s disease. However, with few established laboratory colonies, much remains to be discovered regarding optimal housing, disease control, and veterinary care of the species.

HISTORY AND TAXONOMY

Taxonomy

Octodonts are members of the family Octodontidae, the infraorder Hystricognathi, the suborder Hystricomorpha, and the order Rodentia (Lavocat, 1974; Myers et al., 2006;

Pine et al., 1979; Redford and Eisenberg, 1992; Wilson and Reeder, 2006). Hystricomorph rodents are identified anatomically as being both hystricomorphous (having enlarged infraorbital foramina) and hystricognathous (having a mandibular angle in a latero-external position). The family Octodontidae is indigenous to South America. The term “octodont” is a reference to indentations resembling a figure-of-eight found on the surface of their molars (Woods and Boraker, 1975). The *Octodon* genus is comprised of four species: *O. degus*, *O. pacificus*, *O. bridgesi*, and *O. lunatus*.

Geographical Distribution

The four species of octodonts are distributed through several geographical regions. *O. degus* is found in northern and central Chile, *O. bridgesi* and *O. lunatus* are both found in central Chile and *O. pacificus* is found only on Isla Mocha in central Chile (Contreras et al., 1987; Hutterer 1994; Redford and Eisenberg 1992; Zunino and Vivar, 1986). The species also differ by habitat, with *O. degus* occupying open terrain, *O. bridgesi* occupying forest terrain, *O. lunatus* occupying terrain characterized by dense shrub cover, and *O. pacificus* inhabiting a small coastal island. *O. degus* is one of the most common mammals in central Chile and the cause of numerous agricultural problems (Meserve et al., 1984). *O. degus* is the most widely distributed of the four species and is sympatric with *O. lunatus* and possibly also with *O. bridgesi* in some areas.

Origin and Domestication

Prior to 1970 there were miscellaneous reports on the anatomy and biology of degus and their limited use in biomedical research. Little information on laboratory maintenance was provided in these reports. The first description of an established laboratory colony of degus was by Weir who reported on a colony at the Wellcome



FIGURE 44.1 The adult degu.

Institute of Comparative Physiology at the Zoological Society of London (Weir, 1970). Woods and Boraker also made note of two large colonies maintained at the University of Vermont and at the Instituto de Medicina Experimental del Servicio Nacional de Salud at the Universidad de Chile (Woods and Boraker, 1975).

Genetics

Very little information is available on the genetics of *O. degus*. The diploid chromosome number is 58 and the karyotype has been described (George and Weir, 1972). There are no inbred or unique strains described. Honeycutt et al. (2003) analyzed the phylogenetic relationships among members of the superfamily Octodontoidea using nucleotide sequences from mitochondrial RNA and showed that members of the genus *Octodon* are monophyletic but closely related to *Spalacopus* and *Aconaemys*.

The National Center for Biotechnology Information (NCBI) Nucleotide database (<[http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide&cmd=Search&dopt=DocSum&term=txid10160\[Organism%3AAnoexp\]/>](http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide&cmd=Search&dopt=DocSum&term=txid10160[Organism%3AAnoexp]/>)) includes a number of nucleotide sequences from *O. degus*. A small number of these are partial or complete sequences of known genes while the remaining 40 sequences included sequences with no known coding function, several unknown genes, microsatellite sequences, and retroposons.

Similarly, a small number of proteins from degus were recorded in the NCBI Protein database (<[http://www.ncbi.nlm.nih.gov/sites/entrez?db=protein&cmd=Search&dopt=DocSum&term=txid10160\[Organism%3AAnoexp\]/>](http://www.ncbi.nlm.nih.gov/sites/entrez?db=protein&cmd=Search&dopt=DocSum&term=txid10160[Organism%3AAnoexp]/>)). All but three have been identified.

ANATOMY, PHYSIOLOGY, AND BEHAVIOR

Introduction

Although the degu has been utilized in research for more than 40 years, there has not been a systematic assessment of its anatomy and physiology. Instead, those organ systems and physiological processes that have made the degu a useful animal model in various areas of research have been described in detail while other areas have received little or no attention.

External Features

The body morphology of degus is similar to that of other rodents used in biomedical research. In fact, degus resemble gerbils but are larger. Adult degus measure 250–310 mm in body length and have a tail of

75–130 mm. Weight ranges from 170–300 g. The dorsal fur is yellow-brown, the underbelly is creamy yellow, and the feet are white to gray. The tail is covered in yellow-brown fur and ends in a black brush, leading some to refer to them as “trumpet-tailed rats”. The external pinnae are pigmented and have little fur. The feet have four well-developed digits with a fifth digit that is reduced in size (Nowak, 1999; Woods and Boraker, 1975).

Musculoskeletal System

Very little information has been published on the skeletal anatomy of the degu. Woods and Boraker (1975) provide a brief review. They note that degus have prominent auditory bulla and that the proximal ends of the tibia and fibula are fused. Degus have skeletal characteristics consistent with other members of the infraorder Hystricognathi and the suborder Hystricomorpha. Specifically, in hystricomorphous rodents, the infraorbital foramen is very large and the medial masseter muscle passes through it. In hystricognathous rodents the angular process of the mandible, to which the masseter muscle is attached, is flared laterally (Myers et al., 2006).

Woods (1972) conducted an extensive analysis of the jaw, hyoid, neck, pectoral girdle, and forelimb musculature of 13 genera of hystricognathous rodents for the purpose of determining taxonomic relationships. Degus did not display any unusual muscular anatomy.

Degus and other hystricognath rodents exhibit differences in the anterior nasal skeleton compared to other rodents. Specifically they lack a continuous anterior nasal floor, the nasal septum has a ventral keel below the lamina transversalis anterior, and the lamina transversalis anterior and nasal septum are connected at the same level as the ventral border of the paries nasi (Mess, 1999).

A brief survey of radiographic images of the degu has been published (Capello et al., 2008).

Digestive System

Degus have a total of 20 teeth. The dental formula is incisors: 1/1, canines: 0/0, premolars: 1/1, and molars: 3/3. When viewed from the occlusal surface, the cheek teeth have a figure-of-eight appearance as indicated by the genus name (Edwards, 2009). As with other hystricognathous rodents, the incisors and cheek teeth are continuously erupting (Jekl et al., 2008).

A review of the nutrition of the degu has recently been published (Edwards, 2009). The degu has a simple glandular stomach. There are no descriptions in the literature of the small intestine. The cecum possesses both taenia and diverticulations. Fermentation occurs in the cecum similar to many other rodents. The colon has taenia but lacks diverticulations. On an ad libitum

diet, the gastrointestinal transit time is 5.1 hours for solids and 5.2 hours for fluids. Degus are coprophagic. In a 24-hour period, 38% of feces are reingested, primarily at night (Kenagy et al., 1999b).

Absorption of water occurs in the colon. Three aquaporins (AQP-1, AQP-3, and AQP-8) are expressed in the colon of the degu (Gallardo et al., 2002). By immunohistochemical staining AQP-1 was identified in epithelial cells on the surface and in the crypts of the colon. Blockage of AQP-1 with p-chloromercuribenzenesulfonic acid caused a reduction in fluid absorption rate. AQP-8 was found only in the cytoplasm of surface epithelial cells. AQP-3 was not found in epithelial cells but was identified in the subepithelial area and muscularis of the colon. The roles of AQP-3 and AQP-8 in water regulation are not clear (Gallardo et al., 2002).

Circulatory System

A systematic study of the vasculature of degus has not been conducted. As reviewed by Woods and Boraker (1975), the brain is supplied by the vertebral artery. There are external carotid arteries but the internal carotid arteries are absent. Left and right anterior vena cavae are present.

The arterial supply of the abdomen and pelvic regions of degus has been described (Ventura et al., 1996). The arterial anatomy of the degu is similar to that of the guinea pig as both degus and guinea pigs have a celiacomesenteric trunk arising from the aorta that is the origin of the cranial mesenteric and celiac arteries. The celiacomesenteric trunk has not been described in sciurognathous rodents. In contrast to sciurognathous rodents, these two hystricognathous rodents also have a cranial abdominal artery that supplies the transverse and oblique abdominal muscles. In sciurognaths, these muscles are supplied by the deep circumflex iliac artery, a vessel that is not present in the degu or guinea pig.

The hematology of degus was reported by Murphy et al. (1978). Male degus had a hematocrit of 42.1% and hemoglobin of 12 g/dl. The hematocrit in females measured 40% and the hemoglobin was 11.7 g/dl. Red blood cells numbered $8.7 \times 10^6/\mu\text{l}$ in males and $8.9 \times 10^6/\mu\text{l}$ in females; and leukocytes numbered $8.5 \times 10^3/\mu\text{l}$ in males and $8.2 \times 10^3/\mu\text{l}$ in females. Total protein measured 5.7 g/dl in males and 5.6 g/dl in females. Red blood cell indices in males and females, respectively, were MCV (49.4 and 49.5 fl), MCH (14.1 and 14.4 pg), and MCHC (28.8 and 29.6 g/dl). The male leukogram consisted of 40% neutrophils, 1.2% eosinophils, 0.5% basophils, 38.1% lymphocytes, and 0.6% monocytes. The female leukogram was similar and measured 37% neutrophils, 1.2% eosinophils, 0.5% basophils, 59.1% lymphocytes, and 1.3% monocytes (Murphy et al., 1978).

TABLE 44.1 Hematology Parameters of University of Michigan Colony Degus

Parameter	Mean	Range
WBC	$6.23 \times 10^3/\mu\text{l}$	$1.84\text{--}11.36 \times 10^3/\mu\text{l}$
Neutrophils	$3.37 \times 10^3/\mu\text{l}$ (54.1%)	$1.34\text{--}6.32 \times 10^3/\mu\text{l}$
Lymphocytes	$2.00 \times 10^3/\mu\text{l}$ (32.1%)	$0.27\text{--}5.36 \times 10^3/\mu\text{l}$
Monocytes	$0.77 \times 10^3/\mu\text{l}$ (0.12%)	$0.13\text{--}1.87 \times 10^3/\mu\text{l}$
Eosinophils	$0.06 \times 10^3/\mu\text{l}$ (0.01%)	$0\text{--}0.34 \times 10^3/\mu\text{l}$
Basophils	$0.02 \times 10^3/\mu\text{l}$ (0%)	$0\text{--}0.07 \times 10^3/\mu\text{l}$
RBCs ($\times 10^6/\mu\text{l}$)	8.76	7.07–12.64
Hemoglobin (g/dl)	13.87	11.2–20.4
Hematocrit (%)	39.10	32.2–55.4
MCH (pg)	15.9	14–18.5
MCV (fl)	44.7	41.5–52.2
MCHC (g/dl)	35.5	33–38.8

TABLE 44.2 Clinical Chemistry Parameters of University of Michigan Colony Degus

Parameter	Number of Animals Evaluated	Mean	Range
Total bilirubin (mg/dl)	23	0.49	0.2–1.2
AST (U/l)	24	139.54	21–546
ALT (U/l)	23	21.83	8–103
Alkaline phosphatase (U/l)	18	136.50	57–224
Glucose (mg/dl)	27	192.41	93–305
Urea nitrogen (mg/dl)	28	41.54	16–99
Creatinine (mg/dl)	26	0.39	0.2–0.7
Protein (g/dl)	23	5.94	4.8–8.3
Albumin (g/dl)	23	3.90	2.7–4.9
Sodium (mEq/l)	23	150.70	142–161
Potassium (mEq/l)	24	5.59	3.9–8.1
Chloride (mEq/l)	25	110.92	101–131
Calcium (mg/dl)	16	10.71	9.1–11.6
Phosphorus (mg/dl)	17	6.19	4.5–9.9

We have surveyed the hematology and clinical chemistry of degus in the University of Michigan colony. Values are given in Table 44.1 and Table 44.2 and represent the values obtained from 24 adult males. Results for female degus were not significantly different.

Pulmonary System

There is only one report on the pulmonary system of degus (Wallau et al., 2000). The morphology of the

lungs of a number of rodent species was compared for the purpose of taxonomic classification. Using 23 characteristics of pulmonary morphology such as presence or absence of various lung lobes, number of bronchi, and airway distances and diameters, the authors described ten lung types in rodents. The octodon type was one of the ten. The right lung of degus had four lobes and the left lung had three. Histologic examinations were not performed.

Urinary System

Degus have been the subject of studies in comparative renal physiology because, like many rodents from arid and semi-arid environments, they have well-developed adaptations for conserving water. At the upper elevations in northern and central Chile where degus are found, the climate consists of hot, dry summers and cold, rainy winters. Several studies have examined the urine-concentrating ability of degus. Under laboratory conditions, the urine osmolarity of degus subjected to food and water restriction exceeded 4000 mOsmol/kg (Fonda and Horst, 1976). More recently, Bozinovic et al. (2003) examined water flux rate, urine osmolarity, and the expression of kidney aquaporins in free-ranging degus. They reported that the mean urine osmolarity in the summer was 3137 ± 472 mOsmol/kg and 1123 ± 472 mOsmol/kg in the winter. The higher urine osmolarity in summer is consistent with the scarcity of water.

Recent studies have explored cellular physiology associated with the urine-concentrating ability of the degu. The aquaporin family of proteins is involved in transmembrane water transport in a wide variety of organisms and cell types (Gomes et al., 2009). Field studies conducted with *O. degus* have demonstrated changes in expression of aquaporin-2 (AQP-2) in the kidneys of degus captured in summer and winter months. Using immunohistochemical labeling, the renal medulla of degus captured in the summer had greater AQP-2 staining compared to winter-captured degus. Also in the summer, there was increased labeling of AQP-2 in the apical cell membranes and increased AQP-2 protein production by immunoblotting. No changes were observed for AQP-1 (Bozinovic and Gallardo, 2006; Bozinovic et al., 2003; Gallardo et al., 2005).

Reproductive System

Male Reproductive System

The adult male degu has a perianal circle measuring 25×15 mm made up of thin, pale hair (Contreras and Bustos-Obregon, 1980). The penis, which measures 16 mm in length, is at the anterior border of the perianal

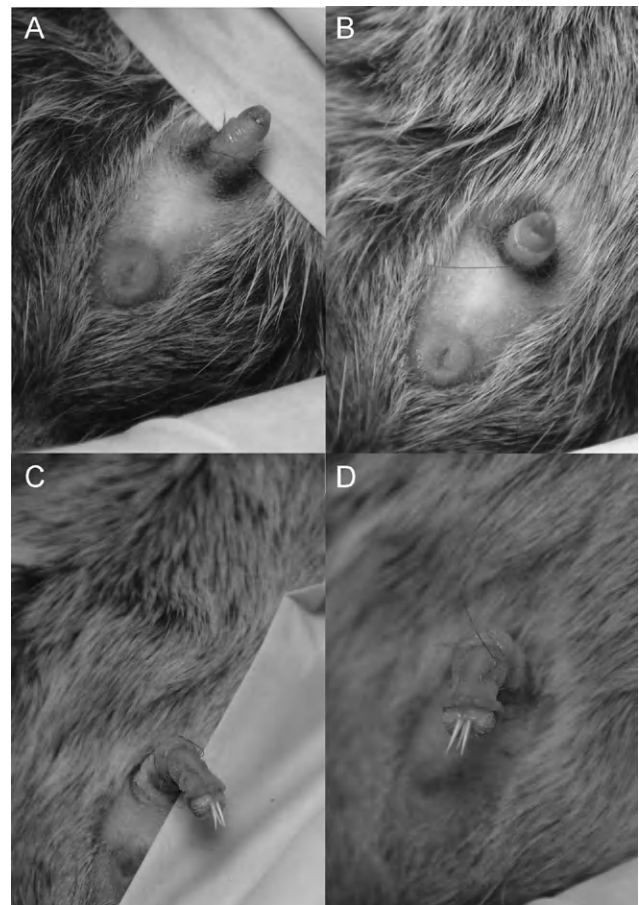


FIGURE 44.2 Genitalia of the male degu. (A and B) Juvenile male demonstrating the absence of cornified spikes; (C and D) adult male with cornified spikes protruding from the intromittent sac.

circle and points posteriorly at rest and anteriorly when erect (Contreras and Bustos-Obregon, 1980; Contreras et al., 1993). The glans penis measures 10×3.5 mm and is covered in multitoothed spines (Contreras and Bustos-Obregon, 1980; Contreras et al., 1993). There are two external openings in the glans. The dorsal opening is the urethral opening and the ventral is the opening to the intromittent sac (Contreras and Bustos-Obregon, 1980; Contreras et al., 1993). The intromittent sac is a structure common to hystricomorph rodents. The sac is an invagination of the glans that everts during sexual activity. In the adult, the surface of each sac is covered in spines and at the base of each sac are two cornified spikes (Figure 44.2) (Contreras et al., 1993). There is an os penis dorsal to the urethra (Contreras et al., 1993). Parallel to the penis and just beneath the perianal circle are two subcutaneous muscular pockets, the cremasteric sacs. The caudal epididymis lies inside the sacs. The testes are intraabdominal (Contreras and Bustos-Obregon, 1980). Accessory sex glands include the prostate, seminal vesicles, and bulbo-urethral glands (Contreras and Bustos-Obregon, 1980).

The spermatozoa of *O. degus* have been described (Berrios et al., 1978). The head is flattened dorso-ventrally and measures $7.7\mu\text{m}$ long by $5.9\mu\text{m}$ wide. The tail measures $41\mu\text{m}$ long. The rim of the acrosome is U-shaped.

Male castration is frequently used to prevent breeding of pair- or group-housed degus as well as for select research models. The method of castration employed must functionally close the degus' wide, open inguinal canal through which the testicles normally move in and external to the abdominal cavity (Powers et al., 2008). Specific techniques for degu castration have been described (Capello, 2005; Fehr et al., 1994).

Female Reproductive System

The anatomy of the reproductive tract of female degus has not been described comprehensively. However, some components have been studied in detail. There is a vaginal membrane covering the opening of the vagina (Figure 44.3) (Reynolds and Wright, 1979). The vaginal membrane is open only during proestrus and estrus (Labyak and Lee, 1995). The uterus is bicornuate (Woods and Boraker, 1975). There are four pairs of mammary glands.

The placentation of the degu has been described and is similar to that of the guinea pig. Degus have a chorioallantoic placenta that is hemomonochorial and labyrinthine (Bosco, 1997; Bosco et al., 2007; Mess, 2007a). Caviomorphs, in general, are useful models of human placentation because they also have a hemochorial placenta similar to humans, a single trophoblast layer, and trophoblast invasion (Mess et al., 2007). Caviomorphs have labyrinthine and highly lobulated placenta that is different from the villous placenta of humans. In contrast to other caviomorphs, the degu has a simpler placenta that is not highly lobulated, a characteristic that makes it more useful as a model of human placentation (Mess, 2007b). Recent studies on the pathogenesis of preeclampsia in humans suggest that this condition is associated with a failure of fetal trophoblasts to invade the maternal tissues in the formation of the placenta (Cudihiy and Lee, 2009). Trophoblast invasion in the degu placenta shows similarities to that seen in the human placenta (Mess et al., 2007a).

Nervous System

Wright and Kern (1992) developed a stereotaxic atlas of the degu brain using adult male degus. Despite differences in body weight, the atlas is applicable to female degus as well since brain size was found to be equal in both sexes. The atlas contains 38 transverse sections and two sagittal sections through the prosencephalon, mesencephalon, and rhombencephalon (Wright and Kern, 1992).

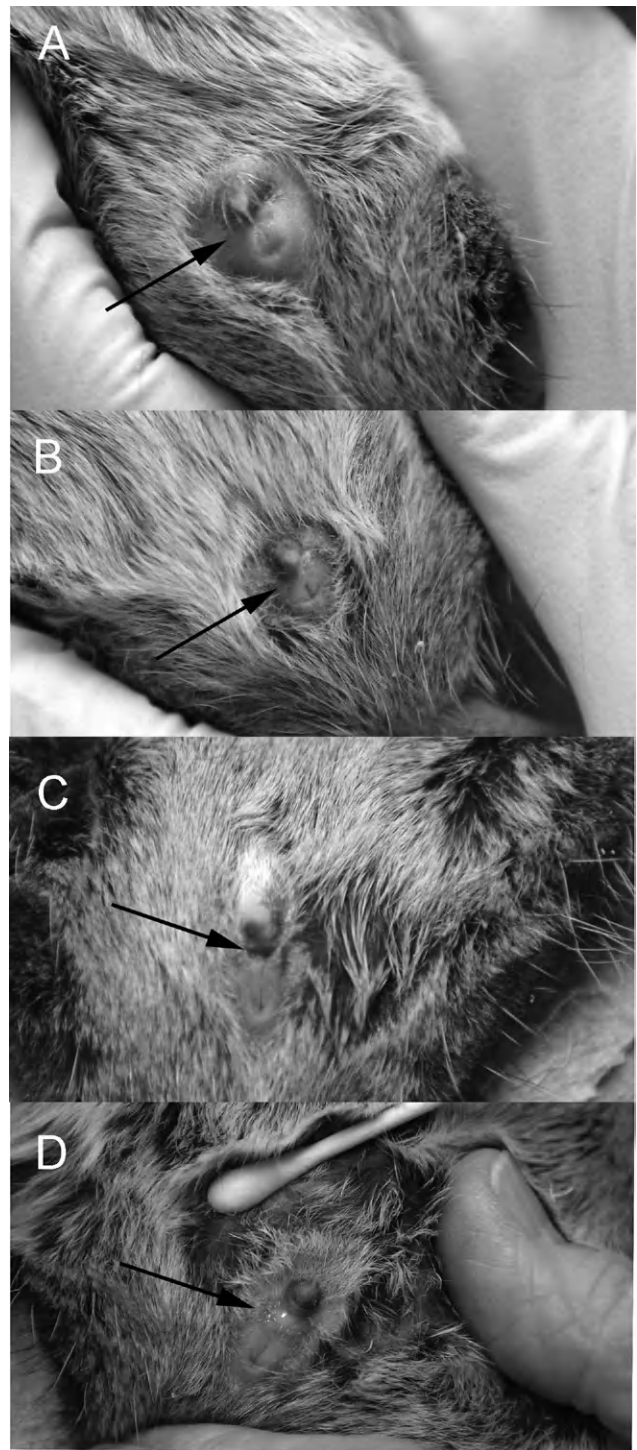


FIGURE 44.3 Adult female degu vaginal area. (A and B) A closed vaginal membrane; (C) perforated vaginal opening; (D) vaginal opening with presence of vaginal secretions. Arrows indicate the vaginal opening.

The brain weight was 2.52g in males and 2.42g in females. The rostral-caudal length of the cerebrum measured 16.6mm in males and 16.3mm in females with a width of 17.0mm in males and 16.7mm in females (Wright and Kern, 1992).

Wright and Kern (1992) compared the forebrain and midbrain of degus, guinea pigs, and rats. Some minor differences (1–2 mm) in the precise location of brain structures (i.e., amygdaloid complex, hypothalamus, and thalamus) relative to the rostral and caudal commissures were seen. In the degu and guinea pig, the hypothalamus appears more compact than in the rat. The degu mesencephalon is more compact than in the guinea pig and rat. This leads some structures (e.g., trochlear nerve, red nucleus) to be situated more rostral than in the guinea pig and rat. The hindbrain of degus and rats are similar although the rat brainstem tends to be wider and flatter. As noted in the midbrain, some structures in the degu brain are either slightly more rostral or caudal than in the rat brain.

The morphology of the degu pineal gland and its role in the synthesis and secretion of melatonin have been described (Lee et al., 2009; Uria et al., 1992). The degu pineal gland is similar to that of other rodents. It is adjacent to the third ventricle and is 0.68–0.95 mm in length, 0.36–0.52 mm in width, and 0.34–0.46 mm in height. No sexual dimorphism is observed. The gland contains three cell types: type I cells are likely pinealocytes, similar to those seen in other rodents; type II cells correspond to astroglial cells, also described in rodents; and type III cells do not resemble pinealocytes and are closely associated with vascular structures. Their significance has not been determined.

Endocrine System

The degus' adrenal glands are relatively large when compared to other rodents of similar size. The predominant glucocorticosteroid produced by the degu is cortisol (Kenagy et al., 1999a). During lactation, female degus produce cortisol titers of 1000 ng/ml, declining to 500 ng/ml just prior to breeding. Similar cortisol levels are noted in males (Kenagy et al., 1999a).

Special Senses

Auditory System

The morphology of the middle ear of degus is very similar to that of the chinchilla and guinea pig (Argyle and Mason, 2008). In all three, the head of the malleus is elongated and the malleus and incus are typically fused. Although in the early literature, the malleus and incus were reported not to be fused (Woods and Boraker, 1975). Other commonalities between the three species include a bony spicule passing through the intercrural foramen of the stapes and the absence of a pars flaccida in the tympanic membrane (Argyle and Mason, 2008). The middle ear cavity is septated in the degu and chinchilla but not in the guinea pig.

The guinea pig and chinchilla have a stapedius muscle but this structure is absent in the degu (Argyle and Mason, 2008).

Visual System

In mammals, the ratio of rods to cones and their distribution throughout the retina varies with habitat and lifestyle. For nocturnal rodents, rods are the predominant photoreceptor; although cones are also present (Chavez et al., 2003). The retina has cones that are sensitive to various wavelengths of light (long (L), middle (M), short (S), and ultraviolet (UV)). Rodents exhibit three main cone patterns, M/S, M/UV, and M only. There are about 3 million cones in the degu retina consisting of M and UV types (Jacobs et al., 2003). The M type predominates with the ratio of M : UV being 13 : 1. There are approximately 6.5 million rods per eye. The ratio of cone types is similar to other mammals and the spectral absorption properties of the cones and rods in the degu are similar to other rodents. Degus can discriminate between some colors (Jacobs et al., 2003). They likely have dichromatic vision based on the presence of green-sensitive M cones and UV-sensitive S cones (Chavez et al., 2003). An age-related increase in optical density was observed in the lens of the degu (Jacobs et al., 2003). Additional adaptations have been noted of the degu's visual system, presumably in response to its diurnal lifestyle (Jacobs et al., 2003). As occurs in other precocial species, neonatal degus have well-developed vision at birth although the visual system continues to mature until adulthood (Delgado et al., 2009).

The histology of the harderian glands has been described (Antolin-Gonzalez et al., 1993). In female degus the secretory cells contain numerous basophilic granules and clusters of lymphocytic and plasmacytic cells. These structures are absent in the harderian glands of male degus.

Behavior

Degus in their natural habitat live in social groups and utilize a variety of sensory modalities in communication. Tactile communication through group huddling and mutual grooming is frequently observed among familiar and related individuals (White et al., 1982; Wilson, 1982; Wilson and Kleiman, 1974). Several visual signals have been identified in association with male–male aggression, mating, and play behaviors (Davis, 1975; Wilson and Kleiman, 1974). A variety of vocalizations are produced, all apparently within the range of human hearing (Long, 2007; Reynolds and Wright 1979). Degus produce specific courtship calls; contact calls between mother and young; loud alarm calls; and a variety of squeaks, whistles, snorts, and whines, which are apparently related to varying levels of arousal but the specificity of which is still unknown

(Braun and Scheich, 1997; Kleiman et al., 1979; Poeggel and Braun, 1996). They also produce tooth-chattering and foot-thumping sounds typical of other rodents during aggressive interactions. Likely, the most highly developed communication method is the olfactory system (Fischer et al., 1986; Fulk 1976). Degus produce highly individual and recognizable (by other degus) odors in the urine and with specialized scent glands (Fischer and Meunier, 1985). Both odor sources are used by both sexes to mark their territory and members of the group (Fischer and Meunier, 1985). Meetings between individuals include extensive olfactory investigation, mutual sand bathing on marked areas, and spraying of one another. Interestingly, in degus, unlike many other species, the female engages in more scent marking, more investigation, and demonstrates greater ability to identify individual odors than does the male (Kleiman, 1975).

A variety of other behaviors, many related to their territorial, semi-fossorial, multi-adult groups, have also been studied (Ebensperger et al., 2004; Quispe et al., 2009). These include their hoarding behavior (Quispe et al., 2009), social foraging behavior for avoidance of predators (Ebensperger and Hurtado, 2005; Ebensperger and Wallem, 2002; Ebensperger et al., 2006a; Quirici et al., 2008), locomotor patterns (Vasquez et al., 2002), maternal-offspring nursing interactions with a group of adults (Ebensperger et al., 2007), exclusivity of the maternal relationship in the shared burrow (Ebensperger et al., 2006b; Jesseau 2004; Jesseau et al., 2008, 2009), and spatial distribution of adults (Hayes et al., 2007; Milstead et al., 2007). A wide variety of studies have examined the impact of environmental conditions (e.g., temperature, humidity, cover, time of day, predator presence, nutrition) on behavior and physiology. Due to their unique physiology and behavioral patterns, degus are frequently used as an animal model to study many aspects of human behavior.

MANAGEMENT, HUSBANDRY, AND COLONY HEALTH

Introduction

A critical component of the management of the degu colony is maintaining sufficient social contact during development for the animals to mature with normal behavioral patterns. Poeggel et al. (2003) reports severe deficits as a function of maternal deprivation. In an early experiment that required young animals be housed alone with a running wheel, the authors discovered that when adults they were terrified of other degus, and were aggressive to humans. Interestingly, single housing of animals that have already reached maturity does not produce the same problems,

particularly if there is environmental enrichment (Poeggel et al., 2003). Without enrichment, some single-housed animals are lethargic and not very attentive.

Housing Systems

Caging

At the University of Michigan, degus are housed with parents and littermates in large polycarbonate solid-bottom cages ($42.5 \times 46 \times 19.5$ cm) until weaning. Degus are weaned between 5 and 6 weeks of age and housed in opaque $42.5 \times 22 \times 19$ cm cages with 2–3 same-sex siblings. When individually housed, we use opaque polycarbonate cages ($42.5 \times 22 \times 19$ cm). We equip cages with Nalgene running wheels (9×34.5 cm). Cages are stored on stainless steel racks in open rooms, or in ventilated, light and temperature controlled housing units that hold 4–8 cages.

Bedding

We found that pine shavings sometimes cause irritation to the skin of the animals, so we use only inert bedding such as ground corn cobs. Degus do not readily build nests, although some will keep new litters in shelters.

Environmental Conditions

The following describes the environmental conditions provided by the authors in support of a long-established breeding colony at the University of Michigan.

Ventilation

Animal rooms are provided with a minimum of 7–10 fresh air exchanges per hour. Animal rooms are maintained under negative pressure with respect to hallways. Exhaust air from the animal rooms is not recirculated, but directly discharged from the building.

Illumination

The entire colony, including breeding groups, is maintained on a 12 : 12 hour light:dark cycle. When housed in an open room, the light intensity within cages may vary significantly (e.g., 50–200 lux). When animals are housed in light-controlled, ventilated isolation chambers the light intensity can be varied from 10–250 lux, as needed. Under some experimental conditions animals are housed in constant darkness (DD), dim red light, or constant light (LL). Circadian rhythms free-run under such conditions and do not become disorganized under LL, as occurs with some nocturnal species. We find that females continue having estrous cycles in all conditions, suggesting that degu reproduction is not strongly photoperiodic.

Temperature and Humidity

Degus have difficulty thermoregulating and may suffer dehydration when at temperatures of greater than 32°C. The breeding room is maintained at 20°C ± 2°C. Cages that contain a litter have a heating pad set on low under one corner of the cage to provide additional warmth for the pups, if they choose. When animals are provided with running wheels, the room is maintained at 17–18°C. When housed at higher temperatures and placed in running wheels, many animals demonstrate masking (i.e., altered circadian pattern without a change in suprachiasmatic nuclei (SCN) function), and their activity is crepuscular or nocturnal (Kas and Edgar, 2001; Kenagy et al., 2002a, 2002b). Room humidity is not tightly controlled and ranges from approximately 30–60%.

Enrichment

We provide all animals not in experiments with alfalfa hay once or twice per week. Breeding pairs and juvenile degus receive alfalfa hay 3–4 times per week as a dietary supplement. We have tried providing individually housed and group-housed degus with large PVC tubes and “nesting boxes”. They never preferred to sleep or nest in either device, typically using them (if at all) as a location for defecation. Nest building was not observed with any of multiple bedding substrates provided. The provision of a dust bath is recommended by some (Johnson, 2002), however colonies maintained over multiple generations without access to dust baths have demonstrated no apparent negative effects (Lee, 2010). Degus are very active animals and will run thousands of revolutions in a running wheel in a day, sometimes even in an hour. When degus must be isolated for experimental reasons, the addition of a running wheel to the cage seems to maintain healthy activity and eating patterns. Whereas degus housed alone for more than a few weeks sometimes become lethargic and relatively unresponsive to the presence of a human. Female degus can readily be introduced as a living partner with any other female, and fighting rarely ensues. Once males have reached maturity, however, we find that they can only be re-housed after isolation with a male sibling, and sometimes that is also not feasible.

Individual housing of young animals has been associated with the development of severe behavioral and neural deficits (Poeggel et al., 2003) and difficulty handling (Lee, 2004). To avoid the developmental disruption induced by social isolation, young animals are housed alone with running wheels for no more than 4 weeks, and then returned to co-housing with a same sex sibling (Hummer et al., 2007). Through the first 6 months of life we maintain social contact between related degus as much as possible. With this protocol,

the behavior of the mature animals is normal, and the behaviors we study were not different between adults that were never tested during development and those that were tested periodically during development (Hummer et al., 2007).

Sanitation

Cages are changed once a week during the light phase, and changed under dim red light every other week when animals are housed in constant conditions for the purpose of studying undisturbed endogenous circadian rhythms. This rate of cage change is acceptable because degus urinate very little, and we house them in cages with considerably more floor space than is required for other comparably sized rodents (Institute of Laboratory Animal Resources (US). Committee on Care and Use of Laboratory Animals, 2011).

Nutrition

Field Studies

The diet of the degu in its natural environment in Chile has been studied in detail. In the wild, the degu consumes a diet of grass, shrub, forb foliage, flowers, and seeds (Meserve 1981; Meserve et al., 1983, 1984; Simonetti and Montenegro 1981). The wet and dry seasons affect the availability of specific plants and hence dietary intake. Simonetti and Montenegro (1981) demonstrated that degus prefer new rather than mature matorral shrubs when given the choice, and do not discriminate between new leaves of different species. In controlled laboratory studies, degus exhibit a strong preference for low-fiber, high-nitrogen plants over high-fiber plants (Bozinovic, 1995; Gutiérrez and Bozinovic, 1998). Other studies confirm that the degu will select high-quality foods when available (Kenagy et al., 1999; Torres-Contreras and Bozinovic, 1997). During the dry season, the degu can compensate for lower-quality food by increasing consumption, whereas in the laboratory the degu can lower its metabolic demands when food quality declines (Bozinovic et al., 1997; Mabry et al., 1988; Veloso and Bozinovic, 1993). Sakaguchi and Ohmura (1992) found that the degu has reduced digestive ability for fiber compared to the guinea pig and that the degu does not appear to readily consume an alfalfa-based diet.

Laboratory Maintenance

The nutritional requirements of degus have not been studied. Several diets are mentioned in the degu literature, ranging from (low-fiber, high-energy) rodent diet (Mabry et al., 1988; Popovic et al., 2009; Wright and Kern 1992); rodent diet supplemented with vegetables and fruit (Poeggel and Braun, 1996); rodent chow with cereal seeds, sunflower seed, and hay (Braun and

TABLE 44.3 Commercial Diets Evaluated for Effect on Reproductive Performance

Designation	Diet Name	Relative Dietary Composition	Manufacturer
Rodent Diet (RD)	LabDiet® 5001	High-protein, medium-metabolizable-energy, low-fiber	PMI International, St Louis, MO
Rodent Breeder Diet (RBD)	Prolab® RMH 2000	Moderate-protein, high-metabolizable-energy, low-fiber	PMI International, St Louis, MO
Guinea Pig Diet (GPD)	LabDiet® 5025	Low-protein, low-metabolizable-energy, high-fiber	PMI International, St Louis, MO
Chinchilla Diet (CD)	Mazuri® Chinchilla Diet 5E05	High-protein, low-metabolizable-energy, high-fiber	PMI International, St Louis, MO

Scheich, 1997); guinea pig pellets (Long and Ebensperger, 2010); and rabbit pellets (Becker et al., 2007; Bozinovic et al., 1997; Veloso and Kenagy, 2005). Edwards (2009) described and provided an analysis of the diet used for a colony of non-reproductive female degus at the Smithsonian National Zoological Park as consisting of a high-fiber herbivore pellet supplemented with greens, roots, and hay. The compositions (fiber, protein, fat, vitamins, and minerals) of several other diets used in degu colonies were reported.

Unlike the closely related guinea pig, the degu does produce the hepatic enzyme L-gulonolactone oxidase and does not have a dietary requirement for ascorbic acid. Therefore, diets supplemented with ascorbic acid are not required for maintenance of this species (Edwards, 2009).

At the University of Michigan (UM), we evaluated reproductive performance, growth, and maintenance of degus maintained on different commercial diets in our colony (Rush, 2010). Specifically, we assessed the fertility of breeding females, litter survival rates, growth patterns of pups, and maintenance of healthy body size in adults. The diets we compared (RD, RBD, GPD, and CD) are summarized in Table 44.3.

RD was selected because it had been used in the UM colony for many years. GPD and CD were selected because of the guinea pig's (a caviomorph rodent) and chinchilla's (a hystricomorph rodent) close taxonomic relationship and similar dietary habits to the degu. Finally, RBD was selected because of its largely wheat-based composition, which was thought to better represent the degu's natural diet. The biggest difference between RD and RBD is the use of corn and soybean in RD while RBD used wheat as the primary ingredient. In addition, RBD was the only diet with no added sugar.

The two high-fiber alfalfa-based diets (GPD and CD) and the high-protein, low-fiber, medium-energy RD were found to be inappropriate for degu reproductive success. CD was insufficient to support gestation (36% of pups were stillborn), caused a high mortality rate

in young degus, produced the smallest animals, and caused the largest weight gain in adult animals. The high-protein, low-fiber, medium-energy RD was also found to be an insufficient diet for the reproductive degu, with a large percentage (33%) of pups being stillborn. Stillbirths in dams fed GPD were not increased but post-weaning mortality in pups fed GPD was significantly higher than for other diets.

Pups were weaned when they reached a weight of 50 g, usually between 4–6 weeks of age. Pups fed RD or RBD reached the weaning weight significantly earlier in development than pups fed CD or GPD. However, only 56.3% of pups fed RD survived to weaning, although the surviving animals appeared to do quite well on RD once past weaning.

Animals raised on RBD had the largest and healthiest pups, with a high survival rate. During the period between weaning (4–6 weeks of age) and 10 weeks of age, mortality rates for pups fed RBD (2% mortality) were significantly lower than for litters on GPD (23% mortality) and CD (14% mortality).

In general, we found that lactating mothers and juvenile degus have different dietary needs than non-breeding adults. We found that RBD produced the best combination of litter size, pup survival, and growth rate. Our results are also supported by findings from Veloso and Bozinovic (2000) who reported that pregnancy and lactation are the most energy-demanding periods for the female degu and that low-quality (high-fiber, low-protein) feed correlates with lower litter mass and size.

Given the literature and our data, we recommend that breeding adult degus and juveniles (up to 10 weeks to 6 months of age) be sustained on RBD. However, without access to exercise wheels, adult degus become overweight on RBD. After 10 weeks of age, a change to RD suffices for juveniles and non-breeding adults, since the animals' response to the RD was not significantly different from that of the RBD after weaning and provided good adult body maintenance and prevented obesity.

Water

Water metabolism has been studied in the degu (Bozinovic and Gallardo, 2006). In their natural environment, degus obtain water primarily through consumption of food. In captivity, water is generally provided ad libitum. In the University of Michigan degu colony, the average 24-hour water intake for an adult degu is 23 ml.

Breeding

Estrous Cycle

Degus are spontaneous ovulators, have regular estrous cycles, and maintain functional corpora lutea. Earlier literature suggested that degus were induced ovulators and required the presence of a male for cycles to occur (Weir 1970, 1974). However, vaginal patency, increased wheel running activity, increased body temperature, and sexual receptivity (estrus) all occur in the same 1–2-day period and occur in a cyclical and regular basis (Labyak and Lee, 1995). The average cycle length in adult females is 21.1 ± 0.56 days and can range from 16–26 days (Labyak and Lee 1995; Mahoney et al., 2005). Cycle length is uniform within an individual and thus one can reliably predict when the next day of estrus will occur using physiological information. Vaginal opening can be detected easily by examining the anogenital region of the female daily or every other day. Degus, like other hystricognathous rodents, have a vaginal membrane that is typically closed throughout the cycle but becomes open, or perforated, at the time of estrus (Figure 44.3) (Addo et al., 2007; Brookhyser and Aulerich, 1980). The opening is present for only 1–3 days each cycle, thus vaginal smears cannot be used to detect changes in the stages of the estrous cycle in this species, as can be done with laboratory rats and mice (Marcondes et al., 2002). At the time of estrus one can also detect associated changes in the perineum and it may appear more swollen and pink in color. Degus may also produce whitish vaginal secretions or plugs around the time of estrus. This is an unreliable indicator of estrous cycle stage as it does not always appear each cycle, and the number of days it is detected varies within and between individuals.

Hormone data and ovarian histology confirm that degus have spontaneous ovulation and corpora lutea formation. On the day of vaginal perforation and the increase in wheel running, (day 1) estradiol concentrations in the blood are also relatively high then drop on days 4, 8, and 16. In contrast progesterone concentrations are elevated on the 8th and 16th day following estrus when compared to the day of estrus. Correlated with these endocrine data, ovaries collected on the day of estrus contain Graafian follicles, secondary follicles, and corpus albicans. Ovaries collected from animals

on day 6 relative to the daily rise in wheel running had well-developed corpora lutea. Primary follicles are also evident at this time but have not progressed to secondary follicles. Sixteen days after the detection of estrus, within a week of the next estrus, ovaries contain both primary and secondary follicles, and corpora lutea are becoming atretic and reduced in number compared to day 6. It should be noted that other laboratory rodents (rats and mice) do not have a spontaneous luteal phase; these species require vaginal stimulation or mating to induce corpora lutea formation (Schwartz, 2000). Thus, degus are a unique and advantageous laboratory model for a variety of research programs, as they are smaller than an adult rat or guinea pig, long-lived, relatively slowly developing (when compared to other rodents), readily adjust to laboratory conditions, and have an estrous cycle which resembles that of primates or sheep.

The pattern of luteinizing hormone (LH) release has not been fully characterized in the degu. Presumably the high concentration of estradiol on the day of estrus triggers a preovulatory surge of luteinizing hormone from the pituitary, as occurs in other mammals. It is also unknown whether the circadian timing system regulates the timing of events such as mating and the LH surge in animals with a long luteal phase (e.g., common laboratory rodents). In rats, mice, and hamsters, the LH surge occurs 6–15 h prior to ovulation and both events occur at precise circadian intervals (Alleva et al., 1971; Christian et al., 2005; Legan and Karsch 1975). The role of the circadian system in species with a true luteal phase has been examined only in sheep and rhesus monkeys. In those species, there is no evidence of a circadian control of luteinizing hormone release (Karsch et al., 1973; Nett et al., 1984). It is unclear at this time whether the lack of a circadian timed release of luteinizing hormone in sheep and rhesus monkey is because they have a spontaneous luteal phase or because of another physiological feature they do not share with most rodents.

Mating and Pregnancy

In our laboratory we house breeding degus in male–female pairs or as groups of one male and two females. Females are reproductively mature at 4 months of age but we do not pair them until they are at least 6 months old to allow the animals to reach adult body weight (170–210 g is typical for females in our laboratory). Breeding animals are housed in 20 in $\times$ 20 in $\times$ 8 in cages (double-width rat boxes). The sire remains with the dams after litters are born so as to have the opportunity for females to mate during postpartum estrus. Males do not direct any aggressive or cannibalistic behavior towards the young; in fact males have been seen huddled over the young. Females of this species are also unusual as they do not exhibit any signs of maternal aggression

when their pups are handled by an investigator. We are able to remove and handle young and return them to their home cage without being attacked by the dam, although she may show some signs of anxiety.

Females exhibit behavioral receptivity (estrus) at the time of vaginal patency and elevated wheel running. Females housed continuously with males throughout their estrous cycle will typically begin mating around the time of their rise in activity. Receptivity does not appear to be strictly regulated by the light:dark cycle. Mating activity continues throughout the day and night with the exception that animals are quiescent for a 3-hour window between 6 and 9 hours after lights off. Mating bouts may be observed periodically for 7–24 hours but copulation does not occur as frequently as seen in lab rats and hamsters. Animals mate only once or twice an hour. Females exhibit a brief lordotic posture when males mount them; males thrust once or twice with no copulatory lock.

Degus have a 90–95-day gestation (Lee, 2004; Weir, 1974). Implantation starts by day 7 and is complete by day 12 (Mess, 2007a). Litter size can range from 1–12 pups (Long and Ebensperger, 2010). The first litter a female produces typically contains 4–6 pups and subsequent litters have 6–10 pups. If dams have more than this number it is difficult for her to provide adequate nutrition to all the young. If it is critical that the pups from a large litter all survive, they can be handfed with commercially available “kitten milk”.

If females breed successfully after delivering young, they may have up to four litters per year (every 3 months). If they do not mate and become pregnant during the postpartum estrus, then females will not mate until after the young are weaned (Lee, 2004). Although degus in the laboratory can live until 5 years of age or more, female reproduction begins to decline after 4 years as we see longer intervals between pregnancies. Thus we stop breeding females by 4 years of age. Breeding animals, their pre-weaned young, and weaned animals up to 6 months of age may be given Prolab RMH 2000 5P06 diet (Lab-Diet®, PMI International, St. Louis, MO) as it improves health and weight gain in lactating females and growing pups although we have recently started providing the RMH diet for only 10 weeks after birth as young were observed to reach mature weights and undergo puberty earlier than desired. We also supply slightly acidified water (2.5 ml of 3.7% HCl solution per gallon of tap water) to prevent diarrheal infections in the pups. Lastly we supply a large handful of fresh or dried alfalfa twice a week to supply additional nutrition for lactating females.

In the wild, degus live in groups, usually with more than one female sharing a burrow system (Ebensperger et al., 2004; Fulk, 1976). Breeding typically occurs once a year but in some populations two litters may be produced in a particularly rainy season (Fulk, 1976;

Meserve et al., 1984). Litters range from 3–10 pups (Weir, 1970). Breeding may be triggered by production of rain and/or green leafy vegetation becoming more abundant (Meserve et al., 1984).

Care of the Young

At birth pups are precocial, fully furred, and their teeth are erupted. Their eyes are typically open within 1 day of birth and their ears are open by postnatal day 3 (Lee, 2004; Reynolds and Wright, 1979). Pups are mobile within the first week of life and begin to nibble on solid food by 2 weeks of age (Lee, 2004). In the wild, young are seen emerging from their burrows at 4 weeks of age (Fulk, 1976). Dams lactate and nurse their young for 4–5 weeks, even though the young are eating solid food (Kleiman et al., 1979; Veloso and Kenagy, 2005). Pups may lay on their backs while nursing from the dam. Males may huddle in a protective way over the young.

Lactation lasts 4–5 weeks (Kleiman et al., 1979; Veloso and Kenagy, 2005). The concentration of solids, lipids, proteins, and ash in degu milk remain constant throughout lactation while carbohydrate concentrations decrease from early to late lactation (Veloso and Kenagy, 2005). Lipids are the main component of degu milk. Total solids average $26.9 \pm 5.8\%$, lipids average $17.3 \pm 5.5\%$, protein average $4.4 \pm 0.4\%$, and ash average $2.7 \pm 0.8\%$. Carbohydrates range from a high of $3.1 \pm 0.3\%$ early in lactation to a low of $1.0 \pm 0.3\%$ late in lactation (Veloso and Kenagy, 2005).

We typically wean pups at 5 or 6 weeks of age, when they weigh 60–70 grams. Weaned pups are housed in same sex social groups; brothers housed together from the time of weaning usually do not show aggression towards one another in adulthood. At the time of weaning, unrelated animals (males or females) can be placed into the same cage without any antagonistic interactions. As mentioned above, weaned young are given acidified water and Prolab RMH 2000 5P06 diet (Lab-Diet®) (Lee 2004) until they are 10 weeks to 6 months of age.

Puberty and Sexual Maturation

In our laboratory, under our feeding program, male and female degus reach sexual maturity around 3 months of age. When animals were fed a less optimal diet, animals did not reach puberty until 4–6 months of age. Males begin to mature at 2 months of age and the majority of males are mature at 6 months of age. Sexual maturity can be assessed by determining the presence and size of the spikes on the glans of the penis, a feature found in the hystricognathous rodents (Contreras et al., 1993). In degus, since the testes are located intra-abdominally, they are less accessible for measurement than the penile spines. Sexual development can be determined by inverting the young animal and gently evert the glans penis to reveal the spikes. Spikes appear at 2.5 months

of age, approximately 60% of males exhibit spikes at 3 months of age and all males exhibit spikes at 3.5 months of age (Hummer et al., 2007). Testes growth also begins at 2 months of age (average 0.2 gram paired testes weight), paired testes weights are about 1.2 grams at 4 months of age. Testes reach their adult size at 6 months of age and on average are about 1.6 grams (Hummer et al., 2007). In parallel with testes growth, spermatogenesis also begins to be detectable in 2-month-old males, then plateaus at 4 months of age.

In female degus sexual maturity can be assessed by determining when vaginal opening occurs. This is done by using a gloved finger to examine the anogenital area for signs of invagination and the color of the perineum. The urethral cone can be gently moved anteriorly to examine this area but care must be taken not to prematurely rupture the vaginal membrane by too much manipulation. The majority of females will exhibit their first vaginal opening between 3 and 3.5 months of age (Hummer et al., 2007). Females may exhibit an opening as early as 1–2 months of age, but this is rare. Intermediate vaginal openings (partial opening, dimpling) may be detected 17–21 days prior to the first full vaginal perforation. Some females will also exhibit an increase in activity or wheel running at the time of these partial signs of vaginal opening. In all animals, after the initial vaginal perforation is detected, it will continue to occur in a cyclical manner. Elevated wheel-running activity typically precedes or coincides with the beginning of full vaginal opening.

Colony Health Monitoring

General recommendations on monitoring animal colonies for the presence of disease have been published (Barthold, 2008; Carty, 2008; International Committee of the Institute for Laboratory Animal Research, 2000). In the case of species other than rats and mice, monitoring for infectious diseases presents a challenge since specific agents may not be known and immunologic reagents may not be available (Weisbroth et al., 1998). In such cases, periodic necropsy assessment combined with the use of mice as sentinel animals may be the best approach. Use of sentinel mice would at least allow detection of agents that can infect mice in other facilities at the institution. This approach has been used with the University of Michigan colony, and no mouse or rat pathogens have been detected.

HANDLING AND RESTRAINT

Physical

Degus should never be picked up by their tail. They will rapidly spin around leaving the sheath of the tail

in the restrainer's hand. The tail bleeds very little after such an event and readily heals, but the animal is left with a stump and other injuries can occur if the animal is dropped. Animals should be picked up by scooping them up with two hands or with a scoop. They are uncomfortable when their feet are not on a stable surface. Carrying them braced against the arm or body, or in a container, will maintain a calm state.

Degus fight aggressively to return to an upright position when they are turned ventral side up. Thus, if it is necessary to turn them over, it is wise to wear a sturdy pair of leather gloves to protect the hands from their nails and teeth. If one does not need access to their ventrum, they can be wrapped in a towel and will be relatively quiet while their ventral or dorsal surfaces are examined.

We have found that giving injections is made much simpler by placing the animal in an empty cage. The animal can be blocked into a corner and will rarely object to the lifting of skin on the back/neck region for subcutaneous injections. To give an intraperitoneal injection the animal's hind legs can be lifted while the front legs remain on the surface of the cage and the needle inserted in the lower, lateral region of the abdomen (Weir, 1970). Degus typically remain calm during this procedure and are not subject to the stress induced by restraining and turning them over to expose the ventral abdomen.

Mechanical

We have successfully used three restraint devices as part of our research program: plexiglass restraining tubes for short-term, full-body restraint; head tethers for select continuous monitoring; and wire-mesh cage dividers for partial isolation of an animal. Plexiglass restraint tubes must be large enough in diameter to accommodate the animal's width, but too small for the animal to turn around. This form of restraint does act as a mild stressor. However, although cortisol rises after animals are placed in the tube, they readily enter the tube repeatedly, suggesting it is not aversive of itself. Animals readily adapt to head tethers that are necessary for collecting EEG data or protecting microdialysis probes. We found that the circadian cortisol patterns return to baseline after 3–5 days of habituating to the tether. Very few animals demonstrate any behavioral signs of stress with the tether attached. When experiments require social, but not physical contact, we use a wire-mesh divider in a 42.5 × 46 × 19.5 cm cage, dividing the cage into two equal sides that are nearly the same size as a single cage. Each animal has independent food and water, and is able to see, hear, and smell another animal through the mesh. This cage adaptor allows us to collect running wheel activity data on both animals, if needed.

Chemical

Chemical restraint can be achieved through use of injectable or inhalant anesthetics. Additional information can be found in the Anesthesia and Analgesia section of this chapter.

SAMPLING TECHNIQUES

Blood Collection

Methods of blood collection in the degu are similar to those used in other rodents and include distal tail transection, surgical exposure and cannulation of the jugular vein, percutaneous cannulation of the anterior vena cava, infraorbital sinus puncture, and cardiac puncture. However, some individuals report poor success in use of tail transection and cannulation of the jugular and anterior vena cava. As with other rodent species, a general recommendation is to withdraw no more than 10% of an animal's total blood volume within a short period of time (e.g., every 2 weeks) without the administration of replacement fluids.

The method of blood collection to be selected is highly influenced by the volume of blood required. Distal tail transection can be repeatedly employed to acquire microliter volumes of blood from a single animal. However, the authors have had great difficulty reliably collecting blood with this technique.

Greater volumes of blood (approximately 1–2 ml) can be obtained from anesthetized animals through puncture of the infra-orbital sinus with a glass pipette or surgical exposure and cannulation of the jugular vein. To access the jugular vein, the ventral neck region is surgically prepared and an approximate 1-cm incision is made over and perpendicular to the jugular furrow of the neck. Tissues are then bluntly dissected, exposing the jugular vein from which blood can be drawn through a needle into a syringe. However, the vein can be fragile and may easily collapse when blood is aspirated. The surgical site is then closed with use of sutures or skin clamps.

Similarly large volumes of blood (approximately 1–2 ml) can be collected from the anterior vena cava of anesthetized individuals. Isoflurane anesthesia may be initiated in an induction chamber and then maintained with use of a face mask. Alternatively, injectable anesthetics can be used although the authors have noted difficulty maintaining an adequate plane of anesthesia followed by successful anesthetic recovery in their colony animals administered injectable anesthetics. The degu is placed in dorsal recumbency with its head extended so as to flatten the ventral surface of the neck, the hair shaved over the left and right thoracic inlets, and the skin disinfected. The front leg on the side of the neck selected for blood collection is extended somewhat

laterally. At this point, it may be possible to visualize the pulsation of the vessel through the skin. A $\frac{3}{4}$ ", 22-g needle attached to a 3-cc syringe is introduced through the skin approximately 1 mm cranial to the clavicle and 1 cm lateral to the midline. The needle is advanced approximately 2 mm along a 45° angle relative to the midline and 10–20° from the horizontal plane of the ventral neck. Gentle suction is applied to the syringe as the needle is slowly withdrawn until blood is visualized in the needle at which time the needle is stabilized and the desired volume of blood collected (Rush et al., 2000). This procedure can be performed repeatedly on a single animal although it does require a significant amount of skill and practice on the part of the technician and can occasionally result in significant complications for the animal (e.g., uncontrolled hemorrhage, unintended lung puncture).

When cardiac puncture is performed, it is recommended that running wheels or other enrichment devices that may promote strenuous exercise be removed or made non-functional for approximately 5–7 days thereby facilitating tissue healing.

Urine Collection

Methods of urine collection are identical to those used with mice and rats (e.g., use of a metabolic cage, bladder expression). However it should be noted that degus infrequently urinate when restrained thereby making restraint an unreliable method for urine collection.

COMPOUND ADMINISTRATION

Recommended sites and maximum volumes for subcutaneous, intramuscular, intravenous, intraperitoneal, and intraosseous injections are listed in Table 44.4.

Oral gavage can also be performed with a maximum recommended gavage volume of 3–5 ml (Johnson-Delaney, 2006b). However care must be taken to avoid the timing of the gavage to occur while a significant volume of stomach contents is present as this may result in gastric rupture (Johnson-Delaney, 2006b).

ANESTHESIA AND ANALGESIA

Recommended dosages of anesthetics and analgesics for use in the degu have been published (Johnson-Delaney, 2006a, 2006b; Visticot, 2002;) although it should be noted that many recommendations are based on anecdotal observations, not pharmacodynamic studies.

Both injectable and inhalation anesthesia are frequently used with the degu. Select, published anesthetic regimens are listed in Table 44.5.

Use of inhalant anesthetics delivered through use of a precision vaporizer is often preferred to use of injectable anesthetics due to the fast induction and recovery periods as well as the ease of anesthetic level manipulation associated with inhalant anesthetics. Also, animals have been noted to develop resistance to some injectable anesthetics (e.g., ketamine and xylazine combination). However, use of select other inhalant anesthetics is contraindicated due to induced adverse effects. For instance, animals administered halothane or methoxyflurane gas anesthesia have been reported to develop

acute centrilobular hepatic necrosis resulting in an approximately 50% mortality rate within 4–7 days of the anesthetic episode (Lee, 2004, 2010). Evidence of liver damage has not been observed in animals administered isoflurane that, in the degu, provides a suitable level of anesthesia for multiple surgical manipulations with quick and smooth anesthetic recoveries (Lee, 2004). Use of an alternate or novel inhalant anesthetic should be considered with caution until its use and potential side effects can be evaluated in this species.

Commonly used analgesics and their recommended dosages are listed in Table 44.6.

In addition to their uncommon physiologic response to halothane and methoxyflurane, the degu has been shown to be unique in its response to morphine administration. The degu has an extremely high tolerance to the pharmacological effects of morphine when compared to that of the Wistar rat and guinea pig (Gaule et al., 1990; Letelier et al., 1985; Villanueva et al., 1980). Its increased ability to metabolize the drug is at least partially due to the high activity level of select hepatic enzymes and possibly due to a unique enzymatic system of drug metabolism (Gaule et al., 1990). The degu's high metabolism of morphine may extend to other drugs utilizing the same or similar hepatic enzyme systems (Letelier et al., 1985). Due to the degu's documented resistance to morphine, it has been proposed as an in vivo model for the study of opiate action and receptors (Paeile et al., 1984). Unlike the closely related guinea pig, the degu does not develop ileal stasis following morphine administration (Pelissier et al., 1989b). In addition, a lower degree of respiratory depression (Paeile et al., 1984), hypotension, bradycardia, and

TABLE 44.4 Recommended Injection Sites and Volumes in the Adult Degu

Injection Route	Injection Site(s)	Recommended Volume
Subcutaneous	Supra/intrascapular	5–10 ml/site
	Dorsal thorax	
	Flank	
Intramuscular	Anterior thigh	0.5 ml
Intraperitoneal	Caudal abdomen	5–8 ml
Intraosseous		1–2 ml bolus
Intravenous and intracardiac	Saphenous vein	1–2 ml bolus
	Cephalic vein	
	Cardiac ventricle (general anesthesia required)	

Table adapted from Johnson-Delaney, 2006b; and Lee, 2010.

TABLE 44.5 Reported Anesthetic Drug Dosages in the Degu

Drug	Dose and Route	Comments	Reference
Ketamine	44 mg/kg IP		(Johnson, 2002)
Ketamine + acepromazine	40 mg/kg + 0.5 mg/kg IP		(Johnson, 2002)
Ketamine + diazepam	40–50 mg/kg + 0.8–1.0 mg/kg IM	Do not give to gravid females as it may cause spontaneous abortion	(Mabry et al., 1988)
Ketamine + medetomidine with supplemental isoflurane administered via face mask	20 mg/kg + 70 µg/kg IM		(Capello, 2005)
Ketamine + xylazine	30 mg/kg + 5 mg/kg IP	May produce inconsistent levels of anesthesia and prolonged recoveries	(Lee, 2004; Rush, 2010)
Isoflurane administered through a precision vaporizer	To effect		(Lee, 2004)
Isoflurane administered in an induction chamber	300 µl isoflurane in a 500 ml volume induction container	For short-term anesthesia (less than 1 minute); isoflurane must not directly contact the animal	Routinely used at the University of Michigan
Pentobarbital	30 mg/kg IV; 35–40 mg/kg IP		(Johnson, 2002)

IP, intraperitoneal; IV, intravenous; SC, subcutaneous.

TABLE 44.6 Recommended Dosages of Select Analgesics, Sedatives, and Tranquilizers

	Drug	Dose, Route, and Frequency
Analgesics	Buprenorphine	0.05 mg/kg SC q 8–12 hours
	Butorphanol	0.4–2.0 mg/kg SC q 8–12h
	Carprofen	1–4 mg/kg SC q 24h
	Meloxicam	0.1–0.2 mg/kg PO or SC q 24h
	Meperidine	10–20 mg/kg SC q 2–3h
	Morphine	2–5 mg/kg SC q 4h
	Naloxone (opioid antagonist)	0.05 mg/kg SC or IP once
	Oxymorphone	0.2–0.5 mg/kg q 6–12h
Sedatives and tranquilizers	Butorphanol	1–5 mg/kg SC q 4h
	Diazepam	1–5 mg/kg IM, IP, IV
	Medetomidine (Atipamazole for reversal)	0.1 mg/kg SC (1 mg/kg SC, IP, IV)

Table adapted from Johnson-Delaney, 2006b.

IP, intraperitoneal; IV, intravenous; SC, subcutaneous.

histamine release occurs in the degu than in the Wistar rat following morphine administration (Pelissier et al., 1989a, 1989b). It has been postulated that many of the differences observed between the degu and Wistar rat are secondary to the activity of the degu's adrenal gland that is proportionally larger (Pelissier et al., 1989a).

EUTHANASIA AND NECROPSY

As in other small mammals, common methods of euthanasia include an overdose of inhaled carbon dioxide or other gas anesthetics and intravenous or intracardiac injection of a high dose of an anesthetic (e.g., pentobarbital). To eliminate associated pain and stress, intracardiac injections and cervical dislocation should be performed only in a fully anesthetized animal.

DISEASES

Non-Infectious Disease

Limited data exist regarding naturally occurring disease in captive-bred populations while more clinical information is available for wild-caught animals either studied in the field or captured and then maintained in a laboratory setting. Given this, it can be difficult to fully understand the level of influence that an animal's

environment and level of nutrition may have on its health status.

However, one association has been clear: the influence of diet on the development of diabetes mellitus and cataract formation in captive-held colonies (Donnelly and Quimby, 2002). Spontaneous development of diabetes mellitus is not uncommon (Fox and Murphy, 1979). However the rate of diabetes mellitus and cataract development can be elevated through provision of diets high in unstructured carbohydrates. The normal blood glucose level of an adult degu is approximately 120 mg/dl or less. Degus will develop cataracts with blood glucose levels of 150 mg/dl, a level lower than that required to induce cataract formation in other species including the rat (Tripathi et al., 1991). Degus may not develop clinical symptoms of diabetes mellitus at this blood glucose level. The most common histopathologic lesions noted in captive diabetic degus include cataracts, fatty metamorphosis of hepatocytes, amyloid deposits in the pancreas, and hyperplasia of islets of Langerhans with an increase in both the number and size of the islets (Murphy et al., 1980). In the University of Michigan degu colony, elimination of fruit and sunflower seed supplements from the diet and use of an exclusive low-fiber, high-energy rodent chow (Prolab® RMH 2000, PMI International, St Louis, MO) reduced the incidence of diabetes and cataracts to near zero (Rush, 2010).

The increased propensity of the degu to form cataracts is due to the presence of high aldose reductase activity in their lens. Aldose reductase converts glucose or galactose to polyol compounds that then accumulate in lens fibers causing them to swell and rupture ultimately resulting in cataract formation (Datiles and Fukui, 1989). The degu has a higher aldose reductase activity in the lens than the gerbil, rat, and human (Datiles and Fukui, 1989). Inhibition of aldose reductase activity delays or prevents cataract development. Histopathic observation of degu cataract development has been described (Worgul and Rothstein, 1975).

In addition to the association with the development of cataracts and diabetes mellitus, the provision of an inadequate or unbalanced diet has been associated with other conditions. Diets high in non-structural carbohydrates (e.g., sugar and starches) may negatively affect reproductive performance (Brown and Donnelly, 2001), however it is unclear what influence associated cataract development may play in this process. In addition, consumption of high-carbohydrate diets may adversely affect the microbial balance of the hind gut and has been associated with the development of obesity (Edwards, 2009).

It has been reported that a change in the color of degu incisors from their normal yellow/orange to white can indicate an underlying nutritional imbalance (Najecki and Tate, 1999; Vanderlip and Earle-Bridges, 2001) although the responsible biologic process is unclear.

Dental disease including malocclusions, caries, elodontomas, and malocclusions are frequently reported in some pet populations (Jekl et al., 2006, 2008).

Kidney disease including nephrosis, pyelonephritis, glomerulonephritis, polycystic kidney disease, and renal failure have been observed (Cadillac et al., 2003) although they are not commonly cited in the literature.

Dystocia and sudden death during pregnancy have been observed infrequently by the authors in captive-bred populations. When they occur, these complications are most frequently noted in primiparous females with a small number of unusually large pups. Histopathologic liver lesions consistent with pregnancy toxemia have been reported in females with sudden death during late gestation (Murphy et al., 1980).

Traumatic lesions have also been reported including one case of a tibial fracture in a 3-month-old degu that was successfully repaired through medullary fixation (Beregi et al., 1994).

Bacterial and Viral Disease

Few bacterial or viral diseases have been reported in laboratory degus and remarkably little is known regarding the pathogenesis of the diseases in their animal hosts. Significant mortality has been associated with *Pseudomonas aeruginosa* septicemia in multiple colonies. Partially as a result, laboratories may choose to acidify drinking water for all or select life stages (Lee, 2004; Mabry et al., 1988). Evidence of a reovirus had been detected in at least one colony and was associated with increased mortality around the period of weaning (Rush, 2010). Similarly, a cytomegalovirus has been found in association with insulinitis and diabetes mellitus in a colony of degu. In this colony, cytomegalovirus inclusion bodies were identified in the pancreas and/or salivary glands of both animals with and without clinical signs of diabetes (Fox and Murphy, 1979). The biological significance of both the reo and cytomegalovirus is presently unclear.

Parasitic Disease

A survey of wild-caught degus from Chile revealed infection with multiple species of helminthes (e.g., *Aprostotandrya octodonensis*, *Graphidioides taglei*, *Longistriata degusi*, *Physaloptera* spp., *Trichuris bradleyi*, *Octodontoxys gigantean*, and *Heteroxynema chilensis*) and one arthropod (*Linguatula serrata*) (Babero and Cattán, 1975). Although these were wild-caught and therefore likely exposed to organisms not normally present in a laboratory setting, it does demonstrate that degus can be parasitized by these species and may be present in animals recently introduced into the lab. Parasites of known or suspected zoonotic importance

have been identified in degus and include *Physaloptera* spp., *Trichuris bradleyi*, *Echinococcus granulosus*, *Echinococcus* sp., *Trypanosoma cruzi*, *Linguatula serrata*, and *Giardia* sp. (Babero and Cattán, 1975; Campos et al., 2007; Najecki and Tate, 1999; Visticot, 2002). In one colony, *Giardia* infections have been diagnosed with a fecal ELISA. Animals from this infected colony with clinical signs of diarrhea were successfully treated with a 100mg/ml solution of fenbendazole at a dose or 25mg/kg PO once a day for 5 consecutive days (Najecki and Tate, 1999).

Neoplasia

The prevalence of neoplasia in degu populations is believed to be low. In one study of 189, 2–60-month-old degus who had died or were euthanized in a colony, seven animals with neoplasias were identified on histopathologic exam. All but one case was found in aged animals and four neoplasias were benign (Murphy et al., 1980). Tumor types reported in the degu include renal transitional cell carcinoma with concurrent choristoma (Lester et al., 2005), primary bronchioloalveolar carcinoma with renal and hepatic metastases (Anderson et al., 1990), parathyroid adenocarcinoma with metastasis and pulmonary adenocarcinoma (Smith et al., 2000), reticulum cell sarcoma of a cervical lymph node, hepatocellular carcinoma with pulmonary and renal metastases, hepatoma, splenic hemangioma, and mesenteric lipoma (Murphy et al., 1980).

THE DEGU AS AN EXPERIMENTAL MODEL

Introduction

When the degu is chosen as an animal model, it is often chosen due to one or more of its following characteristics (Reynolds and Wright, 1979; Weir, 1974):

- individuals are diurnal, tending to be more active in the day and less active at night;
- the young are precocious at birth with open and functioning eyes and ears;
- postnatal development is slow with late weaning and sexual maturity when compared with laboratory mice and rats;
- degu display complex familial and social structure including stable family groups and use of a highly developed vocal repertoire;
- minimal inbreeding has occurred and genetic diversity has been maintained as they have only recently been maintained in laboratory settings;
- individuals are relatively long-lived when compared to many other laboratory rodents.

Circadian Rhythm

As a diurnal species with slow postnatal development and a long lifespan, the degu has been used extensively in the study of human circadian function and rhythm (Hummer et al., 2007; Labyak and Lee, 1995; Lee et al., 2004; Popovic et al., 2009). Degus and humans have similar circadian activity and temperature rhythms, including interindividual and sexually dimorphic variations in rhythmicity. In the degu, sexual dimorphisms develop in the presence of pubertal hormones and the sex differences do not appear until after gonadal puberty is complete (Lee, 2004).

Degu have grown in popularity as a mammalian animal model for the study of behaviors and physiology that may differ in a diurnal niche from nocturnal or crepuscular activity habits (Lee, 2004; Smale et al., 2003). These studies have included examining the sleep patterns of degus (Kas and Edgar, 1998, 1999; Perryman et al., 2006, 2007), impact of social interactions, stress and hormones on recovery from phase shifts of the light:dark cycle, also known as jet-lag (Goel and Lee, 1995, 1996, 1997; Goel et al., 1998; Governale and Lee, 2001; Hagenauer and Lee, 2008; Jechura et al., 2000, 2003, 2006b; Mohawk and Lee, 2005; Mohawk et al., 2005), and the mechanism by which light entrains diurnal patterns (Kas and Edgar, 2000; Labyak and Lee, 1997a; Labyak et al., 1997b; Lee and Labyak, 1997). In addition, they have been useful in demonstrating that adolescent changes in circadian rhythms in humans are not unique, but also occur in other mammals (Hummer et al., 2007; Jechura et al., 2006a; Lee, 2004).

Developmental Biology

Due to many of their reproductive and behavioral characteristics, both those similar and dissimilar to the human, the degu is used as a model for the study of many areas within the field of developmental biology and neurobiology. With a relatively long gestation as compared to mice and rats, the degu has been used in the study of embryonic development (Rojas et al., 1982). They are also frequently used in studies of early behavioral and neurologic development due to their precocial state at birth that facilitates the identification and observation of voluntary behaviors that are not displayed in altricial species of similar ages (Helmeke et al., 2001; Poeggel and Braun, 1996; Reynolds and Wright, 1979). Degus have been used to study the effects of disrupted social interactions early in life on behavior and brain development (Braun et al., 2000, 2009; Ebensperger et al., 2007; Helmeke et al., 2009; Jezierski et al., 2006; Ovtcharoff and Braun, 2001; Poeggel et al., 2003; Seidel et al., 2008). Degus display species-specific and possibly individual-specific vocalizations within the range

of human hearing. This characteristic makes them especially valuable in the study of developmental neurobiology of the auditory system and auditory learning (Braun and Scheich, 1997; Poeggel and Braun, 1996). Degus are also used to study cellular and molecular mechanisms of cognition and can be trained to use a tool (Okanoya et al., 2008).

Reproduction

Placental Development

As in the human, the degu develops a hemochorial placenta with a single layer of syncytial trophoblast separating fetal and maternal blood supply. Unlike in the human, degus have labyrinthine placentation with separate circulatory units supplying each lobe of the placenta (Mess, 2007a). However, due to their simply arranged placental architecture, the degu is useful in the study of placental extension and growth processes and has the potential to serve as a model of placental development in human pregnancy (Mess, 2007a, 2007b) and human placental pathologies (Bosco and Buffet, 2008).

Male Reproduction

The degu has been used as a model of male aging and testicular function (Bustos-Obregon and Ramirez, 1997) including the influence of temperature on spermatogenesis (Bedford et al., 1982). Like the human and sheep, the male germinal line develops to the spermatogonium stage while the degu is still a fetus (Mabry et al., 1988). The degu is preferred by some in place of many more traditional laboratory rodents due to its genetic diversity (Bustos-Obregon and Ramirez, 1997). Degus have also been used as a model when evaluating potential male contraceptives (Moyad, 1987).

Thymic Research

Degus possess select anatomic and physiologic characteristics that make them convenient or unique models for a wide range of research areas. For instance, the presence of two anatomically separate thymus glands (cervical and mediastinal) facilitates their use in thymic research (Science News, 1974). The cervical thymus can easily be removed without complication or negative long-term effects (Science News, 1974). While the mediastinal thymus regresses as the animal ages as is common in many other species, the cervical thymus retains its size and structure throughout the animal's life (Phillips and Boraker, 1975; Woods and Boraker, 1975).

Diabetes

The degu is used as a model for diet-induced diabetes (Spear et al., 1984) partially due to the fact that the

degu, as well as multiple other hystricomorphs (e.g., the guinea pig), possess a unique insulin structure when compared to that of other mammals (Nishi and Steiner, 1990; Opazo et al., 2004). Like humans, they may develop islet amyloidosis (Nishi and Steiner, 1990). The oral glucose tolerance test had been applied to use in the degu (Opazo et al., 2004).

Cataract Development and Prevention

Cataracts develop more consistently and more quickly in the degu than in the rat with most degus developing nuclear opacity within 10–12 days and mature cataracts within 4 weeks following diabetes induction with streptozotocin administration (Datiles and Fukui, 1989; Varma et al., 1977). Cataract development can be noted in 1-day-old offspring of adults with cataracts (Tripathi et al., 1991).

Administration of a potent aldose reductase inhibitor following diabetic induction prevented all ophthalmologic and histopathologic signs of cataract formation while administration of a less potent aldose reductase inhibitor, quercitrin, delayed but did not prevent cataract formation (Datiles and Fukui, 1989; Varma et al., 1977). Due to the role of aldose reductase on the cataract development, degus may serve as a model for evaluating aldose reductase inhibitors for use in human diabetic patients (Varma et al., 1977).

Alzheimer's Disease

The neuropathologic markers associated with Alzheimer's disease have not been identified in wild-type mice or rats. Therefore most previous in vivo models of Alzheimer's disease have necessitated either the creation of transgenic animals or intracerebral injections of amyloid aggregates into the animal host. Degus are being explored as a wild-type rodent model for the neurodegenerative processes associated with Alzheimer's disease as aged (but not young) animals have been shown to develop neuropathological signs of Alzheimer's disease including the presence of amyloid aggregates in multiple regions of the brain and acetylcholinesterase-rich neurons in the cerebral cortex (Inestrosa et al., 2005). The onset of disease is influenced by the source of the animals with the disease onset occurring later in laboratory-maintained than wild-caught animals (van Groen et al., 2009).

Production of Antisera

Degu have been used as a species for preparation of antisera as they have been shown to exhibit a stronger immunologic response to select viruses than is seen in guinea pigs (Phillips and Boraker, 1975).

Atherosclerosis

The degu has been reported as a potential model for atherosclerosis research, as animals fed a high-cholesterol diet developed extensive atherosclerosis with lesions occurring throughout the aorta (Homan et al., 2010).

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Naked Mole Rat

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Naked mole rats are mouse-sized rodents that in recent years have become an important animal model in biomedical research in addition to their unique mammalian role in behavioral and ecophysiological research of life underground.

HISTORY

Naked mole rats (*Heterocephalus glaber*) were first described by Ruppell in 1842 and for many years his taxonomic description was refuted and they were

considered to be either neonates of a much larger species or mammals that had lost their fur as a consequence of disease. Subsequent collections and descriptions were reported in the 1890s through 1957 and are summarized by Brett (1986). Most of those studies focused upon the unusual anatomy of this species and morphological adaptations to life underground. Jennifer Jarvis began studying these animals in the late 1960s and was the first person to bring these hairless rodents into the laboratory. She observed that these animals were highly social and appeared to work together to excavate tunnels and find food. She also noted that she seldom found pregnant animals in the wild and even in captivity had little success with the formation and maintenance of breeding colonies. Subsequently, a group of biologists primarily working with Jarvis (Brett, Buffenstein, Clarke, Faulkes) began studying and reporting on their unique biology. In the mid 1970s, Alexander, an evolutionary biologist whose research focused on the evolution of eusociality (a colonial lifestyle with a strict division of labor culminating in the presence of a single breeding female), described the ideal hypothetical eusocial mammal. At that time no eusocial mammals had yet been identified and only the wasps, ants, and bees were known to be eusocial. He described his hypothetical eusocial mammal as strictly subterranean, living in hard soils and cooperatively foraging on large underground tubers. A colleague and friend of Jarvis, TL Vaughan, told Alexander that he had aptly described the naked mole rat, rodents that Vaughan had seen in the office of Jarvis. Alexander, his doctoral student Sherman, and Jarvis thereafter began an extensive collaborative study on eusociality and behavior in the naked mole rat.

Biologists Bennett, Clarke, Faulkes, O'Riain, and Sherman have contributed greatly to the understanding of naked mole rat behavior. Buffenstein studied with Jarvis and accompanied her on her trip to Kenya in 1980 to assess if naked mole rats in the wild also live in colonies with only one breeding female. Jarvis published this seminal finding in *Science* in 1981 (Jarvis, 1981). Her team published extensively on ecophysiological responses which allow naked mole rats to survive and thrive in their dark, dank environment in the arid and semiarid regions of northeast Africa. These responses include mineral metabolism in the absence of sunlight (Buffenstein et al., 1994), kidney function (Urison and Buffenstein, 1994), thermoregulation (Buffenstein and Yahav, 1991; Withers and Jarvis, 1980), gastrointestinal function (Yahav and Buffenstein, 1991), microecological aspects of the naked mole rat (Bennett and Jarvis, 1995), and finally the biological characteristics that contribute to the exceptional longevity of this, the longest-lived rodent known. Naked mole rats have been documented to live longer than 30 years in the lab (Buffenstein et al., 2008). More recently, neurophysiologists (Catania, Comer, Crish,

Goldman, and Park) have analyzed the nervous system of these animals and have identified many unique neurological features that allow these animals to thrive in their environment. One of the interesting aspects of the naked mole rat nervous system is their lack of ability to sense chemical pain (capsaicin and acid) and inflammatory pain (Park et al., 2008), although they respond normally to acute pinch and heat (Kanue and Hole, 1990; Kanui et al., 1993; Park et al., 2008; Towett et al., 1993).

TAXONOMY

Naked mole rats belong to the order Rodentia in that they have two incisor teeth on the upper and lower arcade that continuously grow. They are in the family Bathyergidae which includes six extant genera (Kock et al., 2006), and includes solitary (Bathyergus; Georchus; Heliophobius), and social taxa (Cryptomys, Fukomys and Heterocephalus). The Suborder is difficult to classify since the criteria for classification are the origination of the masseter muscle and the degree to which the muscle passes through the infraorbital foramen. The Bathyergidae have a small infraorbital foramen and the masseter muscle does not enter into it. Nonetheless, the lateral angle of the jaw relative to the plane of the incisor teeth appears more hystricomorph-like than Myomorph or Sciurormorph. In addition, the Bathyergidae share several Hystricognath features which include similar fetal membranes and sacculus rethralis, a fused malleus and incus, an anterior opening in the pterygoid foss, multiseriate incisor enamel, the loss of the internal carotid artery, and albumin immunology (Honeycutt et al., 1991). Taxonomists thus classify the Bathyergidae in the suborder Hystricognathi. This suborder consists of three super families (the Phiomorpha (e.g. mole rats, rock rats, and cane rats), Caviomorpha (e.g., guinea pigs), and Hystricidae (e.g. porcupines). Interestingly, most hystricognath rodents are found in the western hemisphere, and there are no close relationships between members of the Bathyergidae family and other hystricomorph families.

Molecular genetic data suggest that the naked mole rat is a sister lineage to the group comprising all other genera in the family. *Heterocephalus* forms a monotypic genus with no morphological variation nor variation in chromosome number ($n = 60$). Earliest fossil records of this species were found in East Africa and date back to the early Miocene ~26 million years ago.

GENERAL DESCRIPTION

Good descriptions are available in the literature (Jarvis et al., 1991; Lacey and Sherman, 1991). Naked mole rats are cylindrical-shaped with short limbs and a dorsally



FIGURE 45.1 Photograph of typical naked mole rats. Their elongate tubular shape allows them to move through tunnels. Teeth are exterior to the mouth, and the ears and the eyes are tiny with thickened eyelids and minute eyelashes. The auditory meatus is raised slightly and pinnae are absent. The masseter muscles are prominent.

arched back over lumbar and sacral regions (Hamilton, 1928) (Figure 45.1). The head is cone-shaped and blunted anteriorly in the area of a horseshoe-shaped region that surrounds the nares. Procumbent incisor teeth extend well outside the mouth and are characteristic of the Bathyergidae. Naked mole rats have either two or three molar teeth. The eyes are tiny with thickened eyelids and minute eyelashes. The auditory meatus is raised slightly and pinnae are absent. The masseter muscles are prominent.

The skin is loose, wrinkled, and brownish pink in color. The body is for the most part absent of hairs with the exception of tactile hairs that are regularly arranged throughout the body and which are particularly prominent around the face and to a lesser extent on the tail. The outer edges of the hind feet are fringed with stiff hairs that increase the surface area of the foot. There are five toes on each foot. The tail is about half the length of the body.

Naked mole rats are sexually monomorphic; the external genitalia of males and females appear very similar. Males have internal testes and most females have unperforated vaginas. Males and females have similar-looking external genitalia, ano-genital distance, perineal muscles, perineal motoneurons, brain, and spinal cord (Holmes et al., 2007; Lacey and Sherman, 1991; Peroulakis et al., 2002; Seney et al., 2009) as well as femoral bone morphology and microarchitecture and strength (Pinto et al., 2009). However, these traits differ among breeders and subordinates. There is no sex difference in adult body weight.

Animals raised in captivity have body weights that vary with age, food availability, colony composition, and social and reproductive status (Jarvis, 1985; Jarvis et al., 1991; Lacey and Sherman, 1991; O'Riain and Jarvis, 1998; O'Riain et al., 2000). Most mature animals range from 25–50 grams in captivity but can get as large as 110 grams. This large range in adult body mass is uncommon among small mammals (Jarvis, 1991).

Naked mole rats are one of the slowest-growing mammals and reach stable body mass by the end of the first year of age. Individuals born in the first few litters of a newly established breeding female grow more rapidly than those of later litters (O'Riain and Jarvis, 1998). By 2 years of age all non-breeding animals have stopped growing. Should a dominant animal die or be removed from the colony many individuals will have a growth surge, and this is most pronounced if the breeding female dies.

Any animal within the colony is capable of becoming a breeder and individuals as young as 6 months or as old as 24 years have changed their social status. Should a female change her social status in the colony and become a breeder; she is capable of significant vertebral growth regardless of her age. Indeed a female that became a breeder for the first time at 24 years of age still underwent a prolonged period of lumbar vertebral growth.

NATURAL HABITAT

The members of the Bathyergidae family are all African rodents and are found in distinct geographic locations from the southern-most tip of the continent to about 10°N of the equator. Bathyergids are found in several distinct climatic zones, at different altitudes and in a wide variety of soil types. Naked mole rats are found in the arid and semi-arid regions of eastern equatorial Africa, commonly found in Kenya, Ethiopia, and Somalia. Their habitat gets irregular rain, but it is generally hot and dry with little seasonal or daily variation in climatic conditions, followed by intense short periods of high rainfall. The soil in which they burrow varies from sandy to hard clay soils. The only evidence of the presence of naked mole rats below the surface is small volcano-like mounds of soil excavated by the mole rats during burrow extensions.

Naked mole rats live in an extensive maze of underground tunnels and chambers. Depending upon the size of the colony these may extend more than 1 mile (1.6 km) in length and may reach depths of up to 2 meters. There are multiple layers of interconnected tunnels and chambers. Some tunnels are more permanent (highways) while others near the periphery and surface are opened and closed depending on the needs of the colony. The more superficial tunnels are used for gathering food from tuberous plants, and may be used during behavioral thermoregulation by animals basking in warmer regions of the burrow system. These near-surface tunnels also serve as a path to eliminate soil from new burrows. The deepest tunnels generally open into football-sized nest chambers in which most of the mole rats will huddle together, often resting with many animals piled up on top of each other. The nest area is lined with tufts of grass roots, skins of tubers and bulbs, and other soft detritus encountered in

the burrow system. Often near the nests are deep, blind-ending tunnels that are presumed to be bolt holes and which may possibly serve as drainage systems during periods of heavy rainfall. Interconnecting tunnels that run at a slant are thought to play an important role in ventilation of the colony. Adjacent to the nest chambers in the deeper confines of the burrow system is a blind-ending toilet chamber in which naked mole rats generally urinate and defecate. When these become full, the chamber is plugged with soil and a new toilet chamber excavated. Similarly food hoards or pantry-like chambers are located near the nest; here small bulbs, corms, and roots that have been located during foraging are carried back to the nest and stored or shared among the colony.

Mole rats maintain their burrows all year round, but predominantly extend their burrow system shortly after rains occur when the dry sun-baked soils are moistened and no longer hard and impenetrable.

A sealed subterranean burrow complex protects the inhabitants from predation and also protects against climatic extremes. While surface temperatures above ground may become unbearably hot, the thermal conductivity properties of soil result in attenuated fluctuations in burrow temperatures such that burrow temperatures in the warm confines of equatorial Africa vary by less than 1°C seasonally (Bennett et al., 1988). While superficial burrows may heat up during the day and remain considerably warmer than deeper burrows, burrows at depths greater than 10 cm are thermally stable.

The gaseous atmosphere in the burrow system, and in particular in the deep nests, is extremely challenging (Bennett and Faulkes, 2000). The burrows are usually sealed and gas exchange through soil is limited. This coupled with so many organisms respirating (naked mole rats, commensals such as insects and lizards and microfauna) result in an hypoxic (10–15% oxygen) and hypercapnic (~5% CO₂) atmosphere almost saturated with water, even when soils are dry. Air movement through the burrow is extremely limited and primarily facilitated by animals in tight burrows acting like pistons pushing air in front of them as they move through tunnels. Respiration in such atmospheres, especially in animals that are metabolically active or resting in large communal groups may be especially problematic. Furthermore the impact of high levels of ammonia or methane in nests and latrines could exacerbate respiration and acid-base balance.

BEHAVIOR

Naked mole rats are eusocial, strictly subterranean mammals in the wild. The term eusocial describes species that live in colonies of overlapping litters in which

one or a few individuals produce all the offspring and the rest serve as functionally sterile helpers in colony maintenance, rearing juveniles and protecting the colony (Michener, 1969). The only other mammal that meets these criteria of eusociality is the naked mole rat's close cousin the Damaraland mole rat (*Fukomys damarensis*). A typical naked mole rat colony has only one breeding female (queen) and a few (1–4) breeding males. The remainder of the colony is loosely categorized by body size. The larger animals tend to be the first captured in field studies and are thought to be the defenders of the colony. The smaller animals function as food gatherers and burrow diggers. With the exception of the “dispersomorphs,” animals that occasionally leave their natal colony, presumably to outbreed and form new colonies, naked mole rats only come onto the surface if flooded out of a burrow system or if their burrow system has been breached.

Despite their evolution in a formidable dark, dank environment, naked mole rats thrive in a laboratory setting and display many of the social behaviors that make them unique and of considerable interest for biological and biomedical research. In the wild, colonies of naked mole rats may vary from a few animals to an average of 70 animals to as many as 295 or more animals (Brett, 1991). In an institutional setting, colonies may be formed from breeding pairs and despite being housed in much smaller burrow systems may reach comparable sizes to wild colonies. Average colony size in captivity is ~50, with maximum colony size exceeding 120 individuals. Naked mole rats are extremely xenophobic and readily attack and kill conspecifics from foreign colonies. Members of a colony may roll in the excreta within the toilet to confer a colony scent. This enables colony members to identify each other as members of the group or strangers.

Naked mole rats are strictly herbivorous, feeding on roots, bulbs, corms, and tubers which they locate by excavating tunnels until they stumble across a large tuber or a patch of small bulbs. This is an energetically costly and blind process. NMRs dig/forage in working groups. Typically a lead member digs and passes the soil back to other members who move the soil to other areas of the tunnel system or to the surface. This effort has been described as a chain of diggers. Occasionally members will move the soil to the surface and deposit it in volcano-like structures. This is the only time tunnels are open to predation.

Mole rats generally carry back to the nest small foods (roots, bulbs) they encounter and bites of the large tubers they find. The large tubers may weigh as much as 50 kg and tend to be “farmed” in that animals will gnaw at parts of the tuber, carefully removing the often poisonous outer layers and then plug the regions they have consumed with soil, thereby enabling the tuber

to regenerate. Such large tubers may sustain the colony during the dry season when burrow excavations are energetically prohibitive (Lovegrove and Wissel, 1988). Given the high cost associated with random foraging in regions where food is patchily distributed, a single animal may easily starve to death before foods are found. By working in foraging groups; animals are able to search for food in multiple areas at once and the colony increases its chances of finding a patch of food or a tuber that is large enough to sustain the entire colony. New food sources are communicated to the group through an odor trail (Judd and Sherman, 1996) and cheep-like vocalizations.

Naked mole rats have an extensive vocal communicate repertoire. Pepper et al. (1991) described 18 different vocalizations that include alarm calls, food recruitment calls, mating calls, toilet-assembly calls, and vocalizations specific to pups. These vocalizations range from 65 Hz to 12.8 kHz at 60 dB (Hefner and Hefner, 1993), but the majority of noises are low frequency (1–9 kHz) (Judd and Sherman, 1996). Lower-frequency noises propagate better underground and NMRS take advantage of this. The soft chirp is characteristic of individual members of the group and may be important in identifying individual members (Yosida et al., 2007). There are also several distinct alarm calls employed during cooperative predator avoidance and the defense of the colony.

Colony defense is an important behavior in the colony. If a major predator or member of a foreign colony is encountered in the tunnels, naked mole rats produce a pronounced alarm call and an obnoxious odor, and defenders are recruited in mass to attack the offender. Indeed even snakes may be killed by the wall of teeth they encounter, should they not capture the first mole rat they come upon.

Young pups in the colony employ many different kinds of begging behavior and emit various vocalizations to induce an appropriate response from the breeders and older siblings. One of the most common types of these behaviors is the begging for feces from adults. Adults respond by voiding feces which are directly consumed from the anus.

REPRODUCTIVE BEHAVIOR

Captive colonies of naked mole rats normally contain one breeder female, and she can retain this position for many years (Jarvis, 1991). The breeding females may produce more than 1100 offspring during their breeding reign and litter size tends to increase sometime after the breeders become established. On rare occasions there may be an additional female breeding within the colony. The level of dominant female aggression appears to influence whether this occurs and in most cases the

offspring of the second breeder in the colony seldom survive.

Reproductive suppression occurs by the queen's dominance-related behavioral aggressiveness and requires direct contact between the queen and subordinates (Faulkes and Abbott, 1991, 1997; Margulis et al., 1995). The direct contact takes the form of pushing and shoving. Once dominance is established subordinates readily display subordinate behaviors, they allow the breeders to eat first and they generally tend to be walked over when they encounter a breeder in the tunnel. Aggression in a colony occurs mainly between the queen and other challenging females. Often challengers attack and kill the breeding female when she is at her most gravid or during parturition. If the dominant female dies, succession appears to be rapid, but it may take months to years for colonies to stabilize. Succession appears to come from animals that tend to be larger. This is usually the only time when fighting within a colony occurs. Typically, when the queen identifies a colony member to attack the subordinate animal submits and may even be killed.

Captive colonies of naked mole rats have several breeding males. In contrast to females, there is no evidence of breeding competition in males (Clarke and Faulkes, 1999). Both dominant breeding males and females have higher levels of sex steroid hormones than the non-reproductive colony members.

Lordosis and mounting behavior are commonly observed in the queen and breeding male respectively during the periovulatory period as is seen in other rodent species (Goldman et al., 2006). The female is restless during estrus and initiates courtship behavior by thrusting her rear end into the face of the male and lordosing. Mating occurs several times between a 2–24-hour period and often occurs in the tunnels. Anogenital nuzzling in breeders is also observed and occurs at all phases of the estrus cycle. In this behavior the queen and a breeding male lie side-by-side in the naso-anal position. This latter behavior is not dependent on androgen status in the male, and is most commonly observed in the nest. Naso-genital stimulation of the breeding males is thought to play an important role in elevating and maintaining active hypothalamic/pituitary/gonadal axes compared to non-breeders. The latter have low levels of testosterone and extremely low levels of luteinizing hormone and furthermore are unresponsive to administration of exogenous gonadotropin releasing hormone (Faulkes et al., 1991).

Spermatogenesis occurs in both breeding and non-breeding males. Non-breeding males tend to be sterile, producing fewer and predominantly non-motile sperm (Endo et al., 2002; Faulkes et al., 1994). Similarly subordinate females for all intensive purposes appear to be sterile. Endocrine and anatomical studies reveal that these females show no signs of ovarian cyclicity or

ovulation and that their ovaries are in a functionally quiescent state. This state may change should the subordinate female be isolated from the breeding female of her colony.

Since usually only one female breeds in a naked mole rat colony, the majority of animals (males and females) spend their entire lives in a suspended sterile "prepubescent" reproductive state. These subordinates spend their entire lives maintaining the colony and its burrow systems and generally helping raise the young.

A breeding female breeds continuously throughout the year and may have as many as four litters per year. The average litter size is 12, but may be as small as one pup and as large as 29. In captivity, the gestation period ranges from 64–74 days and there is a 6–11-day post partum estrus, such that the inter-birth interval is approximately 80 days. The first sign of pregnancy is a marked increase in body temperature of the breeding female (Urison and Buffenstein, 1994) and during this time the breeding female spends considerable time basking under heat lamps or in the warmer confines of the burrow. The female becomes obviously pregnant at about 40 days gestation, when her weight begins to increase significantly. Similarly in the last trimester of pregnancy the very gravid queen spends more time in the nest or basking under heat lamps, and other females and males in the colony show signs of nipple enlargement and some females even show perforate vaginas. By the end of pregnancy a female may more than double her non-pregnant body mass. Both pregnancy and lactation are energetically costly processes, increasing metabolic rate 1.5–3-fold (Urison and Buffenstein, 1995). The young weigh about 1.0–2.5 g. The queen nurses the pups for 6 weeks, although they start eating solids and begging for and eating feces from older siblings at 14 days. Fecal consumption provides the appropriate microfaunal inoculum necessary to digest fiber.

Naked mole rats have the highest inbreeding coefficient known for wild mammals (Faulkes et al., 1997; Honeycutt et al., 1991) with a mean coefficient of relatedness among colony members estimated at 0.81 (Reeve et al., 1990) and there is considerable evidence that most new colonies form by fission. There is, however, strong evidence that there is an outbreeding dispersomorph caste with a morphologically, physiologically, and behaviorally distinct phenotype (O'Riain et al., 1996). This disperser caste may occasionally promote outbreeding in naked mole rats, especially in those colonies that are not successfully reproducing. These dispersers are unwilling to engage in colony maintenance, are laden with fat, especially in the neck and pelvic regions, exhibit elevated levels of luteinizing hormone, and solicit mating behaviors with non-colony members and as such are readily accepted by foreign colonies. In captivity, these animals are often seen standing on their hind limbs, trying to

escape from the colony and it seems that if there is a colony escapee it is usually a member of this caste.

GENERAL PHYSIOLOGY

Naked mole rats have low metabolic rates, approximately 70% of that of similar-sized rodents (Buffenstein and Yahav, 1991). Outside the confines of their warm, humid equatorial burrows, naked mole rats cannot regulate their body temperature over a broad range of environmental temperatures (12–37°C) and are thus considered poikilotherms (Buffenstein and Yahav, 1991; Hislop and Buffenstein, 1994). Indeed both their metabolic and body temperature profiles at temperatures below 28°C, closely track that expected for a similar-sized lizard. At temperatures above 29°C, they display a typical mammalian endothermic pattern with the highest metabolic rate at the lowest temperature and a decline in metabolic rate, with the lowest rate at temperatures between 31–34°C; temperatures considered the apparent thermoneutral zone (McNab, 1966). The relative increase in metabolic rate from 29–32°C is similar to that observed in mice housed within their thermoneutral zone and in a refrigerator at 4°C. Hence, given their lack of insulatory pelage, naked mole rats have a much narrower window in which they can thermoregulate relative to other mammals. Indeed it is only at subzero temperatures that mice show the similar hypothermic "reptilian" response of a decline in metabolism and body temperature as conditions become colder. At temperatures above 29°C, naked mole rats employ non-shivering thermogenesis (Hislop and Buffenstein, 1994) initiated by catecholaminergic innervation of interscapular brown fat (Daly et al., 1997). Brown fat is also found in the neck, inguinal, axillary, and perirenal areas. This can generate heat and may maintain a constant body temperature if the animals are housed in large groups in a humid environment that prevents evaporative cooling. Naked mole rats naturally have low thyroid hormone levels, in keeping with the warm temperatures they encounter in burrows or under institutional housing conditions. However, they show increased thyroid activity if housed in the cold for prolonged periods, thus conforming to general mammalian cold acclimation trends (Buffenstein et al., 2001).

Although naked mole rats do not enter a daily or seasonal torpor, like many small mammals do, metabolic rate decreases following prolonged food restriction and may be a mechanism to cope with sporadic and restricted food supplies (Goldman et al., 1999).

Circadian locomotor activity was not observed in general for animals monitored while they were in a colony setting (Riccio and Goldman, 2000a). However, nocturnal rhythms were noted in four males in the colony that were thought to be dispersomorphs. For

individually housed naked mole rats, 65% showed robust circadian rhythms in locomotor activity, and three of four naked mole rats showed circadian rhythms of body temperature and metabolic rate (Riccio and Goldman, 2000b).

ANATOMY AND PHYSIOLOGY OF ORGAN SYSTEMS

Although good descriptions (Hill et al., 1955) of naked mole rat anatomy have been published, very little is known about the basic physiology of naked mole rats. The following are some salient features known about the systems biology of the naked mole rat relative to above ground dwellers.

Digestive System

A unique identifying feature of naked mole rats is the large incisor teeth that are external to the mouth. Having the teeth external enables the animals to dig without the earth coming inside the mouth. Naked mole rats have either two or three molar teeth. The mandibles connect at their anterior extent by a flexible mandibular symphysis, allowing the lower incisor teeth to move independently and provide a greater range of movements and dexterity than is typical of most mammals (Catania and Remple, 2002). The incisor teeth appear to be greatly involved in somatosensory perception (Henry et al., 2006). The parotid, submandibular, and sublingual salivary glands are all well developed (Hill et al., 1955). The esophagus of the naked mole rat has keratinized stratified squamous epithelium and smooth muscle surrounding it. The stomach is monogastric. The length of the small intestine is relatively short, and leads into a very large cecum (Rechkemmer et al., 1988). The intestinal mucosa does not have distinct subdivisions based on duodenal, jejunal, or ileal villar length. The cecum accounts for 8% of the body weight (Buffenstein and Yahav, 1991). Despite the fact that members of the Bathyergidae are strictly herbivorous and eat a diet high in fiber, they nevertheless exhibit the highest digestive efficiency of all mammals (Bennett and Jarvis, 1995). This is primarily due to comparatively long retention times in the gut and highly efficient microbial fermentation processes in the cecum (Buffenstein and Yahav, 1991; Porter, 1957) coupled with coprophagy.

The cecum has a mixed microbial population of ciliate and holotrich protozoa, biflagellates, and fungi as well as an assortment of rod- and cone-shaped bacteria (Buffenstein et al., 1994; Porter, 1957) (Figure 45.2). These abundant microorganisms within the hindgut, provide naked mole rats with high-energy compounds in the form of volatile fatty acids (such as acetic and butyric

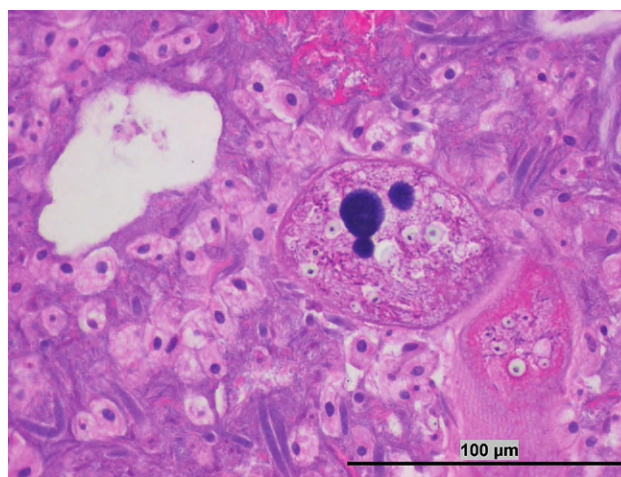


FIGURE 45.2 Cecal contents (H&E): The hindgut fermentation of the naked mole rat in health is associated with protozoa and bacteria. This high-power photomicrograph demonstrates several large protozoal ciliates, likely *Balantidium* spp., as well as several smaller flagellates. Also noted are large fusiform organisms.

acid) that are rapidly absorbed into the bloodstream and provide the host with more than 60% of their basal energy requirements (Parra, 1978). To further maximize energy extracted from the diet, NMRs practice coprophagy which provides a good source of proteins in the form of digested microbes.

Fermentation processes in the gut of the naked mole rat function most efficiently at their low body temperature of 32°C. Surprisingly, because fermentation processes are generally considered to be endothermic, cecal temperature in the mole rat is cooler than core temperature (Yahav and Buffenstein, 1992). The scientific reason for this phenomenon has not yet been explained.

The liver has four principal lobes and a number of minor ones, the main lobes being separated by deep fissures extending in most cases completely throughout the hepatic substance, and a gall bladder is absent (Hill et al., 1955).

Respiratory System

The anatomy and physiology of the respiratory system in subterranean animals is of great interest because their environment can have high levels of carbon dioxide (7–8%) and low levels of oxygen (6%) (Arieli, 1979; Contreras and McNab, 1990; Kennerly, 1964; Nevo, 1999). Naked mole rats have three turbinates (maxillo, ethmo, and sphenoidal), three lung lobes on the right and left sides, and a caudate lobe on the right side (Hill et al., 1955). Histologically, they have a preponderant double pulmonary capillary arrangement, incomplete differentiation of alveolar pneumocytes, and extension of the cuboidal epithelium to the immediate vicinity

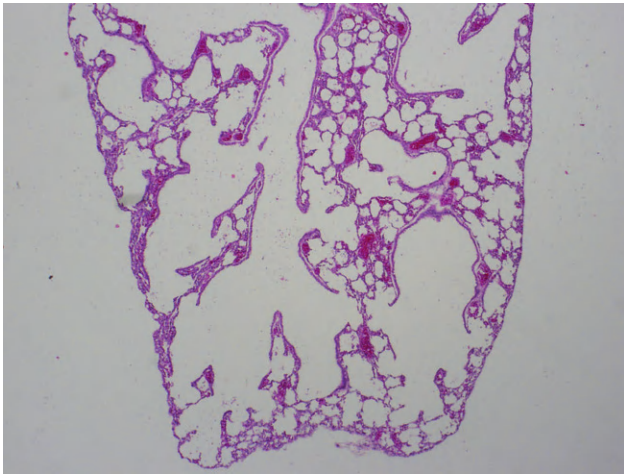


FIGURE 45.3 Lung (H&E) section of diaphragmatic portion of the naked mole rat lung. In contrast to rat and mouse lungs, the terminal bronchiole is ectatic; and alveolar ducts are short or non-existent, allowing alveolar sacs to branch almost directly from the terminal bronchioles. Terminal bronchioles are lined by cuboidal epithelium, the alveoli by flattened type I pneumocytes. Thus the most distal alveolar spaces abut the pleura and connect with the terminal bronchioles. The lungs thus have an “emphysematous” appearance at low magnification. The relation of this design to the low oxygen needs of the naked mole rat is not understood.

of the alveoli (Maina et al., 1992, 2001) (Figure 45.3). Observations between field animals and animals maintained in the lab were similar, suggesting the naked mole rat is intrinsically intractable to environmental shifts in its habitat. Consistent with their overall reduced metabolism, the respiratory rate of naked mole rats is about half the respiratory rate of mice (Blass et al., 2009).

Circulatory System

Subterranean rodents have increased capillary densities in skeletal, cardiac, and pulmonary tissues, as well as increased fractional volumes of mitochondria in tissue (Arieli and Ar, 1981; Widmer et al., 1997). Also, elevated myoglobin levels have been reported in other subterranean species (Ar et al., 1977). In addition, lower resting heart rate and greater heart mass have been reported in ground squirrels and chipmunks (Burlington et al., 1970; Jones and Wang, 1976). In the naked mole rat, the left precaval vein is retained and is larger than the right precaval vein (Hill et al., 1955). However, little else is known about the cardiovascular system of this species. The left ventricular free wall and interventricular septum are thicker in relation to the right ventricular free wall than seen in rats and mice.

Hematopoietic System

Naked mole rats have the typical variety of leukocytes (Johansen et al., 1976) as seen under light

microscopy, but there are no studies that define cells by markers or immunological function. Erythrocytes are similar to those of other mammals. The spleen is in the typical rodent location and is more slender than typical rodent spleens. Extramedullary hematopoiesis is noted in the spleen of young and pregnant animals.

The hemoglobin molecule has higher affinity for oxygen compared to most other mammalian species, and this is viewed as an adaptation for living under chronic low-oxygen conditions in subterranean burrows (Boggs et al., 1984). Naked mole rats exhibit levels similar to those in other mammals for 2,3-diphosphoglycerate in red blood cells (Johansen et al., 1976).

Integumentary System

The skin of the naked mole rat has many features which are well-suited to its warm, subterranean niche. These include the lack of an insulating layer, and a loosely folded morphological arrangement, both contributing to poikilothermic responses to changing temperatures (Daly and Buffenstein, 1998). The presence of pigment-containing cells in the dermis, rather than the epidermis, which is typical of most mammals, also reflects an environment devoid of sunlight. The lack of fur is compensated by a thicker epidermal layer and a marked reduction in sweat glands. In contrast to having fur composed of numerous short hairs, the naked mole rat has an array of sparsely distributed long vibrissa-like hairs (Crish et al., 2003; Daly and Buffenstein, 1998). The follicles of these body hairs are exceptionally large and well-innervated, similar to guard hair of furred species (Park et al., 2003) (Figure 45.4). The role of these sensory hairs is discussed below under somatosensory perception. The arrectores pilorum muscles are lacking, and the sebaceous glands are degenerate (Hill et al., 1955).

Urinary System

Water balance of naked mole rats is dependent on utilizing the water from food sources only, since pools of water are not normally available in a subterranean environment and even in captivity naked mole rats meet all their water requirements through the consumption of foods. The kidneys of the naked mole rat are unipapillate and unlike other arid-dwelling organisms are capable of only a moderate concentrating of urine (1500 mOsm) (Urison and Buffenstein, 1994). Given the high humidity in their burrows in the wild, this may be adequate for both pulmonary and cutaneous evaporative water loss. However, if high humidity is not maintained under captive conditions, evaporative water loss reaches the highest levels recorded for homeotherms (Buffenstein and Yahav, 1991). Not surprisingly, when water stressed under captive conditions, given their low

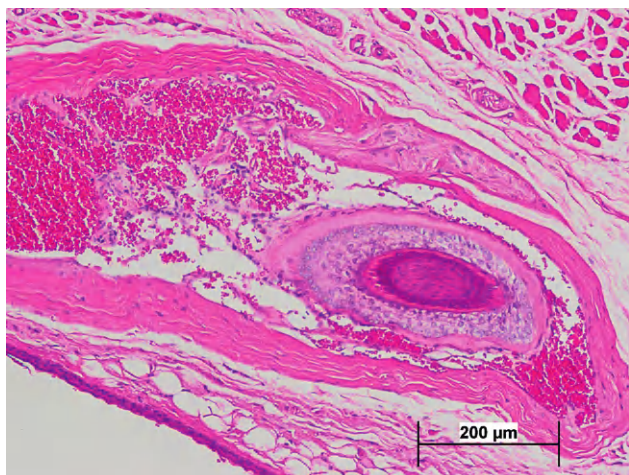


FIGURE 45.4 Photomicrograph of sensory hair (tactile, sinus hair, vibrissa-like hair) (H&E): The most important structure to the naked mole rat sensorium, the tactile hair is found distributed throughout the body surface, with concentration at the face, tail, and feet. These coarse long hairs have follicles that contain prominent vascular sinusoids and nerve endings. Striated muscle can be seen at the upper quadrant.

metabolic rates, metabolic water production is too low to counteract high rates of insensible evaporative water loss and the animals cannot maintain plasma osmolality or body mass.

Reproductive System

Both males and females are able to breed at 6 months of age, should the opportunity arise. The external genitalia show no dramatic difference between the sexes. The males have abdominal testes and there may be a slightly greater ano-genital distance in non-breeding males and females. The only accessory sex gland is the vestibular gland. Non-breeding females have a vaginal closure membrane that is slightly dark in color. Breeding males are generally larger, thinner, and the penis is slightly more raised. Breeding females (queens) are large, elongate (Jarvis, 1991; O’Riain et al., 2000), with a swollen and raised genital area, patent vagina, and large mammary (Sherman, 1999). The uterus is bicornuate, there are no fornices, and the ovarian bursa is absent. The ovary is covered by a greatly expanded infundibulum of the uterine tube that has no fimbriae (Hill et al., 1955). The non-breeding males have hyperplastic testicular interstitial cells, reduced numbers of seminiferous tubules, and a simple epididymus without a tail for capacitation of sperm.

Reproductive suppression of non-breeding female naked mole rats is profound in that the majority of females remain in a pre-pubertal state for their entire lives. Concentrations of urinary and plasma progesterone are consistently low in subordinate naked mole rat females (Faulkes et al., 1990a) yet the pituitaries of

subordinate females are responsive to GnRH (Faulkes et al., 1990b). This suggests that the primary site of reproductive hormone inhibition is at the level of the brain (Holmes et al., 2009). Non-breeding males generally have lower levels of urinary testosterone compared to breeding males, but the difference is not as large as in the females. The rapid rise in urinary progesterone in subordinate females following removal from the colony is probably not an indication of reproductive capacity in that breeding rarely occurs in less than 5–6 months (Holmes et al., 2009).

Other anatomical features associated with reproduction that are sexually monomorphic in naked mole rats include Onuf’s nucleus of the spinal cord, perineal muscles, and brain regions (Holmes et al., 2009).

The induction of ovulation has not been determined in naked mole rats, but the Damaraland mole rats are capable of spontaneous ovulation and the act of coitus may advance the onset of ovulation (Snyman et al., 2006).

Average number of mammary glands in males and females averages 11.6 ± 1.6 (Sherman et al., 1999). Neither total numbers of mammae, nor fluctuation asymmetries in mammary numbers differ significantly between males and females, or between breeders and non-breeders. Also, there is no relationship between litter sizes and numbers of mammae.

Endocrine System

Naked mole rats have an unusual endocrine profile relative to above-ground rodents, although several subterranean-dwelling rodents show similar profiles. As mentioned above, naked mole rats have low levels of sex steroids; however they also have low levels of other steroid hormones.

Living in the dark, it is not surprising that naked mole rats are naturally deficient in the “sunshine hormone”, vitamin D. Levels of 25-hydroxyvitamin D are undetectable and the principal active metabolite is low (Buffenstein et al., 1994). Surprisingly, even when exposed to sunlight for prolonged periods, hormone levels remain low and the enzyme responsible for synthesis of the active metabolite (1-alpha hydroxylase) is rapidly down-regulated. Despite these low levels, naked mole rats are able to tightly regulate serum calcium levels and adequately maintain mineral homeostasis, using passive gastrointestinal absorption and vitamin-D-independent mechanisms to retain calcium, magnesium, and phosphorus within the body (Skinner et al., 1991).

Thyroid hormones and thyroid-stimulating hormones are also maintained at low levels for mammals and may contribute to the low basal metabolic rate of this species (Buffenstein et al., 2001). Thyroid morphology is similar to that of most mammals and the thyroid responds positively to cold stress such that thyroid

follicular cell height and free thyroxine levels were significantly greater in cold-acclimated individuals.

Insulin levels in naked mole rats cannot be detected using standard rodent antibody-based assays, suggesting that naked mole rats either have extremely low levels of insulin or have an insulin molecule with a different protein composition to that of other rodents. Although we have not as yet attempted to sequence naked mole rat insulin, there is considerable evidence that the insulin structure in the hystricognaths (e.g. guinea pigs) is substantially different to that of laboratory mice. Unlike laboratory mice, and in a similar morpholoth, insulin-producing cells are distributed in a mantle around the periphery of the islet, rather than in the more usual location of the central core. Similarly, glucagon-producing cells are not located in the mantle, as previously reported in most vertebrates. Indeed, only horses show a similar pancreatic arrangement. Like most mammals, a greater number of insulin-producing cells than glucagon-producing cells are present, and somatostatin-producing and pancreatic polypeptide-producing cells are considerably more scarce and randomly scattered within the islet (Kramer and Buffenstein, 2004) (Figure 45.5).

Naked mole rats have low fasting blood glucose levels, and show an impaired glucose tolerance test, maintaining elevated glucose levels for more than 2 hours. When given a physiologically determined dose of human insulin, blood glucose rapidly drops to extremely low levels (20mg/dl) and remains there for several hours. The exquisite sensitivity to human insulin coupled with the abnormal glucose tolerance test and undetectable levels of insulin suggest that naked mole

rats naturally only produce small quantities of this hormone and that this is usually sufficient for their needs, given their predominant reliance on volatile fatty acids produced during fermentation for their energy requirements. As such, they are unable to regulate blood glucose when given a supraphysiological dose of sugar.

Little is known about the hormones of the adrenal gland. The right adrenal is medial to the right kidney and the left adrenal is ventro-medial to the left kidney. There are no published data on the adrenal cortical axis in naked mole rats. Mole rats have very low levels of corticosterone compared to mice and rats, but predominantly secrete cortisol. It is not known if these two hormones have identical functions or have differences in their regulatory mechanisms.

Apart from the hormones described above, little if anything is known about other hormone secretions and functions and considerable research is needed to glean a better understanding of the endocrine function of this species.

Nervous System

The naked mole rat brain has the normal anatomy of a typical rodent brain (Xiao et al., 2006). What makes the naked mole rat different is the relative amount of cortex devoted to the different sensory inputs. The somatosensory system occupies one-third of the sensory cortex and appears to be a more important sensory input (Henry et al., 2006).

Unlike the majority of vertebrate species, subordinate and breeding naked mole rats lacked vasopressin innervation of the lateral septum and vasopressin-immunoreactive cells in the nucleus of stria terminalis (Rosen et al., 2007). Instead, the dorsomedial septum contained dense vasopressin-immunoreactive innervation, which did not vary with sex or breeding status. Immunoreactive oxytocin fibers were found in the periaqueductal gray, locus coeruleus, parabrachial nucleus, nucleus of the solitary tract, and nucleus ambiguus (Rosen et al., 2008). The high levels in the nucleus accumbens and preoptic area are associated with maternal behavior in other animals (Francis et al., 2000). Breeders, regardless of sex, had more cells than subordinates in the ventromedial nucleus of the hypothalamus and a larger volume of the bed nucleus of the stria terminalis, paraventricular nucleus, and medial amygdala (Holmes et al., 2007). Non-breeding males and females did not differ on any measure.

Special Senses

Somatosensation

The sensation of touch is an important sense in the naked mole rat. The area of the sensory cortex devoted to the sensation of touch is much larger in comparison

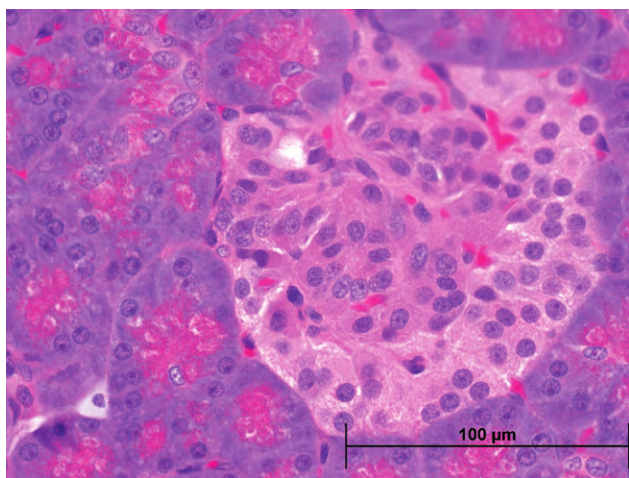


FIGURE 45.5 Photomicrograph of pancreatic islet cells (H&E); The islet distribution of beta and alpha cells which produce insulin and glucagon respectively have a distinct zonal arrangement with the mantle cells of beta cells framing a centrally located mass of glucagon producing cells. Human islets are not organized in this manner, but cells of all types are rather randomly distributed within the islet.

to other mammals (Catania and Remple, 2002; Henry et al., 2006). The queen suppresses reproductive activity via touch by pushing and shoving (Faulkes and Abbott, 1997). Like most mammals, naked mole rats have well-developed facial vibrissae (whiskers). However, they also have a system of vibrissa-like hairs that are regularly spaced in rows over the body, and naked mole rats have a robust and accurate orientating response when these hairs are stimulated (Crish et al., 2003). A large number of vibrissa-like hairs are also present on the tail, consistent with the naked mole rat moving as quickly in reverse as in the forward direction.

Naked mole rats also naturally lack neuropeptides and behaviors associated with signaling of chemical irritants on the skin and nasal cavity (LaVinka et al., 2009; Park et al., 2008) which will be discussed in the Models section.

Olfaction and Gustation

Naked mole rats demonstrate prominent scent-marking behaviors and members of the colony are frequently seen rolling in the toilet areas of housing systems. Yet, reproductive suppression does not appear to be influenced by hormones or pheromones present in the urine from the breeding animals (Smith et al., 1997).

In most vertebrates, chemical (pheromonal) communication involves the vomeronasal organ, a structure within the nasal cavity. For rodents in general, the vomeronasal organ is proportionally large (relative to body size) compared to other mammals (Smith et al., 2005). Yet, the vomeronasal organ in naked mole rats is remarkably small, about ten-fold smaller than vomeronasal organs in social rodents such as the meadow vole and mouse. Also, the naked mole rat is the only known mammal to show no post-natal growth of the vomeronasal organ (Smith et al., 2007). This supports the notion that pheromones and chemical sense do not play a major role in sexual suppression in naked mole rats. With regards to traditional odor sensation involving the olfactory system (apart from the vomeronasal system), naked mole rats perform as well as mice in distinguishing odors in a behavioral laboratory test (LaVinka et al., 2009).

Auditory

Naked mole rats do not have pinnae (important for sound localization), and have narrow external ear canals. The majority of subterranean rodents have ear canals filled with cerumen (Burda et al., 1990). Definitive anatomical descriptions of the auditory system are not available for the naked mole rat with one exception (Hefner and Hefner, 1993). They described the brainstem auditory nuclei as being relatively small but present. Generally though, middle ears are vestigial and the inner ear resembles the guinea pig anatomically more than murid rodents in subterranean rodents (Begal et al., 2007).

In contrast to other rodents, the naked mole rat has hearing and communication calls that are primarily in the low-frequency range (Pepper et al., 1991). This is probably because low-frequency sound propagates well through soil. However, naked mole rats perform poorly in tasks based on sound localization ability (Hefner and Hefner, 1993). This may reflect that these tests are not appropriately designed to match the unusual (tubular) environment and behavior of this species.

Vision

Subterranean rodents live in a light-free environment and as a consequence have very small eyes and reduced or absent visual capacities. The naked mole rat is not an exception. They have a rod-dominated retina and have cones that are S-opsin dominated which is similar to other Bathyergidae and unlike any other mammalian species (Peichl et al., 2004). The eye contains 11-cis retinal, the chromophore of visual pigment. The optic nerve cross-sectional area is 10% of the gerbil and had an ERG response typical of other mammals but extremely attenuated and with much slower kinetics (Hetling et al., 2005). They display a variable degree of retinal detachment which may be associated with the less than gelatinous nature of the vitreous humor (Hetling et al., 2005; Nikitina et al., 2004). The superior colliculus and lateral geniculate nucleus are very small in the naked mole rat (Crish et al., 2006). Collectively these findings show that the retinogeniculocortical system in the naked mole rat is considerably smaller than that of rodents that rely heavily on their visual system, but is nevertheless less regressed than that of the extensively studied blind mole rat; this may facilitate limited responses to visual stimuli (Xiao et al., 2006). Indeed all these findings suggest the naked mole rat eye functions for amorphous light detection that may serve for circadian function and/or for the simple assessment of whether or not the burrow has been breached and requires repair.

Magnetoreception

Magnetoreception is magnetic compass orientation. It has been reported in the African Ansell's mole rat (Burda et al., 1990; Marhold et al., 1997), the blind mole rat *Spalax ehrenbergi* (Middle Eastern mole rat unrelated to the Bathyergidae; Burda et al., 1991), the Siberian hamster (Deutschlander et al., 2003), and the laboratory mouse (Muheim et al., 2006). There are no reports in the naked mole rat.

AGING

Aging, the progressive decline in functional morphology that over time leads to increased mortality risk is markedly abrogated in the naked mole rat. Naked

mole rats are the longest-lived rodents known, living approximately 30 years in captivity; 5.3 times longer than predicted on the basis of body size and nine times longer than similar-sized laboratory mice. In captivity both breeders and non-breeders have similar maximum lifespan, whereas in the wild the more protected breeders live considerably longer (17 years; S. Braude, personal communication) than working, predation-vulnerable subordinates (4 years). Not only do naked mole rats have an extraordinarily long lifespan but they show sustained good health for at least 85% of their maximum longevity and show no age-associated acceleration in mortality risk. From the age of 2 to 20 years naked mole rats maintain lean muscle mass (O'Connor et al., 2002), bone health (Pinto et al., 2009), mitochondrial mass, and enzyme activities (Ungvari et al., 2008). Furthermore they sustain their metabolic rate (O'Connor et al., 2002), level of gastrointestinal absorption (Yang and Buffenstein, 2002), and cardiovascular youthfulness (Csiszar et al., 2007) in addition to their level of activity and reproductive potential well into their third decade (Buffenstein, 2008).

Naked mole rats support the evolutionary theory of aging which posits that species that have evolved in areas of low extrinsic mortality such as thermally buffered, sealed burrows that are protected from predation will evolve mechanisms better-suited for extended tissue maintenance and concomitant longevity; whereas those species living in a dangerous environment fraught with challenging climatic conditions and/or high predation risk will evolve life history traits that facilitate early reproduction and shorter lifespans. Naked mole rats also support the environmentally selected lifespan theory which posits that animals that live where resource availability is unpredictable will exhibit extended longevity. The life history of naked mole rats provides some support for this theory: location of food is a random, blind, and energetically costly process and food resources are patchily distributed. The low metabolic rate of NMRs may help them survive periods of limited resources. Extended longevity, like lower extrinsic mortality, is correlated with group-living, such that cave-roosting bats, social insects, and humans all have long lifespans. The cellular, molecular, and biochemical mechanisms facilitating this extended longevity, good health, and delayed aging are however poorly understood.

Naked mole rats do not support the telomere theory of aging. Telomere length and maintenance will govern the number of times a cell may divide and thereby replace damaged cells. Naked mole rats, like humans, have relatively short telomeres compared with mice and lack telomerase and as such cannot maintain telomeres indefinitely. These will shorten with each replication passage and eventually become too short for further replication events.

Similarly, the naked mole rat only provides equivocal support for the most widely accepted theory of aging, the oxidative stress theory of aging that posits that the characteristic traits of aging are due to accrued damage by unchecked reactive oxygen species (ROS), generated during aerobic metabolism. Naked mole rats produce similar amounts of ROS to those produced by shorter-lived rats and mice (Lambert et al., 2007), do not have a stellar antioxidant defense (Andziak et al., 2006), and accrue more oxidative damage even at a young age than do shorter-lived species (Andziak et al., 2006). However, accrued damage appears to be maintained at steady-state levels throughout their long lives (Andziak and Buffenstein, 2006), suggesting that these animals have a higher tolerance threshold for damage prior to inducing mechanisms to remove and repair accrued oxidation products.

Currently, the mechanisms facilitating prolonged good health and lifespan remain elusive and new studies are focusing on mechanisms that may facilitate protein and genomic stability even in the face of cellular endogenous and environmental insults.

HUSBANDRY

Naked mole rats are popular zoological attractions (Petry, 2003) and are also studied at numerous research institutions (Artwohl et al., 2002). They are typically housed at 28–30°C, and humidity levels at 50–60% relative humidity. If housing temperatures are below 25°C, mole rats will stop breeding (Woodley, 2002). The only way to obtain this environment is to curtail the ventilation into the room significantly. The benefit of turning on the light–dark cycle has not been proven since colony-housed animals have not demonstrated a circadian rhythm. As in most facilities that house breeding rodents, noise should be minimized. Wall-mounted shelves can be used or racks that do not rock and have locking wheels can be used. Some facilities put the rack wheels in sand to reduce vibrations in the floor extending into the rack.

Various bedding material can be provided. All current types of mouse bedding have been used with naked mole rats. Each of these kinds of bedding has different pros and cons. For example, wood shavings from pine and ash may have chemicals or odors that may be irritating, causing scratching of the skin. Unless it is of a fine high quality it can also cause splinters in the feet. The advantage of wood shavings is that the shavings are less dusty and tend not to stick to skin. Vermiculite and corn cob bedding are particularly harsh. Some types of paper bedding produce fine particles of dust that may block noses and irritate eyes of naked mole rats. A highly absorbent material should be

placed in the toilet chamber and paper towel, tissues, and corn husks should be given in addition for nesting. Naked mole rats like to move the bedding material and typically gather materials into the nesting chamber. Occasionally naked mole rats will make a ramp with bedding material that can be used to escape from their enclosure.

No water is generally provided when housing naked mole rats in captivity. Fresh food should thus always be available. Food items such as sweet potato can be soaked in water prior to offering the food. The high humidity in the room will diminish insentient water loss. The staple diet for naked mole rats is sweet potato. Some institutions soak the tuber in 10% bleach or weak acid (lemon/vinegar) for a period of time since fecal organisms can regularly be cultured from them. Sweet potatoes are difficult to disinfect because of their porous nature. Additional fresh foods include apple, grapes, bananas, oranges, melons, squash, cucumbers, lettuce, and peppers. These can be supplemented with frozen beans and corn. Mole rats are particularly fond of melons, however, these water-rich foods lead to frequent urination and the need to clean cages more frequently. A vitamin supplement such as Pronutro (Wadeville, South Africa) is frequently offered.

In a laboratory setting, caging is typically made from caging materials that are readily sanitized in a cage washer. Housing systems consist of single and multiple cages that can be connected via 2-inch-diameter pipe. Sanitizing complex caging that utilizes connecting pipes is more labor-intensive since pipes should be periodically scrubbed. Enrichment for naked mole rats includes offering nesting materials for making a nest, increased cage complexity (Figure 45.6) that would include multiple pipe connections or soils in which to burrow. One strategy is to provide un-husked corn. The animals eat the corn and make a nest out of the husk material. Naked mole rats are relentless chewers and constantly gnaw at the corners of chambers. Blocking tubes with whole corn or yams will stimulate burrowing behavior in the tubes and may possibly reduce repairs to the housing structure. That said, care must also be taken to check for holes and loose tubes or lids to prevent escapes from occurring.

Cages can be spot cleaned daily by removing soiled food and spoiled substrates, particularly in the toilet chamber. The entire system can be cleaned every 2–4 weeks with scent-free mild soaps.

Adult naked mole rats can be easily marked using felt-tipped pens which may last up to 10 days. They do not seem to lick the markings off themselves. Toe clipping and transponders can also be used (Braude and Ciszek, 1998). Subcutaneous implanted transponders however can migrate to different areas of the body and often fall out. Tattoos have also worked in naked mole rats.



FIGURE 45.6 Example of a complex caging system used to house colonies of naked mole rats. Cages are connected by 2-inch vinyl tubes which allow the naked mole rats' functional separations of toilet and nesting areas.

COLONY HEALTH

Neonatal and weanling mortality is common in naked mole rats. Facilities should severely limit traffic and curtail noises in rooms with neonates. Naked mole rats respond to noises and vibrations and can bury or carry neonates as part of their care-taking behavior. Sometimes new litters die simply because the mother does not take notice of the neonates.

Problems associated with the birthing process include dystocia and endometritis. Naked mole rats can have very large litters and uterine fatigue can occur. Oxytocin given at mouse dosages helps this condition. Queens with endometritis typically die a few days after birth. Opportunistic bacteria can be isolated from the uterus.

The queen's aggression is part of the eusocial life of naked mole rats. Aggression is heightened when other females are trying to usurp the queen's control. Fight wounds can be observed when this occurs and are one of the most commonly observed clinical problems. These often result in staphylococcal infections. Wounds should be treated with organic iodines.

Naked mole rats have various non-infectious conditions that occur on occasion. Bloating is not an uncommon sign. It is often associated with weaning and can

be associated with ingestion of less-complex carbohydrate food sources. Usually this sign will abate with time, but occasionally the bloat is debilitating, leading to dehydration and can cause death. Clinical evaluation and treatment are necessary in these cases.

A metastatic calcification condition has been observed (Margulis, personal communication (Figures 45.7 and 45.8). The cause for the condition was probably dietary-related since a higher level of rodent chow was used and naked mole rats typically eat diets low in calcium. Some calcium deposits may be dystrophic since they can be seen in areas of inflammation such as around fight wounds and foot lesions. The foot lesions would be considered painful. The calcium deposits are made of a pasty white material that appears like an abscess, but are often sterile and radio-opaque. Similarly London Zoo reported nephrocalcinosis and mineral deposition in soft tissues in animals fed a calcium-rich diet supplemented with vitamin D.



FIGURE 45.7 Metastatic calcification deposit being expressed. This condition should be considered for any mass in a naked mole rat that appears like an abscess.

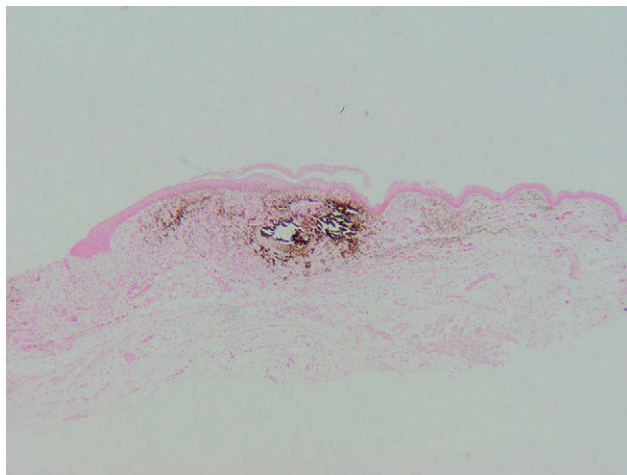


FIGURE 45.8 Photomicrograph (Von Kossa). A focus of cutaneous mineralization which is stained dark.

Few pathogens have been reported in naked mole rats, which is noteworthy considering the preponderance of naked mole rat colonies in zoos and conventional institutional rodent facilities. Zoos are environments that have less control over the spread of pathogens. Naked mole rats seem to be extremely resilient to pathogens and other toxic insults. Perhaps naked mole rats are less susceptible to murine pathogens because of their lower resting body temperature (33°C), or perhaps because naked mole rats are typically housed at higher room temperatures than other rodents, necrosis sets in rapidly when they die such that pathogens and cause of death cannot be easily identified. As such, incidences of pathogenic outbreaks may not be reported. Surveys of zoo pathologists however support the premise that naked mole rats are extremely resilient and resistant to infectious agents.

Ross-Gillespie et al. (2007) described an acute coronavirus epizootic that caused acute diarrhea, dehydration, and severe enteric hemorrhaging that killed 161 of the 365 animals (44.1%). Age (younger animals more susceptible), sex (males were more susceptible), and the degree of inbreeding (inbreeding increased susceptibility) influenced mortality. The source of the coronavirus was not identified.

An acute death syndrome has been noted at multiple institutions and zoos. It is characterized by multiple deaths, sometimes in multiple species (naked mole rats and Damaraland mole rats) in animals that are in good body condition. Sometimes diarrhea is noted. Available tests for clostridial enterotoxin and mycotoxins were negative. Edematous ileum, cecum, and colon were noted histologically. Since naked mole rats have a complex microflora in this region of the intestine, consideration of dysbiosis as described in rabbits or a pathogen that requires special culture conditions should be made for the cause. Naked mole rats occasionally get localized abscesses where opportunistic pathogens such as *Pasteurella*, *Pseudomonas*, or *Staphylococcus* sp. are isolated. *Helicobacter* sp. have not been identified in NMRs.

Small colonies are particularly vulnerable to dehydration and cracked skin in response to low humidity. Give small colonies mini-chambers to increase humidity. If skin is dry add moist cloths and/or supplement nesting with fresh corn husks, lettuce, or fresh (washed) grass. In the past we have rubbed Vaseline intensive care or hypoallergic moisturizer creams onto skin with considerable success. Recently however, a small proportion (up to 10% of a colony) of animals has gone into shock and although we were able to resuscitate them, we have replaced this moisturizing spa treatment with a rub of olive oil. Ring tail has also been observed in naked mole rats (Figure 45.9).

The immune function of naked mole rats has not been described. Consideration for susceptibilities to experimental infections should be considered. Artwohl



FIGURE 45.9 Ringtail in a naked mole rat.

et al. (2009) reported 100% mortality in naked mole rats given a recombinant herpes simplex virus type 1 that was avirulent to mice. The virus was administered via scarification of the foot.

Neoplasia has not been reported in naked mole rats (Buffenstein, 2008).

It is possible but not substantiated adequately by pathology that old naked mole rats may suffer from cardiovascular insults such as aneurysms and possible strokes.

A couple of considerations when treating naked mole rats are that they take a long time to recover from anesthesia, and the reintroduction of animals back into the colony can cause a xenophobic response. Gaseous anesthesia is preferred and dosages of analgesics are much lower than dog dosages. In addition, the greater amount of time the naked mole rat is removed from the colony, the greater the chance of queen aggression on the animal. Gloves should be cleaned before removing the animal, minimal disinfectants should be used that would impart a foreign scent, and post-procedural monitoring should be performed to be sure the animal is not killed after reintroduction. One tactic is to roll the animal in the toilet area before placing it back in the colony. Animals that are mobbed by colony members after reintroduction should be removed again. Some shoving by the queen is acceptable and usually does not last long.

USE IN RESEARCH

Model of Social Cooperation

Because naked mole rats are social and have cooperative behaviors, the study of their behavior has more applicability to people. Functional castes within a

colony have been identified (Bennett and Faulkes, 2000; Sherman et al., 1992), and some means of communication. No wonder they are popular attractions at zoos and in the biology class.

Model of Reproductive Suppression

Naked mole rats are one of only two species of mammals identified as being eusocial. The advantage of studying this species in comparison to the Damaraland mole rats (the other eusocial mammal) is that they are much smaller and colonies reach much higher numbers of animals. The basic mechanisms involved in reproductive suppression have not been elucidated, but there appears to be some psychological aspect to it (Faulkes and Abbott, 1997).

Model of Somatosensory Processing

The sensation of touch is an important sense in the naked mole rat since they inhabit an environment that is devoid of light. The area of the sensory cortex devoted to the sensation of touch is larger in comparison to other mammals (Catania and Remple, 2002; Henry et al., 2006). Like most mammals, naked mole rats have well-developed facial vibrissae (whiskers). However they also have a system of vibrissa-like hairs that are regularly spaced in rows over the body, and naked mole rats display a robust and accurate orientating response when these hairs are stimulated (Crish et al., 2003). The highly organized pattern of the body hairs and the decisive orienting responses to their touch suggest that the hairs convey somatosensory-based spatial information.

Since naked mole rats have no fur, the vibrissa-like body hairs are easily accessed for precise stimulation by investigators. The superior colliculus, a midbrain center involved in directing orientation movements to somatosensory, auditory, and visual stimuli, provides an excellent area for studying the neural basis of orientation to somatosensory stimuli in the absence of vision (which is dominant in the superior colliculi of more visually guided animals) (Crish et al., 2006). Taken together, the tractable number of body hairs (~50), their ease of access for stimulation, and the lack of visual inputs to the superior colliculus make the somatosensory system of the naked mole rat an excellent system for studying somatosensory processing as it relates to orientating behavior.

Model of Pain Insensitivity

Naked mole rats show a unique and remarkable lack of cutaneous pain-related behaviors to two potent chemical irritants, capsaicin (from chili peppers) and acid (Park et al., 2008), and a lack of trigeminal pain-related

behaviors to capsaicin and ammonia (LaVinka et al., 2009). Furthermore, when exposed to inflammatory insults or known mediators (e.g. Freund's adjuvant), naked mole rats do not display sensitization to heat on the inflamed area (thermal hyperalgesia) as do other mammals. In contrast, naked mole rats do display normal (mouse-like) pain behaviors to mechanical stimuli (pinch) and heat.

Naked mole rats display several physiological and anatomical features that explain their insensitivity to noxious chemical and inflammatory stimuli. Most notably, nerve fibers in the skin that normally respond to acid in other animals are completely insensitive to acid in naked mole rats (Park et al., 2008). Also, while nerves in the skin do respond to capsaicin, those nerves lack the neurotransmitters that would usually be released onto spinal cord cells to signal pain (Park et al., 2003). The neurotransmitters that are missing are substance P (SP) and calcitonin gene related peptide (CGRP).

Why do naked mole rats lack pain from chemical irritants? As mentioned earlier, naked mole rats have a very unusual combination of ecological and social characteristics. They are fully subterranean, extremely social, and they live in colonies with many individuals. In other words, naked mole rats live in large numbers in very tight quarters and in very poorly ventilated spaces where metabolic activity increases carbon dioxide (CO₂) and ammonia to extremely high levels. In other mammals, exposure to chronically high levels of CO₂ and ammonia induces pain, especially in the eyes and nose. It appears that the insensitivity to chemical irritants in naked mole rats is an adaptation to living in an otherwise painful environment.

Model of Cutaneous Substance P (SP) and Calcitonin Gene Related Peptide (CGRP) Knockout

In addition to functioning in certain aspects of pain processing, SP and CGRP also play an important role in regulating blood flow, for example in thermoregulation and digestion. The absence of these neuropeptides in naked mole rats may be related to the finding that naked mole rats are the only known mammalian poikilotherms, and they may not require cutaneous vasodilation to regulate body temperature. Cutaneous vasodilation in other mammals is mediated by nerve cells that release SP and CGRP onto small arteries in the skin to increase regional blood flow and heat transfer. Of interest, naked mole rats have SP/CGRP innervation to mesenteric arteries, which presumably subserves regulation of blood flow related to digestion (Park et al., 2003).

SP and CGRP have been implicated in a direct effector role in tissue maintenance. Absence of this peptidergic innervation raises questions about the capacity

of the skin of naked mole rats to respond to insult and could explain the extreme susceptibility to herpes simplex I administered cutaneously (Artwohl et al., 2009).

Model of Delayed Puberty and Prolonged Hypogonadism

Hypogonadism, be it induced by delayed puberty, exercise-induced amenorrhea, or testicular malfunction, impacts upon bone quality, resulting in impaired bone strength with reduced bone mass and area as well as changes in trabecular architecture (Chevalley et al., 2009). Suboptimal skeletal development during puberty affects long-term bone strength, leading to an increase in fracture risk later in life (Yingling et al., 2008). Current animal models to study delayed puberty and hypogonadism have several drawbacks. Ovariectomy models often manifest with high GnRH, since it is inhibition by sex steroids and inhibition is reduced, whereas GnRH antagonists affect not only the entire hypothalamic-pituitary-gonadal axis, but also have a tendency to increase body weight, serum IGF-1 levels, and complete suppression of the onset of puberty is dose-dependent (Roth et al., 2000; Yingling and Taylor, 2008). While these studies have yielded considerable information in this regard, the short-term nature of these studies as well as the extended duration of bone remodeling and turnover, limits these studies in what they can tell us about "catch-up" growth or chronic hypogonadism and begs the question of whether or not there is a natural animal model for long-term hypogonadic effects on bone.

One such novel animal model may be the naked mole rat. They are naturally suppressed from entering puberty and do not show differences in other hypothalamic pituitary hormones or serum IGF-1 levels, a clear advantage to other models. Further, "puberty" can be initiated within 7 days once isolated from the dominant female and levels of the sex steroid hormones in these recently isolated NMRS reach concentrations comparable to those of their long-term breeding counterparts (Clarke and Faulkes, 1997, 1998). This activation of gonadal activity and fertility is extremely rapid, especially when considering that non-breeders may have been reproductively suppressed for many years, and even 24-year-old females are capable of exhibiting rapid changes from reproductive suppression to ovarian cycling. Further studies in which hormone status may be experimentally manipulated in young individuals both prior to and after attainment of adult mass may shed more light as to whether or not bone morphology and mechanical properties are sustained by sex steroid hormone-independent mechanisms. Elucidating the mechanisms facilitating sustained bone structure and strength in hypogonadic subordinates may yield

pivotal insights into potential mechanisms and therapies for sustaining bone in delayed puberty and exercise-induced hypogonadism.

Model of Bone Elongation

Naked mole rat queens have significantly longer lumbar vertebrae for their body size compared with all other colony members (Jarvis et al., 1991; O'Riain et al., 2000). This finding parallels eusocial insects in that the dominant breeder is physically different. The length of the spine is proportional to the number of pregnancies experienced and suggests that hormones related to pregnancy may have a role in bone growth (Henry et al., 2007). Queens no longer experienced net gain in lumbar spine length after eight pregnancies, but did have some transient elongation during pregnancy after the eighth pregnancy (Dengler-Crish and Catania, 2009).

Model of Aging

Few animal models used in aging studies show slow rates of aging with affiliated prolonged good health-span and lifespan. Rather, traditional aging models are chosen primarily because such animals have poor defenses against aging and are short-lived. For example the most common mammal used in aging is the C57Bl/6 mouse that only lives 50% as long as predicted by body size. Use of short-lived species enables rapid evaluation of whether experimental manipulation of environmental (e.g., caloric restriction (CR)) and/or genetic factors alter lifespan (Miller and Nadon, 2000). Although we recognize the many advances in understanding aging processes in these short-lived models, we do not know if their lifespan extension mechanisms are pertinent to long-lived mammals, such as humans, that naturally age slowly. Long-lived species may possess certain traits germane to retarding aging that enable them to attain their impressive longevity (Austad, 2009). Thus, a complementary approach for studying mechanisms of aging, that we as humans would like to use in translational research, would be to exploit the untapped resource of natural variation in longevity and focus on long-lived successfully aging species such as the naked mole rat.

Similarly, since naked mole rats do not show any signs of spontaneous neoplasia during aging and also exhibit negligible declines in cardiovascular function, bone quality, and cognitive function; this unusually long-lived and healthy species may also prove to be useful models in many age-associated diseases. As such this species may reveal novel, potentially causal, mechanisms naturally involved in abrogated aging and age-associated diseases. Such knowledge will enable therapies aimed at activating mechanisms to delay human aging, prevent degenerative disease, and extend quality of life in old age.

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Deer Mice, White-Footed Mice, and their Relatives

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INTRODUCTION

Peromyscus are myomorph rodents belonging to the family Cricetidae, subfamily Neotominae. Neotominae is comprised of 16 genera of New World cricetid rodents including deer mice, woodrats, and their relatives (Musser and Carleton, 2005). The genus *Peromyscus* contains at least 60 species, with *P. maniculatus*, the deer mouse (Figure 46.1), and *P. leucopus*, the white-footed mouse (Figure 46.2), being the most commonly used

in research (Donnelly and Quimby, 2002). While other *Peromyscus* species, such as *P. californicus*, are used in various areas of research, the former two species will be the primary focus of this chapter. This genus has undergone extensive taxonomic revision and requires further study to resolve the nomenclatural proposals. Some systems use the subgenus designations *Haplomylomys*, containing the *californicus* and *eremicus* species groups, and *Peromyscus* containing all other species groups. Others emphasize species group assemblages rather



FIGURE 46.1 *Peromyscus maniculatus gracilis*, woodland deer mouse, Chippewa Co., MI. (Photograph courtesy of Phil Myers, Museum of Zoology, University of Michigan).

than subgenera (Musser and Carleton, 2005). Despite their common names, deer mice and white-footed mice are not closely related to common laboratory mice of the genus *Mus*, or to laboratory rats (*Rattus*) for that matter.

Physical Description

P. maniculatus has a round, slim body typically 119–222 mm in total length. Tail length varies among different populations and ranges from 45–105 mm (Baker, 1983). Body weight also varies among populations and subspecies and ranges from 12–30 g (Jones and Birney, 1988). Characteristic features of this species include a pointed nose with large black eyes, large lightly furred ears, long, prominent vibrissae, and forelimbs which are shorter than the hindlimbs. Like most cricetids, the dental formula of *Peromyscus* is 1/1 0/0 0/0 3/3 and its molars are low-crowned and cuspidate. *P. maniculatus* is grayish to reddish-brown in color with



FIGURE 46.2 *Peromyscus leucopus noveboracensis*, white-footed mouse, Otsego Co., MI. (Photograph courtesy of Phil Myers, Museum of Zoology, University of Michigan).

a white ventrum. The finely haired tail is distinctly bi-colored, dark above and white below, a feature that distinguishes it from other species of *Peromyscus* (e.g., *P. leucopus*) in which the separation of dorsal and ventral tail color is less distinct. The subspecies of *P. maniculatus* segregate into two broad categories, long-tailed, large-eared woodland inhabitants (e.g., *P. maniculatus gracilis*) and small-eared open-country forms (e.g., *P. maniculatus bairdii*) (Baker, 1983; Wilson and Ruff, 1999). In addition to larger ears, the woodland form has a longer tail, skull and hind feet than the open-country form. Despite having sympatric ranges these subspecies do not interbreed, which may be due to lack of contact related to differences in habitat preference (Baker, 1983).

Peromyscus leucopus is similar in size and appearance to *P. maniculatus*, ranging in weight from 17–30 g, with a total body length of 150–205 mm and a tail spanning 65–95 mm in length (Jones and Birney, 1988). Like *P. maniculatus*, the size and coat color of *P. leucopus* vary among geographic populations. *P. maniculatus* is generally more reddish-brown in color in contrast to the grayish-brown coloration of *P. leucopus*, and, as

mentioned above, has a longer, more sharply demarcated bi-colored tail. However, in some geographic regions it may be extremely difficult to differentiate the two species based on external morphology (LTER, 1995). Furthermore, *P. leucopus* has larger hind feet (>22mm), a rounded braincase compared to the flattened and parallel-sided braincase of *P. maniculatus*, and a third upper molar that is approximately equal in width to the first two. This contrasts with the same tooth in *P. maniculatus* which is narrower than the first two upper molars (Jones and Birney, 1988). It should be noted that tail and rear foot length more reliably differentiate *P. leucopus* from the open-country subspecies of *P. maniculatus* as compared to the woodland forms which have hind feet equal in length and longer tails. The ear length of *P. leucopus* (16–18mm) is intermediate between that of *P. maniculatus bairdii* (12–16mm) and *P. maniculatus gracilis* (18–21mm) and can also be used as a means of differentiation (Baker, 1983).

Geographic Range and Habitat

Peromyscus maniculatus has one of the broadest distributions of any small, terrestrial mammal in the New World (Jones and Birney, 1988). It ranges from Alaska and Canada south to central Mexico, although it is absent from the Southeastern United States and some coastal areas of Mexico within this range. *P. maniculatus* occupies a variety of ecological zones within its geographic range. While it most commonly inhabits prairies, bushy areas, and woodlands, it can also be found in alpine habitats, northern boreal forest, desert, grassland, brushland, agricultural fields, southern montane woodland, and arid upper tropical habitats as well as on boreal, temperate, and tropical islands (Baker, 1968, 1983).

Peromyscus leucopus is found throughout the eastern United States. The easternmost part of its range extends north to Nova Scotia and south to Virginia. They range as far west as Saskatchewan and throughout the plains states, extending south through eastern Mexico into southern Mexico and the Yucatan peninsula (Wilson and Ruff, 1999). Like *P. maniculatus*, *P. leucopus* occupies a wide variety of habitats although they most commonly inhabit warm, dry forests and brushlands at intermediate elevations. They are the most abundant small rodent in mixed forests and brushy areas bordering agricultural lands in the eastern United States, nesting in warm dry places such as hollow trees or vacated bird nests (Wilson and Ruff, 1999).

Use in Research

Both *P. maniculatus* and *P. leucopus* readily adapt to the laboratory environment and have been used extensively in the areas of toxicology, epidemiology, ecology,

genetics, physiology, aging, behavior, and ophthalmology (cataracts). Their susceptibility to a variety of zoonotic agents, including hantaviruses, *Borrelia burgdorferi* (Lyme disease), vesicular stomatitis virus, granulocytic and monocytic *Ehrlichia*, *Babesia*, trypanosomes, and *Francisella tularensis* (tularemia), has generated renewed interest in these species (Donnelly and Quimby, 2002; Joyner et al., 1998). *P. californicus* has received attention from research biologists due to their monogamous mating behavior and paternal participation in nurturing young (Joyner et al., 1998). Comparative studies between *Peromyscus polionotus subgriseus* and *P. maniculatus bairdii*, which differ in sexual dimorphism, behavior, and habitat, have demonstrated differences in glucose regulation, particularly among males. *P. polionotus subgriseus* exhibit better regulatory ability compared to *P. maniculatus bairdii*, and studies utilizing interspecies hybrids suggest variations in the Y chromosome as a genetic cause for these differences (Oriel et al., 2008). Crosses between *P. polionotus subgriseus* and *P. maniculatus bairdii* have also been used to study parent-of-origin effects on growth and development which appear to be caused by variation in imprinted loci and control of genomic imprinting (Duselis and Vrana, 2007).

While they adapt well and are easy to maintain under laboratory conditions, less than 25% of the research performed in *Peromyscus* is “biomedical” research, in which *Mus* and *Rattus* are still preferred. A survey of the literature in 1998 for the period 1993–1997 revealed the use of *Peromyscus* in the following areas: community ecology, species interaction, and natural history (23.9%); parasitology and infectious disease (23.2%); physiology and metabolism (13%); systematic, population genetics, and evolution (12.3%); behavior and social organization (9.5%); applied ecology, toxicology, and environmental monitoring (7.1%); molecular biology and cell/organism genetics (6.0%); and reproduction, growth, development, and aging (4.9%) (Joyner et al., 1998). An informal review of the literature from 1998–2009 revealed minor differences in these areas of study, with a few noteworthy changes in the relative proportions of each. Since 1998, infectious disease research accounted for the greatest proportion of publications based on work with *Peromyscus*, followed by community ecology, species interaction, and natural history. Hantavirus studies accounted for the vast majority of articles on infectious disease during this period, followed by *Borrelia burgdorferi*. Physiology and metabolism remained the third most prevalent area of study, while reproduction, growth, development, and aging moved up to fourth on this list.

Two distinct disadvantages of *Peromyscus* as a laboratory species are the limited availability of highly inbred, genetically homogeneous strains and the difficulty in handling due to their quickness and jumping ability.

Both problems might be addressed through various breeding programs. Although a number of inbred lines of *P. leucopus* and *P. maniculatus* have been developed, reduced fertility and viability of inbred animals has limited the success of inbreeding. Selective breeding of some lines of deer mice has resulted in greater docility (Joyner et al., 1998).

Sources

Deer mice were first bred in captivity around 1916 by Francis Summer at the Scripps Institute, and between 1925 and 1975 a particularly large colony was maintained at the University of Michigan. Several contemporary lines can be traced to the Michigan colony. In 1962 the *Peromyscus* colony that would later form the core of the *Peromyscus* Genetic Stock Center (PGSC) was established at the University of South Carolina. The PGSC, formally established in 1985, contains dozens of "wild-type" and mutant genetic stocks and is supported in part by the National Science Foundation as a biological resource (Joyner et al., 1998). While smaller colonies may be maintained at other institutions, the PGSC is the primary source of *Peromyscus* available to the biomedical research community, with several established and thoroughly characterized lines of *P. leucopus* and *P. maniculatus* as well as other *Peromyscus* species.

BIOLOGY

Lifespan

Peromyscus are generally long-lived compared to traditional laboratory rodents (e.g. *Mus*, *Rattus*, and *Mesocricetus*), with a life span of up to 8 years reported for captive specimens. However, in the wild they rarely live longer than 1 year, with some populations undergoing complete turnover annually (Lackey et al., 1985). Their relative longevity in captivity has generated some interest in aging research. Somewhat surprisingly, mortality in *P. leucopus* in the Northeast US is relatively low in the winter and high in spring and early summer. This may be due to increased predation pressure at that time (Wilson and Ruff, 1999). A variety of mammals, birds, and reptiles including foxes, weasels, owls, and snakes prey on *Peromyscus* (Kaufman and Kaufman, 1989).

Genetics

Peromyscus has a constant diploid chromosome number of 48, although karyotypic variation has been reported among species, subspecies, and geographic

populations (Lackey et al., 1985; Rasmussen, 1968). Examination of individual mice representing three subspecies of *P. maniculatus* revealed variability in chromosomal size and centromere position (Rasmussen, 1968). Two distinct karyotypic races of *P. leucopus* have been reported, one in the northeastern and north-central US and the other in the southwestern US. These two populations differ by three euchromatic pericentric inversions. These differences are greater between the two karyotypic races of *P. leucopus* than between closely related species of *Peromyscus* (e.g. *P. leucopus* and *P. gossypinus*). Intermediate karyotypes exist, suggesting extensive hybridization of the two races in some areas (Lackey et al., 1985).

Anatomy and Physiology

Anatomy and physiology of *Peromyscus* is generally similar to that of other cricetid and murid rodents. A detailed account of *Peromyscus* anatomy has been published (Klingener, 1968). For the purposes of this chapter we will focus on a few distinguishing features of this genus. Documented anatomic characteristics include a less distinct line of demarcation between the glandular and non-glandular stomach when compared to Old World rodents (Donnelly and Quimby, 2002). In comparison to most other rodents, the glans penis in *Peromyscus* is structurally very simple. The external surface of at least the proximal portion is covered with spines. Members of the genus *Peromyscus* contain a complete or nearly complete set of accessory sex glands, with documented variation in the preputial glands (Klingener, 1968). These glands are microscopic or absent in the subgenus *Peromyscus*. Notably, *P. leucopus* contains microscopic preputial glands while *P. maniculatus* lacks them altogether. In contrast, the preputial glands are well developed in members of the subgenus *Haplomylomys* (Lackey et al., 1985). Adrenal gland mass, both relative and absolute, has been shown to be larger in *P. leucopus* compared to *P. maniculatus bairdii*. It has been suggested that this difference in adrenal weights reflects fundamental differences in metabolism and behavior (Lackey et al., 1985).

Evaporative water loss from the lungs and skin, a major avenue of water loss among rodents, is moderate to high in *Peromyscus* (MacMillen and Garland Jr., 1989). With regards to their water regulatory efficiency, *Peromyscus* species are considered relatively inefficient compared to many rodent species and of intermediate efficiency when species that subsist on more succulent diets are considered. Water conservation occurs primarily through urine volume reduction (Lackey et al., 1985). The lack of efficient physiologic water regulatory mechanisms is offset by an omnivorous diet which provides adequate intake of both energy and water. Basal

metabolic rates of *Peromyscus* are also considered intermediate among rodents. This general intermediacy is consistent with an omnivorous diet that provides substantial flexibility in meeting both energy and water needs, even in the face of demanding climatic conditions and competitive interactions. This flexibility helps explain the fact that this genus is found over such a far-reaching geographic range and inhabits virtually every terrestrial situation regardless of degree of environmental rigor, often co-existing with rodents that are much more physiologically specialized to handle demanding environmental conditions (e.g. *Dipodomys*). In addition to its omnivorous diet, the employment of torpor as an adaptive strategy allows *Peromyscus* to escape periods of environmental stress (MacMillen and Garland Jr., 1989). Torpor is a physiologic mechanism employed by many small mammalian and avian species to get temporary relief from demanding circumstances related to energy or water regulation. Torpor varies among endothermic species both with regard to temporal patterns and degree of hypothermia. In *Peromyscus* it appears to be very non-specialized in nature, limited to successive diurnal periods and involving only moderate hypothermia (MacMillen and Garland Jr., 1989). Torpor in *P. leucopus* is commonly reported in the colder parts of its range and is associated with drops in body temperature from 35°C to 17°C, and in respiratory rate from 700 breaths per minute in active mice to as few as 60 breaths per minute in torpid mice (Wilson and Ruff, 1999).

Activity and Behavior

Peromyscus are active throughout the year and generally nocturnal. *P. leucopus* is primarily nocturnal, but is occasionally diurnal during the winter (Donnelly and Quimby, 2002; Wilson and Ruff, 1999). *P. maniculatus* is crepuscular and nocturnal, rarely leaving its nest during daylight hours. *P. leucopus* utilizes a significant vertical component within its home range, and is considered semi-arboreal (Lackey et al., 1985; Wilson and Ruff, 1999). Anatomic adaptations to this semi-arboreal lifestyle include a long tail that is used as a prop and balancing organ during climbing. *P. leucopus* is also an adept swimmer which may play a role in population dispersal, especially in island populations. Individuals have been reported to pass between islands up to 233 m apart (Sheppe, 1965a).

Woodland forms of *P. maniculatus* are at home on the ground, but also climb tree trunks easily and adeptly move among limbs and vines. They are likewise skillful in their utilization of rafters, shelves, etc. for traversing areas within human domiciles (Wilson and Ruff, 1999). Like *P. leucopus*, the woodland forms of *P. maniculatus* are equally adapted to terrestrial and arboreal habitats,

often climbing trees as a primary means of escape following release from live traps (Baker, 1983). Food hoarding is documented in both the deer mouse and the white-footed mouse, although *P. leucopus* seems to demonstrate greater geographic and seasonal variation in this behavior.

Peromyscus tend to center their activity around nesting sites and food caches, which may occur in the same location. They also maintain several different home sites and refuges within their home range, varying their primary activity between the sites at different times (Baker, 1968). *P. maniculatus* has a home range of 242–300 square meters. The home range of males tends to be larger than that of females and shows greater overlap with conspecifics. Males use their home ranges for access to feeding and nesting as well as seeking out reproductive females. The home range of males overlaps with those of several females to provide multiple breeding opportunities. Females use their home ranges for feeding, nesting, and rearing young. While males exclude other males from their home ranges, reproductive females tend to be more aggressive in territory defense than males, suggesting a greater investment in the territory (Wolff, 1989). The home range of *P. leucopus* shows significant variation depending on many factors such as population density, age, season, and habitat characteristics. Like *P. maniculatus*, *P. leucopus* is territorial, with females demonstrating a greater degree of aggression toward conspecifics (Lackey et al., 1985; Wilson and Ruff, 1999).

Female promiscuity may be an adaptive mechanism to prevent infanticide by males. Male *Peromyscus* will commit infanticide of unattended young that they did not father. Male *P. leucopus* that have copulated are inhibited from killing any pups within their home range, whereas dispersing males and resident males that have not copulated will kill pups. Therefore, promiscuous mating by females with neighboring and resident males may confuse paternity and prevent infanticide (Wolff and Cicirello, 1991; Wolff, 1989). Male aggression also seems to play a role in dispersal of the previous year's young in the springtime (Baker, 1983; Wolff, 1989).

Peromyscus communicate vocally and visually with associates and intruding conspecifics utilizing buzzing sounds and sharp squeaks, as well as urine odor, fecal deposits, and scented exudates from anal, clitoral, and preputial glands (Baker, 1983).

Nests of both species tend to be located near or slightly below ground level and may be located under logs, in stumps, rocky ledges, hollow trees, dense branches or vines, remodeled bird nests, burrows of other small mammals, under boards and other debris, or in mattresses, cupboards, and drawers of human shelters (Baker, 1983).

Diet and Nutrition

As mentioned above, *Peromyscus* species consume an omnivorous diet in the wild. The diet of both *P. leucopus* and *P. maniculatus* consists primarily of insects and other invertebrates, seeds, nuts, green vegetation, and fruit. The relative proportions of these items vary geographically and seasonally (Lackey et al., 1985; Wilson and Ruff, 1999).

Reproduction

While both *P. maniculatus* and *P. leucopus* breed year-round in the laboratory and in the southern portions of their range, they are seasonal breeders in the northern aspects of their range. A great deal of variation in seasonal breeding is observed among *Peromyscus* and in some cases is restricted to just a few weeks each year. Seasonal breeding occurs in response to a complex combination of environmental cues including photoperiod, temperature, and food availability (Millar, 1989). Sexual maturation in wild populations is dependent on several factors. Reproductive stimulation has been documented in *P. maniculatus*. The presence of females stimulates male reproductive development, as does the presence of female urine. The presence of urine from adult males stimulates vaginal opening and development of the female reproductive tract. Reproductive suppression has been documented in both species. Suppression of female reproductive maturation may be due to such factors as high population density, sibling pairs, and presence of mother or unrelated females. Sexual development in young males may be inhibited by the presence of adult or same-aged males. Data collected from laboratory colonies indicate that all species of *Peromyscus* are capable of maturing at less than 3 months of age (Millar, 1989). *Peromyscus* females are polyestrous and undergo a postpartum estrus, maximizing their reproductive output, especially in populations in which breeding seasons are short. In the wild, most females survive only long enough to produce two or three litters during a breeding season, regardless of the length of the season. However, the frequency of breeding increases with decreasing season length (Millar, 1989). In contrast, females housed under constant environmental conditions in the laboratory environment may produce up to 14 litters per year (Donnelly and Quimby, 2002). In wild populations litter size varies with age, parity, geography, and environmental temperatures during early pregnancy, and gestation has been shown to be longer in lactating females (Millar, 1989).

While males of most *Peromyscus* species are polygynous, *P. californicus* is naturally monogamous. *P. leucopus* and *P. maniculatus* have been reported to be monogamous under certain conditions (Joyner et al.,

1998; Wolff, 1989; Wolff and Cicirello, 1991). Despite the predominance of polygyny, paternal care of young is documented in several species including *P. maniculatus* and *P. leucopus*, although in one study this behavior was reported to be more pronounced in *P. maniculatus* (Millar, 1989; Wolff and Cicirello, 1991).

Studies have been performed to compare reproductive performance between wild-caught and captive colonies. Notable observations were that the semidomesticated colonies (maintained in captivity for at least 25 generations) of *P. maniculatus* bred more readily and had a greater rate of litter production than wild mice, but also had higher rates of cannibalism and desertion of litters (Millar, 1989; Millar and Threadgill, 1986).

HUSBANDRY

Unlike laboratory mice of the genus *Mus* and rats of the genus *Rattus*, *Peromyscus* species are regulated under the Animal Welfare Act. Therefore the care and research use of the species must comply with the regulations and standards described in the [Code of Regulations, Title 9, Subchapter A \(rev. 2002\)](#). USDA space requirements for unconventional rodents mandate only that the enclosure affords each animal the ability to make normal postural and social adjustments with adequate freedom of movement.

Colonies of *Peromyscus* can easily be maintained in rodent cages composed of polypropylene, polycarbonate or polysulfone. Standard cage dimensions allowing 70–75 square inches of floor space are sufficient to house up to six adults. Aspen shavings, processed corncob, and cellulose are all acceptable as contact bedding materials. The addition of pulped cotton fibers (Nestlet™, Ancare Corporation, Bellmore, NY) provides environmental enrichment by permitting species-specific behaviors, including the alteration of environment in captivity. The creation of cotton nests using this material also increases privacy during breeding and neonatal care by dams. Clean cages and bedding should be provided weekly.

Ambient temperatures are not recommended to exceed 33°C; optimal macroenvironmental temperatures range from 22–25°C (Joyner et al., 1998). Relative humidity should be maintained between 30–70%. A 12 : 12h light : dark cycle is adequate to maintain non-breeding colonies of *Peromyscus*. Food and water should be provided ad libitum. Stainless steel wire cage tops with food hoppers and water bottle inserts are suitable delivery methods. A commercially prepared, nutritionally complete laboratory rodent diet is routinely used. *Peromyscus* adapt well to drinking from a water bottle.

Restraint of *Peromyscus* for purposes of examination or experimental manipulation can be achieved by

carefully covering the mouse with the palm of one's hand. With the thumb and forefinger grasp the tail base and lift the animal onto a wire cage top. With the thumb and forefinger of the alternate hand, a *Peromyscus* can now be immobilized by grasping it around the head and shoulders, pulling the skin taut. *Peromyscus* are quick and quite agile. Caution should be exercised during handling as captive-bred mice are reportedly capable of jumping to a vertical height of approximately 30 cm (Botten et al., 2001). To reduce the risk of escaped mice, the bottom half of a plastic sanitizable 55-gallon drum or a changing table with high walls can be used during transfer of mice from soiled to clean caging.

CAPTIVE BREEDING

Several laboratories have demonstrated success in establishing breeding colonies of *Peromyscus* from wild-caught founder stock (Botten et al., 2001; Joyner et al., 1998; Millar and Threadgill, 1987). Mice reach sexual maturity approximately 7–8 weeks after birth but exposure to a light cycle of <12 hours has been shown to delay gonadal development for as many as 7 months (Joyner et al., 1998; Nelson et al., 1997). In addition, shortening of the photoperiod results in ovarian and testicular atrophy in *P. maniculatus* and *P. leucopus* (Dewey and Dawson, 2001). In the laboratory where light and temperature conditions can be controlled mice can be expected to breed throughout the year (Drickamer and Vestal, 1973). Breeding occurs most successfully when *Peromyscus* are maintained under a light : dark cycle of 16 : 8 hours. Reduction of the light cycle to provide a 12-hour photoperiod with incremental advances to 16 hours of light may be beneficial in correcting problems with breeding (Botten et al., 2001). Efficient breeding is best achieved by maintaining monogamous pairs. If a litter has not been produced after 3 months, breeding candidates should be introduced to a new mate. *Peromyscus* have an estrous cycle of 5 days and an average gestational period of 22 days except in lactating females where birth may be delayed 4–5 days (Clark, 1936; Joyner et al., 1998). A postpartum estrus often occurs within 24 hours following parturition. Litter size in *P. maniculatus* and *P. leucopus* ranges from 1–11, averaging approximately five pups per litter (Drickamer and Vestal, 1973; Martin et al., 2007; Price, 1967). Males assist in the care and rearing of neonates (Wolff and Cicirello, 1991). Although nursing ceases at 20 days of age it is recommended not to wean young mice until they reach an age of 25 days (Joyner et al., 1998). Normative data pertaining to reproduction and development are summarized in Table 46.1. As with *Mus* and other rodents, anogenital distance can reliably be used in sexing young deer mice (Donnelly and Quimby, 2002). Newly weaned

TABLE 46.1 Normative data for reproduction and development in *Peromyscus*

Parameters	<i>P. leucopus</i>	<i>P. maniculatus</i>
Puberty (days)		
Male	42–48	35–37
Female	42–48	35–37
Estrous cycle (days)	4–5	4–5
Gestation (days)	22–24	23
Litter size (no. of pups)	1–11 (avg. = 5)	1–11 (avg. = 5)
Birth weight (grams)	1.5–2.4	1.3–2.2
Eyes open (days)	12–15	12–16
Weaned (days)	21–28	21–28

Adapted from Donnelly and Quimby (2002).

Peromyscus can be identified by ear tags or ear punching. [Table 46.1 near here]

DISEASES

Non-Infectious Diseases

Periodontal Disease

Periodontal disease has been described in *Peromyscus* species captured from western North America (Sheppe, 1965b; Wiebe et al., 2001). Originally, the disease was thought to be confined to a small area in British Columbia and Washington, affecting a single species (*P. keeni oreas*), as the disease was not noted in other *Peromyscus* species analyzed (Sheppe, 1965b). However, more recent investigation indicates that the condition may be more prevalent than previously believed. Wiebe et al. (2001) described periodontal disease occurring in three subspecies of *P. keeni* and one subspecies of *P. maniculatus* originating from the west coast of North America. The authors described a scoring system, classifying the disease from I–III based on furcation exposure and degree of bone loss. Incidence of periodontal disease of any class was 3.6% in *P. keeni* and 4.4% in *P. maniculatus*. Animals that were captured on small islands had a much higher incidence of disease compared with those captured on large islands and the mainland (Wiebe et al., 2001). Both reports suggest a genetic component to this disease, and further analysis may prove these mice to be a suitable model for periodontal disease in humans.

Spherocytosis

A recessive mutation causing hereditary spherocytosis has been described in a colony of *Peromyscus maniculatus* descended from mice trapped in Oregon

and California (Huestis and Anderson, 1954; Huestis et al., 1956). The syndrome is characterized by varying degrees of jaundice occurring within a few hours after birth, persisting for 1–2 days, and followed by several days of pallor. Other related findings include splenomegaly, mild hepatomegaly, bone marrow erythroid hyperplasia, and an increased incidence of gallstones. Red blood cell analysis in affected mice revealed an increased proportion of reticulocytes, shortened red blood cell life span, and increased osmotic fragility (Anderson et al., 1960; Fitzpatrick et al., 1963; Huestis and Anderson, 1954; Huestis et al., 1956). Except for the mode of inheritance, the disease was described as identical to hereditary spherocytosis in man, and was thus proposed as a promising model; however, this condition has not been described in recent literature, most likely due to a lack of available mice and replacement with genetically altered inbred *Mus* (Anderson et al., 1960; Rosenblum, 1976).

Neoplasia

Although spontaneous neoplasia occurs at a low frequency in random-bred *Peromyscus* (Parnell et al., 2005), a variety of tumors have been reported in *Peromyscus* species. A report comparing aging and longevity in *P. leucopus* with *Mus musculus* described the occurrence of neoplasias in an established colony of *P. leucopus*. Characteristic tumors included hepatocellular carcinoma, mammary ductal carcinoma, and lymphoreticular and bone tumors. Primary mammary tumors were locally invasive with metastasis to the lymph nodes, in contrast to those of *Mus*, which metastasize primarily to the lung. Other, less frequently observed tumors in this colony included histiocytic lymphoma, papillary adenocarcinoma of the ovary, endometrial adenocarcinoma, choriocarcinoma, and a single subpleural pulmonary tumor of alveolar origin (Sacher and Hart, 1978). A high incidence of highly malignant harderian gland adenocarcinoma was reported in two of three inbred lines of *P. leucopus*; a single case was also seen in a random-bred male. Other neoplasias found in the three described inbred strains included a fibrosarcoma and tumors of giant cell, salivary, and mammary origin (Parnell et al., 2005). Odontomas have been described in a colony of *P. leucopus*. The growths were seen at the root of incisor teeth and were histologically classified as complex-type odontomas. Mice aged 4–5 years were most commonly affected (Finkel et al., 1979). In a colony of hairless *P. maniculatus gambeli*, a low percentage of animals developed spontaneous epidermal papillomas; histological findings were characteristic of benign papillomas. In addition, some mice developed cystic hair follicles, primarily on the dorsum and abdomen (Packchianian and Rigdon, 1970). A single case of granulocytic leukemia and uterine adenocarcinoma has been described

in a wild-caught adult female *P. leucopus* (Davis and Morton, 1993). Thymic lymphoma was also briefly described in one of 12 *P. leucopus* 7 months after undergoing whole-body irradiation (Cosgrove et al., 1974).

Metabolic Disease

Type II diabetes mellitus has been described in a colony of *P. leucopus* that were maintained on a diet containing 25.8% kcal from fat. When these animals were switched to a low-fat diet, serum glucose, triglyceride, and cholesterol levels returned to normal within 6 months, although some animals remained hyperinsulinemic. The mice were initially evaluated for tail and extremity lesions, although the authors concluded that the lesions were traumatic in origin and not related to the presence of a diabetic state (Krugner-Higby et al., 2000).

Miscellaneous

Diarrhea may occur in weanling *Peromyscus*, particularly when stressed. The condition is generally self-limiting, and treatment may be administered in the form of tetracycline following separation from cagemates (Joyner et al., 1998). At the authors' institution, multiple cases of cystic endometrial hyperplasia, mucometra, and mineralization of the ovaries and cervix were noted in a breeding colony of female *P. leucopus*. A review of husbandry practices revealed that the colony had been maintained on a 12 : 12h light:dark cycle. No additional cases were found after corrections to lengthen the light phase of the photoperiod were made. As no controlled studies were performed, a direct cause-and-effect relationship could not definitively be made.

Infectious Diseases

Peromyscus species act as natural hosts or reservoirs of a large number of infectious agents, many of which are zoonotic (Donnelly and Quimby, 2002; Vandegrift and Hudson, 2009). Susceptibility to infection by these organisms is one of the advantages for using this genus in biomedical research. Precautions must be taken when importing these animals into the laboratory, particularly when working with non-captive-bred animals, to decrease the risk of exposure to both handlers and other animals in the colony (Joyner et al., 1998; Mills et al., 1995). This section focuses on those diseases that are known to cause pathology in *Peromyscus* and/or are capable of infecting humans or other laboratory rodents.

Parasitic Diseases

TOXOPLASMA GONDII

As with humans and most mammalian species, *Peromyscus* can act as intermediate or reservoir hosts

for *T. gondii* (Dabritz et al., 2007; Dubey, 1983; Wallach and Boever, 1983). Following infection via oral ingestion of sporulated *T. gondii* oocysts, sporozoites excyst, transform into motile tachyzoites within small intestinal enterocytes, and disseminate to many organs including lymph nodes, heart, lung, spleen, and brain (Dubey, 1997). The organisms then form bradyzoites which subsequently continue to divide, forming tissue cysts; in rodents, these cysts are most numerous in the brain (Baker, 2007; Dabritz et al., 2007). The life cycle is completed by ingestion of these cysts by a felid, the definitive host of *T. gondii*. Disease and clinical signs in infected *Peromyscus* may be silent or include encephalitis, granulomas, pyrexia, lymphadenitis, and/or myocarditis, depending upon location and/or size of tissue cysts (Dabritz et al., 2007; Wallach and Boever, 1983). Of potential importance is the finding that vertical transmission has been demonstrated in *Mus* and *Rattus* species; whether this phenomenon occurs in *Peromyscus* remains to be determined (Dabritz et al., 2007).

GIARDIA

A protozoan that affects both humans and animals worldwide, *Giardia* has a direct life cycle. Trophozoites attach to enterocytes in the host, reproduce via binary fission, undergo encystation, and exit the host in the feces (Baker, 2007). *Giardia* has been isolated from both natural and wild-caught captive populations of *Peromyscus* (Appelbee et al., 2005; Grant and Woo, 1978; Rothenbacher et al., 1970; Wallis et al., 1984; Ziegler et al., 2009). As in many species, infection is generally asymptomatic; however, one report described diarrhea, emaciation, and high mortality resulting from giardiasis in a wild-caught captive colony. Stress and poor-quality diet were suspected as contributing factors to the onset of disease (Rothenbacher et al., 1970; Wallach and Boever, 1983).

SCHISTOSOMATIUM

Peromyscus maniculatus are the natural hosts for *Schistosomatum douthitti* (Malek, 1977; Schwanz, 2006). *Schistosomatum* is a trematode that infects a variety of rodents throughout North America (Malek, 1977). In the natural host, adult schistosomes reside in the mesenteric and hepatic portal veins, producing eggs that pass into the intestines and exit via the feces. Asexual reproduction occurs in a snail intermediate host, which shed cercariae that subsequently burrow through the skin of the definitive host (Price, 1931; Schwanz, 2006). Experimental infection of *P. maniculatus* with *S. douthitti* resulted in diarrhea and bloody feces; enlarged and darkened spleen and liver; increased intestinal mass; and decreased serum alkaline phosphatase and albumin (Schwanz, 2006).

OTHER INTERNAL PARASITES

Peromyscus are host to a large number of other endoparasites that may cause pathology, including *Cryptosporidium* (Appelbee et al., 2005; Perz and Le Blancq, 2001; Ziegler et al., 2007), *Babesia* (Etkind et al., 1980; Hofmeister et al., 1998; Mather et al., 1996; Spielman et al., 1981; Telford and Spielman, 1993), *Hymenolepis* (Munger et al., 1989; Zenchak and Hall, 1971), *Echinococcus* (Carney and Leiby, 1968; Leiby, 1965; Leiby and Kritsky, 1974), *Trichinella spiralis* (Holliman and Meade, 1980; Martin et al., 1968; Meagher and Dudek, 2002), *Pterygodermatites peromysci* (Luong et al., 2009; Vandegrift and Hudson, 2009; Zenchak and Hall, 1971), and *Baylisascaris procyonis* (Kazacos and Boyce, 1989; Page et al., 2001; Sheppard and Kazacos, 1997; Tiner, 1953).

ECTOPARASITES

Peromyscus are natural hosts to a wide variety of ectoparasites, including numerous species of fleas, ticks, and mites (Durden et al., 2004; Glicken and Schwab, 1980; Ritzi and Whitaker, 2003; Storm and Ritzi, 2008). While an extensive list of ectoparasites is beyond the scope of this chapter, it is worth noting that some parasite species that infect *Peromyscus* are important vectors of pathogens that affect humans and other animals. One example is the American dog tick (*Dermacentor variabilis*), which is commonly found on wild-caught *Peromyscus* and is a vector for *Rickettsia rickettsii*, *Francisella tularensis*, and *Coxiella burnetii* (Durden et al., 2004; Norment and Burgdorfer, 1985; Ritzi and Whitaker, 2003; Storm and Ritzi, 2008). Another example is the tropical rat mite (*Ornithonyssus bacoti*), which has been shown to harbor *Rickettsia* spp., *Yersinia pestis*, *Francisella tularensis*, and *Coxiella burnetii* (Baker, 2007; Durden et al., 2004). Additionally, *O. bacoti* itself can inflict a painful bite and cause dermatitis in humans (Baker, 2007; Morishita and Schaul, 2007). Some ectoparasite species that infect *Peromyscus* are well known to infect laboratory mice and rats, including *Myocoptes*, *Radfordia*, *Ornithonyssus*, and *Myobia* (Durden et al., 2004; Ritzi and Whitaker, 2003; Storm and Ritzi, 2008). Depending on the parasite species and burden, clinical signs may or may not be present and can include pruritis, alopecia, anemia, and anorexia (Wallach and Boever, 1983).

Bacterial Diseases

BORRELIA

Peromyscus leucopus is a primary natural reservoir for the spirochete *Borrelia burgdorferi*, which is the causative agent of Lyme disease (Bosler et al., 1983; Burgess et al., 1990; Levine et al., 1985; Steere et al., 1983). Transmission of this microorganism occurs through

the bite of nymph and larval stages of the tick vector, *Ixodes scapularis*, of which *Peromyscus leucopus* is a preferred host (Bosler et al., 1983; Levine et al., 1985; Steere et al., 1983). In *Peromyscus*, the organism has been demonstrated in skin, kidney, spleen, blood, and urine (Levine et al., 1985; Steere et al., 1983). *B. burgdorferi* generally does not cause clinical disease in *Peromyscus*, although systemic disease with neurological signs has been reported in a colony of captive wild-caught *P. leucopus* (Burgess et al., 1990). Contact transmission of *Borrelia* without a vector host has been demonstrated in *Peromyscus* (Burgess et al., 1986).

HELICOBACTER

Peromyscus leucopus has been shown to harbor *Helicobacter* species. A report of wild *Peromyscus leucopus* caught in traps within animal facilities at a large institution demonstrated by PCR analysis the presence of *H. rodentium* and *H. hepaticus* in the cecum, colon, and occasionally the stomach. No lesions were seen on gross examination (Dyson et al., 2009).

OTHER BACTERIA

Potentially zoonotic bacteria and rickettsial organisms that may be present in wild-caught *Peromyscus* but generally do not cause disease include *Francisella tularensis* (Burroughs et al., 1945; Wallach and Boever, 1983; Wobeser et al., 2007), *Yersinia pestis* (Barnes et al., 1974; Gage et al., 1995; Marchette et al., 1962), *Anaplasma phagocytophilum* (Ravyn et al., 2001; Stafford et al., 1999; Walls et al., 1997), *Coxiella burnetii* (Riemann et al., 1979; Wallach and Boever, 1983), and *Rickettsia rickettsii* (Magnarelli et al., 1981, 1984; Wallach and Boever, 1983).

Viral Diseases

HANTAVIRUS

Peromyscus are natural hosts for two hantaviruses found in North America. Sin Nombre virus is a hantavirus endemic in the *Peromyscus maniculatus* population in western North America (Childs et al., 1994; Hjelle et al., 1995). In humans, Sin Nombre causes hantavirus cardiopulmonary syndrome (HCPS), resulting in severe acute pulmonary edema and cardiac depression (Clement, 2003; Lednicky, 2003; Nichol et al., 1993; Schönrich et al., 2008). Zoonotic transmission of hantavirus occurs primarily through inhalation of aerosolized excreta (Hjelle and Glass, 2000; Tsai, 1987). In *P. maniculatus*, the virus localizes to the lung, heart, and brown fat; the animals mount an immune response and become chronically infected (Botten et al., 2000; Camaioni et al., 2001; Green et al., 1998). However, as with most natural hosts of hantaviruses, the animals do not develop clinical disease. Viral replication appears to be low in this species and the infection is not readily transmitted between animals (Green et al.,

1998). Epidemiological studies thus far indicate that the virus is not transmitted vertically (Borucki et al., 2000; Camaioni et al., 2001). New York virus is a more recently described hantavirus and has *P. leucopus* as its natural host. The virus is characteristically similar to Sin Nombre virus and also causes HCPS in humans (Lyubsky et al., 1996). A detailed histological study of *P. leucopus* naturally infected with New York virus demonstrated some pathologic changes consistent with HCPS in humans, including lymphohistiocytic infiltrates in hepatic portal zones and edematous alveolar septae in the lungs (Lyubsky et al., 1996).

Importantly, zoonotic transmission of hantavirus within an indoor laboratory animal facility has been described, and seroconversion in *Peromyscus* during quarantine has been documented (Camaioni et al., 2001; Tsai, 1987). The agents of HCPS are classified as biosafety level 3 (BSL-3) in tissue culture and animal biosafety level 4 (ABSL-4) in rodent hosts permissive to chronic infection (CDC-NIH, 2007). Therefore the Centers for Disease Control and Prevention recommends a quarantine for wild rodents. Published guidelines recommend a 5-week period of seronegativity prior to release from quarantine into the colony (Mills et al., 1995).

OTHER VIRUSES

In an experiment to investigate mouse hepatitis virus infection in *Peromyscus maniculatus*, animals were inoculated intranasally. While seroconversion did occur, clinical disease did not develop and the virus was not transmitted to susceptible laboratory mice (Silverman et al., 1982). Other viral diseases reported in *Peromyscus* include lymphocytic choriomeningitis virus, Western and Venezuelan equine encephalitis viruses, and St. Louis encephalitis virus (Chamberlain et al., 1964; Day et al., 1996; Donnelly and Quimby, 2002).

ANESTHESIA/ANALGESIA

Several inhalant and injectable anesthetic regimens have been used successfully in *Peromyscus*. For brief procedures requiring a greater degree of immobilization than can be afforded with manual restraint, the open-drop method of delivery using isoflurane is effective. Because of its higher vapor pressure along with blood and tissue solubility, concentrated isoflurane should be diluted to a 20% solution in propylene glycol when using the anesthetic in this manner (Itah et al., 2004). The use of precision vaporizers to administer either sevoflurane or isoflurane provides safe and effective anesthesia for surgical procedures or those necessitating a longer duration of immobilization of mice. Induction chambers provide a delivery method

that minimizes animal distress. Once the righting reflex is lost animals can be maintained by nose cone delivery of inhalant vapors of 1–2%. To reduce the risk of waste gas exposure to personnel proper scavenging methods should be utilized at all times.

Injectable agents can also be used to provide effective chemical restraint or anesthesia. Sedation without effective analgesia can be achieved with a ketamine dose of 44–50 mg/kg given intraperitoneally or intramuscularly (Scoles et al., 2001; Silverman and Ingram, 1986). Induction of anesthesia was reported to occur within 1 minute when a combination of ketamine (50 mg/kg) and medetomidine (1 mg/kg) was administered intraperitoneally to laboratory-bred deer mice (Hahn et al., 2005). An intraperitoneal dose of atipamezole (5 mg/kg) provides rapid recovery. Ketamine/medetomidine at these doses does not completely eliminate the response to noxious stimuli. Chloral hydrate was reported to provide effective long-term anesthesia in *Peromyscus* with an intraperitoneal dose of 630 mg/kg (Baumgardner and Dewsbury, 1979). Given its nature as a gastrointestinal irritant and propensity to induce adynamic ileus in several species, the use of chloral hydrate should be limited to non-survival procedures where other available anesthetic regimens are not suitable. At the authors' institution a combination of ketamine (50 mg/kg), xylazine (3 mg/kg), and acepromazine (1 mg/kg) administered intraperitoneally provides sedation suitable for non-invasive procedures. Anesthesia can be achieved with higher doses of intraperitoneal ketamine (150 mg/kg), xylazine (5 mg/kg), and acepromazine (2 mg/kg) allowing for surgeries of 35–55 minutes' duration (Veres, 2010).

The synthetic opioid, nalbuphine, has been used to safely provide analgesia in *Peromyscus* species. Doses similar to published recommendations for *Mus* (5.0 mg/kg) can be administered subcutaneously every 4 hours (Crossland, 2010).

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Dormouse

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INTRODUCTION

Human monkeypox (MPXV) infections were first diagnosed in the United States (US) in 2003 ([Centers for Disease Control, 2003](#)). Consequently, research efforts were directed to develop a rodent model that could be used to study the MPXV, as it was believed that the virus could be a bioterroristic agent and human MPXV infections appear clinically similar to variola (smallpox) virus infections ([Schultz et al., 2009](#)). [Schultz et al. \(2009\)](#) validated that *Graphiurus kelleni*, a species of African dormouse that is commonly known as Kellen's African dormouse ([Holden, 2005](#)), is highly susceptible to lethal infections with MPXV and a valuable animal model for the evaluation of prophylactics and therapeutics against MPXV infections. *G. kelleni* has risen from relative obscurity in the pet population to a validated research animal model. A few institutions currently house and maintain *G. kelleni* colonies to support MPXV research.

TAXONOMY AND HISTORY (TABLE 47.1)

Dormice are the only members of the Gliridae family that are located solely in sub-Saharan Africa. The family Gliridae contains 28 species in nine genera. It can be divided into three subfamilies: Graphiurinae (*Graphiurus*); Leithiinae (*Chaetocauda*, *Dryomys*, *Eliomys*, *Muscardinus*, *Myomimus*, and *Selevinia*); and Glirinae (*Glirulus* and *Glis*) ([Holden, 2005](#); [University of Michigan Museum of Zoology, 2009](#)).

To the author's knowledge, only three institutions currently house dormice for breeding and research applications: Saint Louis University, Arizona State University, and the Intramural Research Program at the National Institute of Allergy and Infectious Disease (NIAID) of the National Institutes of Health. Vendor-maintained dormice are not currently available.

Following initial human MPXV infections in 2003, traceback investigations implicated a shipment of animals from Ghana that was imported to Texas as the

TABLE 47.1 Taxonomic classification of Gliridae

Kingdom: Animalia
Phylum: Chordata
Subphylum: Vertebrata
Class: Mammalia
Order: Rodentia
Suborder: Sciuromorpha
Family: Gliridae

probable source of introduction of MPXV. The shipment contained approximately 800 small mammals of nine different species, including six genera of African rodents. These rodents included rope squirrels (*Funisciurus* sp.), tree squirrels (*Heliosciurus* sp.), Gambian giant rats (*Cricetomys* sp.), brush-tailed porcupines (*Atherurus* sp.), dormice (*Graphiurus* sp.), and striped mice (*Hybomys* sp.) (Centers for Disease Control, 2003).

In an attempt to contain the emerging MPXV outbreak, the Department of Health and Human Services (HHS) announced an immediate ban on importation of all African rodents. The ban also included the distribution, sale, transportation, and release of prairie dogs, which are believed to have carried MPXV to humans as well as the six other rodent genera mentioned above (Centers for Disease Control, 2003).

ANATOMIC CHARACTERISTICS

G. kelleni are relatively small rodents, with an average body length ranging from 7–10 cm, and with body weight ranging between 18 and 23 g. They are generally mouse-like in appearance, but resemble squirrels or chipmunks, as they possess furred tails. Their bodies are covered with thick, soft, golden brown fur (Figure 47.1). Dormice are mainly, but not exclusively, arboreal animals, and are agile and well-adapted to climbing as they possess well-developed footpads. *G. kelleni* are nocturnal and have an excellent sense of hearing, and can signal each other with a range of vocalizations. They are omnivorous, typically feeding on fruits, berries, flowers, nuts, and insects. *G. kelleni* and other dormice are unique among rodents in that they lack a cecum (Baudoin, 1984; Nowak, 1999). *G. kelleni* has a dental formula, I1/1, C0/0, PM0-1/0-1, M3/3 = 16 or 20, that is similar to that of squirrels, although they often lack premolars. The incisors are sharply pointed; premolars and molars are brachydont, and their occlusal surfaces are made up of a series of cusps and basins or parallel enamel ridges. The skull of the dormouse has an enlarged infraorbital foramen through which passes a slip of the medial



FIGURE 47.1 Juvenile Kellen's African dormice (*Graphiurus kelleni*).

masseter, as in other myomorphs. Nerves and blood vessels pass through this foramen as well as muscle. Dormice lack the separate infraorbital foramen, for the passage of nerves and blood vessels, that is found in dipodids. The zygomatic plate is not as strongly developed as in most other members of the group (Wahlert, 1978; Wahlert et al., 1993). Limbs are relatively short; feet are broad; and the toes are tipped with short, curved claws. The forefeet have four functional digits and hindfeet have five (University of Michigan Museum of Zoology, 2009).

HUSBANDRY

The distribution of the genus *Graphiurus* is primarily Eurasian, while *G. kelleni* (Kellen's dormouse) is unique by its African distribution. Consequently, native habitats and environmental conditions vary widely. Most species are considered hibernators (Bentz and Montgelard, 1999). *G. kelleni* seems to be the single exception as there are no observable signs of torporous behavior reported in captive research colonies. Knowledge of native food and conditions is limited for this family and few nutritional studies have been done (Nowakowski et al., 2006). The primary source of information available on husbandry, breeding, and health status is from the few known research colonies of *G. kelleni*.

NUTRITION

Non-captive diets are variable, but are reported to include seeds, fruits (Bentz and Montgelard, 1999) insects, small birds, and eggs (Konjevic et al., 2007). Captive *G. kelleni* obtained from domestic pet suppliers have been successfully maintained and propagated on standard laboratory rodent diets (e.g. PMI Rodent Diet 5001 and Teklad Global 18% Protein Rodent Diet 2018) with no additional supplementation. Water is offered ad libitum in bottles with sipper tubes. At least one research facility (NIAID) supplements the diet of dormice with meal worms and soy nuts as additional sources of protein and environmental enrichment (Kastenmayer et al., 2010).

HOUSING

In the wild, dormice live in small family groups with home ranges that vary widely between species depending upon the availability of food (Baudoin, 1984).

Bedding material used for *G. kelleni* ranges from packing paper (Mrosovsky et al., 1980) to other commercially available products, (e.g. Tekfresh and Diamond Soft (Harlan Teklad, Madison, WI)). Cage sizes range from standard (27 × 17 × 13 cm) to larger caging (37 × 22 × 19 cm) (Mrosovsky et al., 1980). *G. kelleni* anecdotally seems to benefit from enrichment with red plastic, miniature toy igloos (Bio-Serv, Inc., Frenchtown, NJ). Red igloos accommodate the nocturnal nature of dormice and allow them to control their level of light exposure in the home cage. It has been noted that *G. kelleni* hides by burrowing in the litter on the cage floor but will choose to remain in the igloo when available and bring the litter inside the igloo to build nests. Males are generally not aggressive and non-littermates may be successfully co-housed. The life span of dormice is up to 5 years.

ENVIRONMENTAL CONDITIONS

While *Gliridae* are considered to be hibernators under natural conditions, that is not the case under laboratory housing conditions. When *Glis glis*, the edible or fat dormouse, was kept under warm conditions it displayed cycles of torpor and weight loss lasting only a few weeks (Mrosovsky et al., 1980), as opposed to torpor lasting the entire winter in the wild. Laboratory-housed *Graphiurus* (*G. kelleni*) have not been noted to demonstrate torporous behavior (authors' observation; Schultz et al., 2009).

Environmental temperatures in the native habitats of the Eurasian and African species vary widely. Information available regarding temperature requirements of the

African genus is anecdotal from pet breeders. However, it is known (authors' observation; Kastenmayer et al., 2010) that *G. kelleni* will breed and thrive in temperatures between 21 and 27°C. Humidity levels can be maintained between 30 and 70% as for other rodent species.

Currently, there have not been any studies done to evaluate the effect of light cycles and intensity on this species. Dormice are reported to be nocturnal and light levels at facilities successfully maintaining dormice colonies have varied between 250 and 325 lux with the light:dark cycles varying between 12:12 and 14:10 (University of Michigan Museum of Zoology, 2009).

HANDLING

Dormice should not be handled by the tail because they are susceptible to degloving injury. *G. kelleni* is very quick when aroused and can climb slick surfaces easily and hide in small crevices. Manual restraint can be done by grasping the loose skin over the neck and back, and requires quick reflexes from the handler.

Captive dormice maintain prey characteristics in that they demonstrate a high level of fearful behavior when aroused or startled. This trait makes standard husbandry activities more challenging than for most rodent species. Development of techniques which accommodate the reticent nature decreases stress for both the animals and handlers. For example, during the daylight hours the animals will cluster in miniature plastic igloos provided for enrichment. The igloo and its occupants can then be picked up by the use of an enclosed scoop and moved, thus avoiding the fearful stimulus of handling. Igloos are also useful as nests for breeding pairs by allowing movement of the litter without disturbance.

BREEDING

In the wild, *Gliridae* typically breed once to twice a year with a gestation period of 21–32 days and have an average litter size of four pups, though litters can range in number of pups from 1 to 6. The number of pups per litter decreases as the female ages. Young are born altricial and the eyes open around 18 days of age. Dormice typically become sexually mature after the end of their first hibernation, with sexual maturity for the females occurring between 6 and 8 weeks of age as evidenced by the production of offspring (author's observation).

The gestation period of captive *G. kelleni* is similar to that of *Mus*. Breeding pairs consistently produce litters every 5–6 weeks and can maintain that level of fecundity for at least 2 years (author's observation). To date, no prospective studies have been done to determine longevity of

the reproductive state. Dystocia has not been noted in this species, but cannibalization of first litters is not uncommon. Birth defects have not been noted or reported.

Pups can be weaned at 21 days of age (Table 47.1). However, distinguishing the sexes is difficult prior to 6 weeks of age due to the visual similarity of the genitalia and lack of difference in anogenital distance. Manual palpation of the penis is the only reliable method in animals under 6 weeks of age. After 6 weeks the scrotal development provides visual evidence of sexual differentiation.

DISEASES AND ZONOSSES

The isolation of MPXV, a member of the Orthopox genus, from the outbreak in the United States brought the genus *Graphiurus* to world attention as these rodents were initially considered a possible source of infection (Hutson et al., 2007). There was some evidence that the imported animals had long-term exposure to MPXV, which would indicate that the pathogen could be endemic in *Graphiurus*. Other reports of diseases and zoonoses carried by the Gliridae family have been primarily determined from field studies on European species and are summarized here.

Encephalomyocarditis virus was isolated from dormice (*Glis glis*) in Italy in 1995. Although the rat is considered the main natural reservoir, domestic swine have experienced several epizootic outbreaks. In that instance, it was suspected that there was transmission between swine and dormice or other rodents in the area (Amaddeo et al., 1995).

Both edible dormice (*Glis glis*) and garden dormice (*Eliomys quercinus*) have been shown to be reservoir hosts for *Borrelia burgdorferi*, the spirochete responsible for Lyme disease, in central Europe (Matuschka et al., 1999). While there are at least four species of *Borrelia* capable of causing human disease, a novel strain of pathogenic spirochete designated as a “dormouse-associated genospecies” and named *Borrelia spielmani* was identified from the garden dormouse (Richter et al., 2004).

In Switzerland, a human case report of tularemia characterized by systemic illness and isolation of *Francisella tularensis*, sub sp. *holarctica* subsequent to a dormouse bite demonstrated the capacity of dormice to act as reservoir hosts for the causative agent of tularemia (Friedl et al., 2005).

The coccidian parasite *Eimeria* has been found and at least two species identified from the garden dormouse in Europe (Bertolino and Canestri-Trotti, 2005). The nematode parasite *Paraheligmomina gracilis* and cestode parasite *Hymenolepis sulcata* have been identified from *Glis glis* in Britain and several European countries. A weight comparison of infected animals versus non-infected animals showed no difference, implying an asymptomatic

infection. Despite the fact that this dormouse is considered a game animal and is sometimes used as a food source, neither of these parasites has been found in humans (Konjevic et al., 2007).

COLONY HEALTH MONITORING

Sentinel monitoring with the CD-1 strain of mouse using dirty bedding exposure transfer has been used by the authors to evaluate colony health of dormice. Serologic monitoring should include screening for Hantaan viruses along with testing for common pathogens of laboratory rodents. Examination of fecal material and cecal contents for endoparasites, and pelts for ectoparasites is recommended. Microbiological testing should include bacterial culturing of cecal, tracheal, and oropharyngeal surfaces and contents.

Laboratory-reared *G. kelleni* have very few health issues. The authors have not noted dermatitis, malocclusion, or neoplasia even in those dormice surviving for over 3 years. Occasionally the young will have stump tails that are presumed to be the result of trauma earlier in life. The remaining stump may be short or hairless and it does not appear to affect the animal in adulthood.

EXPERIMENTAL APPLICATIONS

As noted previously, the sole research interest in this species as an animal disease model in the result of the 2003 outbreak of human MPXV in the US which was the first report of human MPXV infection outside of Africa (Hutson et al., 2007). Although MPXV transmission between humans is not as efficient as smallpox, the zoonotic potential and the absence of widespread smallpox vaccination of human populations was recognized as a concern for spread of the disease (Parker et al., 2007). Because *G. kelleni* was associated with the initial zoonotic disease in the United States, it was chosen as a model. It does not appear that *G. kelleni* is currently used as a model for other diseases.

MPXV infection in *G. kelleni* appears to be disseminated systemically, with no single tissue noted as best for viral DNA testing. The brain, eyelid, gonad, lymph node, spleen, skin, liver, heart, lung, and tongue all gave DNA recovery rates above 80% (Hutson et al., 2007; Schultz et al., 2009). Histopathological changes associated with MPXV infection are shown in Figure 47.2.

Although a number of genera were found to be capable of carrying the MPXV (Hutson et al., 2007), *G. kelleni* has characteristics which make it one of the most practical for modeling the disease. For example, they can be housed in conventional rodent caging; they reproduce well under laboratory conditions; and they

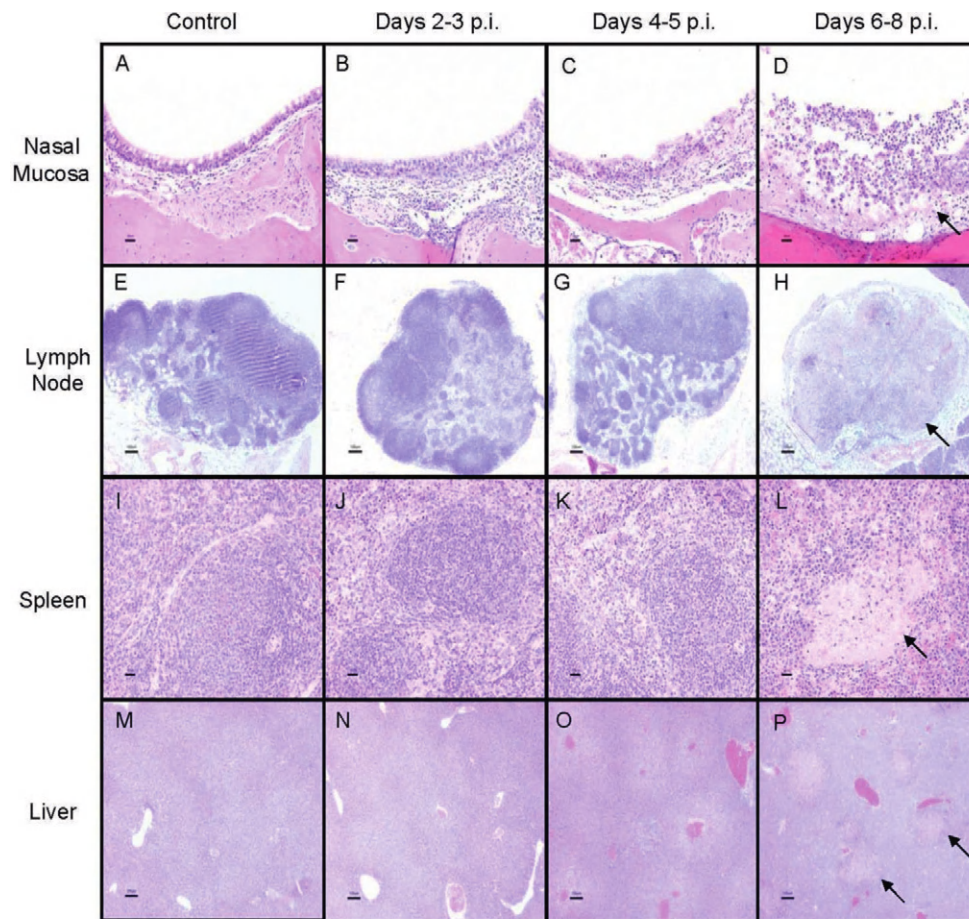


FIGURE 47.2 Histopathologic changes in selected tissues of *G. kelleni* following a lethal intranasal infection with MPXV-ZAI-79. (A–D) Nasal mucosa, bar = 20 μ m. Submucosal inflammation, mucosal necrosis (arrow) and erosion/ulceration were observed with increasing severity over time; (E–H) submandibular lymph node, bar = 100 μ m. Necrosis (arrow) increased in severity and distribution over time; (I–L) Spleen, bar = 20 μ m. Increased death of lymphocytes were followed by the development of patchy areas of necrosis (arrow) over time; (M–P) Liver, bar = 100 μ m. Large necrotic foci (arrows) of hepatic parenchyma were observed at 8 days p.i., which corresponded to maximal viral load in this tissue. With permission from Schultz et al. (2009).

can be maintained free of common laboratory murine pathogens.

For personnel safety, anesthesia of the animals is recommended prior to experimental manipulation with this species. The same anesthetics as those used for laboratory mice can generally be used for *G. kelleni* (Schultz et al., 2009).

Footpad injection has historically been utilized as an infection route in pox virus studies, but its use sometimes results in lesions which are outside acceptable humane limits. In response, intranasal and intradermal infection techniques have been developed and validated (Parker et al., 2007).

BIOMETHODOLOGY

Collection of blood from the submandibular vein using the same technique as that used for laboratory

mice has been used in dormice, but with variable success. Injections sites are the same as for laboratory mice with the exception of intravenous injections since the tail is not an available site in *G. kelleni*. Non-survival blood collection can be performed by cardiac puncture. Euthanasia methods used for laboratory mice may be used in this species successfully.

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Kangaroo Rat

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INTRODUCTION

Taxonomy

The kangaroo rat is a member of the order Rodentia and family Heteromyidae. Heteromyidae contains nine genera and 60 extant species (Carrasco, 2000). The genus *Dipodomys* consists of 21 species, all of which inhabit North America (Donnelly and Quimby, 2002). *D. ordii*, or the Ord's kangaroo rat (Figure 48.1), is the most widely distributed species in the United States (Sjoberg et al., 1984). The Merriam's kangaroo rat (*D.*

merriami) and the bannertail kangaroo rat (*D. spectabilis*) are the most commonly studied species (Donnelly and Quimby, 2002). The diploid number for *Dipodomys* spp. ranges from 52–74 (Patton and Rogers, 1993).

Description

The common name “kangaroo rat” likely originated from descriptions of this animal's hind limb structure and characteristic hopping patterns. The kangaroo rat's hind legs and feet are long and powerful, especially



FIGURE 48.1 *Dipodomys ordii*, the Ord's kangaroo rat, is widely distributed throughout the United States. (Photograph courtesy of Mammal Image Library of the American Society of Mammalogists)

compared to their short weaker forelimbs (Sjoberg et al., 1984). Kangaroo rats have a predominantly bipedal, hopping style of locomotion involving the hind limbs (Alberta Ord's Kangaroo Rat Recovery Team, 2005). The feet of the kangaroo rat can be four-toed (Merriam's and Bannertail) or five-toed (Ord's) (Williams et al., 1993). The forelimbs are used mainly to grasp and manipulate small items (Sjoberg et al., 1984).

The coloring of the kangaroo rat varies with species, ranging from pale yellow to dark brown, frequently with a white belly (Sjoberg et al., 1984). The tail is usually dark in color, sometimes with lateral white lines that are a continuation of white hair from the thighs (Donnelly and Quimby, 2002; Sjoberg et al., 1984). The tail most often ends in a terminal tuft of longer hairs (Sjoberg et al., 1984). The tail of the kangaroo rat can be longer than the length of the head and body combined and is used for balancing during locomotion and as a prop while standing. Tail removal has been shown to result in little to no change in locomotion suggesting that the unusually long tail may have additional functions, such as fat storage (Donnelly and Quimby, 2002; Hafner, 1993; Sjoberg et al., 1984).

The size of the full-grown kangaroo rat varies greatly depending on species, ranging from 35.5–186.3g (Sjoberg et al., 1984). The largest species is the giant kangaroo rat (*D. ingens*), weighing between 133g and 186.3g. In contrast, the average adult Merriam's kangaroo rat weighs between 35.5g and 51.8g, while the Ord's kangaroo rat weighs between 44.3g and 74.0g. The length of the kangaroo rat ranges from 100–260mm. The adult Ord's rat is usually between 230–260mm in length (Alberta Ord's Kangaroo Rat Recovery Team, 2005). The head of the kangaroo rat can be as much as half of the overall length of the animal (Sjoberg et al., 1984).

Kangaroo rats have relatively poor eyesight, despite having unusually large eyes that are located high on

the skull (Sjoberg et al., 1984). Unlike other members of the family Heteromyidae, the cheek teeth of *Dipodomys* grow continuously throughout life (Donnelly and Quimby, 2002). Additionally, *Dipodomys* have external fur-lined cheek pouches, as well as dermal sebaceous glands that are located in the intrascapular region of the back (Donnelly and Quimby, 2002; Williams et al., 1993). See the section *Unique Anatomic and Physiologic Characteristics* for additional information on internal and external morphology of the kangaroo rat.

Distribution

Kangaroo rats are desert rodents. In North America, *Dipodomys* are found from southern portions of western Canada to central Mexico. In the United States, kangaroo rats range from California to central Kansas and Oklahoma (Sjoberg et al., 1984). *D. ordii* are located throughout the Canadian provinces of Alberta and Saskatchewan as well as throughout the western United States. *D. spectabilis* is found in Arizona, New Mexico, and western Texas, while *D. merriami* is located in the southwestern portion of the United States and northern Mexico (Donnelly and Quimby, 2002).

Habitat

The typical habitat of the kangaroo rat is one with high summer temperatures, low rainfall, and high evaporation rates (Sjoberg et al., 1984). Kangaroo rats are nocturnal animals and spend their daytime hours residing in burrows they construct from sandy or gravelly soil (Sjoberg et al., 1984). Peaks of activity occur shortly after sunset and just prior to dawn (Hills and Butler, 1978). Canadian kangaroo rats hibernate during the winter whereas kangaroo rats in the United States and Mexico do not (Alberta Ord's Kangaroo Rat Recovery Team, 2005).

Kangaroo rat burrows are refuges, sites of seed storage, and aid in thermoregulation. These animals compensate for their weak front limbs by burrowing in loose, easily workable soil, and certain species, such as *D. spectabilis*, construct elaborate labyrinths of tunnels (Donnelly and Quimby, 2002; Sjoberg et al., 1984). Burrows are often constructed in sparsely vegetated areas to allow for unobstructed views of the area, which assists animals in identifying predators and aids in defense of their burrows from other kangaroo rats. Adult animals often reside alone with male burrows interspersed among female burrows. Burrow sharing is noted during mating and when young animals are being reared (Sjoberg et al., 1984). An adult male kangaroo rat can have an average home range of one-half acre (21780sq. feet) (Sjoberg et al., 1984).

Behavior

Kangaroo rats are predominantly solitary in nature and spend up to 80% of their time in their burrows (Sjoberg et al., 1984). Animals can hop at speeds of up to 12 mph with individual hop lengths of 24–28 inches (61–71.1 cm) (Muths and Reichman, 1996; Sjoberg et al., 1984). The locomotion of the kangaroo rat helps prevent predation from a number of natural predators, which include great horned owls, barn owls, red-tailed hawks, rattlesnakes, gopher snakes, gray foxes, kit foxes, bobcats, coyotes, and occasionally skunks (Sjoberg et al., 1984). When predators are in close range, kangaroo rats defend themselves by puncturing predators with the nails of their hind feet. Kangaroo rats can be very aggressive both in nature and in the laboratory setting. These animals have been reported to bite handlers, although over time they can become much tamer (Sjoberg et al., 1984).

Foot drumming is another possible anti-predator action for some species of kangaroo rats. The behavior consists of repeated strikes of the hind feet on the ground. The bannertail kangaroo rat is thought to foot drum to alarm and frighten predators, especially snakes (Sjoberg, 1984). In contrast, the Great Basin kangaroo rat (*D. microps*) foot drums before and after mating, similar to what has been noted in gerbils during mating (Randall and Stevens, 1987).

BIOLOGY

Unique Anatomic and Physiologic Characteristics

External Morphology

The head of the kangaroo rat is broad, flat, and triangular with large protruding eyes. The neck is short and the cervical vertebrae are partly fused (Williams et al., 1993). The pinnae are of normal size, and the lining of the external auditory meatus contains ceruminous glands that are sebaceous in nature rather than modified tubular glands, likely an adaptation for water conservation (Webster, 1966). A hypertrophied mastoid region results in a middle ear volume that is greater than the volume of the brain and is the main cause for the triangular appearance of the kangaroo rat's head. The mastoid's large size is thought to narrow the supraoccipital bone in the midline, push the parietal forward, reduce the squamosal in the posterior orbit wall to a small bone, and render the interparietal almost non-existent (Webster and Webster, 1972). The tympanic membrane of a 40-g *D. merriami* was reported by Webster and colleagues (1966) to be approximately 5.5 mm by 1.0 mm with a surface area of 25.9 mm². The manubrium of the

malleus and process of the incus are long in length and this feature, paired with the large middle ear cavity that decreases the damping of the tympanic membrane, allows kangaroo rats to be sensitive to sounds at both low and high frequencies (100–30 000 Hz) (Hafner, 1993). In a field study conducted by Webster and Webster (1966, 1971), the middle ear volume of kangaroo rats was experimentally reduced and the response to predation by owls and rattlesnakes was tested. Sonographic analysis of the sounds produced by snakes and owls during the time of predation fell within the 1000–3000-Hz range. Normal kangaroo rats were more successful in avoiding predation than those with reduced middle ear volume, especially during the dark phase of the moon when lighting was poor.

Kangaroo rats have a highly developed sense of smell that allows them to find hidden food caches and identify reproductively active sexual partners during mating periods (Sjoberg et al., 1984). Fur-lined cheek pouches, similar to those of gophers, allow kangaroo rats to carry seeds to their burrows and arrange them below ground. Transporting seeds in such a manner promotes the growth of a diverse population of fungi and mold in the cheek pouches of the wild kangaroo rat (Wicklow and Rebar, 1988).

Internal Morphology

Midgley (1938) reported on the results of an investigation of the visceral anatomy of *D. microps levipes*, and the reader is directed to his work for detailed pictures and descriptions. Several points of interest will be discussed here.

The gastrointestinal tract of the kangaroo rat includes several unique features that differentiate it from that of other rodents. The small intestine is short, only about twice the length of the large intestine, suggesting that the cells and mucous lining of the small intestine are specialized. The cecum is large and the large intestine is long and highly coiled. Heteromyids, in general, tend to have a distinct colon morphology that includes thick epithelial cells on the mesenteric side that are likely specialized for absorption. Additionally, a limited number of secretory goblet cells have been noted in the colon of the kangaroo rat, along with prominent venous and lymphatic drainages (French, 1993). These characteristics likely allow water to be resorbed from feces, assisting in overall water conservation (French, 1993). Irradiation experiments indicate that the normal intestinal flora of the kangaroo rat is similar to the rabbit and includes *Lactobacillus acidophilus*, other lactobacilli, *Nocardia* spp., *Streptomyces* spp., apocyanogenic pseudomonads, and streptococci (Haley et al., 1960).

The liver has four lobes: the median or cystic lobe, the right lateral lobe, the left lateral lobe, and the caudate or Spigelian lobe. The cystic and right lateral lobes

TABLE 48.1 Developmental Characteristics in Several *Dipodomys* Species

Function	Days after birth					
	<i>D. merriami</i> ¹	<i>D. deserti</i> ¹	<i>D. heermanni</i> ²	<i>D. stephensi</i> ²	<i>D. nitratoides</i> ²	<i>D. panamintinus</i> ²
Incisors appear	2–8	7–10	11–12	8	5	
Ears open	8–10	9–15	16	10	8	13
Eyes open	11–15	11–17	14–15	14	10	17
Solid food eaten	13	15				
Solid feces excreted	17	15				
Nails harden	12–15	12–15				
Sandbath	13–15	17				
Fully haired	14	11–15				
Weaned	17–22	15–25	17–25	18–20	21–24	27–29
Mammae visible	11	21				
Footdrumming	–	33				
Full ear growth	90	<90				
Full tail growth	89	<90				
Full foot growth	50–90	50–90				
Fully mature	90	90				
Attain adult body weight	150–180	150–180				

¹Haley (1964).²Eisenberg and Isaac (1993).

are each further divided into two halves. Kangaroo rats do not have gall bladders (Midgley, 1938).

The parotid and submaxillary glands of the kangaroo rat are large, and additionally, molar glands (close to molars, between skin and external surface of the mandible) are present (Midgley, 1938). The presence and size of such glands correlates with the dry diet and environment of the kangaroo rat and the need for added moisture during mastication (Midgley, 1938). Large Harderian glands are present in the orbit of the kangaroo rat, encircling about half of each eye and running down towards the optic nerves (Midgley, 1938).

The right kidney is slightly larger in size than the left; approximately 181840 glomeruli per kidney with a total glomerular volume per kidney of 8.9mm³ has been reported for the kangaroo rat (Haley, 1964). A long tapering papilla and lobulate glomerulus have also been noted. The glomerular filtration rate of *D. merriami* is 0.77 ± 0.004 ml/min/100g and the collecting ducts are long with cuboidal to flattened to cylindrical-shaped shifting epithelial cell lining (Haley, 1964). Adrenal glands are slightly smaller in males than in females (Midgley, 1938).

The right lung has four lobes: superior, middle, inferior, and medial or postcaval. The left lung has only one lobe. The great blood vessels are similar to most rodents,

with a few slight variations. The aortic arch gives off three branches: left common carotid, left subclavian and innominate branch, which further branches into the right subclavian and the right common carotid artery. The left and right superior venae cavae, the inferior vena cava and the azygous vein enter the right auricle directly (Midgley, 1938). The heart rate of a kangaroo rat is about 420bpm and the respiratory rate about 140bpm (Hills and Butler, 1978). Additionally, the thymus is bilobed (Midgley, 1938).

Development

Developmental characteristics for multiple species of kangaroo rats have been observed in the laboratory setting. Table 48.1 presents information for several *Dipodomys* species.

In Ord's kangaroo rats, both true hermaphrodites and pseudohermaphrodites have been found (Bawdon, 1965; Pfaffenberger et al., 1986). Out of a population of 124 live-trapped Ord's kangaroo rats in New Mexico, 16.1%, or 34% of the non-female animals, were male pseudohermaphrodites. It was noted that the weight of the male pseudohermaphrodite was slightly greater than normal male kangaroo rats of similar age (Pfaffenberger et al., 1986).

TABLE 48.2 Hematology Values for *D. merriami*

Cell type	Blood value (± SE)
Erythrocytes	$8.55 \pm 0.22 (\times 10^6 \text{ mm}^3)$
Platelets	$621 \pm 0.33 \text{ mm}^3$
Hematocrit	$47.3 \pm 0.7\%$
Hemoglobin	$13.2 \pm 0.22 \text{ g}$
Total leukocytes	$7.77 \pm 0.62 (\times 10^3 \text{ mm}^3)$
Lymphocytes	$81.4 \pm 1.49\%$
Monocytes	$3.2 \pm 0.40\%$
Neutrophils	$12.6 \pm 1.23\%$
Basophils	$2.6 \pm 0.36\%$

Table modified from Haley (1964).

Hematological Characteristics

Haley and colleagues (1960) studied the hematological characteristics of *D. merriami* (Table 48.2) and the response of this species to whole-body ionizing irradiation, which was found to be similar to the albino rat. A few findings of note were that lymphocytes constituted about 80% of the leukocyte population, and that despite the various water conservation specializations of the kangaroo rat, hematocrit values for *D. merriami* ranged between 46–48%.

Nutrition

Seeds make-up approximately 78.4% of the diet of the kangaroo rat, while 15.5% consists of insects, and the remaining 1.1–6.1% consists of green vegetation (Reichman, 1975). Seedlings, which are high in the vitamin A precursor carotene, are often the vegetation of choice, and the amount consumed depends largely on the availability of seeds (Reichman, 1975; Sjöberg et al., 1984). Sjöberg et al. (1984) listed the favored vegetation and grasses of the kangaroo rats in California as brome grasses (*Bromus* spp.), filaree (*Erodium* spp.), and fescues (*Festuca* spp.), whereas in the southwest, kangaroo rats preferred grama grasses (*Bouteloua* spp.), needlegrass (*Stipa* spp.), pigweed (*Amaranthus* spp.), fescues (*Festuca* spp.), and plantain (*Plantago* spp.). Forb seeds were the primary seed type consumed by *D. merriami* and other Sonoran Desert rodents (Reichman, 1975).

Kangaroo rats commonly gather seeds and other food items and place them in caches, or temporary storage sites in the soil close to their burrows. Seeds are usually transferred from the cheek pouch of the kangaroo rat to the cache (Sjöberg et al., 1984). Caches are typically located somewhat close to the surface facilitating seed retrieval via smell. A soil depth of 0.25 inches was reported for caches of Merriam's kangaroo rats in Arizona

(Sjöberg et al., 1984). Caching not only provides a safe place for food stuffs, but burying seeds enhances their water content, as the soil promotes water absorption.

Some kangaroo rats regularly practice coprophagy. *D. microps* is a herbivorous kangaroo rat species that ingests close to one-quarter of the feces it produces daily (Kenagy and Hoyt, 1980). In contrast, the granivorous *D. merriami* species rarely ingests feces, suggesting that the extent to which different species of kangaroo rats practice coprophagy depends on their specific diet (Kenagy and Hoyt, 1980). For those species that do ingest their feces, a pattern of coprophagy is observed: all the feces produced during the non-foraging period are ingested, while no feces are ingested during periods of foraging.

Reproduction

The gestation length of the kangaroo rat is 29–33 days, with *D. microps* reported as 31 days, *D. merriami* as 33 days, and *D. ordii* as 29–30 days (Day et al., 1956). Typical litter size is 1–4 offspring, often with fewer than ten offspring born to one dam within 1 year (Wilson et al., 1985). Newborn kangaroo rats are similar in appearance to newborn mice, with notable differences being that the toes of the kangaroo rat are separated at birth and cheek pouches are present as small grooves on either side of the mouth (Eisenberg, 1993). Weaning has been observed in wild *D. spectabilis* at 1 month of age (Jones, 1984). It was speculated that small litter size in these desert animals may conserve body fluids by lessening the lactation demand, though it should be noted that the long hind feet of fetuses may necessitate smaller litters during intrauterine development (Eisenberg and Issac, 1963).

In nature, kangaroo rats practice natal philopatry, or the retention of offspring in natal home ranges past the age of independence from their parents. Both male and female offspring tend to stay with their mothers in their natal mounds for the first 3–4 months of life. This is despite the fact that lactation ends at 1 month of age and that offspring are capable of foraging for themselves at this point in time. Some juveniles have been noted to stay in the natal mound until sexual maturity. These animals may then go on to settle into a secondary mound within the maternal home range or acquire their mother's primary mound when she vacates it or dies. Possible reasons for such behavior include the lack of resources available during the spring when young are born, or that space to build mounds is sparse and requires a great deal of energy expenditure (Jones, 1984).

Sexual maturity is reached at 1 year of age and banner-tail kangaroo rats in nature live through a maximum of three reproductive seasons as adults (Jones, 1984). The estrous cycle for *D. microps* and *D. merriami* is 12

or 13 days long, respectively, with a luteal phase of 1 week. Females are more active during estrous and those in nature are noted to travel more during this period. Vulvar swelling and vaginal opening are signs of estrus. The receptive phase of the estrous cycle is confined to a single day. Slight vaginal bleeding may precede estrus by 2 or 3 days. Vaginal casts consisting of crusty, flaking material have been seen immediately after estrus. Vaginal cytology has been reported to be of little help in determining cycle stages (Jones, 1984). Spontaneous ovulation and corpus luteum function is noted in kangaroo rats, and fertile matings have been noted to occur at first estrus cycling (Daly et al., 1984).

The kangaroo rat placenta is chorioallantoic with trophoblast cells constituting up to two-thirds of the total mass of the placenta (Fuller and Tibbitts, 1973). Young are born without membranes and the umbilical cord is broken by movements of the offspring and the mother. The mother will ingest the placental tissues (Eisenberg and Issac, 1963).

Kooyman (1963) analyzed the milk of a lactating *D. merriami* and found that the milk was relatively low in water content (50.42%). Kangaroo rats spend the hottest portion of the day in their burrows to minimize water evaporation, and it was speculated that offspring may have a lower requirement for water compared to other rodents.

DISEASES

Infectious Diseases

Viral

Five viruses have been reported in wild kangaroo rats, all of which are arboviruses (Whitaker et al., 1993). Powassan virus, Modoc virus, and St. Louis encephalitis (all *Flaviviridae* viruses) have been reported in *D. heermanni*. Bottonwillow virus (*Bunyaviridae*) and Western equine encephalitis (*Togaviridae*) have been reported in *D. nitratoides*. Arthropod vectors are associated with all of these viruses, and clinical disease is uncommon (Whitaker et al., 1993).

Rickettsial

Rickettsia rickettsii infection has been identified in *D. californicus*, *D. microps*, and *D. ordii*. *Coxiella burnetii* infection has been identified in *D. microps* and *D. ordii*. *Coxiella* spp. cause Q fever in humans and are normally carried by the tick *Dermacentor andersoni*. *D. microps* remains infected with *C. burnetii* for up to several months and may therefore represent a reservoir (Whitaker et al., 1993). *R. rickettsii* leads to Rocky Mountain spotted fever and is carried by a number of ticks.

Bacterial

Yersina pestis-positive kangaroo rats were noted in the wild in New Mexico (Whitaker et al., 1993). *Y. pestis* is the causative agent of plague and the true susceptibility of kangaroo rats to *Y. pestis* is unknown. Experimental inoculations of *D. spectabilis* and *D. ordii* showed that these species of kangaroo rat were less susceptible to infection following inoculation than mice (Whitaker et al., 1993).

Francisella tularensis, the causative agent of tularemia or plague-like disease of rodents, has been isolated in *D. ordii* and *D. microps* in Utah (Whitaker et al., 1993).

Parasitic

Various species of protozoa, cestodes, flies, mites, ticks, and fleas have been reported in wild-caught kangaroo rats. Table 48.3 is a partial list of these parasitic infections; the reader is directed to Whitaker et al. (1993) for a more comprehensive list of parasitic agents. There do not appear to be reported cases of parasitism in laboratory-reared kangaroo rats (Donnelly and Quimby, 2002).

Fungal

Mold and fungi were discussed briefly in the *Unique Anatomic and Physiologic Characteristics* section, and the reader is directed to Whitaker et al. (1993) and Wicklow and Rebar (1988) for more information on fungal and mold species noted in the kangaroo rat.

Metabolic/Nutritional Diseases

Suckow et al. (1996) reported an obstructive gastric trichobezoar in a male wild-caught bannertail kangaroo rat. The animal presented clinically as emaciated, listless, and hypothermic. At necropsy, a $1.9 \times 1.2 \times 0.9$ cm dark, hard, brown mass was noted in the gastric lumen. Microscopically, the mass was composed of shafts of hair and proteinaceous material. Excessive oral grooming was listed as a possible cause.

Iatrogenic Diseases

McGinn and Faddis (1997) reported that, like the Mongolian gerbil (*Meriones unguiculatus*), kangaroo rats develop spongiform degeneration of the central auditory system in response to prolonged exposure to low-frequency noise. Wild-caught *D. deserti* and *D. merriami* developed excitotoxic neuronal damage presumably secondary to sustained release of an amino acid neurotransmitter, such as glutamate.

Neoplastic Diseases

Mammary gland adenocarcinoma in a female kangaroo rat housed at a zoo has been reported (Griner, 1983).

TABLE 48.3 Parasites of *Dipodomys*

Parasitic organism	<i>Dipodomys</i> spp.	
PROTOZOA		
<i>Eimeria utahensis</i>	<i>D.californicus</i> ¹	<i>D. microps</i> ¹
<i>Eimeria mohavensis</i>	<i>D. panamintinus</i> ¹	
<i>Eimeria</i> spp.	<i>D. venustus</i> ¹	
<i>Giardia</i> spp.	<i>D. heermanni</i> ²	
FLIES		
<i>Cuterebra polita</i>	<i>D. ordii</i> ⁴	
LICE		
<i>Hoplopleuridae</i>	<i>D. elephantinus</i> ⁶	
MITES		
<i>Sertitympanum contiguum</i>	<i>D. elephantinus</i> ⁶	<i>D. venustus</i> ⁶
<i>Euschoengastia decipiens</i>	<i>D. elephantinus</i> ⁶	
<i>Geomylichus californicus</i>	<i>D. elephantinus</i> ⁶	<i>D. venustus</i> ⁶
<i>Geomylichus texanus</i>	<i>D. elephantinus</i> ⁶	<i>D. venustus</i> ⁶
<i>Androlaelaps fahrenheitzi</i>	<i>D. elephantinus</i> ⁶	<i>D. venustus</i> ⁶
<i>Eubrachyla elaps circularis</i>	<i>D. venustus</i> ⁶	
<i>Ischyropoda armatus</i>	<i>D. venustus</i> ⁶	
<i>Echinonyssus incomptis</i>	<i>D. elephantinus</i> ⁶	<i>D. venustus</i> ⁶
<i>Echinonyssus triacanthus</i>	<i>D. elephantinus</i> ⁶	
<i>Dipodomydectes americanus</i>	<i>D. merriami</i> ⁵	
TICK		
<i>Ixodes jellisoni</i>	<i>D. elephantinus</i> ⁶	
<i>Ixodes pacificus</i>	<i>D. venustus</i> ⁶	
<i>Dermacentor occidentalis</i>	<i>D. elephantinus</i> ⁶	<i>D. venustus</i> ⁶
FLEAS		
<i>Meringis cummingi</i>	<i>D. elephantinus</i> ⁶	<i>D.venustus</i> ⁶
<i>Meringis vitabilis</i>	<i>D. merriami</i> ³	

¹Hill and Best (1985).²Herman (1943).³Eads (1960).⁴Gummer et al. (1997).⁵Fain and Lukoschus (1978).⁶Thomas et al. (1991).

USE IN RESEARCH

Water Conservation

The water conservation specializations of the kangaroo rat make it an interesting model for the study of renal physiology and related topics. Turnover times

for exchangeable body water of the kangaroo rat are long: 13.9–22.1 days for the *D. deserti* on a pearl barley diet (Haley, 1964). The mean exchangeable body water content for *D. deserti* is $62.37 \pm 2.16\%$ (Haley, 1964). Kangaroo rat urine is as much as five times more concentrated than human urine. The kidney structure of the kangaroo rat plays a large part in water conservation in this animal and is described further in the section *Unique Anatomic and Physiologic Characteristics* of this chapter. Antidiuretic hormone also impacts the water-conservation properties and urine-concentrating abilities of the kangaroo rat. The kangaroo rat excretes up to 50 milliunits/ml of antidiuretic hormone in the urine, and levels of 0.9 milliunits/ μg were noted in the posterior pituitary gland (Haley, 1964).

In the lungs, ventilation is decreased to reduce water loss (Sjoberg et al., 1984). Kangaroo rats have few to no sweat glands, thus water excretion is handled by other organs such as the lungs and kidneys, which are adapted for water conservation.

Disuse Osteoporosis

The bipedal nature of the kangaroo rat, paired with the animal's ability to hop great distances, puts large accelerative forces on the hind limb musculature and bones, affecting bone remodeling. Muths and Reichman (1996) reported that routine handling and locomotion of kangaroo rats during short periods of laboratory captivity (7–10 days) resulted in hind limb fractures. Additionally, experimental confinement may weaken the bones of the hind limb and decrease mineral content, making the kangaroo rat a valid model for studying disuse osteoporosis. The fact that kangaroo rats eat predominantly seed diets and can thrive in an environment with little to no free water, may also make them valuable models for space research that focuses on the study of disuse osteoporosis secondary to microgravity (Muths and Reichman, 1996).

Marginal Decompression Sickness

Kangaroo rats have been suggested as an alternative to goats as an animal model to study type I decompression sickness (diver's disease). Hills and Butler (1978) found that kangaroo rats reliably bit their tails during decompression procedures in hyperbaric chambers; and tail biting ceased with reversal of the decompression. Microbubbles were noted between the fibers of tendon bundles in the bitten areas of the tail. Similar gas bubbles are noted in humans at the nerve endings of connective tissue and it is thought to be the cause of "limb bends" in deep sea divers (Hills and Butler, 1978).

LABORATORY CARE

Housing

In the laboratory, kangaroo rats have been housed on sand and soil substrates (1–2 in or 2.5–5.1 cm thick), provided with cotton as nesting material, and given cardboard tubing or dark glass bottles to act as an artificial burrow (Haley, 1964). Polycarbonate rat cages (10.5 × 9.5 × 8 in or 26.7 × 24.1 × 20.3 cm) are adequate for laboratory housing (Suckow et al., 1996). Room conditions of 12:12 light:dark cycles, 71.5°F (21.9°C) and 27% relative humidity have been reported in the literature (Muths and Reichman, 1996). Suckow et al. (1996) reported room conditions of 62–65°F (16.7–18.3°C) and room ventilation of 10–15 room changes per hour of fresh outside air. Severe fighting has been reported among newly combined captured kangaroo rats, including breeding pairs, and gradual, supervised introduction is recommended (Booth, 1943). Life spans of 2–5 years have been reported in kangaroo rats (Hooper, 1956; Sjöberg et al., 1984).

Dust bathing is practiced by kangaroo rats in the wild and dust bath sand, such as the type used with chinchillas, or a similar substrate should be made available to laboratory-maintained kangaroo rats. If not able to dust bathe, body sores and hair mats can develop on the body from a build-up of oily secretions (Nowak, 1999).

Nutrition

Many foods have been offered to kangaroo rats in the laboratory setting. A diet of sunflower seeds, wheat, rolled oats, spinach, or lettuce and fresh water offered ad libitum has been used successfully (Butterworth, 1961; Daly et al., 1984). Others have fed an even more varied diet consisting of rolled oats, rolled barley, meat meal, powdered milk, whole wheat kernels, cod liver oil, sunflower seeds, mixed bird seed, iodized salt, powdered calcium carbonate, lettuce, millet, and dried dog food (Haley, 1964). Eisenberg and Isaac (1963) warn against offering water ad libitum to captive kangaroo rats that are used to periods of seasonal drought. The continual availability can lead to water addiction by these animals, and they can develop a diabetes insipidus-like syndrome (Donnelly and Quimby, 2002). Carrots and lettuce have been recommended as sources of water (Booth, 1943).

Breeding

A variety of kangaroo rat species have been bred in the laboratory setting. The reader is directed to Butterworth's (1964) observations on breeding *D. deserti* and *D. merriami* in captivity.

Daly and colleagues (1984) successfully bred *D. merriami* and *D. microps* in the laboratory under the following conditions. Kangaroo rats were housed in Plexiglas® cages (33 × 17 × 28 cm or 13 × 6.7 × 11 in) at 22°C (71.6°F) with a 13-hour light cycle (lights on at 10:00 pm and off at 11:00 am). *D. microps* were housed separately and males and females only introduced briefly during estrus, due to the high prevalence of fighting noted in this species of kangaroo rat. Introduction was performed in a neutral cage and under close supervision. For the less-aggressive *D. merriami* species, one male and one female could be housed in linked cages joined by a tunnel. At the end of the tunnel was a guillotine door that could join or block the two cages depending on its position. In this set-up, after several gradual introductions of the pair, animals could be combined peacefully for a 24-hour period during estrus.

When combined with a female in estrus, a male kangaroo rat will initially rush, chase, and spare the female, followed by nosing and grooming. He will finally lick the female's perineal region and mount her. Mounting often lasts 1–7 min, and although it can occur several times, only the final mount will result in copulation (Haley, 1964). Observation of a copulatory plug after intromission is indicative of successful copulation. Pregnant females can be supplemented with powdered skim milk added to the diet (Daly et al., 1984).

Daly (1984) weaned pups of both *D. merriami* and *D. microps* at 25 days of age. Kangaroo rats do not appear to have a postpartum estrus and females can conceive up to 4 days later. Cyclic cessation and prolonged anestrus can occur if males and females are kept in continuous proximity (Haley, 1964).

Anesthesia and Euthanasia

Kangaroo rats are more sensitive to combinations of ketamine and xylazine than gerbils or mice. An initial dose of 40 mg/kg ketamine and 10 mg/kg xylazine, followed by supplemental doses of 10/2.5 mg/kg ketamine/xylazine has been used for successful kangaroo rat anesthesia (McGinn and Faddis, 1997). Inhalant agents, such as isoflurane, are another anesthetic option, and may be preferable over ketamine/xylazine. Indirect supplemental heat should be provided to maintain the core body temperature. Optimally, a rectal temperature of 38 ± 0.5°C should be maintained throughout surgery (McGinn and Faddis, 1997).

Euthanasia via cardiac puncture and exsanguinations under inhalant anesthesia, or pentobarbital anesthesia (120 mg/kg administered intraperitoneally) has been reported (McGinn and Faddis, 1997; Suckow et al., 1996).

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Cotton Rat

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INTRODUCTION

Description

The cotton rat, *Sigmodon hispidus*, is a New World rodent with a stocky, robust stature (Figure 49.1). While the cotton rat has been classified in the family Muridae by one source (Niewesk and Prince, 2002), most sources assign it to the family Cricetidae (Faith et al., 1997; University of Michigan, 2010; Wilson and Reeder, 2005). Average adult weight is between 100–250 grams. The name *Sigmodon* derives from the sigmoid enamel loops on the grinding surface of the molar (Faith et al., 1997). Sexual dimorphism is not prominent, but males and females can be distinguished based on the size of the genital papilla and distance from the anus (Faith et al., 1997). The term *hispidus* refers to pelage with coarse guard hairs (Davis and Schmidly, 1997). The cotton rat is distinguished from the Norway rat by its smaller size, shorter tail, and longer grizzled fur (Hawthorne, 1994). Depending on the source there are up to 14 *Sigmodon* species (Musser and Carleton, 2005). There is

little phenotypic difference between species, but there is striking karyotypic diversity, ranging from 22 chromosomes for *S. arizonae* to 52 for *S. hispidus* (Prince, 1994). The lifespan of the hispid cotton rat is less than 6 months in the wild (Cameron and McClure, 1988). In captivity animals have a survival span up to 23 months of age (Faith et al., 1997).

Distribution and Habitat

Cotton rats range over most of the southern United States, Mexico, Central America, and into Columbia and Venezuela (Hawthorne, 2005; Prince, 1994). For the most part, cotton rats reside in dense, grassy cover such as fencerows and marshy fields. They nest in burrows approximately 3–5 cm in diameter and no longer than 8 meters in length, or in above-ground mounds of grass, weeds, or brush (Davis and Schmidly, 1997). Cotton rats can be nocturnal, diurnal, or crepuscular in the wild, but can change from nocturnal to diurnal under laboratory conditions (Faith et al., 1997; Niewiesk and Prince,



FIGURE 49.1 *Sigmodon hispidus* adult.

2002). In the wild, the cotton rat diet centers around primarily seeds and plants. However cotton rats are known to eat small birds, eggs, and insects (Hawthorne, 2005). In the wild, lifespan is short- between 4–6 months (Faith et al., 1997).

Sources

Cotton rats are available from commercial sources in both Europe and the United States. These colonies were derived originally from inbred stock held at the National Institutes of Health but are currently maintained as outbred lines (Harlan Laboratories, 2010; Niewiesk and Prince, 2002).

BIOLOGY

Anatomy and Physiology

Physical features, general biology, clinical pathology, and physiologic features of the cotton rat have been summarized by Faith et al. (1997), and Sharp and La Regina, 1998. Selected hematologic parameters and organ weights are summarized in Tables 49.1 and 49.2. It has been reported that cotton rats convert 7-alpha-hydroxycholesterol to 7-alpha-hydroxy-4-cholesten-3-one similar to the laboratory rat, but which differs from the predominant pathway in hamsters (Maeda et al., 2006).

Phenotypic hair coat mutants have been described by Niewiesk and Prince (2002). These include a white pelage with black eyes, a partially white hair coat, and a white-footed variety. Some of these variations appear

TABLE 49.1 Hematology Data for Cotton Rats

Total protein (g/dl)	6.7–7.2
WBC ($\times 10^3/\mu\text{L}$)	3.9–11.9
Lymphocytes ($\times 10^3/\mu\text{L}$)	3.2–7.4
Neutrophils ($\times 10^3/\mu\text{L}$)	2.2–9.2
Eosinophils ($\times 10^3/\mu\text{L}$)	0.1–0.8
Splenocytes ($\times 10^6$)	135 (18)
Splenocytes/mg spleen ($\times 10^3$)	927 (175)
RBC ($\times 10^6/\mu\text{L}$)	3.9–6.2
PVC (%)	33.3–43.5
HGB (g/dL)	10.4–17.1
MCV (mm^3)	59.4–71.3
MCH (pg)	19–27.8
MCHC (g/dL)	31.3–32.6
PLT ($\times 10^5$)	4.9–8
Basophilic stippling	0.17–0.18
NRBC/100 WBC	0.91–1.25

Range of data compiled from Faith et al., 1997; McMurry et al., 1995; Sharp and La Regina, 1998.

TABLE 49.2 Selected Organ Weights of Cotton Rats

Parameter	7-Week Data (Standard Deviation)	13-Week Data (Standard Deviation)
Relative Organ Weight (% body Weight)	n = 11 (Liver, n = 10)	n = 12
Liver (g/g)	3.6 (0.4)	2.9 (0.3)
Kidneys (mg/g)	655.0 (27.8)	563.4 (23.4)
Adrenals (mg/g)	30.9 (4.8)	22.5 (1.6)
Thymus (mg/g)	28.9 (6.5)	43.3 (8.9)
Spleen (mg/g)	97.7 (9.1)	103.5 (7.3)
Popliteal lymph nodes (mg/g)	3.4 (0.7)	2.5 (0.3)
Absolute organ weight	n = 6	n = 6
Testes (g)	2.23 (0.13)	1.56 (0.32)
Seminal vesicles (g)	1.53 (0.23)	0.89 (0.31)
Epididymes (mg)	550.6 (36.8)	376.7 (89.7)
Coagulating gland (mg)	288.7 (53.0)	57.1 (18.9)
Ovaries (mg)	36.6 (7.3)	35.9 (3.3)
Uterus (mg)	264.3 (92.1)	159.7 (36.0)

Combined data from male and female control animals on 7- and 13-week toxicology study (McMurry et al., 1995).

to be recessive lethal mutations. Cotton rats have been described to have two left and three right lung lobes and 4–13 Peyer's patches in the intestine (Niewiesk and Prince, 2002). However, Faith et al. (1997) list one left and four right lung lobes. It is likely that this discrepancy is related to anatomical terminology, as necropsy performed on animals from the authors' institution revealed one left and three right lung lobes, and one accessory lobe. Like many small rodents, the cotton rat has a squamous and glandular region of the stomach, a large cecum, and is coprophagous. The dental formula is I(1/1) C(0/0) P(0/0) M(3/3). Normal rectal temperature is 36.5°C. The tail is an important organ for thermoregulation.

Data for hematological parameters and normal adult organ weights are presented in Tables 49.1 and 49.2, respectively.

Reproduction and Growth

Cotton rats are generally solitary in the wild (except for mothers with nursing litters) with males and females only coming together to mate. In the wild, males are dominant to females, and adults are dominant over juveniles (Faith et al., 1997). However, in the laboratory environment, they can be maintained as breeding pairs and aggression is seen by females directed toward males. To minimize this problem, some sources indicate breeding pairs should be established at 3 weeks of age (Harlan Laboratories, 2010; Prince, 1994). Animals are not sexually mature at this age, and the first litter is not seen until approximately 6–7 weeks after mating. However, others recommend separating animals by gender at weaning, and not mating until 5 weeks (Ward, 2001) or 7–8 weeks of age (Faith et al., 1997). Both systems seem to be effective, as reproductive parameters are reported to be similar by all sources. When mating, it is recommended that the female be introduced to the male's cage to minimize the risk of fighting (Niewiesk and Prince, 2002; Ward, 2001). Harem mating with one male and two females can be performed, but this increases the risk of fighting (Niewiesk and Prince, 2002). It is not recommended to separate the male and female at the birth of the litter, or to re-pair animals if one of the pair dies, as aggression is seen in these situations (Faith et al., 1997).

Cotton rats are reported to be spontaneous ovulators and breeding is not seasonal in the laboratory environment (Faith et al., 1997). The estrous cycle duration is 7–9 days, gestation is 27–28 days and cotton rats produce on average of five pups per litter, with a range of 1–12. A post-partum estrus occurs within 12 hours of parturition (Faith et al., 1997; Harlan Laboratories, 2010; Niewiesk and Prince, 2002; Sharp and La Regina, 1998; Ward, 2001). Some sources discourage handling the



FIGURE 49.2 A 1-day-old cotton rat (*Sigmodon hispidus*) demonstrating semi-precocial features.

pups immediately after parturition, as this can be associated with maternal cannibalism (Faith et al., 1997). Weaning is typically performed at 2–3 weeks of age. The interval between litters is approximately 1 month for continuously mated pairs (Mitchell, 2009; Niewiesk and Prince, 2002).

Birthing has been described by Ward (2001). Animals normally birth during the night, but can become acclimated enough to staff for birthing to be observed during the day. Experienced females build nests prior to parturition. The animal becomes restless and muscular fasciculation can be observed along the animal's side. Grooming of the perineum occurs right before delivery. The female may assist with pulling out and opening the fetal sac. The young are born semi-precocious with a full hair coat (Figure 49.2). Eyelids are closed at birth and are generally considered to open at 3 days (Faith et al., 1997; Niewiesk and Prince, 2002; Watkins, 2010). However, Sharp and La Regina (1998) list that eyes open at 1 day. Incisors erupt at 5 days. Niewiesk and Prince (2002) and Faith et al. (1997) indicate that young can survive by this age.

First estrus in females and testicular descent in males is reported at 20–30 days, with puberty at 30–40 days (Faith et al., 1997). Niewiesk and Prince (2002) report sexual maturity at 6 weeks (42 days), while Sharp and La Regina (1998) list a range of 30–50 days.

Diet, social environment, and photoperiod can affect sexual maturation. In one study, onset of estrus was not seen until 95 ± 5.88 days on a 4% protein diet, while estrus was seen at 63.9 ± 6.62 days on an 11% protein diet. In this study, animals on the 4% protein diet did not become pregnant. It was noted that estrus occurred at a similar body mass regardless of the diet fed (64.6–66.3 g body weight) (Cameron and Eshelman, 1996).

Social environment has an effect on the age of sexual maturation. Co-housing juvenile females with males affected the age of vaginal perforation and first estrus with younger males inducing sexual maturity at a younger age than older males. The presence of a second juvenile female during maturation was significantly associated with early vaginal opening (39.6 ± 1.7 days) but not with early first estrus (Evans and McClure, 1986).

Exposure to a shortened photoperiod (10 hours light versus 14 hours) delayed maturation of the testes and seminal vesicles, and vaginal opening in juvenile cotton rats, but not spermatogenesis or ovulation. Shortened photoperiod did not affect sexually mature males (Johnston and Zucker, 1979).

Cotton rats have been reported to grow at a rate of between 0.59 and 1.35g/day up to 12 days of age on an 11% protein diet (Cameron and Eshelman, 1996). They have been reported to weigh 20–25g at 3 weeks of age, 60–70g at 6 weeks of age, and 140–160g as adults (McIntire et al., 1944). However, more recent references give adult weight ranges of between 70 and 310g (Faith et al., 1997; Harlan Laboratories, 2010; Niewiesk and Prince, 2002; Sharp and La Regina, 1998). Body length is reported to range between 125 and 200mm, with an additional 75–166mm tail length (Faith et al., 1997; Niewiesk and Prince, 2002). The lifespan of cotton rats in the laboratory is reported as between 2 and 3 years (Faith et al., 1997).

Immunology

The immunology of the cotton rat has been well-defined through its use as a model for infectious disease research. Lymphocytes respond to stimulation by concanavalin A, pokeweed mitogen phytohemagglutinin, and lipopolysaccharide (Faith et al., 1997). Immune response may be affected by age, as control values for splenocyte proliferation indices to concanavalin A and pokeweed mitogen have been reported to be slightly lower after 13 weeks of study compared to 7 weeks of study (McMurry et al., 1995). Cotton rats also develop classic delayed-type hypersensitivity and humoral immune responses, but are considered highly resistant to bacterial endotoxin (Faith et al., 1997). An inversion of the lymphocyte to neutrophil ratio is seen between juvenile and adult animals (Faith et al., 1997; Niewiesk and Prince, 2002). The number of lymphocytes found in the spleen ($5\text{--}10 \times 10^7$) is more similar between cotton rats and mice than rats. The lymph nodes are smaller than those in rats and a lack of sacral lymph nodes has been reported (Niewiesk and Prince, 2002).

HUSBANDRY

Husbandry for cotton rats is generally adapted from that used for other laboratory rodents. Shoe-box-style



FIGURE 49.3 Cotton rat (*Sigmodon hispidus*) housing using contact bedding, nesting material and a nest tube.

rat cages with contact bedding are commonly used (Hill, 2009; Mitchell, 2009; Watkins, 2010;) (Figure 49.3). To prevent boredom and fighting, numerous enrichment objects have been used for cotton rats. Items that have been tried include hazelwood sticks, petite Nylabones, nest boxes, and PVC or cardboard tubing (Hill, 2009; Mitchell, 2009; Niewiesk and Prince, 2002; Ward, 2001; Watkins, 2010). Apples may also provide enrichment as well as a water source (Ward, 2001). Cage space guidelines have not been established, but one commercial source has had good reproductive success providing approximately 120in² for a breeding pair and litter, 12in² per animal from weaning to approximately 4 weeks of age, and 24in² per animal for young adults in same-sex groups (Mitchell, 2009; Watkins, 2010).

Intraspecific aggression is not uncommonly seen with cotton rats. Cotton rats demonstrate organized social behavior and an increased level of agonism was seen for the first 24 hours after introduction to unfamiliar animals (Wolfe and Summerlin, 1968). At one facility, best success with same-sex housing was achieved by co-housing only littermates after weaning, limiting cage groups to no more than two males or four females, providing nesting enrichment, and changing gloves between cages. For males, providing a separate nesting tube for each animal was determined to be critical (Hill, 2009).

While cotton rats have been established as a laboratory strain, they are not docile. Protective gloves are recommended for general handling, and use of nesting tubes provides a convenient method for moving animals without the need for direct handling (Figure 49.4). Cage changing may be performed inside a deep tub or barrel to prevent escape, as cotton rats will readily jump out of an open cage (Mitchell, 2009; Watkins, 2010). If direct handling is required, scruffing the skin over the neck is recommended, as tail restraint may result in injury to the animal or the handler (Niewiesk et al., 1997; Ward, 2001).



FIGURE 49.4 An adult cotton rat (*Sigmodon hispidus*) being restrained manually using protective gloves.

Nutritional requirements for cotton rats were studied by McIntire and colleagues (1944). In general, nutritional requirements were found to be similar to the laboratory rat. However, a requirement for nicotinic acid and inositol was found that was not noted at the time to be a requirement in rats. One commercial source feeds an extruded-type 19% protein, 9% fat balanced diet in breeding colonies (Harlan Laboratories, 2010). Cameron and Eshelman (1996) showed that growth and reproduction were improved in animals fed 11% protein compared to 4% protein in the diet, but differences were not seen between the use of sucrose or dextrin as a carbohydrate source at 3.85 kcal/g diet. Animals on this diet consumed approximately 0.3 g/g body weight/day.

Cotton rats can be maintained on acidified and chlorinated drinking water without reports of taste aversion (Mitchell, 2009; Ward, 2001; Watkins, 2010). One author suggests that water consumption of cotton rats is higher than for laboratory rats (Niewiesk and Prince, 2002). McMurry et al. (1995) report average daily water consumption of approximately 24 ml for adult animals measured over a 7 or 13 week period, beginning at a body weight of approximately 170 g. Taste preference in cotton rats for salt was determined to be 0.005 M and for sugar 0.1 M (Nevitt and Harriman, 1977). While this was determined for use as an incentive in behavioral research, these data may have practical applications for medication administration.

DISEASES

A review of health monitoring data by the authors from commercial colonies (Harlan Laboratories, 2010) showed relatively few incidents of clinical disease.

Proteus mirabilis and *Klebsiella oxytoca* are occasionally reported as normal cecal microflora, while *Staphylococcus aureus*, *Proteus mirabilis*, and swarming *Proteus* spp. may be found in the nasopharyngeal cavity in the absence of clinical signs. Isolation of *Proteus* from the nasopharynx may be associated with transient increase in fecal exposure, such as may occur during transportation (Franklin, 2010). Peribronchiolar lymphoid hyperplasia is seen with some frequency in histologic sections of lungs from healthy animals. There was one report of multiple pulmonary arterial thrombi, and another case of foreign body pneumonia (inhaled bedding), neither with associated clinical signs.

The authors are aware of no reports of naturally occurring infections with common murine pathogens. However, cross-reactivity of anti-mouse and rat antibody conjugates from a commercial multiplex fluorescent immunoassay with cotton rat IgG is low, which could result in under-reporting of infections (Steffen, 2009). One study suggested that the gastrointestinal flora of the cotton rat is very different than the gastrointestinal flora of other rodents in that anaerobic bacteria are the dominant flora in the stomach and intestines of the cotton rat, in contrast to guinea pigs, hamsters, rats, and mice where lactobacilli are the predominate bacteria (Itoh et al., 1989).

Wild cotton rats have been reported to be susceptible to naturally occurring infections from a number of organisms. Reports of bacterial infections include *Bartonella* spp. (Abbot et al., 2007), and *Borrelia burgdorferi* (Burgdorfer and Gage, 1987; Oliver et al., 2003). Viral infections include West Nile virus (Dietrich et al., 2005), Hantavirus (Mantooth et al., 2001), and vesicular stomatitis virus (Jiménez et al., 1996). Nematode infections include *Hepatozoon* spp. (Breshears et al., 2009; Johnson et al., 2007, 2008), *Hypocristata tertercera* (Durette-Desset and Guerrero, 2006), *Strongyloides* sp. (Elangbam, 1990), and *Trichinella spiralis* (Holliman and Meade, 1980). Protozoan infections include *Trypanosoma cruzi* (de Lima et al., 2006), a low incidence of *Toxoplasma gondii* (Smith and Frenkel, 1995), and *Cryptosporidium* sp. (Elangbam et al., 1993). Arthropod infestations that have been reported include *Polygenis gwyni* fleas (Abbot et al., 2007), *Dermacentor variabilis* ticks (Cooney et al., 2005; Gage et al., 1990), sucking lice, chiggers, and mites (Durden et al., 2000).

There are limited reports of non-infectious diseases in laboratory-reared cotton rats. Spontaneous enterochromaffin-like (ECL) cell carcinomas have been reported in an inbred laboratory colony of cotton rats (Martinsen et al., 2003). Spontaneous gastric adenocarcinoma has been reported (Kawase and Ishikura, 1995). Intravascular thrombosis associated with cardiomyopathy and exophthalmos was reported in a small inbred colony (Sorden and Watts, 1996). Adenocarcinomas and a high incidence

of chronic nephropathy and degenerative cardiomyopathy that was associated with exophthalmos have also been reported by others (Faith et al., 1997; Niewiesk and Prince, 2002). Cholesterol cholelithiasis was reported as an incidental finding in wild cotton rats (Pence et al., 1978). Other lesions reported by Faith et al. (1997) include steatitis in the mesenteric and perirenal fat, seminiferous tubule atrophy and aspermatogenesis, a 22–45% incidence of salivary gland mineralization and associated chronic inflammation, and occasional inflammatory or degenerative changes in the lung, liver, gastrointestinal tract, and reproductive organs. Fatty vacuolization of centrilobular hepatocytes has been described in control animals on a toxicological study (McMurry et al., 1995).

Physical injuries can be seen secondary to escape behavior. Posterior paralysis, presumably secondary to spinal fracture, has been observed in animals that were jumping up against the top of their cage (Mitchell, 2009).

EXPERIMENTAL METHODS

Blood sampling is best performed retro-orbitally (Niewiesk, 2009; Prince, 1994; Ward, 2001) since the cotton rat does not have easily accessible tail or other peripheral vasculature. Cardiac collection is also used in some instances. Dosing may be performed by methods commonly used for other rodents (Ward, 2001). Inhalant anesthesia can be performed using isoflurane (Niewiesk, 2009; Niewiesk and Prince, 2002). The use of methoxyflurane has also been reported (Prince, 2004). Injectable anesthetics include ketamine HCl (25mg/kg) and acepromazine maleate (2.5mg/kg) (Prince, 1994) or a mixture of xylazine (7.5mg/kg), ketamine (25mg/kg), and acepromazine (2.5mg/kg) (Ward, 2001). Euthanasia may be performed by an overdose of carbon dioxide gas.

A variety of immunologic reagents and methods have been developed for cotton rats. Macrophage isolation from the peritoneal cavity 2–3 days after injection of bacteria or bacterial lysates, and induction of granulocyte-macrophage progenitor cells from bone marrow have been reported (Niewiesk and Prince, 2002). Chromium uptake of fibroblasts and macrophages in T-cell cytotoxicity systems is lower than that seen in mice, and spontaneous release or leakiness is high. The mouse lymphoma YAC-1 line has been used for NK activity assays (Niewiesk and Prince, 2002). These authors also report that cotton rat T-cells derived from the spleen or lymph nodes adhere to nylon wool columns at over 90%, which should be taken into account when purifying T-cells from these samples. In addition, they note that cotton rat sera should be incubated at 4°C for enzymatic B-cell response assays to prevent non-specific findings, and that B-cells do not proliferate well after in vitro stimulation with LPS unless

membrane-bound immunoglobulins are cross-linked with antiserum.

Cell lines that are available include the CCRT osteogenic sarcoma line that expresses MHC I but not MHC II antigens, and CR-T1 and CR-T2 lymphoid cell lines that express cotton rat T-cell markers (Niewiesk and Prince, 2002). Monoclonal antibodies against cotton rat IgA, IgM, IgG, CD4 cells, and cotton rat macrophages have been developed. In addition, antibodies to human β -2 microglobulin have been shown to cross-react with cotton rat MHC I, while a mouse MHC II antibody cross-reacts with cotton rat MHC II (Niewiesk and Prince, 2002). Cytokine assays for IL-1, IL-2, IL-3, IL-4, TNF, and IFN- γ have also been developed. Reagents and gene sequences for a wide variety of cytokines and cellular markers are available commercially.

USE IN RESEARCH

The cotton rat (*Sigmodon*) species has long been recognized as a significant animal model for diseases caused by a variety of human and rodent pathogens. One of the first uses was by the NIH in 1937 for polio research in wild-caught animals. It was also used as a model in Britain to develop a typhus vaccine for British troops in Southeast Asia during World War II (Prince, 1994) and for early experiments on dental caries (Howell et al., 1948; Wakeman et al., 1948). *Sigmodon hispidus* has been the primary species of interest with *S. fulviventer* less frequently used as a research model (Faith et al., 1997).

The primary research use for cotton rats is for studies into infectious disease and immunology. The immune response of the cotton rat was characterized by Pfau et al. (2001) and Wilson et al. (2003) and is reviewed by Niewiesk and Prince (2002). In addition to polio and typhus research, a wide variety of human and animal pathogens have been studied in the cotton rat, including the measles virus (Wyde, 2000); and development of a measles vaccine was conducted using the cotton rat (Haga et al., 2009; Pasetti et al., 2009). It is also a valuable model for the study of human respiratory pathogens and diseases such as severe acute respiratory syndrome (SARS) (Watts et al., 2008), Everglades virus (Coffey et al., 2004), Metapneumovirus (Williams et al., 2005), human influenza (Jones et al., 2009; Ottolini et al., 2005; Trias et al., 2009), respiratory syncytial virus (Boukhvalova et al., 2009; Niewiesk and Prince, 2002; Nokes et al., 2008), herpes virus (Niewiesk and Prince, 2002), and genital herpes infections (Yim et al., 2005). Other uses have included infectious disease studies of human immunodeficiency virus (Blanco et al., 2009; DeNoon, 1998), *Helicobacter pylori* (Mahter et al., 2005), *Hepatozoon americanum* (Johnson et al., 2007), and nasal colonization of *Staphylococcus aureus* (Kokai-Kun, 2008;

Kokai-Kun et al., 2003). Cotton rats have been evaluated as a model for latent and active tuberculosis infection (Elwood et al., 2009) and Venezuelan equine encephalitis virus infection (Carrara et al., 2007). The cotton rat is the only known rodent model of human adenovirus type 5 (Jackson, 1997). Further, they have also been used for studies of adenovirus-based gene therapy (Niewiesk and Prince, 2002) and as a model for adenovirus-induced ocular disease (Tsai et al., 1992). Cotton rats implanted with syngeneic tumors have been used for the study of oncolytic adenovirus infection (Steel et al., 2007).

The cotton rat has been used for the study of environmental pollution (Paranjpe et al., 1994; Reddy et al., 2000; Tannenbaum, 2009), studies of wildlife population dynamics (Bowne et al., 1999; Mabry et al., 2003; Reed and Slade, 2008), and in nutritional research (Webb et al., 2005; Wilson et al., 2009).

Acknowledgments

The authors thank Lisa Mitchell and David Watkins of Harlan Laboratories and Dr. Lorraine Hill, Baylor College of Medicine for contributions on the biology and husbandry of the cotton rat.

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Pocket Gopher

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INTRODUCTION

North American pocket gophers have received limited use as a laboratory model due to the challenges of accommodating a fossorial animal in captivity. However, the evolved physiological and behavioral characteristics required for life underground render the pocket gopher valuable for a variety of investigations. For example, restrictions imposed by the subterranean ecotop make pocket gophers ideal subjects to examine the impact of reduced sensory modalities

upon communication and energy costs associated with burrowing. Further, the solitary lifestyle of the pocket gopher allows for a model system to investigate parasite–host relationships where transmission rates are relatively low. Recent advances in the husbandry of pocket gophers now make large-scale laboratory studies feasible. Within the following chapter, the current and potential uses of pocket gophers as laboratory animals are discussed. Information concerning the biology of pocket gophers and diseases to which they are susceptible is provided as well.

Taxonomy and Natural History

Pocket gophers (commonly known as “gophers”) comprise the family Geomyidae within the order Rodentia. Their distribution extends from Central America to Canada, including the western, central, and southeastern United States. Patchy distribution patterns and limited dispersal of the pocket gopher facilitate rapid speciation, resulting in a fluctuating number of confirmed species. However, the physical characteristics common to all species of pocket gopher include: a cylindrical body with a thick-set anterior; broad head; short neck; external fur-lined cheek pouches (from which the name “pocket gopher” is derived); large incisors; small, round ears; tiny eyes; short, heavily clawed forelimbs for digging; lesser clawed, relatively small hindlimbs; and a short, nearly naked tail (Figure 50.1). Pelage above is soft and varies from shades of brown to slate gray and pales ventrally. Patches of white pelage along the bodyline are common and occasional melanistic animals are reported.

The pocket gopher is an aggressive, solitary rodent that lives within individual burrow systems constructed a few centimeters beneath the ground surface (Andersen, 1987). Each tunnel system is actively defended against conspecifics (Hansen and Miller, 1959) and has discrete areas designated for nesting, waste deposition, and food caching (Reichman et al., 1982). As the pocket gopher extends its tunnel system to feed on roots and tubers, mounds of excavated soil are pushed to the ground surface. Considering the pocket gopher is rarely seen above-ground, the presence of these mounds can aid the researcher in locating individuals for capture.

Use in Research

Numerous aspects of the ecology, taxonomy, and social structure of the pocket gopher have been studied extensively in the wild (reviewed in Patton, 1990).



FIGURE 50.1 Baird's pocket gopher, *Geomys breviceps*.

In comparison, laboratory studies of the pocket gopher are limited, but diverse. Captive pocket gophers have served as models to investigate the energetic costs associated with burrowing (Gettinger, 1975; McNab, 1966; Vleck, 1979); factors influencing activity patterns (Hickman, 1984; Werner et al., 2005); swimming ability (Best and Hart, 1976); breeding behavior (Andersen, 1978; Schramm, 1961); estrous cycles (Desy and Druecker, 1979); gestation (Andersen, 1978; Schramm, 1961); neonate growth and development (Andersen, 1978; Barrington, 1942); aggression (Baker, 1974; Gregory et al., 1987); and methods of communication (DeVries, 2007; DeVries and Sikes, 2008; Heffner and Heffner, 1990). Details of such studies are discussed in subsequent sections.

Sources

Pocket gophers must be captured from the wild. Live traps for pocket gophers are not currently available for purchase, but can be readily constructed from numerous design descriptions (Baker and Williams, 1972; Connior and Risch, 2009a; Hart, 1973). State permits and approved institutional protocols (e.g., Institutional Animal Care and Use Committees) for the capture and care of wild animals should be acquired and followed.

Upon capture, pocket gophers should be examined for anatomical or behavioral abnormalities and transported to the laboratory in individual plastic cages lined with 6–10 cm of soil from their respective burrow system. Stress during transport is greatly reduced by covering cages with dark cloth. A 2-week quarantine period for new captures is recommended when bringing pocket gophers into captivity (DeVries and Sikes, 2009). Within quarantine, individuals should be introduced to caging systems designed for pocket gophers (see below), topically treated with insecticidal powder, and administered weight-appropriate dosages of ivermectin to terminate any existing intestinal parasite infections (DeVries and Sikes, 2009). Individuals showing symptoms of advanced parasitic infection (e.g., distended abdomen) require euthanization. Weight at capture should be recorded and monitored throughout the quarantine period. Subcutaneous pit-tags can be used for permanent identification. Pocket gophers are difficult to age, but it is possible to distinguish juveniles from adults (criteria described below). The sex of juveniles can be determined by palpating genitalia for the presence of a baculum. As adults, males are usually larger than females.

Pocket gophers are not commercially available. However, *Thomomys bottae* (Schramm, 1961) and *Thomomys talpoides* (Andersen, 1978) have bred successfully in captivity, suggesting that a breeding colony of pocket gophers could be established within the laboratory.

Laboratory Management and Husbandry

Housing

To encourage caching and nesting behaviors performed as in the wild, pocket gophers should be housed separately in simulated subterranean tunnel systems (DeVries and Sikes, 2009). Each system is constructed of three or more polycarbonate cages (46 by 23 by 20 cm) interconnected with polyvinylchloride (PVC) tubing (Figure 50.2). Stainless steel hose clamps attached to the ends of the PVC tunnels provide stability. Bottoms of cages should be flat as slatted floors can damage claws. Diameter and length of PVC tubing used is dependent upon the size of the pocket gopher species housed. No food dishes or water bottles are provided.

Pocket gophers are accustomed to living in almost total darkness, which can be accommodated in captivity by covering one chamber of the simulated tunnel system with an inverted plastic container. Rims of containers are trimmed to allow a close fit over PVC tunnels. This practice allows individuals to select a microenvironment of darkness without altering the photoperiod of the laboratory.

If provided only soil as a substrate, pocket gophers can die of exhaustion from continuous digging (Hanley, 1944). Therefore, it is recommended that in addition to soil, wood chips and hay should be supplied within each simulated tunnel system. Ideally, these different substrates should be provided in separate containers upon cage changing. This procedure permits the animal choice of container, cover, and substrate in which to construct a nest and provides behavioral enrichment by allowing each individual to mix and move substrates at will. With these additional substrates, pocket gophers quickly construct nests in the laboratory (Figure 50.3) similar to those seen in the wild (Figure 50.4). Individuals typically drag hay and wood chips through the tunnel system to construct nests within darkened chambers. Nests serve as sleep locations and food caches. Providing a small amount of

soil (6 cm) supports moderate digging behavior. Pocket gophers will also spread soil along the bottoms of cages and tunnels (Figure 50.5) to reduce contact of their claws on plastic surfaces. Soil can be obtained from the



FIGURE 50.3 Pocket gopher nest in captivity.



FIGURE 50.4 Pocket gopher nest in the wild.



FIGURE 50.2 A simulated subterranean tunnel system for housing pocket gophers.



FIGURE 50.5 Soil packed within a PVC tunnel.

field and autoclaved or purchased sterile in bagged form. Caging systems should be cleaned weekly.

A diet of fresh vegetation should be provided as pocket gophers do not drink free water (Hanley, 1944). Placing food items in chambers not containing a nest encourages foraging behavior and allows for assessment of general appearance. Individuals drag or store food items in cheek pouches to cache within nests. Pocket gophers are coprophagous and fecal pellets are also cached (DeVries and Sikes, 2009; Wilks, 1962). To prevent overgrown incisors, fruit bark should be provided for gnawing. Claws can be trimmed with pet nail clippers as needed.

In the wild, ambient temperature and humidity of the pocket gopher's tunnel system remain relatively stable (Kennerly, 1964) and efforts should be made to achieve similar results in captivity. An ambient temperature of $22 \pm 2^\circ\text{C}$ is recommended for the laboratory. Humidity should be maintained between 50–70%. Photoperiod can be maintained to correspond with season.

Identification

Temporary forms of identification are not readily used on pocket gophers. However, fur dyes could probably be applied on individuals not undergoing molt. Fur clipping would be less effective due to pelage length and texture.

Passive integrated transponder (PIT) tags are the most efficient means of permanently identifying pocket gophers. PIT tags are injected subcutaneously with a large-bore hypodermic syringe and do not require anesthesia. Once injected, PIT tags should be gently pushed under the pelage away from the point of insertion (Gannon et al., 2007). Syringes should be sterilized between animals.

Radiotransmitters can also be used to permanently identify pocket gophers (Cameron et al., 1988; Connior and Risch, 2009b; Zinnel and Tester, 1991). All radiotransmitters must be surgically implanted and weigh less than 5% of the mass of the pocket gopher. Subcutaneous insertion of radiotransmitters is quicker and less invasive than implantation within the peritoneal cavity (Connior and Risch, 2009b). After lightly anesthetizing animals with isoflurane, the hair is shaved, the skin disinfected, and a small incision made between the scapulae. Radiotransmitters are then inserted between the skin and muscle. The incision is closed with surgical adhesive and antibiotic ointment is applied topically (Cameron et al., 1988). Surgical instruments should be disinfected between surgeries.

Toe clipping of the forelimbs severely hampers mobility and should be avoided (Gannon et al., 2007). Ear tags and ear notches are also unsuitable, given the reduced size of the pocket gopher's pinnae.



FIGURE 50.6 Restraint technique for pocket gophers, with face and claws of the animal facing forward.

Handling and Restraint

Pocket gophers can bite with substantial force. Cowhide leather palm gloves prevent bite wounds without sacrificing hand dexterity. Animals can be grasped and held within a gloved hand, leaving the opposite hand free for minor manipulations (e.g., external examination, palpations, etc.). With the gloved hand, individuals should be pinned to the ground ventral side down. The thumb and middle finger are wrapped around the body under the forelimbs and the index finger is placed behind the head to prevent neck movement (Figure 50.6). Stress of the handling process can often be lessened by providing individuals with a substrate to chew on, such as fruit bark. Complex procedures (e.g., subcutaneous injections, vaginal smears, nail clipping, etc.) require the assistance of a second researcher. Following handling, pocket gophers frequently exhibit displacement behavior in the form of excessive grooming or teeth chattering.

Anesthesia

Surgical procedures on pocket gophers require chemical restraint and inhalation anesthesia is delivered easily. Isoflurane is recommended for short surgeries and is administered by placing an individual within a plexiglass chamber containing a cotton ball that has absorbed 40–80 μl (1–2 drops) of the anesthesia (Connior and Risch, 2009b). Respirations should be monitored closely and once abdominal breathing is observed (usually 1–2 min after initial exposure), the individual is ready for surgery. If necessary, additional doses of

anesthesia can be delivered through placing the cotton ball within a rostral cone. Following short surgeries (<25 min), animals fully recover from anesthesia within 1 hour of the procedure (Cameron et al., 1988; Connior and Risch, 2009b). These methods have proven effective for both juvenile and adult pocket gophers (Connior and Risch, 2009b).

Physical Examination

When assessing the health of pocket gophers in captivity, many observations should first be made without handling. Discharges from the eyes and ears, labored movements, nail length, and excessive weight fluctuations can be detected with no disturbance to the animal. Incisor length can be assessed by observing animals chew on fruit bark. Behaviors performed within simulated tunnel systems should be monitored closely (e.g., food caching, nest construction, etc.). Loss of appetite, reduced mobility, and a reluctance to build nests usually indicate a serious underlying condition.

Closer examination should be made following reproductive attempts or aggressive encounters. Handling is required to examine individuals for bite wounds, skin irritations, and to perform palpations.

Clinical Techniques

TOPICAL MEDICATIONS

Medicated powder can be applied to dorsal and ventral surfaces. Provided that care is taken to avoid the eyes, individuals can be lightly dusted from above without handling. To medicate the ventral surface, individuals should be grasped with a gloved hand and dusted with the other. Powder should be thoroughly rubbed within pelage in areas prone to skin irritation, such as the pelvic or axillary regions. For additional application, powder can also be mixed within hay or wood chips.

INJECTIONS

Two individuals are needed to deliver subcutaneous injections to pocket gophers. One person should restrain the animal while the other lifts the skin and delivers the injection.

VAGINAL CYTOLOGY

Vaginal smears are an effective technique to monitor the estrous cycle of female pocket gophers. Smears can be obtained through either vaginal lavage or swabbing. Performing this procedure also requires two individuals: one to restrain the animal and another to obtain the smear.

MEASUREMENT OF BODY TEMPERATURE

Body temperatures of pocket gophers have been obtained with rectal thermometers (Darden, 1972; McNab, 1966) and temperature radiotelemeters (Vleck,

1979). Accuracy of rectal temperature is questionable due to excessive struggling of the animal during the procedure (Vleck, 1979). Body temperatures obtained through radiotelemeters are lower than reported rectal temperatures (Vleck, 1979), but require surgical implantation, which can result in additional stress for the animal.

BIOLOGY

Unique Anatomic and Physiological Characteristics

Metabolism

Many aspects of the annual metabolic cycle of the pocket gopher have yet to be investigated. However, it has been established that pocket gophers are characterized by low basal metabolic rates and low thermal conductance (McNab, 1966; Vleck, 1979). In the wild, tunnel systems must be continuously lengthened as pocket gophers forage. Digging is an energetically expensive form of locomotion that can require 360–3400 times the energy needed to travel aboveground (Vleck, 1979). Thus, it has been proposed that a low resting metabolism is an adaptation of fossorial rodents to reduce overall energetic costs (McNab, 1966; Vleck, 1979). For example, mean rates of oxygen consumption of *Thomomys bottae* during burrowing ($2.8\text{--}7.1\text{ cm}^3\text{O}_2/(\text{g}\cdot\text{h})$) are much greater than the average resting metabolic rate at the same temperature ($0.84 \pm 0.16\text{ cm}^3\text{O}_2/(\text{g}\cdot\text{h})$; Vleck, 1979). Activity patterns alternate between short bursts of activity and periods of rest. Yet, circadian rhythms of oxygen consumption and activity do not appear to exist within the laboratory (Vleck, 1979).

Due to the relative stability of the subterranean ecotop, pocket gophers have a narrow range of thermoneutrality and cannot tolerate excessive heat. Pocket gophers effectively maintain a body temperature of approximately 36°C when exposed to ambient temperatures ranging from $5\text{--}25^\circ\text{C}$, (McNab, 1966; Vleck, 1979). Body temperatures elevate at ambient temperatures greater than 25°C . Ambient temperatures above 33°C can be fatal (McNab, 1966; Vleck, 1979). Signs of heat stress are easily identifiable. When exposed to excessive heat over a prolonged period, pocket gophers will modify their resting position. A stressed individual will rest either on its side or back. An unstressed animal usually rests on its feet, with its head and tail curled under its body.

Hydration

Pocket gophers do not consume free water and depend upon a diet of fresh vegetation for hydration. Further, they excrete very concentrated urine, similar

to that described for desert rodents (e.g., *Dipodomys ordii*; Howell and Gersh, 1935). Like desert rodents, it is probable that the production of such concentrated urine is an adaptation for life in an environment void of standing water (i.e., underground). Although the physiological processes responsible for water conservation in the pocket gopher are unknown, increased resorption within the urinary bladder or various ducts of the kidney are plausible. Similar mechanisms of water conservation have been described for numerous rodents inhabiting xeric environments (reviewed in Lawler and Geluso, 1986).

Pocket gopher urine is pale yellow in color with a thick, milky appearance. Animals will urinate frequently within the same location and the amount of urine produced can be modified by altering the amount of fresh vegetation provided. Dried urine can be difficult to remove from cage bottoms, requiring some additional effort on the part of the technician when cleaning tunnel systems.

Unique Natural Microenvironment

The microenvironmental conditions of the pocket gopher burrow differ from atmospheric conditions aboveground. Relative humidity of a tunnel system is frequently greater than 80% and oxygen content may be slightly lower than the atmospheric level (Kennerly, 1964). Further, carbon dioxide levels are exceedingly high and can range from 10–60 times atmospheric concentration (Kennerly, 1964). Captive pocket gophers experience no difficulty acclimating to standard laboratory conditions (0.04% CO₂; 21% O₂; humidity > 50%). However, if performing carbon dioxide euthanasia, pocket gophers require longer exposure to the gas than most laboratory rodents.

Behavior

Within captivity, patterns of activity and food consumption of the pocket gopher do not appear to change seasonally. Weight loss ranging from 1–18% of total body mass is common in newly captured animals. After a few weeks in captivity, weights at capture are usually surpassed and only slight weight fluctuations (2–5 g) are subsequently observed (DeVries and Sikes, 2009). Sick animals appear lethargic and will not perform routine behaviors (e.g., nest building, food caching, etc.).

Regardless of handling frequency, pocket gophers are aggressive rodents that do not become docile in captivity. Aggressive behavior does not appear to fluctuate seasonally or differ between the sexes. However, due to their greater size, males can be more difficult to handle than females. Individuals must be housed separately and interactions between pocket gophers should only occur under supervised conditions.

Normal Values

Longevity

From the author's experience, pocket gophers can be successfully maintained in captivity for 2–3 years with a maximum life expectancy of 3.5–4 years (unpublished data, 2009). In the wild, maximum longevity is 4 years and it has been suggested that females live longer than males (Howard and Childs, 1959).

Genetics

The confirmed number of pocket gopher species is in constant fluctuation (Steinberg and Patton, 2000). Many species are characterized by a diploid (2n) number ranging from 38–76 and a fundamental number varying from 68–102. Diploid and fundamental numbers differ among species and within species complexes (i.e., subspecies). In addition, hybrids between species occur frequently.

Hematology and Clinical Chemistry

Blood cell parameters and serum chemistry results have not yet been reported for pocket gophers.

Nutrition

Exact nutritional requirements have not yet been determined for pocket gophers. However, a diet of fresh vegetation effectively sustains animals in captivity (DeVries and Sikes, 2009). Dietary components include: sweet potatoes, Russet potatoes, carrots, apples, and bean sprouts. Considering pocket gophers depend upon the supplied diet for hydration, fresh vegetation must be provided daily. Alfalfa hay and rodent chow (Purina 5012) are also readily consumed and provide important trace minerals and vitamins. This recommended diet promotes seasonal molts, adequate fat deposition, and normal muscle development in juvenile and adult pocket gophers (DeVries and Sikes, 2009).

Reproduction

Overview and Breeding Season

Many species of pocket gopher have extended periods of reproduction (5⁺ months). Exact lengths of breeding seasons differ among taxa and can vary geographically within species. Males of some species (e.g., *Geomys bursarius*, *Geomys breviceps*; Pitts et al., 2005) are capable of reproducing year-round, but female receptivity appears to be restricted to December–October (Howard and Childs, 1959; Pitts et al., 2005). Pregnancy rates peak during February–April. Gestation periods vary from 18–50 days, depending upon species. Litter sizes range from 1–13 young (3–5 average) and most females give birth to only one litter per year. However, it has been suggested that older females in southern populations produce a

second litter at the end of the summer (Sealander and Heidt, 1990).

Male and female pocket gophers do not reach sexual maturity until approximately 8 months of age (Vaughn, 1962). Sex determination of individuals prior to sexual maturation (juveniles and subadults) is difficult. Palpating genitalia for the presence or absence of a baculum is the most effective method of quantifying the sex of immature individuals. Once sexually mature, the sexes are more readily distinguishable. Sexually mature males have a scrotal region and are larger in size than females. During the breeding season, testicular descent from the abdomen into the scrotum is common (Vaughn, 1962). Mature females have an open pubic symphysis and well-developed mammary glands. Sexual receptivity is indicated by the presence of a swollen vulva, open vagina, and cornified epithelial cells in vaginal smears (Andersen, 1978).

Male Reproduction

In the wild, most male pocket gophers are in breeding condition 2–3 months before females (Vaughn, 1962). Enlargement of the seminal vesicles, epididymides, and testes occurs in December–February. The scrotal region enlarges to accommodate testicular descent and/or the swollen epididymides. Unlike most mammals, the position of the testes might not be an accurate indicator of breeding condition in pocket gophers. Even though scrotal development occurs, many males have abdominal testes during the peak of the breeding season (Vaughn, 1962). Rather, testes size is a more reliable measure of reproductive potential. Within geomyids, testes range in length from 3–21 mm and the size at which spermatozoa are produced varies among species. For example, in *Geomys breviceps*, testes larger than 11 mm contain spermatozoa while in *Geomys bursarius*, testes must be 12.5 mm for sperm production (Pitts et al., 2005; Vaughn, 1962). Spermatozoa are produced until testicular regression, which usually occurs during the late summer and early fall. However, the onset of male breeding condition can be asynchronous within populations, resulting in the potential for year-round reproduction.

If maintaining a seasonal light cycle within the laboratory, most males will come into breeding condition during the late winter and early spring, similar to those in the wild. Reproductive organs enlarge and scrotal development occurs. Individuals with these physical characteristics might be viable candidates for reproductive attempts.

Female Reproduction

Vaginal swelling occurs and ovaries and uteri enlarge as females approach breeding condition (Howard and Childs, 1959; Vaughn, 1962). A swollen vulva and perforated vagina usually indicates a reproductively

receptive individual. Reduced genital swelling and a closed vagina indicate anestrus.

Stages of the estrus cycle can be quantified with vaginal smears. Smears taken during proestrus contain many nucleated epithelial cells and few leukocytes. Estrus smears are dominated by cornified epithelial cells. During metestrus, some cornified cells are present and leukocytes greatly increase in number. Diestrus smears are characterized by moderate leukocyte numbers and an increase in nucleated epithelial cells (Desy and Druecker, 1979). Smears can be obtained by removing cells from the walls of the vagina with a moist tapered swab or by flushing the vagina with distilled water. Vaginal solutions should be transferred to a slide and stained for microscopic observation.

Mating is a lengthy process for pocket gophers. If pairs are tolerant of one another, copulatory behavior is exhibited almost immediately (within 5 minutes) upon introduction. During intromission, males mount females and perform rapid pelvic thrusts. Soft vocalizations, such as purrs and/or squeaks, are frequently emitted (DeVries and Sikes, 2008). Intromission attempts have been reported to range from 5–29 within an hour period with each attempt lasting 15–30 seconds (Andersen, 1978; Schramm, 1961). Repeated copulation might be necessary to induce ovulation in pocket gophers as has been suggested for other solitary subterranean rodents (Bennett et al., 2000). Copulatory plugs have been reported in some species (Howard and Childs, 1959).

During copulation, males will exhibit forceful, but non-aggressive behavior, such as grasping females with their teeth and/or forelimbs. However, intolerant pairs will attempt to fight and should be separated immediately. Aggressive behavior may also be displayed at the conclusion of a copulatory period, requiring separation of the pair. Consequently, supervision is necessary during reproductive attempts and all individuals should be thoroughly examined following such encounters.

Laboratory Breeding and Female Fertility

Large breeding colonies of pocket gophers have yet to be established. However, several litters of *Thomomys bottae* (Schramm, 1961) and *Thomomys talpoides* (Andersen, 1978) have been produced in captivity, suggesting that the establishment of breeding colonies is possible.

HOUSING AND PAIR SELECTION

Breeding in captivity has been achieved by pairing scrotal males with receptive females (see above) in neutral locations (e.g., cage, terrarium, etc.). Some pairs are intolerant of one another and exhibit aggressive behavior (Schramm, 1961). Pairs that are tolerant usually copulate. Copulatory behavior can be performed by novel individuals (Schramm, 1961), but, it has been suggested that repeated introductions of potential pair

members could increase breeding success (Andersen, 1978). Considering that olfactory cues could be important in coordinating reproduction in pocket gophers (DeVries, 2007), copulatory behavior might be stimulated by placing feces and urine of receptive females within the simulated tunnel systems of males. After reproductive attempts, pair members should be separated and returned to their individual tunnel systems.

PREGNANCY AND PARTURITION

Once females have copulated, they should be provided sufficient nesting material and subjected to minimal handling. Late-stage pregnancy can be detected by palpation and the presence of enlarged mammary glands. Gestation periods are highly variable among taxa, but of the species that have bred successfully in captivity, parturition occurs 18 days after mating (Andersen, 1978; Schramm, 1961). Females remain within nests for the 1–2-hour process of parturition (Barrington, 1942; Schramm, 1961).

NEONATE GROWTH AND DEVELOPMENT

Pocket gophers give birth to 1–13 altricial young per litter. Newborn pocket gophers are hairless with pinnae follicles present on the head. Eyes, ears, and cheek pouches are closed and forelimbs are not clawed. Newborn weights range from 2.7–6.5 grams, depending on species (Andersen, 1978; Schramm, 1961). Birth weight is negatively impacted by increased litter size (Andersen, 1978).

Neonatal growth is rapid. Offspring of *Thomomys talpoides* acquire juvenile pelage and foreclaws within the first 2 weeks of development and can eat solid food at 17 days old (Andersen, 1978). Eyes, ears, and cheek pouches are open at approximately 3 weeks of age. Litter mates require separation at 2 months and individuals are approximately adult size and begin acquiring adult pelage by month 3 (Andersen, 1978).

NEONATAL MORTALITY

Pocket gophers born in captivity often die due to exposure as females appear hesitant to retrieve young that leave the nest (Andersen, 1978). Many of these litters were housed in open cages with little nesting material (Andersen, 1978; Barrington, 1942). Housing litters within simulated tunnel systems might better protect offspring from exposure and encourage more natural behavior from the mother. Further, adequate nesting material should be supplied and handling of newborn individuals kept to a minimum.

NUTRITION

Fresh vegetation appears to meet the nutritional needs of pregnant and nursing females and developing offspring. No dietary alterations have been described.

DISEASES

Colony Health

Being a solitary mammal, pocket gophers do not readily transmit disease. Minor health issues common to newly captured animals, such as ectoparasite infestations or skin irritations, are eliminated during quarantine. Existing endoparasite infections are also terminated before introducing individuals into the colony. Injuries can occur during reproductive attempts, but most are not severe if individuals are not allowed to exhibit excessive aggression. Dental problems, such as incisor breakage or malocclusions, can occur. Malocclusions are rare, but can cause serious threat to an individual's health. If properly clipped, nail injuries are minimal.

Once established, maintaining the health of a pocket gopher colony is not difficult. For example, a large colony of *Geomys breviceps* (26 animals; 11 males, 15 females) was maintained by the author for nearly 3 years using many of the aforementioned husbandry techniques. Upon termination of the colony, all individuals were determined to be free of internal parasites, with no indication of infectious, degenerative, or neoplastic disease processes at necropsy.

Infectious Diseases

Due to their solitary nature, pocket gophers are not readily susceptible to bacterial or viral disease. However, parasitic infections are common in newly captured individuals.

Parasitic Disease

Wild pocket gophers harbor numerous external and internal parasites (Hafner et al., 2000). Common parasites include fleas, mites, chewing lice, roundworms, and tapeworms. Existing infestations are terminated before new individuals are introduced into the colony. Ectoparasites are eliminated through the application of insecticidal powder. Weight-appropriate dosages of ivermectin appear to exterminate internal parasites. Housing animals within individual caging systems helps prevent subsequent infections. Parasites to which colony-born individuals are susceptible are unknown.

PROTOZOA

Coccidia, such as *Eimeria thomomysis* and *Eimeria fitzgeraldi*, have been found in the fecal pellets of multiple geomyids (Gardner, 1983; Todd et al., 1971).

NEMATODES

Both *Ransomus rodentorum* and *Trichuris fossor* have been found in the cecum of wild pocket gophers (Gardner, 1985; Todd et al., 1971). *Trichuris fossor* has also

been identified within the large intestine (Gardner, 1985; Todd et al., 1971). *Longistriata vexillata* (Todd et al., 1971) and *Heligmosomoides thomomyos* (Gardner, 1985) are harbored within the small intestine and *Protospirura ascaroidea* within the stomach (Todd et al., 1971). *Capillaria hepatica* is occasionally found in the liver (Todd et al., 1971).

CESTODES

Several tapeworm species have been identified in pocket gophers. Taxa residing in the small intestine include: *Catenotaenia linsdalei*, *Paranoplocephala variabilis*, and *Paranoplocephala infrequens* (Todd et al., 1971), as well as *Hymenolepsis horrida* and *Hymenolepis tualatinensis* (Gardner, 1985).

EXTERNAL PARASITES

Pocket gophers are host to many species of ectoparasites and new parasitic taxa are continuously discovered. Those reported include: chewing lice (Trichodectidae: *Geomydoecus*, *Thomomydoecus*), fleas (Ceratophyllidae: *Dactylopsylla*, *Foxella*; Pulicidae: *Pulex*), mites (Haemogomastinae: *Ischyropoda*; Pygmephoridae: *Pygmephorus*; Listrophoridae: *Geomylichus*), and ticks (Ixodidae: *Amblyomma*, *Haemaphysalis*, *Ixodes*). Many external parasites of pocket gophers are host-specific, providing the potential for host-parasite coevolution (Hafner et al., 2000). Consequently, the relationships of pocket gophers and their ectoparasites receive much investigative attention.

Traumatic Disorders

Bite wounds frequently occur during reproductive attempts. Most are not severe and can be treated with topical disinfectants. In addition to bite wounds, incisors can be broken and foreclaws can rupture eyes, resulting in blindness. Consequently, all measures should be taken to reduce aggressive interactions.

Dental Problems

Broken incisors and malocclusions occasionally occur. Such problems can result in difficulty eating and thus, poor condition. Unfortunately, malocclusions are difficult to diagnose and are often not detected until necropsy.

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White-Tailed Rat

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INTRODUCTION

Mystromys albicaudatus is a rodent species rarely used in contemporary research. This species is also declining in the wild. While the status of *Mystromys* has not been evaluated and is not included in the Convention on International Trade in Endangered Species (CITES) appendices, it is listed as an endangered by the International Union for Conservation of Nature (IUCN) due to declining populations and loss of habitat (IUCN, 2006). This chapter is included to provide historical information as well as update any new findings published for this species. Recognizing that *Mystromys* can be maintained in captivity and given the declining wild populations, the information provided in this chapter may also be useful to maintain captive-bred populations for conservation purposes in addition to biomedical research purposes.

TAXONOMY, HISTORY, AND GENETICS

Mystromys albicaudatus is commonly referred to as the white-tailed rat or less frequently the white-tailed mouse. This species is the only living species in the

genus *Mystromys* and the subfamily Mystromyinae. Recent phylogenetic studies place this species in the family Nesomyidae and show that this species does not belong in the hamster family Cricetinae (Steppan et al., 2004). The common name, South African hamster, is now considered a misnomer for this species. Wild populations of *Mystromys* are found in the savanna grasslands of South Africa where it lives in burrows in the ground or shelters in cracks in the soil.

The first research colony was established in 1941 by Davis at the South African Institute of Medical Research (Hall et al., 1967). Animals from this colony were subsequently transferred to the Armed Forces Institute of Pathology (AFIP) in 1962 and 1969 (Howell and Hall, 1977). Additional colonies were established from dissemination of the AFIP colony to several other institutions in the United States. However, the last research publication in the US utilizing this species occurred in 1987 and several research colonies established in the 1960s and 1970s in the US are no longer in existence. Research colonies may still exist in Africa, although most current publications are based on wild-caught animals.

Mystromys albicaudatus has 32 chromosomes consisting of 30 autosomes and the two sex chromosomes X and Y (Srivastava et al., 1974). The X chromosome

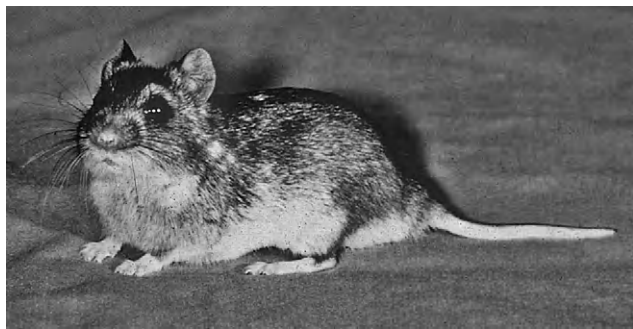


FIGURE 51.1 *Mystromys albicaudatus*.

is the longest and the Y chromosome is the smallest of the 32 chromosomes. G banding patterns have been established (Srivastava et al., 1974). While earlier studies focused on trying to establish genetic relationship of *Mystromys* to other hamster species through G-banding analysis, newer sequencing studies of five independent genes have resulted in the placement of *Mystromys* in the family Nesomyidae (Gamperl et al., 1978; Jansa et al., 1999; Steppan et al., 2004).

ANATOMY, PHYSIOLOGY, AND
BEHAVIOR

In the wild, *Mystromys* is a burrowing rodent that is nocturnal and solitary (Nowak, 1999); however, it adapts well to captivity and is described as quick, active and occasionally acrobatic (Hall et al., 1967). It is a medium-sized rodent with gray soft luxurious textured fur with shades of brown over the back and shoulder and a creamy-white-to-gray undercoat on the tail, feet, and belly (Figure 51.1). *Mystromys* has large rounded erect ears, lacks cheek pouches, and has eyes that are dark and prominent. Body weight for adult females averages 95 g (75–125 g range); and males average 145 g (105–185 g range) (Hall et al., 1967). The tail is short and is about 30% of the body length. Life span is relatively long compared to other rodents with an average lifespan of 2.4 years with an upper limit of 6.2 years.

The most unique anatomical features of *Mystromys* include the lack of a gall bladder and the anatomy of the stomach. The stomach is divided into two distinct compartments by an annular muscular band (Hall et al., 1967; Mahida and Perrin, 1993; Vorontsov, 2003). The forestomach is covered with tall keratin papillae having the gross appearance of a rumen, and the stomach was thought for several decades to have a fermentation function. Mahida and Perrin (1994) determined that fermentation occurs in the cecum, classifying *Mystromys* as a hind-gut fermenter (Mahida and Perrin, 1994). The papillae arise from the horny layer of the stratum



FIGURE 51.2 Stomach of *Mystromys albicaudatus*. Arrowhead denotes smooth glandular region and arrow denotes non-glandular region containing tall keratin papillae.

TABLE 51.1 Organ Weights for Female and Male *Mystromys Albicaudatus*

Organ	Female ^a	Male ^a
Body weight	82.90 (16.10)	110.00 (23.80)
Heart	0.39 (0.08)	0.45 (0.08)
Liver	4.37 (.98)	5.10 (1.46)
Spleen	0.13 (0.04)	0.14 (0.03)
Kidney	0.37 (0.06)	0.43 (0.08)
Ovary/testes	0.05 (0.02)	1.77 (.250)
Brain	1.33 (0.08)	1.36 (0.08)
Lung	0.69 (0.18)	0.79 (0.19)
Pancreas	0.32 (0.09)	0.38 (0.08)

^aMean organ weight in grams with standard deviation in parentheses.

corneum and consist of stratified squamous epithelium and the periphery of the papillae are heavily laden with bacteria. It is thought that this adaptation might be more important to fat digestion and may compensate for the absence of a gall bladder in this species (Mahida and Perrin, 1993). The glandular portion of the stomach is similar to that found in other rodents (Figure 51.2).

Mystromys has two pairs of inguinal mammae that the young attach to soon after birth and to which they remain attached until the third week of life (neonatal tenacious nipple attachment). A large subcutaneous sebaceous gland is posterior to the mammae and extends to the vulva. Females have a rudimentary prostate and males have an os penis (Hall et al., 1967). Typical organs weights are found in Table 51.1; and

TABLE 51.2 Hematology Values for Female and Male *Mystromys albicaudatus*

	Female ^a		Male ^a	
	3–5 Months	13–24 Months	3–5 Months	13–24 Months
Erythrocyte count ^b	4.88 (0.56)	4.56 (1.02)	6.27 (0.72)	5.40 (0.70)
Hemoglobin (g/dl)	14.0 (1.0)	12.9 (1.4)	14.6 (0.9)	14.4 (1.3)
PCV (%)	43.4 (3.0)	39.7 (3.1)	45.5 (2.3)	45.7 (2.6)
WBC count ^c	10.2 (4.0)	9.6 (4.9)	12.5 (3.8)	9.1 (2.8)
Neutrophils ^c	1.2 (0.9)	1.9 (2.4)	1.5 (1.3)	1.5 (1.2)
Lymphocytes ^c	8.5 (3.2)	7.1 (2.6)	10.4 (2.7)	7.1 (2.3)
Monocytes ^c	0.1 (0.1)	0.1 (0.1)	0.2 (0.2)	0.1 (0.1)
Eosinophils ^c	0.4 (0.1)	0.4 (0.2)	0.4 (0.2)	0.4 (0.2)
Basophils ^c	0	0	0	0

^aMean value with standard deviation in parentheses.^bCell count $\times 10^{12}$ cells/L.^cCell count $\times 10^9$ cells/L.**TABLE 51.3** Serum Chemistry Values for Female and Male *Mystromys albicaudatus*

	Female ^a	Male ^a
Serum chloride (mEq/l)	107.6 (3.45)	104.0 (2.75)
Serum sodium (mEq/l)	145.1 (2.72)	147.3 (1.74)
Serum potassium (mEq/l)	5.43 (0.92)	5.31 (0.78)
Serum calcium (mEq/l)	9.27 (1.03)	9.50 (0.43)
Serum glucose (mEq/l)	99.05 (26.80)	91.0 (18.8)
Serum urea nitrogen (mg/dl)	19.6 (3.94)	19.0 (1.41)
Total protein (g/dl)	6.91 (0.46)	6.86 (0.56)

^aMean value with standard deviation in parentheses.^bmEq/l.^cCell count $\times 10^9$ cells/L.**TABLE 51.4** Urinalysis Values for *Mystromys albicaudatus*

Urine	Mean (Standard Deviation)
pH	8.52 (0.69)
Protein (mg/dl)	6.17 (2.77)
Refractive index	1.3469 (0.005)
Specific gravity (g/ml)	1.036 (0.014)
Volume (ml/h/g body wt)	1.9×10^{-3} (1.0×10^{-4})

Tables 51.2–51.4 contain normal hematology, serum chemistry and urinalysis values (Becker and Middleton, 1979; Becker et al., 1979; Cantrell and Padovan, 1987).

MANAGEMENT, HUSBANDRY, AND COLONY HEALTH

Mystromys can be housed in standard caging sufficient for rats. Animals are generally housed in monogamous lifetime breeder pairs, as mixing of adults results in fighting. Pairs are monogamous and fighting may occur when new mates are introduced if a partner is removed or dies. Offspring can be weaned at 25 days of age and typically weigh 25g. Weaned animals from different litters can be co-housed. New animals should not be introduced to cages after 2 months of age, as fighting occurs with new introductions after this age. Recommended housing space is 70 sq inches per breeding pair and 200 sq inches per four weaned offspring (Hall et al., 1967). Overcrowding results in increased fighting.

Environmental conditions are typical of those for other rodents. A light cycle of 12 hours light and 12 hours dark has been utilized for breeding colonies. In the wild, the diet consists mainly of seeds with also vegetable material and insects. In captivity, *Mystromys* can be fed standard rodent chow and water ad libitum (Hall et al., 1967). Parents will eat offspring that die.

Mystromys become sexually mature between 4–7 months of age and the estrous cycle occurs at 4–9-day intervals. *Mystromys* are considered to be non-seasonal breeders and new litters can be born year-round. Gestation averages 38 days with a range of 36–39 days. *Mystromys* offspring are born hairless and the eyes open around days 16–20. Soon after birth the young attach to mammae and remain attached for approximately 3 weeks. During this time the female drags the young around (Figure 51.3). After this 3-week period they continue to nurse intermittently for another 2–3 weeks. If allowed to remain with the parents, the female will naturally wean the young at 5–6 weeks old, and offspring will weigh around 40g at the time of natural weaning. They can be weaned as early as 25 days of age or at 25g (Hall et al., 1967). This early weaning facilitates regrouping animals from multiple litters. Average litter size is three pups with a range of 1–5 offspring. Typical survival rate at weaning is 80%.

BASIC EXPERIMENTAL METHODS

Mystromys has been used infrequently in research. The majority of studies currently utilizing *Mystromys* are behavioral and biology-related studies not requiring experimental manipulation. Animals should not



FIGURE 51.3 *Mystromys albicaudatus* female with attached offspring. Offspring remain firmly attached to nipples even when female is picked up and the offspring weight is only supported by nipple attachment.

be handled by the tail as the tail is short and the skin is thin and easily damaged, which can result in tail necrosis and/or tail slip. Animals should be grasped from above, around the thorax or abdomen. While animals are not prone to biting, heavy gloves are recommended for handling because they may resist handling. Blood collection techniques listed for *Mystromys* include cardiac collection and tail vein collection utilizing a vacuum apparatus (Hall et al., 1967; Packer et al., 1970). Pentobarbital anesthesia induced by an intraperitoneal route has been described (Padovan, 1985).

DISEASES AND VETERINARY CARE

Very little information has been reported for infectious diseases occurring naturally in *Mystromys*. Hall

et al. (1967) reported a single case each of septicemia, granulomatous retinitis, granulomatous pneumonitis, and suppurative pneumonitis, but no etiologic agent was identified in these four affected animals. Cilia-associated respiratory (CAR) bacillus has been noted in *Mystromys* with chronic pulmonary lesions. However, it is unknown if this CAR bacillus species is the same species infecting laboratory mice and rats, or if it is a separate species (Institute for Laboratory Research, 1991). *Mystromys* has been experimentally infected with *Clostridium piliforme*, indicating that *Mystromys* is susceptible to infection (Waggie et al., 1986). Experimentally infected animals had lesions characterized by focal necrosis and inflammation in the intestine, liver, heart, and brain.

Neoplasia is an infrequent finding in *Mystromys*, and was generally found in animals 3 years of age or older. Tumors found in one colony studied over a number of years included perianal squamous cell carcinoma, adnexal tumor of the skin, osteosarcoma, leiomyosarcoma, hepatic adenocarcinoma, hepatomas, and pituitary gland adenoma (Rantanen and Highman, 1970). *Mystromys* appear to be relatively resistant to chemically induced neoplasia as compared to rats, mice, hamsters, and *Mastomys* (Roebuck and Longnecker, 1979; Yamamoto et al., 1972).

Several other non-infectious and non-neoplastic diseases have been reported in *Mystromys*. Ringtail has been described in pre-weanling aged animals, and is associated with low environmental humidity (Stuhlman and Wagner, 1971). A fatal enteric syndrome associated with topical triple antibiotic ointment containing polymyxin B sulfate, bacitracin, and neomycin sulfate has been documented in *Mystromys* (LaRegina et al., 1978). Affected animals had ruffled fur, anorexia, and increased respiration. Necropsy findings consisted of congestion and inflammation of the cecum, dilation, and petechia in the ileum and colon.

Genetically related disorders reported in *Mystromys* include partial oculocutaneous albinism and diabetes mellitus. Partial oculocutaneous albinism is characterized by a decrease in melanin pigment in the structures of the eye and skin of the head (Priour et al., 1979; Rodrigues et al., 1971). Diabetes mellitus has been noted to occur in colonies at varying incidences ranging from less than 3% to 22% (Hallett and Politzer, 1972; Packer et al., 1970). Affected animals are polydipsic and polyuric, and show abnormally wet cages. Diabetes is not related to obesity and onset occurs by 4 months of age, suggestive of type I diabetes. Diabetes has been linked to other secondary diseases in *Mystromys*, including cataracts and renal glomerular lesions, making this a suitable model for type I diabetes mellitus research (Stuhlman, 1979).

EXPERIMENTAL MODELS

Mystromys was first used in research primarily to determine susceptibility of the species to a number of viral, bacterial, and parasitic pathogens. Viremia does not occur with Wesselsbron, Spondweni, Pongola, West Nile, or Crimean-Congo hemorrhagic fever viruses but occurs and lasts 5 days or less with Bunyamera, Chikungunya, Rift Valley Fever, Middleburg, and Simbu viruses without clinical illness (Jupp et al., 1981; McIntosh, 1961; Shepherd et al., 1989). *Mystromys* is resistant to infection with *Mycobacterium leprae* and *Mycobacterium microti*, but susceptible to *Yersinia pestis* and *Clostridium piliforme* (Davis, 1963; Grasset et al., 1946; Waggle et al., 1986). *Mystromys* is also susceptible to infection with some species of *Leishmania* and is a suitable model for cutaneous leishmaniasis caused by *Leishmania braziliensis* (Beacham et al., 1982; Franke et al., 1985; McKinney and Hendricks, 1980; Sayles et al., 1981). *Leishmania donovani* can be propagated in *Mystromys* (Mikhail and Mansour, 1973). *Mystromys* has also been used in *Schistosoma mansoni* and *Schistosoma haematobium* studies (Pitchford and Visser, 1960, 1962).

Mystromys has been used in dental caries research in which it was found that a combination of a high-sucrose diet with a caries-associated strain of *Streptococcus* was necessary to produce dental lesions (Larson and Fitzgerald, 1968). However Larson indicated that *Mystromys* was more difficult to handle, had smaller litter sizes, and less flexible breeding habits when compared to rats and did not offer any compelling reason for use when compared to rats and hamsters for dental caries studies. *Mystromys* has also been proposed for studies utilizing mitogen-induced T-lymphocyte cultures due to the robust response to mitogen stimulants (Howell and Hall, 1977).

Diabetes mellitus has been extensively characterized in *Mystromys* and at one time *Mystromys* was utilized as a model for human diabetes mellitus type I research (Packer et al., 1970; Stuhlman, 1979; Stuhlman et al., 1974). In *Mystromys*, diabetes is unrelated to obesity or diet, and is characterized by polydipsia, polyuria, hyperglycemia, and glycosuria (Packer et al., 1970). Pancreatic lesions in affected animals include vacuolization and glycogen deposits in beta cells. In more severe cases of hyperglycemia, pancreatic beta cells exhibit degeneration, nuclear pyknosis, and beta cell death (Goeken et al., 1972; Stuhlman et al., 1975). Glomerular changes include capillary basement membrane thickening and mesangial proliferation (Riley et al., 1975). Other changes include skeletal muscle capillary microangiopathy and cataract development (Stuhlman, 1979; Yesus et al., 1976). Thus diabetes mellitus in *Mystromys* has a number of features similar to diabetes mellitus

type I in humans. While this animal model may be useful for the study of human diabetes, the colony at the University of Missouri in which the diabetes was characterized is no longer in existence. Diabetes incidence in other *Mystromys* colonies has been reported to be less than 3%, and this model may be difficult to re-establish.

There are no biomedical research publications for *Mystromys* since the late 1980s and current publications focus more on the comparative biology of *Mystromys*. Swimming behavior studies, thermal biology characterization, and micromammal remains of *Mystromys* as a model of evolution related to environmental conditions have been described for *Mystromys* in more recent publications (Avery, 2001; Downs and Perrin, 1995; Hickman and Machine, 1986).

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Gerbils

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INTRODUCTION

The introduction and development of the Mongolian gerbil, *Meriones unguiculatus*, as a laboratory animal is recent, compared to other rodents. The gerbil is usually non-aggressive and is one of the easiest rodents to maintain and handle. Its disposition, curious nature, relative freedom from naturally occurring infectious diseases, and adaptability to its environment have contributed to its popularity as a laboratory animal (Wagner and Farrar, 1987). It has several interesting anatomical and physiological characteristics that make it a useful model in biomedical research. Several other species of gerbils (e.g. *Meriones libycus*, *Meriones crassus*) have been used as experimental animals (Belhocine et al., 2010; Khokhlova et al., 2009). However, the Mongolian gerbil (*Meriones unguiculatus*), on which this chapter will focus, is the most common species of gerbil used in biomedical research (Schwentker, 1963).

TAXONOMY AND HISTORY

Taxonomy

It should be noted that mammalian taxonomy is a rapidly changing field. The following description outlines a classification of the Mongolian gerbil based on morphology as well as more recent molecular techniques. Gerbils are in the class Mammalia and order Rodentia. Rodents are divided into five major suborders (Musser and Carleton, 2005). Gerbils are part of the suborder Myomorpha, originally so named because the deep and lateral masseter muscles attach to the front of the muzzle, giving it a forward thrust. Myomorphs also lack premolar teeth (Hurst, 1999). Gerbils belong to the superfamily Muroidea, family Muridae, and subfamily Gerbillinae based on morphology and on evaluation of nuclear protein coding sequences of the Lecithin Cholesterol Acyl Transferase gene and the von Willebrand factor gene (Michaux et al., 2001; Robinson, 1975). The genus *Meriones* was first described by Illiger in 1811, and *Meriones unguiculatus* was first identified in

1867 by Milne-Edwards (Robinson, 1975; Thiessen and Yahr, 1977). Chaworth-Musters and Ellerman (1947) provided a comprehensive description of the genus *Meriones* which was updated by Ellerman and Morrison-Scott (1951), Corbet (1978), and Pavlinov et al. (1990). The genus name, *Meriones*, was derived from a Greek warrior who wore a battle helmet decorated with boar tusks (Robinson, 1975). The species name, *unguiculatus*, is Latin for clawed or fingernail leading to one of the common names, clawed jird. The name gerbil is from the Arabic word, yarbu, which refers to saltatorial, desert-inhabiting rodents. Yarbu was translated into Latin as gerbo and into English as gerbil (Robinson, 1975).

Origin, Domestication, and Geographical Distribution

Approximately 15 genera and 81 species of gerbils are known (Agren, 1986). Gerbils are found in deserts and semi-arid geographical regions of the world (Field and Sibold, 1999; Thiessen and Yahr, 1977). They are native to northern Africa, India, Mongolia, southwestern and central Asia, northeastern China, and regions of Eastern Europe (Robinson, 1976). Because wild gerbils live in arid habitats they dig burrows that extend between 50cm and 1.5m below the surface so the temperature is relatively constant throughout the day and night. The burrows may be small and simple with one entrance or complex with eight to 14 entrances (Agren, 1986; Brain, 1999). The domestication of the Mongolian gerbil is a relatively recent occurrence. They were found by a French missionary, Father Armand David, who traveled extensively in Mongolia and China in the 1860s. Some wild-caught stock were sent to Japan, where they were bred freely (Alderton, 1986). The Mongolian gerbils that are available today originated from 20 pairs of captured animals that were maintained in 1935 by Dr. C. Kasuga in a closed, random-bred colony at the Kitasato Institute in Japan. In 1949 a sub-colony of gerbils was established at the Central Laboratories for Experimental Animals in Tokyo by M. Nomura. In 1954 Dr. V. Schwentker imported 11 pairs from Japan. Five females and four males from this group were

successfully bred and formed a foundation colony which produced gerbils for distribution throughout the United States (Robinson, 1979). This foundation colony was established at Tumblebrook Farm at Brant Lake, NY, founded by Dr. Schwentker. After his retirement in 1971, the Brant Lake facilities were phased out, and the Tumblebrook Farm gerbil production colony was relocated to new facilities in West Brookfield, MA under the ownership of D.G. Robinson. The name "Tumblebrook Farm" was retained (Robinson, 1974). A sub-colony established from seven pairs of animals by Dr. J.H. Marston at the Worcester Foundation for Experimental Biology in Shrewsbury, MA during 1961–62 was the origin of the sub-colony established at the University of Birmingham, England in 1964. This breeding stock produced the animals used in laboratories throughout the United Kingdom and Europe. Charles River Laboratories purchased Tumblebrook Farm in 1996 giving rise to the Crl:(MON)BR strain.

Genetics

There is a low amount of genetic variability present in laboratory gerbils compared to wild gerbils as a result of their origination from a few founder animals (Razzoli et al., 2003). Recently, Neumann et al. (2001) identified the first polymorphic dinucleotide repeat loci in Mongolian gerbils by a microsatellite technique. These studies demonstrated that there has been minimal genetic variability identified in the Mongolian gerbil used in research, even though there are several different lines or strains. Razzoli et al. (2003) confirmed these results using an amplified fragment length polymorphism technique to characterize and detect strain-specific polymorphisms in gerbils. Prior to these studies, relatively few genetic studies had been performed.

The common agouti or mixed brown Mongolian gerbil stock is used in research and sold as pets. The agouti has given rise to black (Cramlet et al., 1974; Waring and Poole, 1980), albino (Hedges, 1977), piebald, dove, cinnamon, and a "hairless" mutant (Alderton, 1986; Matsuzaki et al., 1989b). An inbred strain (Mon/Tum) is also available (Brain, 1999). There are also seizure-sensitive and seizure-resistant lines of gerbils (Loskota et al., 1974).

At the time of publication, Mongolian gerbils are commercially available in the United States from Charles River Laboratories, Inc., Wilmington, MA and Harlan, Indianapolis, IN.

ANATOMY, PHYSIOLOGY, AND BEHAVIOR

Basic Features

Gerbils have several unique anatomical and physiological features. Mature gerbils are smaller than rats, but



FIGURE 52.1 Size comparison between an adult rat (top), gerbil, and mouse.



FIGURE 52.2 Male and female gerbils are easily differentiated by the male's greater anogenital distance and pigmented scrotum (left, female; right, male).

larger than mice (Figure 52.1). Adults of both sexes vary between 11.5–14.5 cm in body length with males weighing an average of 100 grams and females weighing an average of 87.5 grams (Kramer, 1964). Body weights vary among colonies and this variation may be a genetic phenomenon as well as a dietary factor (Arrington et al., 1973). In adults, the sexes are easily differentiated by the male's longer anogenital distance, prominent testicles, and pigmented scrotum (Figure 52.2). Females have four pair of mammae, two inguinal and two thoracic, and the urethra is located outside the vagina (Wagner and Farrar, 1987).

External Features

External anatomical features are depicted in Figure 52.3. Cranial characteristics are consistent with saltatorial rodents. They have broad, short heads with prominent ears and large, black, slightly bulging eyes. Most

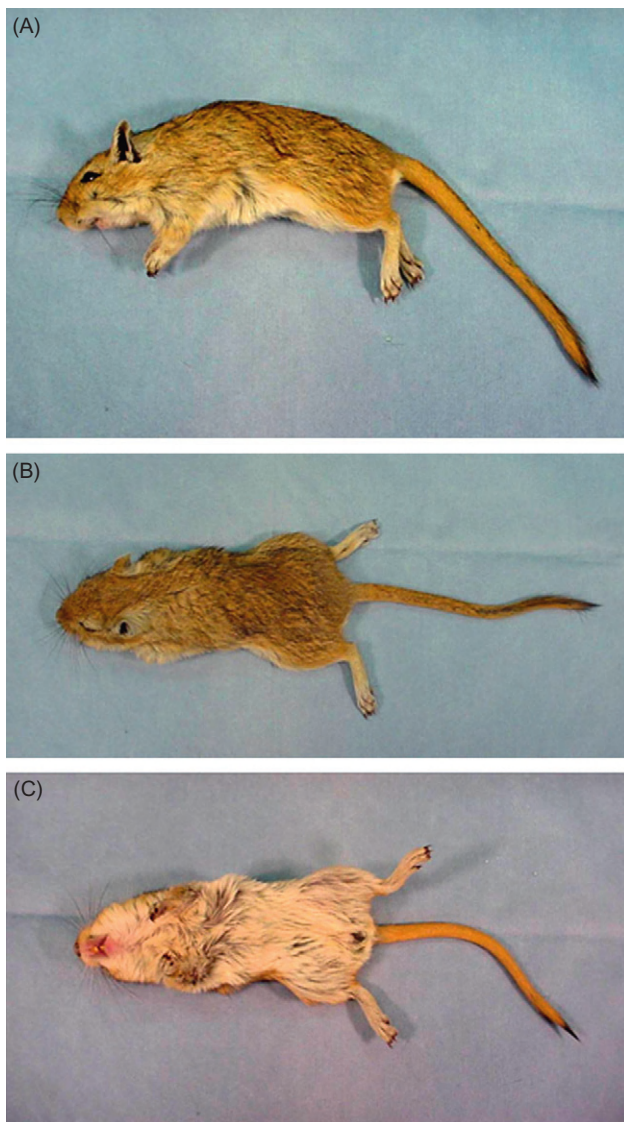


FIGURE 52.3 External anatomy of the gerbil. (A) Lateral view. (B) Dorsal view. (C) Ventral view.

mice and rats have longer, extended faces. The large eyes are an adaptation found in nocturnal animals, and their vision is very well developed (Alderton, 1986). Gerbils show active periods during day and night, and use both rod- and cone-based vision.

Gerbils generally have reddish brown fur with black outer tips on their backs, but coat color can vary from tan to gray, with a gray or creamy white undercoat. The tail is covered with fur that is short at the base and progressively becomes longer and bushy toward the tip of the tail. The tail length ranges from 10.0–19.3 cm (Norris and Adams, 1972c).

Musculoskeletal System

The hindlimbs are elongated, and the forefeet are relatively small. The hindlimbs are very muscular, and

aid in the gerbil's ability to jump considerable distances in proportion to their size. Gerbils use jumping or hopping as a form of ambulation (Brain, 1999). Gerbils' hind paws are not equipped with friction pads or opposable toes, and the soles are covered with fur; therefore, they cannot climb like mice (Roger and Polioudakis, 1977).

Special Senses

The gerbil's sense of smell is very keen and well developed. Gerbils have been shown to use a variety of odors as discriminative social cues from urine, ventral sebaceous glandular secretions, and Harderian gland secretions (Halpin, 1974; Thiessen et al., 1976; Thiessen and Yahr, 1977). Mongolian gerbils are attracted to saliva and use salivary cues to discriminate between siblings and non-siblings, and females use oral cues in the selection of sociosexual partners (Smith and Block, 1991). The Harderian gland functions as a potential site for immune response, serves as a part of the retinal–pineal axis, acts to cushion the eyeball, secretes lubrication for the eye, and is a source for pheromones and thermoregulatory lipids (Johnston et al., 1983; Sakai, 1981). Harderian gland secretions, comprised of lipids, proteins, and protoporphyrin, are carried by an excretory duct to the medial aspect of the nictitating membrane. These secretions bathe the eye and conjunctival space and are transported down the nasolacrimal duct, exiting at the external nares. The Harderian secretions are mixed with saliva and spread over the pelage during grooming (Thiessen, 1977).

Compared with mice, gerbils possess a much higher proportion of cones to rods. Since the gerbil retina is not exclusively rod-dominated, it is a valuable model for *in vitro* studies of retinogenesis (Bytyqi and Layer, 2005).

The tympanic bullae are prominent, giving the gerbil remarkable hearing with a high frequency peak of 50 kHz (Johnson and Marcotti, 2008).

Digestive System

The dental formula is 2 (incisors 1/1, canines 0/0, premolars 0/0, molars 3/3) = 16. The incisors are hypsodont (long crown) and elodont (continuously growing and erupting teeth that do not develop anatomical roots). The molars in gerbils are brachydont (short crown), rooted, anelodont (limited growth period) and cease to grow in mature animals. They are prone to dental caries, periodontal disease, and can develop incisor malocclusion (Field and Sibold, 1999).

The Mongolian gerbil's liver enzymes which are involved in cholesterol metabolism and hepatic cholesterol ester storage differ from those found in other rodents and make the gerbil an excellent model for hypercholesterolemia research (Norris, 1987; Temmerman et al., 1989). The gerbil's serum cholesterol

level is very sensitive to increased dietary cholesterol. Gerbils are resistant to atheromatous changes on high-cholesterol diets, but they can develop hepatic lipidosis and cholesterol gallstones when fed these diets (Vincent et al., 1979).

Circulatory System

Approximately 40% of Mongolian gerbils have an incomplete circle of Willis that allows for a reliable development of focal cerebral ischemia which is used to study the pathophysiology and treatment of ischemic stroke (Başkaya et al., 1999). When one carotid artery is ligated, a cerebral infarct on the side ipsilateral to the ligation is formed (Vincent et al., 1979). Gerbils have less collateral blood supply to the brain compared to mice and rats (Vincent et al., 1979).

Respiratory System

The respiratory system is very similar to other rodents in that gerbils do not appear to have respiratory bronchioles in the lung (Bal and Ghoshal, 1988). The right lung is composed of four lobes, while the left lung has three lobes (Williams, 1974).

Genitourinary System

Since they are desert animals, gerbils have several characteristics that have allowed them to adapt to dry environments. Gerbils have an excellent ability for thermoregulation, and they have a high level of heat tolerance. They have a unique water metabolism in that they require very little water to function (Winkelmann and Getz, 1962). Gerbils can obtain sufficient water from their diet and their kidneys have a highly efficient urine-concentrating capacity to ensure adequate hydration (Goyal et al., 1988). The ratio of long-loop nephrons to short-loop nephrons in gerbils is high. Ninety-six percent of their nephrons are long loop which allows them to efficiently concentrate their urine (Ichii et al., 2006). The digestive system is also very efficient at absorbing and retaining water, and water can be stored in fat cell layers. Gerbils produce and excrete a small amount of concentrated urine and dry feces per day (Alderton, 1986); therefore they require less frequent cage changing than other laboratory rodents.

Other Characteristics

The internal anatomy of Mongolian gerbils was investigated and described in detail by Williams (1974). He demonstrated that the anatomy of the gerbil and the albino laboratory rat is similar except for some minor

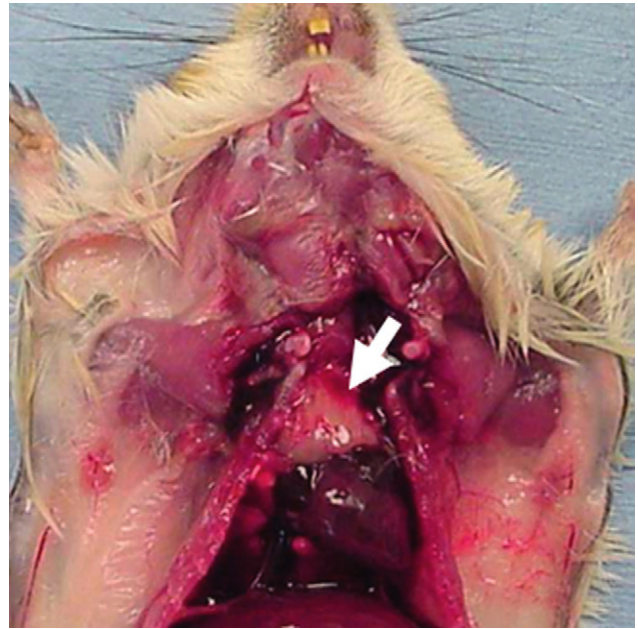


FIGURE 52.4 The thymus persists in adult gerbils (arrow).

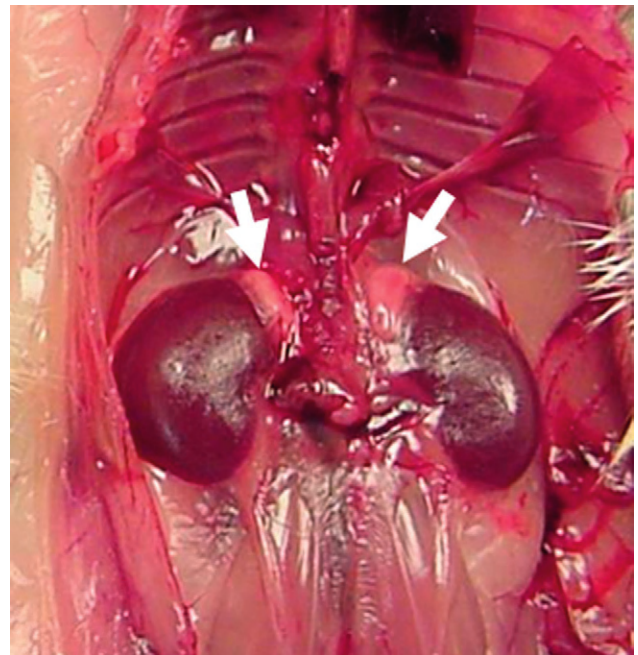


FIGURE 52.5 Gerbils have large adrenal glands for their weight when compared to rats and mice (arrows).

variations. These include a lack of a preputial gland, the presence of a gall bladder, and 12 pairs of ribs in gerbils (Williams, 1974).

Gerbils have an unusually large thymus and adrenal glands compared to other rodents of similar size (Figures 52.4 and 52.5). The thymus is persistent in adults (Wagner and Farrar, 1987). The adrenal gland weight



FIGURE 52.6 There is a significant sex difference in the size of the ventral sebaceous gland, located in the shaved areas of these gerbils (left, female; right, male).

when compared to the body weight is approximately three times the size of the adrenal gland in rats (Cullen et al., 1971; Holmes, 1985). The significance of these anatomically enlarged structures is not known (Schwentker, 1963), although the enlarged adrenal glands are thought to contribute to their unique water conservation ability (Wagner and Farrar, 1987). The adrenal cortex produces equal amounts of corticosterone and 19-hydroxycorticosterone (Drummond et al., 1988). Unlike a rat, an adrenalectomized gerbil cannot be maintained by providing supplemental sodium (Cullen et al., 1971).

Both sexes have distinct mid-ventral abdominal sebaceous glands, which are used for territorial marking. The male sebaceous gland is twice the size of the female gland (Figure 52.6). At puberty in males, the gland becomes orange and produces an oily, musky scented secretion (Clark, 1984).

Physiology

Selective normal mature gerbil physiological parameter and hematological and serum chemistry values are listed in Tables 52.1, 52.2 and 52.3, respectively. The data reported are compiled from literature using average values with sexes combined. There is a limited amount of hematological and clinical chemistry data available in gerbils. Ruhren (1965), Mays (1969), Dillon and Glomski (1975), and Gattermann (1979) conducted studies and reported original data in gerbils. The blood volume of a Mongolian gerbil is approximately 7.7 ml/100 g. The red blood cell half life is approximately 9.9 days, which is short in comparison with that of other rodents (Womack, 1972). Young gerbils can have up to a 40% increase in circulating, stippled red blood cells and reticulocytes compared to adults (Ruhren,

TABLE 52.1 Basic Biological Parameters of Gerbils

Chromosome number	44
Life span (years)	2–5
Number of mammary glands	4 pairs: 2 thoracic, 2 inguinal
Birth weight (g)	2–4
Mature body weight (g)	65–100 (males) 55–85 (females)
Body temperature (°C)	37–38.5
Dental formula	2 (1/1, 0/0, 0/0, 3/3)
Food intake (g/day/100 g weight)	5–7
Water intake (ml/day/100 g weight)	4–7
Urine volume (ml/day)	Few drops to 3–4 ml
Heart rate	260–600
Respiratory rate	70–120
Sexual maturity	9–12 weeks for both sexes
Estrous cycle	4–6 days; postpartum estrus is present
Gestation	24–26 days
Number of litters in lifetime	6 or more
Litter size	1–12 (average 5)
Eyes open	16–20 days
Weaning	21–24 days

(Adapted from Clark, 1984; Field and Sibold, 1999; Harkness and Wagner, 1983).

1965; Smith et al., 1976). Gerbils have lower hematocrit values than mice, and have higher blood lipid values than rats (Mays, 1969). Sexual dimorphism in hematocrit, hemoglobin, total leukocyte count, and lymphocytes has been reported (Dillon and Glomski, 1975; Mays, 1969). Mays (1969) demonstrated that there were significant differences between males and females in phosphorus and uric acid levels. Serum cholesterol levels are higher than other rodents, even when gerbils are fed diets containing normal levels of fat (Roscoe and Fahrenbach, 1962).

Select Normative Values

Basic biological values are presented in Tables 52.1, 52.2, and 52.3 (adapted from Clark, 1984; Field and Sibold, 1999; Harkness and Wagner, 1983; Wagner and Farrar, 1987). Similar and additional data are presented in the sections on Normative Values; and Clinical Biochemistry and Hematology.

Behavior

Gerbils are quiet animals that are generally docile, easy to handle, and seldom bite. They are quite

TABLE 52.2 Hematological Parameters

Red blood cells ($10^6/\mu\text{l}$)	7–10
Hemoglobin (g/dl)	13–16
Hematocrit (%)	44–49
MCH (pg)	16–19
MCV (fl)	46.6–60
MCHC (%)	30.6–33.3
Reticulocytes (%)	2.0–5.4
Platelets ($10^3/\text{mm}^3$)	400–600
White blood cells ($10^6/\mu\text{l}$)	7.3–15.4
Neutrophils ($10^6/\mu\text{l}$)	1.3–5.2
Lymphocytes ($10^6/\mu\text{l}$)	5.1–11.8
Eosinophils ($10^6/\mu\text{l}$)	0.07–0.32
Basophils ($10^6/\mu\text{l}$)	0.1–0.28
Monocytes ($10^6/\mu\text{l}$)	0.03–0.25
Blood volume (ml/kg)	60–85
Adult total blood volume (ml)	4.4–8.0

(Adapted from Clark, 1984; Field and Sibold, 1999; Harkness and Wagner, 1983).

TABLE 52.3 Clinical Chemistry Parameters

Albumin (g/dl)	1.8–5.5
Total protein (g/dl)	4.3–12.5
Globulin (g/dl)	1.2–6.0
Blood urea nitrogen (mg/dl)	17–32
Creatinine (mg/dl)	0.64–1.12
Glucose (mg/dl)	50–135
Sodium (meq/L)	143–157
Chloride (meq/L)	105
Calcium (mg/dl)	3.6–6.0
Potassium (meq/dl)	3.9–5.2
Phosphorus (mg/dl)	3.7–7.1
Total bilirubin (mg/dl)	0.2–0.6
Cholesterol (mg/dl)	90–151

(Adapted from Clark, 1984; Field and Sibold, 1999; Harkness and Wagner, 1983).

curious as demonstrated by their increased activity in an open field test and decreased thigmotrophic or “wall hugging” behavior compared to mice (Oldham and Morlock, 1970). In nature, gerbils live in family groups in complex burrows. Their compulsive burrowing behavior extends into the laboratory setting as well, and in many cases they will scratch at the sides and bottoms of cages in their attempts to burrow (Brain, 1999; Field and Sibold, 1999). Other normal activities include gnawing,

making nests, and nibbling on food continually to support their bursts of energy (Bradley and Pence, 1995). While primarily nocturnal, they are active during daylight hours, too, alternating periods of intense activity with sleep or rest (Brain, 1999; Thiessen and Yahr, 1977). They do not hibernate, but may exhibit estivation, depending on the species (Bradley and Pence, 1995).

Gerbils are territorial, and will mark almost any object in their environment by depositing a pheromone with their ventral sebaceous gland (Thiessen and Gray, 1971, 1974) (Figure 52.6). This enlarged, specialized gland is under the control of gonadal hormones (Glenn and Gray, 1965). Animals of both sexes which have higher androgen levels are more frequent markers and have an advantage in hostile encounters (Swanson et al., 1978; Thiessen et al., 1968a). In males, the gonadal androgens act on the medial preoptic area of the brain (Commings and Yahr, 1984; Thiessen and Yahr, 1970). In the female there is contradictory information regarding the influence of estrogen and progesterone on marking behavior (Thiessen and Lindzey, 1970; Wallace et al., 1973; Whitsett and Thiessen, 1972). Male gerbils scent mark more frequently than females (Lindzey and Carbonell, 1968) and gerbils with black coat color tend to mark more often than those with brown coat color (Turner and Carbonell, 1984). Marking behavior in males is used for exploration, establishing social dominance, and identifying territory (Thiessen and Yahr, 1970). In females, scent marking is used for exploration and is also important for identification and for the attraction between mother and young (Dagg and Windsor, 1971; Wallace et al., 1973). Males also urinate and defecate to mark their territory. Only groups of males that are raised together are compatible as adults and will defend their territory as a group. Once a family unit (male and female, or parents with a litter) is established, the gerbils will viciously attack intruders.

Pair bonding between male and female gerbils occurs; however, gerbils may not be completely monogamous (Brain, 1999; Robinson, 1978). Mating usually occurs at night, and the male will mate with the female several times. Both males and females will build a nest and care for the young (Elwood, 1975).

Gerbils make a drumming sound by thumping their hindlegs rapidly on the cage floor to signal a warning or excitement, or during courtship by the male (Brain, 1999).

Food hoarding is also a natural behavior in gerbils with females serving as the primary food hoarders. Castration of male gerbils will increase hoarding behavior indicating that hoarding is inversely related to androgen levels. In one experimental setting where a pair of gerbils was provided with a ten foot square room with a closet, the female gerbil gathered and stored approximately 50 pounds of food in the closet (Thiessen and Yahr, 1977).

MANAGEMENT, HUSBANDRY, AND COLONY HEALTH

Maintaining a well-managed gerbil colony requires attention to many aspects of animal care. Animal well-being is dependent on appropriate housing conditions, from the micro-environment (cage) to the macro-environment (holding room and facility). Animals must receive appropriate nutrition, and veterinary oversight must be provided to ensure an effective preventive medicine and health-monitoring program. Finally, documentation is essential to ensure that procedures are being followed as designed and to comply with national regulations.

Housing Systems

Caging designed for other rodents (e.g. hamsters and rats) is generally adequate for gerbils, and solid-bottom, bedded caging is preferred (Brain, 1999; Field and Sibold, 1999; Harkness and Wagner, 1983; Moore, 1995; Olfert and Cross, 1993). Recommended cage dimensions vary and may be subject to national regulations. For example, the Canadian Council on Animal Care (Olfert and Cross, 1993) proposes a floor area per animal of 116cm² and 15cm cage height, while the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) in India recommends a floor area of 64.5cm² (up to 60g) to 122.5cm² (over 100g) and a height of 12cm. Although the gerbil is a United States Department of Agriculture (USDA)-covered species, neither the Animal Welfare Act Regulations (9CFR Part 3 nor the *Guide for the Care and Use of Laboratory Animals* (Guide)) (National Research Council, 2011) provide standards for cage sizes for gerbils in the United States. Others have recommended that adult gerbils have 36in²/230cm² of floor space per animal and 6in/15cm of internal cage height and that breeding pairs with litters should have 180in²/1300cm² of floor space (Harkness and Wagner, 1983).

Bedding types used for gerbils are the same as those used for other rodent species. These include chopped corncob, hardwood chips, cellulose fiber, and other commercially available beddings. In addition to absorbing wastes, bedding may be used as a burrowing substrate, or a separate substrate such as sand may be provided. Pine bedding is not recommended as it causes matting and greasiness of the fur (Brain, 1999; Field and Sibold, 1999) as well as the induction of hepatic drug-metabolizing enzymes (Vesell, 1967).

Gerbils do best when housed in groups established prior to puberty. Introducing new cagemates into an established group, or re-introducing former cagemates who have been separated for more than 14 days, may precipitate fighting (Harkness and Wagner, 1983; Moore, 1995; Olfert and Cross, 1993). One method of pairing

unfamiliar adults is to allow them to recover from anesthesia in a neutral cage (Harkness and Wagner, 1983). Male gerbils raised in isolation exhibit increases in social sniffing, aggression, and anxiety compared with group-reared males (Shimozuru and Kikusui, 2008).

Environmental Conditions

Recommendations for environmental conditions for gerbils are similar to those for rats and mice. Temperature recommendations range from 64–79°F/18–26°C (typically 72 ± 2°F/22 ± 1°C). A 12-h light: 12-h dark cycle is appropriate for general housing, but a longer 14-h light: 10-h dark cycle is desirable for breeding colonies (Field and Sibold, 1999; Moore, 1995). Cold temperatures and/or short light cycles (e.g. 5°C, 8-h light: 16-h dark) may elicit physiological changes related to winter survival mechanisms such as increased basal metabolic rate and non-shivering thermogenesis (Li and Wang, 2005). Relative humidity should be maintained at 30–70% RH; however, rough hair coats have been reported to occur at humidity levels greater than 50% RH (Field and Sibold, 1999; Harkness and Wagner, 1983; Moore, 1995). Housing areas should be kept quiet, because sudden noises may elicit epileptic seizures. The *Guide* (National Research Council, 2011) recommends 10–15 air changes per hour as a general standard for animal holding rooms.

Cage enrichment devices for gerbils that are not made of a sturdy material will be quickly destroyed by the gerbils' natural chewing activities. Stainless steel and PVC materials have been recommended in the form of "toys", cups, tubes, and other shelters (Field and Sibold, 1999). However, it has been suggested that providing shelters may result in differences in behavior, maturation rate, and adrenal weights (Moore, 1995). Nesting material is used readily but may be chewed and incorporated into the cage bedding. Heavier material such as thick cardboard tubing can provide shelter and a chewing substrate which will last longer than other nesting materials (Batchelder, personal communication). Naturalistic environmental enrichment such as sand and stones has been recommended. Sand or deep bedding allow expression of the animals' burrowing behaviors (Field and Sibold, 1999). Sand bathing is an important part of the gerbil behavioral repertoire (Tortora et al., 1974), but providing sand may not always be practical in the laboratory setting.

Gerbils in solid-bottom caging generally have the bedding replaced once or twice a week, depending on factors such as cage density or the presence of newborn pups. Since gerbils are a desert species, they produce little urine and dry feces, which may allow a decreased frequency of bedding changes relative to other species (Moore, 1995). Typically, bedding is changed and solid-bottom cages are sanitized on the same schedule,

while animals in wire-bottom cages may have the cage sanitized less often. The *Guide* recommends that primary enclosures and accessories (including lids and feeders) be sanitized at least every 2 weeks (National Research Council, 2011).

Sanitization may be performed by hand or in automated equipment. Detergent and hot water (143–180°F/62–82.2°C) are used to remove soil and disinfect the equipment. Care must be taken to rinse all chemical residue from the caging before use (Committee for the Purpose of Control and Supervision on Experiments on Animals, 2010; National Research Council, 2011). Cages, accessories, and bedding may be autoclaved if a sterile environment is desired (Field and Sibold, 1999).

Nutrition

Gerbils are adapted to live in arid conditions in the wild. Their diet is primarily seeds, and a large portion of their water intake is derived from their food. Although they can live for extended periods without supplemental water, provision of fresh water is generally recommended for animals in captivity. Reproduction may be decreased if water is restricted (Yahr and Kessler, 1974). Supplementation with natural feeds such as vegetables and grains is not necessary, but they may be provided as a treat.

Zeman (1967) proposed a semi-purified diet as a basis for research into the nutritional requirements of gerbils. However, very little work has been published since then, and the specific nutritional requirements of gerbils remain largely undetermined. They are generally considered to be similar to those of the rat, and gerbils are usually maintained by ad libitum feeding of commercial diets formulated for rats or mice. Diets should contain at least 16% protein. Harkness and Wagner (1983) recommend 22% protein and 2–5% dietary fat. Gerbils appear to have a higher need for magnesium than rats, as levels below 1 g/kg diet may result in alopecia and increased susceptibility to seizures; 1.5 g/kg diet is recommended. Gerbils are also susceptible to developing elevated blood and liver cholesterol when fed an excess of fat or cholesterol in the diet (Brain, 1999; Subcommittee on Laboratory Animal Nutrition, 1995). Gerbils are not coprophagic when fed a nutritionally complete diet (Field and Sibold, 1999; Otken and Scott, 1984).

Weanling gerbils may have difficulty reaching food provided in a cage-lid feeder. If necessary, food may be placed in a dish on the cage floor.

Breeding

It is relatively easy to determine the sex of adult gerbils. The male is distinguished by its longer anogenital

distance, prominent genital papilla, and pigmented scrotum (Figure 52.2). Gerbils become sexually mature at 9–12 weeks of age. Females have a 4–6-day estrous cycle and are spontaneous ovulators. They are sexually receptive for 12–15 hours, and mating often occurs several times. Males thump their feet as a courtship display. A vaginal plug is present after ejaculation, but usually disappears by the following morning (Brain, 1999). Gestation is 24–26 days, and litter size averages five pups (range 1–12). Larger litters may necessitate fostering some of the pups to another lactating female gerbil or even to a lactating rat (Brain, 1999; Moore, 1995). Both parents participate in building a nest and caring for the young, although during post-partum estrus the male may be more interested in mating than in child-rearing (Prates and Guerra, 2005). Arrington et al. (1973) reported that 47% of females become pregnant at this time. As in mice, gestation during lactation may be prolonged due to delayed implantation (Brain, 1999; Norris and Adams, 1981). Pups are about 2.5 g at birth, are altricial, begin eating solid food at about 14 days of age, and are weaned when they reach 21–24 days of age. Handling and cage changing should be minimized during the breeding and peri-partum periods to reduce the chance of females abandoning their pups.

Gerbils may be bred as pairs or as harems with 2–3 females per male. However, they may form monogamous pairs, mating for life. After the loss of one mate, it may be difficult to introduce a new one to the survivor (Norris and Adams, 1972a). Pairs are usually established at about 6–7 weeks of age (Moore, 1995), although Brain (1999) suggests animals reproduce sooner if paired at 2–3 months of age. Animals may fight or fail to mate if they were not previously housed together. Females which mature earlier (vaginal opening before 25 days of age) have been reported to be more productive breeders than later-maturing females (Brain, 1999). Female gerbils housed as a single-sex group show decreased incidence of estrus whereas pairing with a male will result in estrus by day 3 in 44% of females (Meckley and Ginther, 1974). Pseudopregnancy has been known to occur, lasting a shorter period (14–18 days) than pregnancy (Brain, 1999). Gerbils have a relatively high incidence of cystic ovaries which may affect breeding production (Norris and Adams, 1972b). Wu (1974) has described artificial insemination in the gerbil, and Mochida and Wakayama (2005) describe gerbil embryo cryopreservation with subsequent implantation and live births.

Colony Health

Maintaining a healthy gerbil colony is a matter of good husbandry and sanitation, screening of any new animals brought into the colony, and appropriate veterinary medical care and oversight. Routine health monitoring is

recommended to detect any unwanted agents that may arise in the colony.

Animals being brought into an established colony should be obtained from a reliable source and have documentation that they do not carry any infectious agents excluded by the institution. Shipping containers should be disinfected before bringing into the facility, in case the outside surfaces were contaminated during transport. Animals should be checked for general health upon receipt, and in some cases – especially if obtained from a non-commercial or untested source – may be subjected to a quarantine period to ensure absence of unwanted infectious agents before introduction into the general population.

Similarly, any tissues or biologicals that will be introduced into the colony should be screened for infectious agents prior to use (Rehbinder et al., 1996). This can be done by Mouse Antibody Production testing or by screening with a panel of polymerase chain reaction (PCR) tests.

Gerbils have not been reported to be susceptible to spontaneous viral diseases. Nevertheless, they can be screened for viruses that may infect other species, including zoonotic viruses. These include lymphocytic choriomeningitis virus (LCMV), pneumonia virus of mice (PVM), minute virus of mice (MVM), parainfluenza virus, hantavirus, coronavirus, reovirus, Sendai virus, and simian virus 5 (Charles River Laboratories, 2009; Rehbinder et al., 1996).

Health monitoring for agents likely to cause disease in gerbils is primarily directed toward bacterial and parasitic agents. It is recommended that gerbil colonies be screened for *Clostridium piliforme* (Tyzzer's disease) and for *Salmonella* spp. They may also be screened for *CAR* bacillus, *Bordetella bronchiseptica*, *Pasteurella* spp., *Beta Streptococcus* spp., *Streptococcus pneumoniae*, *Pseudomonas* spp., *Klebsiella* spp., *Helicobacter* spp., *Citrobacter rodentium*, and *Corynebacterium kutscheri*, which may less commonly cause disease in gerbils, or be transmitted to other species (de la Puente-Redondo et al., 1999; Glage et al., 2007). Screening for *Staphylococcus aureus* may generate positive results without disease being present, but the organism can be associated with some conditions such as nasal dermatitis (Charles River Laboratories, 2009; Rehbinder et al., 1996; Solomon et al., 1990).

Screening should also be performed for the more common parasites including *Demodex merionis* mites and pinworms such as *Syphacia* spp. or *Dentostomella translucida* (Wightman et al., 1978). Other possible parasitic agents include *Hymenolepis diminuta* and *Hymenolepis nana*, *Giardia* spp., *Entamoeba muris*, *Eimeria* spp., *Encephalitozoon cuniculi*, *Toxoplasma gondii*, *Spiroplasma* spp., and intestinal flagellates.

Screening may be performed using colony animals and/or using dedicated sentinel animals, most often

animals exposed to soiled bedding and/or caging from colony animals. Mice have been used as sentinels in gerbil colonies as they are susceptible to lymphocytic choriomeningitis virus, coronavirus, Sendai virus, pneumonia virus of mice, and Reovirus 3, as well as to *Syphacia* and bacterial pathogens (Batchelder, personal communication).

Record Keeping

Several types of records are important in the maintenance of a gerbil colony, including animal records, facility records, breeding records, and regulatory records.

Identification records and health records are two types of animal records. Each animal should be able to be identified as to source, birth date, receipt date, sex, animal use protocol, responsible investigator name, and any identifying information such as an ear tag or microchip number. These records help ensure that the appropriate animal, and the correct animal, is used in an experimental procedure. Health records can include examination records at the time of receipt, surgical and experimental procedure records, and records of any individual health issues and treatments the animal may have received.

Facility records consist of documentation that appropriate husbandry and sanitization procedures have been followed. These are commonly kept as a check-off sheet that lists daily, weekly, and other scheduled tasks which are marked off as they are completed. Other facility records include cage wash and autoclave validation, feed receipt and expiration dates, pest control documentation, environmental parameters such as light, temperature, and humidity, etc.

Breeding records are essential to the maintenance of any breeding colony. An animal's genealogy should be able to be traced, and records should be kept on the breeding success of both males and females. Daily records, such as birth and weaning dates, and long-term records such as planned breeder replacement are necessary to keep breeding at top efficiency. Unless inbreeding is desired, careful records will help avoid mating close relatives.

Additional records may be required by regulatory agencies in the country where the animals are being kept. Such records might include animal receipt and transfer records, disposition records, and documentation concerning the research for which the animals were used. Animals which are sick or which die unexpectedly may be expected to have additional documentation such as individual medical or necropsy records. Records may be needed to document research oversight activities such as approval of protocols involving gerbils, facility inspections, and animal care program reviews. Regulations may also stipulate how long such records need to be

retained. Those involved with research using gerbils should be familiar with the regulations for their particular location.

BASIC EXPERIMENTAL METHODS

Gerbils have been used as experimental models in a number of areas of biomedical research. They are relatively easy to maintain and to handle. Experimental methods used for gerbils are similar to those used in other rodents such as mice and rats.

Handling and Restraint

Gerbils are generally non-aggressive and can easily be handled with little risk of being bitten. They may be moved using cupped hands, or may be lifted gently by the base of the tail. Care must be taken to grasp the tail base only (Figure 52.7), since the skin on more distal parts of the tail may pull off easily (Donnelly, 1997). If more secure restraint is needed, gerbils may be grasped by the loose skin on the back of the neck (Figure 52.8). With this grasp, they may be oriented in any position; however gerbils dislike being held on their backs and may struggle in this position (Moore, 1995).

Mechanical restraint devices designed for mice or small rats may be used for gerbils. Again, care must be taken to grasp the tail only at the base when placing the animal in the device to prevent a degloving injury.

A number of chemical agents used in other species are also used to sedate gerbils for handling. These include diazepam (5mg/kg IM or IP), ketamine (100–200mg/kg IM), medetomidine (100–200µg/kg IP or SC), midazolam (5mg/kg IM or IP), or xylazine (2mg/kg IM)

(Flecknell, 2009; Hawk et al., 2005). Acepromazine has been reported to induce convulsions in gerbils (Harkness and Wagner, 1983) and is not recommended in this species. A short-acting inhalant anesthetic such as isoflurane or sevoflurane may also be used to effect for brief chemical restraint. Atropine (0.02–0.05mg/kg SC, IM, or IV) or glycopyrrolate (0.01–0.02mg/kg IM or SC) may be used to decrease salivary and bronchial secretions (Hawk et al., 2005).

Identification

Gerbils may be identified by cage cards, ear tags or notches, indelible markers, or microchip implantation. Tattooing is another option, but is less useful in gerbils than in mice and rats due to the presence of hair on the paws and tail.

Sampling Techniques

Several sites may be used for blood collection in the gerbil. Use of the lateral tail vein may be facilitated by warming the tail to promote blood flow and by placing pressure on the vein at the base of the tail. Methods for



FIGURE 52.7 When restraining a gerbil by the tail, only the base of the tail should be grasped to avoid degloving injuries.



FIGURE 52.8 Gerbils may be restrained by the scruff of the neck.



FIGURE 52.9 Blood collection from the retro-orbital sinus.



FIGURE 52.10 Blood collection by cardiac puncture.

bleeding from the lateral saphenous vein and from the submandibular vein have also been reported (Golde and Gollobin, 2005; Hem and Smith, 1998). For a small amount of blood, a toenail may be clipped short, or the tip of the tail (1–2 mm) may be transected. Care should be taken to control hemorrhage, the tail tip should only be used once or twice to avoid damage to the coccygeal vertebrae, and anesthesia is recommended. Larger volumes of blood may be collected by retro-orbital puncture (Figure 52.9), jugular venipuncture (Palm and Hollander, 2007), or cardiac puncture; all of these procedures should be performed under anesthesia, and cardiac puncture (Figure 52.10) should be terminal because of the risk of pericardial hemorrhage and cardiac tamponade. The maximum volume for blood withdrawal which has the least scientific impact on the gerbil's physiologic response is 0.77 ml/100 g body weight (Diehl et al., 2001; Moore, 1995).

Urine collection is difficult due to the very small volume of urine produced by the gerbil, variously reported as a few drops to 3–4 ml/day (Brain, 1999; Moore, 1995). If urine cannot be collected by expressing the bladder or by free catch, a metabolism cage may be used. Care should be taken that the small amount of urine does not evaporate from the collection chamber.

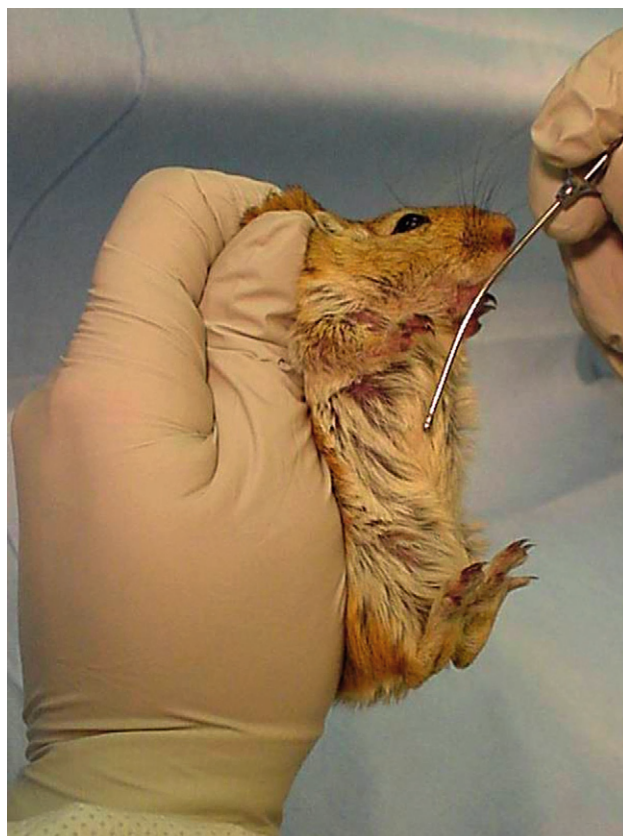


FIGURE 52.11 Measuring the gavage needle to the level of the last rib.

Compound Administration

Oral administration of compounds is most commonly accomplished with the use of a ball-tipped gavage needle, as used in other small rodents. The length of the needle should be checked against the side of the animal to the level of the last rib to ensure it is not so long as to bypass the stomach (Figure 52.11). The needle should slide easily into the stomach (Figure 52.12); any resistance may indicate the needle is located in the trachea and it should be withdrawn and redirected. Animals may also be dosed orally by the use of medicated feed or water. If this method is used, the animals' food and water consumption should be monitored to ensure that the drug is not causing the feed or water to be unpalatable. Decreased consumption may lead to underdosing.

The most common site for intravenous (IV) injection in the gerbil is the lateral tail vein (Figure 52.13). Injection may be facilitated by warming the tail to promote blood flow and by placing pressure on the vein at the base of the tail to dilate the vessel. A 23-gauge or smaller needle should be used (Field and Sibold, 1999). Cutdown procedures have been described for intravenous dosing using the jugular vein (Palm and Hollander, 2007) and the femoral vein (Pérez-García et al., 2002).



FIGURE 52.12 Oral gavage using a ball-tipped dosing needle.



FIGURE 52.14 Intramuscular injection into the thigh muscles.

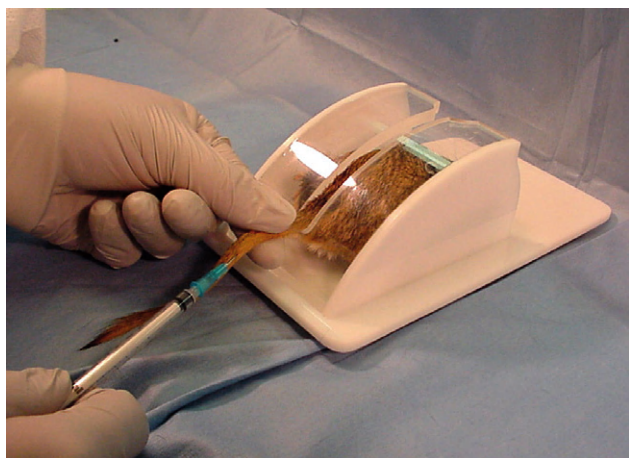


FIGURE 52.13 Intravenous injection in the lateral tail vein.



FIGURE 52.15 Subcutaneous injections may be given under the loose skin over the gerbil's back by tenting the skin and inserting the needle parallel to the body wall.

Intramuscular (IM) injections are given in the muscles of the thigh ([Figure 52.14](#)), with care being taken to avoid the sciatic nerve. The volume of the IM injection should be small enough to avoid muscle damage and pain. Volumes over 0.1 ml should be split between two sites.

Most subcutaneous (SC) injections are given under the loose skin over the neck and shoulders of the gerbil ([Figure 52.15](#)). The skin is tented and the needle is inserted parallel to the body wall to avoid injecting into deeper tissues.

Gerbils may be dosed intraperitoneally (IP) in the caudal left or right abdomen, similar to other small rodent species. However, because gerbils may struggle if held on their backs, they should be restrained in a

vertical position while dosing ([Figure 52.16](#)). Extra care should be taken to draw back on the syringe to ensure the needle has not entered the bowel or bladder prior to injecting.

Other routes of dosing include topical, intradermal, intranasal, and cerebroventricular. Any substance used for parenteral dosing should be sterile and pyrogen-free ([Moore, 1995](#)).

If dosing is performed repeatedly, the animal should be monitored closely for any complications. Depression, dyspnea, hunched posture, abdominal splinting, or signs of pain or irritation at an injection site should be brought to the attention of the veterinary staff.



FIGURE 52.16 An intraperitoneal injection is given while holding the gerbil in a vertical position.

An alternative to repeated parenteral injections is the implantation of an osmotic pump. These devices are designed to be implanted subcutaneously or intraperitoneally, and will subsequently provide a steady-state dose of compound for up to several weeks.

Euthanasia

Methods for euthanasia should be selected based on the recommendations of the AVMA ([American Veterinary Medical Association, 2007](#)), the experimental parameters, and operator comfort with the procedure. Common methods include carbon dioxide inhalation, overdose of an anesthetic agent (e.g. pentobarbital 100–150 mg/kg IP), use of a commercial injectable euthanasia agent, or physical methods such as decapitation or cervical dislocation. The latter two methods should be performed under anesthesia unless scientifically justified and approved by the Institutional Animal Care and Use Committee. Less commonly used methods include microwave irradiation and exsanguination/perfusion under anesthesia. All methods require training and proper equipment in order to be performed humanely.

VETERINARY CARE AND DISEASES

Veterinary Care

Gerbils are excellent subjects for laboratory animal research as they are susceptible to bacterial, viral, and parasitic pathogens that affect humans and other species, yet they have very few of their own diseases. Some of the most common conditions seen in laboratory gerbils are related to handling, husbandry, or keeping the gerbils until old age. In general, veterinary practices used in providing health care for other laboratory rodents are also appropriate for laboratory gerbils.

Meeting gerbils' specific housing and husbandry needs will help keep them healthy. Gerbils' hind legs are longer than their front legs and they like to stand erect on the hind legs. To accommodate this behavior, the floor to lid height of gerbil cages needs to be sufficient for the gerbil to stand. Gerbils should be housed in solid-bottom caging with bedding at least 3–5 cm deep to allow for burrowing behavior. Gerbils also exhibit gnawing behavior and need to have non-toxic, hard materials on which to gnaw.

Gerbils must be picked up by the base of the tail to avoid the skin on the tail degloving. They should have their incisors and claws checked periodically to make sure that both are being worn down. Claws can be clipped if they are too long. A rough-surfaced object such as a stone or a bathroom tile can be placed in the cage for the gerbils to wear down their claws as well as to use for normal marking behaviors. Gerbils exhibit shredding behavior and should be provided with autoclaved cardboard, hay, straw, paper, or tissue. Gerbils are burrowing animals and sometimes will resort to stereotypic digging in the corner of the cage. A man-made burrow may be used in a laboratory setting. One example utilizes clear and opaque sections with an access tunnel ([Waiblinger, 2010](#)).

Gerbils need to have a substrate to remove the lipids that they place on their hair coat during self-grooming. In the wild, gerbils use sand baths, and they will use them in the laboratory if provided (Tortora et al., 1974). If gerbils exhibit a dirty, ungroomed hair coat, consider changing the type and amount of bedding, make sure the relative humidity is less than 50% and consider lowering the temperature of the room.

Common Diseases

Epilepsy

Gerbils may have spontaneous seizures secondary to stress such as handling, cage change, abrupt noises, or changes in the environment. Generally, gerbils will recover from these seizures after 30–90 seconds without treatment, and appear to have no long-lasting effects.

Tail Slip

Gerbils that are picked up by the tip or middle of the tail are very susceptible to skin degloving (Donnelly, 1997). Gerbils should always be picked up by the base of the tail or by cupping the hands. Tails that have been degloved can be surgically amputated.

Nasal Dermatitis (Sore Nose, Facial Eczema)

This is a common skin condition of gerbils which likely has a multifactorial etiology. Clinical signs start as erythema at the nares and develop into facial alopecia, dermatitis, and scab formation. Some cases can develop into a moist dermatitis involving the head, forelimbs, and chest. This condition has been reported to be most common in young weanling gerbils (Field and Sibold, 1999).

Gerbils release a complex mixture of pigments and lipids from the Harderian gland during self-grooming. The material is excreted at the nares and is mixed with saliva and spread over the gerbil's hair coat. Harderian exudates are normally removed from the gerbil's pelage by sand bathing (Harriman and Thiessen, 1983; Thiessen, 1988). Two studies have shown that Harderian secretions which act as primary skin irritants are a factor in the development of nasal dermatitis. Removal of Harderian glands or housing animals on sand either prevented lesions or caused improvement in existing lesions, while animals with intact Harderian glands and reduced opportunity to groom or sand bathe developed or maintained existing lesions (Farrar et al., 1988; Thiessen and Pendergrass, 1982).

Staphylococcus spp. have been implicated in the development of this condition. One paper reports *S. aureus* as the bacteria most commonly cultured from the nasal area of affected animals, although *S. aureus* was also cultured from non-affected gerbils (Bresnahan et al., 1983). Another study reported that *S. xylosus* was the predominant species isolated from all gerbils, most commonly from the nasal area, and the only species isolated from the nasal area of gerbils with clinical signs of nasal dermatitis (Solomon et al., 1990).

Cystic Ovaries

Cystic ovaries are seen commonly in female gerbils over 1 year of age (Norris and Adams, 1972b). Clinical signs include symmetrical alopecia, abdominal swelling, lethargy, anorexia, dyspnea, and reduced fertility. In 1982, Norris and Adams showed that female gerbils older than 700 days had a 79% rate of cystic ovaries in gerbils with two ovaries and a 40% rate of cystic ovaries in gerbils with one ovary. They also showed that removing one ovary in a gerbil does little to affect overall reproductive performance (Norris and Adams, 1982).

Less Common Diseases

Bacterial

Although there have been reports of bacterial diseases in laboratory gerbils in the past, animals can be purchased today that are free from antibodies to *Clostridium piliforme* and are culture-negative for *Salmonella* spp., *Bordetella bronchiseptica*, and *Pasteurella pneumotropica*. The most common bacterial threat to a high-quality research colony is helicobacter.

There have been reports of laboratory and pet gerbils infected with *Clostridium piliforme*, the etiologic agent for Tyzzer's disease (Motzel and Gibson, 1990; Port et al., 1971; Vincent et al., 1975). Gerbils usually exhibited diarrhea or sudden death. Necropsy findings included multi-focal hepatic necrosis. In 1984, Waggle et al. experimentally induced Tyzzer's disease in gerbils and showed that they were highly susceptible to the disease. Strittmatter (1972) eliminated the disease in gerbils by cross-fostering gerbil offspring to unaffected mice.

In 2005, Bergin et al. reported a case of two laboratory gerbils with fatal *Clostridium difficile* enteritis. The gerbils were being treated for helicobacter infection with antibiotic wafers containing amoxicillin, metronidazole, and bismuth subsalicylate. There is also one report of a fatal epidemic of *Citrobacter rodentium* affecting nine gerbils (de la Puente-Redondo et al., 1999).

In 2009, Tappe et al. reported a new species of the genus *Streptococcus* which was cultured from the oropharynx of Mongolian gerbils. The new name proposed for these Gram-positive, catalase-negative, chain-forming cocci is *Streptococcus merionis* sp. nov. There were no clinical signs reported in the gerbils.

Gerbils are natural carriers of multiple species of helicobacter (Bergin et al., 2005). Glage et al. (2007) reported rederivation of *Helicobacter hepaticus*-infected gerbils by Caesarean section and cross-fostering on non-infected mice.

Viral

No naturally occurring viral diseases of gerbils have been published.

Parasitic

Parasites rarely cause clinical problems in the laboratory gerbil, and are excluded from major commercial gerbil suppliers. Wightman et al. (1978) reported finding *Syphacia obveleta* and *Dentostomella translucida* in gerbils. They showed that *S. obveleta* can be transmitted from gerbil to mouse, mouse to gerbil, and gerbil to gerbil, while Ross et al. (1980) showed that *Syphacia muris* is similarly easily transmitted between gerbils, rats, hamsters, and mice. Wilkerson et al. (2001) reported that using fenbendazole-medicated feed was the only practical and reliable way to eradicate pinworms in

gerbil colonies. In 1970, Lussier and Loew reported a naturally occurring case of *Hymenolepis nana* in Mongolian gerbils, while in 1975, Vincent et al. reported the recovery of *Hymenolepis diminuta* from gerbils. *Demodex merioni* mites have been reported in gerbils with alopecia, dry skin, and ulcerations. Animals who are already debilitated are at most risk for developing mite infestations (Rollin and Kesel, 1995).

Other Diseases

Antibiotic Sensitivity

Gerbils are very sensitive to dihydrostreptomycin. Wightman et al. (1980) reported that an injection of 50 mg caused mortality in 80–100% of gerbils weighing 55–65 grams. Two gerbils given a commercially available treatment to eradicate helicobacter developed a necrohemorrhagic enteritis. It was determined that the amoxicillin component caused an overgrowth of *Clostridium difficile* (Bergin et al., 2005).

Neoplasia

There are a number of surveys about neoplasia in laboratory colonies of Mongolian gerbils (Meckley and Zwicker, 1979; Ringler et al., 1972; Vincent and Ash, 1978; Vincent et al., 1975). Neoplasia has been most often reported in gerbils over 3 years of age (Matsuoka and Suzuki, 1995). Commonly reported tumors are squamous cell carcinoma of the ventral marking gland in males and ovarian granulosa cell tumors in females. In surveys, adrenocortical tumors, cutaneous squamous cell carcinoma, malignant melanoma, and renal and splenic hemangiomas are the next most commonly reported tumors. Other tumors that have been reported include astrocytoma, craniopharyngioma, and systemic mastocytosis (Guzman-Silva, 1997; Guzman-Silva et al., 1988; Kroh et al., 1987; Rembert and Johnson, 2001). In 1997, Campos et al. reported a variety of epithelial-type neoplasias in the ventral prostrate of 18-month-old gerbils. Tumors included prostatic intraepithelial neoplasias, microinvasive carcinomas, and adenocarcinomas.

Chronic Interstitial Nephritis

This condition is considered an old-age change. Gerbils will exhibit clinical signs of polyuria, polydipsia, and weight loss. On gross exam the kidneys will be shrunken and pitted (Bingel, 1995).

Aural Cholesteatoma

Older gerbils can spontaneously develop cholesteatomas in the ear canal which resemble the human condition. Clinical signs include scratching, head tilt, and circling. The incidence appears to be age-related (Fulghum and Cole, 1985; Schiffer et al., 1986).

GERBILS AS EXPERIMENTAL MODELS

Gerbils have unique characteristics which make them appropriate for a number of animal models. Classically, gerbils have been used in research involving stroke, parasitology, infectious diseases, epilepsy, brain development and behavior, and hearing.

Stroke (Cerebral Ischemia)

In mammals the circle of Willis is comprised of a communication of arteries at the bottom of the brain consisting of the internal carotid arteries, anterior cerebral arteries, anterior communicating arteries, posterior communicating arteries, posterior cerebral arteries, and basilar arteries. This structure provides for alternate blood flow to the brain in case one artery becomes occluded. Levine and Payan (1966) first identified gerbils as having an anatomical anomaly of the circle of Willis with no communication between the posterior cerebral arteries. This has been termed an incomplete circle of Willis. Researchers found that ligating the left carotid artery of the gerbil caused acute cerebral ischemia much more consistently than when performing the same ligation in the rat (Wexler, 1972). In 1974, Levy and Brierley performed a study on gerbils to assess the cerebrovascular anatomy and communications in the gerbil brain by injecting colored dye into the aorta. Based on evaluating ten male gerbils from an unspecified source, these authors concluded that each of the gerbil's carotid arteries is divided into an anterior, middle, and posterior cerebral artery. They found that the basilar artery communicated with the carotid artery in all gerbils, that the posterior cerebral artery was a branch of the carotid, and that there was no single posterior communicating artery.

As gerbils were more frequently used for stroke research, more information about their cerebrovascular anatomy became available. It was determined that not all gerbils have an incomplete circle of Willis and that "stroke-prone" and "stroke-resistant" gerbils could be identified (Delbarre et al., 1988; Kitagawa et al., 1989; Pelliccioli et al., 1995). Considerable variability was found both in the extent of damage after bilateral artery occlusion and in the percentage of stroke-prone gerbils in a population. The percentage varied between the sexes (males 42.9% stroke-prone, females 26.7%) (Hall et al., 1991) and by the source of the animals (Breuer and Mayevsky, 1992; Laidley et al., 2005; Seal et al., 2006).

Methods to define stroke-prone gerbils include examination of retinal blood flow after ligation of the carotid artery (Delbarre et al., 1988), measurement of the diameter of the common carotid artery before and after temporary ligation (Kitagawa et al., 1989), survival

after unilateral carotid artery occlusion (Pelliccioli et al., 1995), and determination of the presence and diameter of the posterior communicating artery (Seal et al., 2006).

Despite the fact that the anatomy of the gerbils' circles of Willis cannot be considered uniformly lacking in posterior communicating arteries, gerbils continue to be used as a common animal model for stroke, with the two-vessel occlusion being considered the simplest (Small and Buchan, 2000). Laboratory animal veterinarians that work with gerbils as stroke models need to be aware that this difference in cerebral vascular anatomy could lead to variable or conflicting results.

Parasitic Diseases

Gerbils seem to be very well suited to host parasitic infections from other species and are therefore a popular animal model for a wide variety of parasites. Many parasitic genera have been described in experimental studies of infection, susceptibility, pathology, immunology, and chemotherapy.

Cestodes

Gerbils are alternative definitive hosts for *Echinococcus granulosus* (Conchedda et al., 2006). Gerbils have also been used as an animal model for *Echinococcus multilocularis* (Kamiya and Sato, 1990) as well as *Echinococcus vogeli* (Matsuo et al., 2000).

Although the Mongolian gerbil is not a natural host for *Rodentolepis nana*, it can be experimentally transmitted to gerbils that are given dexamethasone daily (Vianna and deMelo, 2007). Gerbils have also been experimental hosts for *Hymenolepis diminuta*, *Taeniasolium*, *Taenia saginata*, *Taenia asiatica*, and *Taenia crassiceps* (Avila et al., 2005; Chang et al., 2006; Johnson and Condor, 1996; Kamiya and Sato, 1990; Sato et al., 2000).

Nematodes

Gerbils can also serve as experimental hosts for a number of nematode parasites. These include *Haemonchus contortus*, *Toxocara canis*, *Strongyloides stercoralis*, *Strongyloides papillosus*, *Aspiculuris tetraptera*, *Syphacia obvelata*, *Dentostomella translucida*, and *Baylisascaris procyonis* (Akao et al., 2003; De Jesús-Gabino et al., 2009; Lok, 2007; Pinto et al., 2003a, 2003b; Zanandrea et al., 2008).

One of the most important uses of gerbils in biomedical research is for the study of filarid nematodes, which include *Brugia malayi*, *Brugia pahangi*, *Loa loa*, and *Litomosoides sigmodontis* (Lim et al., 2004; Mand et al., 2006; Shigeno et al., 2006; Wanji et al., 2002). Gerbils have been experimentally infected with these filarid nematodes to study the immunology, antigenicity, and life cycles of the parasites. Serologic tests and treatment

regimens have been developed by using these models (Hübner et al., 2009; Lim et al., 2004; Shigeno et al., 2006).

Trematodes

Gerbils have been used to study all aspects of the trematode life cycle including infectivity and immunology as well as to study potential treatments. These trematodes include *Schistosoma mansoni*, *Schistosoma japonicum*, *Schistosoma haematobium*, *Schistosoma magreboweie*, *Echinostoma caproni*, and *Opisthorcis viverrini* (Adam et al., 1993; Boonmars et al., 2009; Chisty et al., 2002; Mahler et al., 1995; Ogbe, 1983; Sato and Kamiya, 2001). Gerbils are also a good host for the avian schistosome, *Austrobilharzia variglandis* (Bacha et al., 1982).

Protozoa

Gerbils are good animal models for intestinal *Cryptosporidium parvum* infection and for gastric infection with *Cryptosporidium andersoni* and *muris* (Kvác et al., 2009). Immunosuppressed gerbils have been reported to be infected with *Cryptosporidium hominis* (Baishanbo et al., 2005).

Gerbils have been identified as a model for infection with *Giardia lamblia* (Belosevic et al., 1984; Faubert et al., 1983). Gerbils were used to develop the fecal antigen test for giardiasis (Moss et al., 1990). Gerbils can also be experimentally infected with *Giardia duodenalis* (Araújo et al., 2008).

Viral Diseases

Borna Disease

This is caused by a negative-strand RNA neurotropic virus which causes acute or subacute encephalitis in horses and sheep. This virus has been linked with schizophrenia and other affective disorders in humans (Rott et al., 1985). Borna disease is considered a model for studying neuronal plasticity, as the virus can persist in the CNS and cause changes in brain cell function (Gonzalez-Dunia et al., 2005). The gerbil is the definitive animal model for Borna disease. Newborn gerbils are very susceptible to intracranial infection with Borna virus resulting in inflammatory reactions in the brain as well as evidence of viral persistence in the brain (Nakamura et al., 1999).

Swine Hepatitis Virus

In 2009, gerbils were identified as the first animal model for swine hepatitis virus. Gerbils inoculated intraperitoneally with hepatitis E virus became infected, with viremia and virus shedding in the feces for 4 weeks. Additionally, the virus could be found in the liver of the infected gerbils (Li et al., 2009).

La Crosse Virus

This is an arbovirus that is one of the most common causes of pediatric arboviral infection in the US (Haddow and Odoi, 2009). In 1996, researchers at the University of Wisconsin discovered that Mongolian gerbils were an ideal animal model for La Crosse virus studies. Gerbils were susceptible to infection, survived, and developed viremia and neutralizing antibody titers following exposure by intramuscular injection and by the bite of infected mosquitoes. Moreover, they are attractive to mosquito vectors (Osorio et al., 1996).

Other Viruses

Gerbils are able to be infected with encephalomyocarditis virus, with viral replication evident in the heart and pancreas (Matsuzaki et al., 1989a). The young gerbil has also been identified as the first animal model of Rift Valley fever encephalitis that was uniformly fatal without causing lesions outside of the CNS (Anderson et al., 1988). During the summer of 2003, three gerbils were shown to be infected with monkey pox virus after being exposed to the virus from an infected Gambian giant rat (Kulesh et al., 2004). Newborn gerbils have been shown to be a better model for Puumala virus transmission than rats or mice (Lokugamage et al., 2003). Newborn gerbils can also be experimentally infected with reovirus 3 with histopathologic lesions noted in the brain and pancreas (Yukawa et al., 1993).

Bacterial Diseases

Helicobacteriosis

The gerbil is favored by many researchers as an animal model for *Helicobacter pylori* research because, when infected, the gerbil develops severe gastritis and gastric ulcers. These ulcers do not heal spontaneously but do respond to therapeutic treatment. This is similar to human infection with *Helicobacter pylori* (Peek, 2008). Gerbils also can develop gastric adenocarcinoma secondary to chronic helicobacter infection (Franco et al., 2008; Tsukamoto et al., 2007; Watanabe et al., 1998).

Listeriosis

The Mongolian gerbil is an animal model for listeriosis, the disease resulting from infection with *Listeria monocytogenes* (Blanot et al., 1997). The gerbil has two cell receptors which are similar to the human, InlA-E-cadherin and InlB-Met. These cell receptors work with *L. monocytogenes* surface proteins to facilitate entry into the cells (Disson et al., 2009).

Leptospirosis

The gerbil has been identified as a good animal model to study pulmonary hemorrhage as a consequence of

severe leptospirosis infection. More importantly, the gerbil mimics the human in its response to the infection by producing increased amounts of platelet-activating factor acetylhydrolase (Yang et al., 2009).

Borreliosis

Gerbils have been used in research on vaccines for *Borrelia burgdorferi*, the etiologic agent for Lyme disease (Preac-Mursic et al., 1992).

Epilepsy (Seizures)

Gerbils are considered a good model for inherited epilepsy. Gerbils will display spontaneous, recurrent generalized seizures beginning at about 6 weeks of age (Buckmaster and Wong, 2002; Loskota and Lomax, 1975). Mongolian gerbils were first identified as a new animal model of inherited seizures in 1968 (Thiessen et al., 1968b). Immediately thereafter a number of papers were published regarding gerbils and seizures (Kaplan and Mizejeskic, 1972; Loskota et al., 1974, 1975).

In the 1970s Loskota started a selective breeding program at UCLA to develop seizure-sensitive and seizure-resistant gerbils. Selective breeding of three pairs of seizure-sensitive gerbils, originating from Tumblebrook Farm in Massachusetts, for 18 generations resulted in the creation of the seizure-sensitive gerbil. The seizure-sensitive gerbils were identified as WJL/UC and seizure-resistant gerbils as STR/UC (Loskota et al., 1974).

Mongolian gerbils continue to be used as a model of inherited epilepsy. It is now known that the seizure-sensitive gerbils have abnormalities that involve the GABAergic synaptic transmission in the brain (Hwang et al., 2004; Kang et al., 2001; Kwak et al., 2005). The entire mechanism of how and why seizures occur continues to be under investigation.

Brain Development and Behavior

Since the 1980s, the gerbil has been widely used in studies of brain growth and development, neural plasticity, behavior, and normal and pathological aspects of aging (Cheal, 1986). Examples include studies of changes in the muscarinic receptors in the gerbil thalamus from age 6–36 months (Pilar-Cuellar et al., 2008), effects of periadolescent sensory stimulation on neural development (Lehmann et al., 2009), and ontogeny of the dopamine innervations in the nucleus accumbens (Lesting et al., 2005). Behavioral studies include reports of neonatal separation that results in behavioral and biochemical differences in adult gerbils (Jaworska et al., 2008) and studies of the effects of the father in gerbil developmental behavior (Piovanotti and Vieira, 2004). Lee et al. (2009, 2010a, 2010b) have studied neurochemical changes in the gerbil brain, especially the hippocampus, as it relates

to aging. As gerbils are an important model for hearing research there is also interest in the development of specific parts of the brain that relate to hearing, such as age-related changes in the medial nucleus of the trapezoid body and in the lateral superior olive (Gleich and Strutz, 2002; Gleich et al., 2004).

General Neuroscience

Gerbils have been used extensively in neuroscience research. It has been found that gerbils are more homologous to humans in the tachykinin NK-1, 2, 3, receptor activity and affinity as compared to rats and mice (Griffante et al., 2006; Leffler et al., 2009). A new model, gerbil foot tapping, has been developed as a fear-related response. Gerbils injected with NK-1 agonists in the brain develop a fear-related foot-tapping behavior (Bristow and Young, 1994; Sundqvist et al., 2007). Gerbils have also been used as animal models of anxiety in the elevated plus maze and Black/White Box tests (Bridges and Starkey, 2004; Heldt et al., 2009), and to evaluate the anxiolytic properties of rose odor inhalation (Bradley et al., 2007). Gerbils have been used in the forced swim test which has predictive validity for the evaluation of novel anti-depressants (Wallace-Boone et al., 2008). In 2009, Gaese et al. published a report using Mongolian gerbils for acoustic startle and prepulse inhibition studies.

Auditory Research

Mongolian gerbils are a common animal model in auditory research as gerbils have human-like low-frequency hearing (Engel, 2008; Wetzel et al., 2008). Gerbils have been used as an animal model of auditory neuropathy and it has been shown that there is the potential for use of stem cells to cure some hearing loss (Matsuoka et al., 2007).

Miscellaneous Disease Models and Research Uses

Iron Overload

The gerbil is the first animal model found for the human disease hemochromatosis. In the experimental gerbil model histopathologic lesions in the heart and liver are very similar to the human disease. The model is created by once- or twice-daily injections with iron dextran (Carthew et al., 1993). The gerbil iron-overload model continues to be used to further elucidate the mechanisms of the disease as well as to explore potential therapies (Al-Rousan et al., 2009; Kaiser et al., 2003; Otto-Duessel et al., 2008).

Cholesteatoma and Otitis Media

Just as gerbils have been used widely in acoustic and auditory basic research, they have also been found to be an animal model for cholesteatoma, a benign tumor of the middle ear. The animal model is most commonly created by surgical ligation of the gerbil's auditory duct (Choufani et al., 2007; Kim and Chole, 1998). Gerbils are also commonly used for studies of infectious otitis media. In the induced model the gerbils are usually infected with *Streptococcus pneumoniae* (Soriano et al., 2000), although *Haemophilus influenza* (Ponte et al., 1999) and *Moraxella catarrhalis* (Fulghum and Marrow, 1996) have also been used.

Prostate Gland Biology

Gerbils have been used as an animal model for the study of androgen receptors in the prostate gland (Campos et al., 1997; Cordeiro et al., 2008).

Dental Research

Gerbils are used as experimental models to correlate the periodontium's biological response to various mechanical stresses, as the periodontal ligament was shown to be highly sensitive to occlusal alterations (Iyomasa et al., 2008). Gerbils have also been used as animal models for dental caries (Fitzgerald and Fitzgerald, 1966).

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Egyptian Fat-Tailed Jird

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INTRODUCTION

The Egyptian fat-tailed jird (jird) (Figure 53.1) has been housed in zoological collections since its discovery in 1880. It has only been within the last decade or so that the species has become increasingly popular among exotic pet hobbyists. Many pet owners are intrigued by the unusual appearance of the tail and the gentle disposition of this species. Captive-bred jirds are now available for purchase in pet stores within the United States and Europe, where they may be referred to as “duprasi” or “fat-tailed duprasi.” Despite the jird gaining recognition and movement globally, much remains unknown regarding basic biologic and physiologic data. These rodents have a rounded body with long fluffy agouti-colored fur on the dorsum and white on the ventrum. They have a highly distinctive, blunt, sparsely haired, club-shaped tail which has been established as an effective site for the development of cutaneous leishmaniasis.

History

Pachyuromys duprasi were first discovered in Algeria by French zoologist Fernand Lataste in 1880 (Lataste, 1880). The Egyptian subspecies (*P.d. natronensis*) was first described in 1903 by William Edward De Winton.

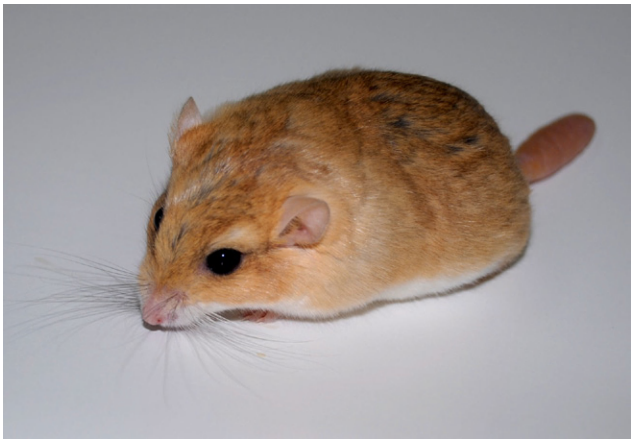


FIGURE 53.1 Adult Egyptian fat-tailed jird, *Pachyuormys duprasi natronensis*.

Taxonomy

The jird is in the genus *Pachyuromys*, subfamily Gerbillinae, family Muridae, and order Rodentia. *P. duprasi* is the only species within this genus. *Pachyuromys* appear phylogenetically closely related to *Desmodilliscus* (Brauer’s gerbil) and together they comprise a new tribe taxonomically (Chevret and Dobigny, 2005). Although there is considerable debate among taxonomists, currently there are three geographically distinct forms of this species: Libyan (*P. duprasi*), Egyptian (*P. d. natronensis*), and Algerian (*P. d. faroulti*). The Egyptian subspecies can be distinguished from the nominate (Algerian) form by its lighter hair coat. The Greek roots of the genus name, *Pachyuromys* (“Pachy” = “thick”, “uro” = “tail”, “mys” = “mouse”), provides those who have never seen this animal an idea of what it might look like and highlights the animal’s most distinguishing physical feature.

Geographical Distribution

Jirds are found in North Africa from Algeria to Egypt. The Egyptian subspecies lives along the Mediterranean coastal desert. They usually inhabit rocky or sandy areas with sparse vegetation and live in simple burrows which they excavate to a depth of 1m (Figure 53.2).



FIGURE 53.2 Wild jird burrow (upper left) with desert beetle exoskeletons (lower right) in Wadi Natroun, Egypt.

Jirds have also been observed residing in tunnels excavated by other rodent species. They are described in the wild as being nocturnal mammals which become active at dusk (Osborne and Helmy, 1980). Much remains unknown about the breeding and dietary habits of wild jirds but they are believed to be omnivorous, eating both plants and at least two species of desert beetles from the Tenebrionidae family (*Adesmia* sp. and *Pimelia* sp.) (Osborne and Helmy, 1980).

Origin

The Naval Medical Research Unit No. 3 (NAMRU-3) in Cairo, Egypt began work in December 1996 to establish a purpose-bred jird colony to mitigate the confounding potential of unknown variables (such as age, health status, and nutritional factors) associated with wild-caught research subjects, and to avoid harming the local ecosystem with ongoing trapping. Wild-caught jirds were used from the Wadi El Natroun area of the western margin of the Nile Delta to found the colony. A government-authorized local hunter caught jirds using either surface live traps or by excavating their tunnels. Initially, 24 juvenile jirds (12 males and 12 females) were brought to NAMRU-3 and served as the foundation colony. Additional wild-caught jirds were added to the colony in 2000 and 2003. Since 2003, NAMRU-3 has had a closed, self-sustaining colony.

ANATOMY, PHYSIOLOGY, AND BEHAVIOR

Anatomy

External Features

The Egyptian fat-tailed jird is mouse-like with well-developed hind legs and forelegs that can be used as hands. The eyes and ears are large and vibrissae well-developed. The coat is very dense and soft. The palmar and plantar aspects of the feet are partially haired. Dorsal hairs are pale agouti with blackish tips. The lateral aspects are agouti and both the ventrum and feet are white.

The average jird lifespan is 2–4 years. The body of the adult jird is 148–183 mm long, and its tail is 55–62 mm long. In the wild, their adult body weight typically ranges from between 22–44.6 g (Hoath, 2003; Osborne and Helmy, 1980). Captive adult males are approximately 25% heavier than adult non-gravid females (Table 53.1). Adult captive jirds are approximately twice the size of their wild counterparts. This could result from several factors, including ad libitum feeding in captivity, access to calorie-dense feed instead of calorie-restricted natural diets, decreased exercise, genetic selection, and metabolic

changes related to environmental conditions such as temperature and humidity.

The tail is relatively thick and club-shaped, bicolored, and like the body has an agouti dorsal surface and white ventral surface, and no apical brush. Histologically, the tail is well-vascularized and comprised of abundant adipose tissue. Because of the sparse hair on the tail, skin color can readily be assessed. Thus the tail is a good location to assess for oxygenation and capillary refill time (CRT). The tail also is usually “plump” in healthy adult animals and “thinner” in immature animals, lactating females, and sick animals. The skin over the tail is taut in well-hydrated animals and “wrinkled” in dehydrated jirds. Although fighting is uncommon, the tail is usually the site towards which bite wounds are directed.

Dentition

Dental morphology has been studied in detail (Osborne and Helmy, 1980; Petter, 1956) and the overall dentition pattern is similar to that of murid rodents. Some noteworthy features of *Pachyuromys* which help distinguish it from other Gerbilline genera include the presence of grooves on the anterior surfaces of the upper incisors and simple third molars which lack folds. The adult enamel pattern has been noted to be more similar to *Meriones crassus* than *Gerbillus* sp.

Skeletal System

The morphology of the skull of jirds has been well-characterized (Hoath, 2003; Osborne and Helmy, 1980). The skull is elongate with very large and prominent auditory bullae. The external auditory meatus is also large. A large suprameatal triangle is a distinguishing characteristic of this species. The meatal lip is prominent, and an accessory tympanum is present. Other identifying features of the skull of the jird include presence of complete zygomatic processes and a poorly differentiated supraorbital ridge.

TABLE 53.1 Biological Data for the Captive Egyptian Fat-Tailed Jird (*Pachyuromys duprasi natronensis*)

Adult body weight (male)	80–82 g
Adult body weight (female)	55–61 g
Birth weight	2.4–2.6 g
Life span	2–4 y
Adult food consumption	5 g/d
Adult water consumption	5 ml/d
Gestation period	20–22 d
Litter size	1–7 pups (3.1 pups on average)
Weaning age	21–28 d

Mammary System

Conspicuous rows of nipples (four pairs: one thoracic, one abdominal, and two inguinal) are visible on the ventrum of the female jird by 16 days of age. Nipples are not visible on males. This dimorphic feature can be used in proper sex determination.

Genitourinary System

Male jirds lack preputial glands and have a single pair of unusually small ventral prostate glands. Vesicular glands are lobed medially and along the acutely edged greater curvatures. The subterminal flexures of the vesicular glands are rounded and smooth (Voss and Linzey, 1981). Jirds are reported to produce post-copulatory vaginal plugs but unlike other common laboratory rodent species, plugs can be challenging to visualize consistently and their absence should not imply an unsuccessful breeding attempt (Héron-Royer, 1891; Lataste, 1882).

Physiology

Serum Biochemistry Parameters

Serum biochemical parameters have been determined for a number of analytes, using a serum chemistry analyzer, in healthy adult male and female jirds under chemical sedation (Table 53.2). Serum concentrations of amylase and glucose are significantly different in male versus female jirds. Amylase was significantly higher in males (mean, 1380 μ /L) than in females (mean, 1214 μ /L). Mean blood glucose was significantly lower in male jirds (193 mg/dl) compared with females (247 mg/dl). Values for globulin, creatinine, alanine aminotransferase, alkaline phosphatase, albumin, and total protein were not significantly different in male and female jirds. In captivity, despite ad libitum feeding of the same diet to both sexes, adult males grow to about 25% greater average body weight than adult non-gravid females (Felt et al., 2008). Weight difference among wild jirds of different sexes had not been noted previously (Capello, 2003; Hoath, 2003; Osborne and Helmy, 1980).

Hematological Parameters

The normal parameters have been determined for a number of hematological indices for adult male and female healthy jirds under chemical sedation (Table 53.3). Leukocytic and erythrocytic indices are not significantly different between the sexes. Platelet counts are similar between the sexes as well, but mean platelet volume (MPV) is significantly higher in females than males. The baseline hematological laboratory reference ranges along with serum biochemical established parameters permit for individual and overall colony health monitoring.

Behavior

Wild jirds tend to live in small groups but have also been found to live solitarily. They are described as nocturnal, becoming active at dusk (Hoath, 2003; Osborne and Helmy, 1980), but in captivity they appear to sleep and awaken intermittently throughout the day and night. Captive jirds spend considerable time burrowing. They tend to build a simple cup-shaped nest which they usually share with all of their cage mates. PVC plastic mouse tunnels (Bio-Serv, Inc., Frenchtown, NJ) are readily used as shelter when provided.

When a male and female are ready to breed, they will focus their attention on one another almost exclusively. They often vocalize emitting high-pitch squeaks, and they stand on their hind legs and appear to wrestle. It remains unclear if this behavior is a component of the courtship process or the copulation act itself. Internal cage height should be at least 6 inches to accommodate "standing" if breeding is desired. Unlike *Meriones* sp., which have a strong pair bond and are often described as monogamous, proven jird breeders will accept and successfully mate with unfamiliar conspecifics (Donnelly and Quimby, 2002).

In the wild, omnivorous jirds are successful hunters. Captive jirds will display active hunting behavior when offered live food items, such as mealworms or crickets.

Cannibalism has been reported to be a common expected behavior encountered in captive animals (Osborne and Helmy, 1980). This occurs very rarely in laboratory-reared animals and is probably confined to primiparous females.

MANAGEMENT, HUSBANDRY, AND COLONY HEALTH

Housing Systems

Caging Systems

Jirds are successfully housed and bred in conventional and individually ventilated rodent caging systems. Cages with dimensions of 12.1 in long $\times$ 12.1 in wide $\times$ 7.4 in high have been successfully used. Breeding pairs, nursing dam, or four males or females can be co-housed without aggressive behavior in a 144-sq-inch solid-bottomed cage. Smaller cage sizes have not been evaluated. They adapt well to sipper tube-equipped water bottles.

Bedding

Unlike many gerbilline species, jirds can be housed on sterilized hardwood bedding without access to a sand substrate and will remain free of any dermatological health problems. Jirds produce small quantities

TABLE 53.2 Serum Chemistries for Egyptian Fat-Tailed Jirds (*Pachyuromys duprasi natronensis*)

Indicators/ animals	ALB (g/dl)	ALP (U/L)	ALT (U/L)	AMY (U/L)	TBIL (mg/dl)	BUN (mg/dl)	Ca (mg/dl)	PHOS (mg/dl)	CRE (mg/dl)	GLU (mg/dl)	Na (mmol/L)	K (mmol/L)	TP (g/dl)	GLOB (g/ dl)
FEMALES (N = 20)														
Range	1.8–2.3	50.0– 250.0	84.0– 583.0	869.0– 1779.0	0.4–0.7	13.0–37.0	9.3–10.1	3.1–6.6	0.2–0.6	113.0– 457.0	140.0– 154.0	3.5–4.7	4.9–5.9	3.1–3.7
Mean	2.0	112.7	168.6	1213.7	0.5	25.7	9.7	4.8	0.4	246.5	147.9	4.1	5.5	3.5
SD	0.2	49.8	115.6	210.3	0.1	6.1	0.2	1.0	0.1	80.9	3.0	0.3	0.2	0.2
Population confidence interval for mean	0.1	21.8	50.7	92.2	0.0	2.7	0.1	0.4	0.0	35.5	1.3	0.1	0.1	0.1
MALES (N = 19)														
Range	1.7–2.4	47.0– 201.0	70.0– 631.0	1073.0– 1893.0	0.1–0.9	17.0–30.0	9.0–10.5	2.4–6.9	0.2–0.5	132.0– 302.0	143.0– 160.0	3.0–5.2	5.2–6.2	3.3–3.9
Mean	2.0	107.8	197.9	1380.4	0.5	25.1	9.8	4.4	0.4	193.4	149.5	3.9	5.6	3.6
SD	0.2	45.2	139.0	185.8	0.2	3.7	0.4	1.2	0.1	40.4	3.6	0.6	0.3	0.1
Population confidence interval for mean	0.1	20.3	62.5	83.6	0.1	1.6	0.2	0.5	0.0	18.2	1.6	0.3	0.1	0.1
P	0.7	0.8	0.9	0.014	0.2	0.7	0.7	0.3	0.8	0.013	0.1	0.5	0.1	0.0

*ALB, albumin; ALP, alkaline phosphatase; ALT, alanine transaminase; AMY, amylase; TBIL, total bilirubin; PHOS, phosphorous; CRE, creatinine; GLU, glucose; TP, total protein; GLOB, globulin.

TABLE 53.3 Hematological Values of the Egyptian Fat-Tailed Jird (*Pachyuromys duprasi natronensis*)

	Females (n = 18)		Population confidence interval for mean (+/-)	Males (n = 17)		Population confidence interval for mean (+/-)	p value by t- test at 95% confidence level
	Range	Mean ± SD		Range	Mean ± SD		
WBC (K/UL)	2.00–13.46	8.21 ± 2.76	1.28	2.64–13.22	8.52 ± 3.16	1.5	0.84
NE (K/UL)	0.18–3.32	1.49 ± 0.91	0.42	0.33–3.89	1.67 ± 1.11	0.53	0.55
LY (K/UL)	1.74–9.26	6.37 ± 1.99	0.92	2.26–9.08	6.42 ± 2.17	1.03	0.92
MO (K/UL)	0.06–0.49	0.24 ± 0.12	0.06	0.04–0.51	0.30 ± 0.15	0.07	0.22
EO (K/UL)	0.01–0.39	0.09 ± 0.11	0.05	0.00–0.26	0.09 ± 0.09	0.04	0.96
BA (K/UL)	0.00–0.09	0.03 ± 0.03	0.01	0.00–0.10	0.03 ± 0.03	0.01	0.69
NE (%)	1.95–28.51	17.24 ± 6.75	3.12	4.48–32.78	18.38 ± 7.64	3.63	0.58
LY (%)	67.18–95.42	78.60 ± 7.46	3.45	61.97–92.40	76.94 ± 8.95	4.25	0.52
MO (%)	1.9–4.15	2.86 ± 0.61	0.28	1.56–5.57	3.35 ± 1.09	0.52	0.11
EO (%)	0.11–2.89	1.00 ± 0.86	0.4	0.07–2.15	0.99 ± 0.82	0.39	0.85
BA (%)	0.00–0.69	0.30 ± 0.25	0.12	0.00–0.94	0.34 ± 0.31	0.15	0.81
RBC (M/UL)	4.47–7.00	5.96 ± 0.63	0.29	4.39–7.37	5.93 ± 0.93	0.44	0.99
Hb (g/dl)	6.40–10.30	9.13 ± 0.94	0.43	6.80–11.20	9.34 ± 1.49	0.71	0.57
HCT (%)	26.00–42.30	37.24 ± 3.79	1.75	27.60–46.70	37.90 ± 5.95	2.83	0.63
MCV (fL)	55.40–67.20	62.51 ± 3.05	1.41	58.10–70.10	64.03 ± 3.47	1.65	0.21
MCH (pg)	14.30–16.60	15.33 ± 0.73	0.34	13.80–16.70	15.74 ± 0.74	0.35	0.13
MCHC (g/dl)	23.40–25.80	24.53 ± 0.67	0.31	22.70–26.10	24.63 ± 1.01	0.48	0.74
RDW (%)	15.20–18.80	16.57 ± 0.95	0.44	14.40–19.20	16.64 ± 1.43	0.68	0.89
PLT (K/UL)	79.00–623.00	283.11 ± 152.02	70.23	75.00–736.00	257.94 ± 168.58	80.14	0.8
MPV (fL)	4.90–5.70	5.29 ± 0.23	0.11	5.20–6.40	5.60 ± 0.33	0.16	0.01

of urine daily, despite ad libitum availability of water. Their pelleted stool, although very hard and dry, is similar in shape and size to that of rodents of a comparable size. The bedding remains odorless for several days and cage changes are typically performed once a week.

Environmental Conditions

Ventilation

Ventilation requirements are similar to those for the mouse and other rodent species. Use of individually ventilated cage systems does not negatively impact reproductive capabilities.

Temperature and Humidity

Jirds can be housed at temperatures of 17.8–22.2°C (64–72°F), similar to those used for other rodents. They are sensitive to temperatures above 35°C (95°F). Humidity range of 35–50% is well-tolerated, but humidity values greater than 50% have been reported to result in a rough

and damp hair coat in *Meriones* sp. (Harkness and Wagner, 1995). Ringtail has not been a condition identified in jirds. Nasal dermatitis, which has been associated with high humidity in other gerbilline species, has not been observed in captive *Pachyuromys duprasi*.

Illumination

A typical light:dark cycle of 12:12h throughout the year is adequate for both the breeding colony and the research animals. Incandescent light (approximately 400lux) 1 meter above the floor has not induced any clinical or histological evidence of vision or reproductive problems.

Enrichment

Nestlets (Ancare Corp., Bellmore, NY) and/or cotton balls are readily used by jirds to line the nest in which they sleep and give birth. Co-housed jirds usually share the nest. PVC plastic mouse tunnels (Bio-Serv, Inc., Frenchtown, NJ) can also be used as shelter.

Sanitation

The routine sanitation practices used in laboratory animal facilities are adequate to ensure healthy laboratory jirds. Cage changes are typically performed weekly, unless pups are present. In this case, spot cleaning may be performed as needed and complete cage changing should be delayed until pups are at least 10 days old.

Nutrition

Feed Type

Jirds are successfully maintained on pelleted rodent feed with 18% protein and 11% fat (Purina LabDiet, St. Louis, MO).

Nutritional Requirements

Jirds thrive on commercial rodent ration. Fresh vegetables such as carrots and cucumber can be placed on the cage floor once a week as part of the enrichment program. Like hamsters, some jirds prefer food on the floor and may refuse to take it from the hopper even if it is the only available food source in the cage. After weaning, young animals are given food both on the cage floor and in the hopper. Animal care staff must ensure that young jirds are eating during the transition from floor feeding to hopper feeding. Most animals transition to using a feed hopper by the time they are 5 weeks old. If an approved supplier is available, meal worms and crickets can be offered; these seem to be preferred food items.

Water

Jirds adapt well to sipper tube equipped water bottles filled with acidified water (pH 2.8). Water should be provided *ad libitum*. Unlike other desert-dwelling rodents such as the kangaroo rat (*Dipodomys* sp.), compulsive water drinking behavior and a subsequent diabetes insipidus-like disease have not been observed in this species (Donnelly and Quimby, 2002).

Breeding

Reproductive Biology

Jirds are paired according to compatibility, health status, and size. Jirds are placed together in a clean, neutral cage and must be observed for several minutes to ensure compatibility. If fighting is observed within the first few minutes, animals should be immediately separated. Animals from unsuccessful matings have been successfully paired with other mates. Compatible pairs are kept together for approximately 1 week. The male is removed and placed in same-sex group housing or individual housing. Females are allowed a minimum of a 4-week rest interval between weaning and subsequent mating attempts. Unlike *Meriones* sp., which have



FIGURE 53.3 Adult male jird.



FIGURE 53.4 Adult female jird.

a strong pair bond and are often described as monogamous, proven jird breeders will accept and successfully mate with unfamiliar conspecifics. After weaning, the offspring can be housed with same-sex littermates or individually, if littermates are not available.

Like other rodents, males have a greater distance between the urogenital and anal openings than do females (Figures 53.3 and 53.4). When adult males are held upright, there is a prominent bulge of the caudal body due to the large descended testicles within the scrotum (Figure 53.3). Only females have visible nipples after 16 days of age; these are also helpful in sex determination.

Unlike wild jirds which have been described as having a seasonality to breeding (Osborne and Helmy, 1980), captive jirds breed successfully all year. Females and males reach sexual maturity at 8 weeks of age and reach peak performance at 1 year. Compatible pairs have been reproductively successful for 3 years.



FIGURE 53.5 Newborn captive jirds in a cup-shaped nest.



FIGURE 53.6 Pair of 10-day-old jird pups. Furred with adult hair pattern, non-ambulatory, and eyes still closed.

Breeding System

Random breeding methods are used to maintain outbred stock. Inbreeding has not been initiated; instead, systematic mating of cousins is used in an attempt to preserve heterozygosity, since this is thought to be better than random mating in smaller populations (Festing, 2003). The jirds can be divided into four groups and a circular mating system used. Members of one group are mated with members of another group, and their offspring constitute a third group. As more generations are produced, inbreeding and homozygosity will gradually increase within the colony.

Care of Young

Jirds have a gestation period of 20–22 days with litters of 1–7 pups (average is three pups per litter). Newborns weigh 2.4–2.6g (Figure 53.5); they double their weight by day 7, and triple their weight by day 14. Hair growth begins by week 1, and the adult hair coat pattern (Figure 53.6) becomes obvious by week 2. Pups usually begin to crawl by day 12 and open their eyes by day 16. Pups begin to eat pelleted food, if offered on the cage floor, by 21 days of age and are fully weaned by 28 days. More than 90.2%

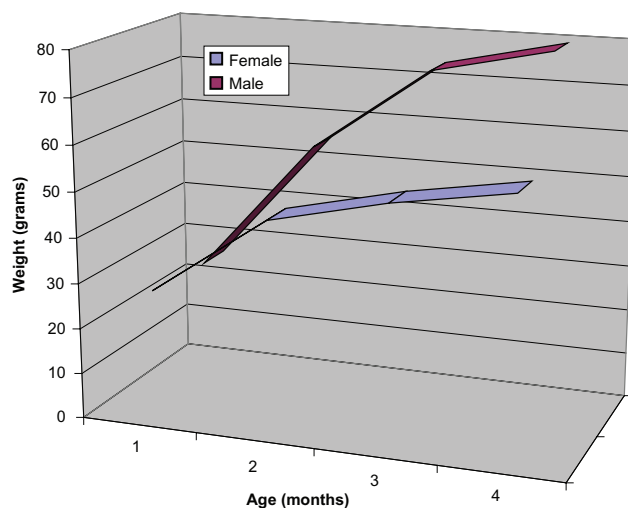


FIGURE 53.7 Typical growth rate of captive male and female jirds.

of pups reach weaning age. Juveniles continue to gain weight until they are 16–20 weeks old (Figure 53.7).

Colony Health

Preventive Medical Strategies

Routine traditional rodent quality assurance and sentinel methods can readily be applied to this species and colony health can be monitored through the placement of dirty bedding sentinels (jirds) in each room and periodic scheduled serological, microbiological, parasitological, and pathological screening.

Record Keeping

Conventional cage cards and breeding records adapted from those used for mice have proven satisfactory for maintaining jird colony records.

DISEASES

Introduction

Properly maintained laboratory jirds, in general, lead lives that are largely free of infectious diseases. Dental disease and neoplasia are the most common health issues encountered and seem to be confined to aged animals. Both can result in significant morbidity and mortality.

Infectious Diseases

Viral Infections

Serological screening for several murine viral pathogens (Sendai pneumonia virus, mouse hepatitis virus, minute virus of mice, GD-7, reovirus 3, ectromelia,

K virus, mouse cytomegalovirus, mouse parvovirus, pneumonia virus of mice, polyomavirus, rotavirus (enzootic diarrhea of infant mice), hantavirus, mouse thymic virus, lymphocytic choriomeningitis virus and mouse adenovirus-1 (FL) and -2 (K87)) have been conducted on sentinel and wild-caught jirds repeatedly for several years. To date, serological evidence of infection has not been documented.

Bacterial Infections

Ciliated-associated respiratory bacillus (CAR) and *Mycoplasma pulmonis*, using serology and culture, have not been detected in the jird colonies despite long-term active surveillance. Specific screening for Tyzzer's (*Clostridium piliformis*), staphylococcal dermatitis or salmonellosis, which are known to occur in the Mongolian gerbil (*Meriones unguiculatus*), has also not yielded any positive animals.

COLIBACILLOSIS

Escherichia coli has been isolated from jirds that have died of septicemia. These animals were either found dead in their cage or severely moribund. Onset was very acute in all cases and there were no signs of external trauma. Suppurative metritis, typhlocolitis, and myocarditis were present in at least two of the cases.

KLEBSIELLOSIS

Klebsiella pneumoniae has been isolated from the blood, spleen, and liver of a jird that died suddenly and had evidence of hepatomegaly and splenomegaly. No other abnormalities were noted on necropsy.

SERRATIOSIS

Serratia marcescens was isolated from the blood and muscle tissue of a jird that died acutely. Severe rhabdomyolysis was noted in one of its distal hind legs. No other gross lesions were noted on necropsy or histopathology.

BARTONELLOSIS

A single case of severe acute pulmonary hemorrhage occurred in an adult wild-caught jird, which acutely developed severe dyspnea and was euthanized. *Bartonella* sp. was cultured from the blood. The organism is likely a novel species and seems to most closely genetically resemble a species isolated from the Tanezumi rat, *Rattus tanezumi flavipectus* (Ying et al., 2002). The affected jird's cagemates were also screened for the organism using blood culture and tested negative.

Parasitic Infections

PROTOZOA

Routine serological screening for *Encephalitozoon cuniculi* has not detected the presence of that pathogen

in jirds. An uncharacterized protozoal organism has been identified in the intestinal mucosa of several wild-caught jirds. In all cases, no lesions could be attributed to infection.

CESTODES

Adult *Hymenolepis diminuta* were found in the small and large intestines of wild-caught jirds. Eggs were detected by direct fecal smear and by fecal flotation with zinc sulfate. Parasites were eliminated by treating infected jirds with a single oral dose of niclosamide (10mg/100g body weight). The potential zoonotic health risk of this parasite should be considered when bringing wild-caught jirds into a facility.

NEMATODES

Adult pinworms, unspecified to date, have been found intraluminally in the gastrointestinal tracts of wild-caught jirds. Pinworm eggs can be isolated using a fecal flotation technique with zinc sulfate. Perianal tape tests of infected animals did not yield any eggs. Infected animals appeared healthy and no lesions were attributed to infection in animals that were screened by gross necropsy and histologically. Infection was eliminated using a subcutaneous dose of ivermectin (200µg per kg body weight) weekly for 3 weeks.

Eggs resembling *Strongyloides* have been detected occasionally on direct smears of feces from wild-caught jirds.

MITES

Wild-caught jirds have been found to be susceptible to colonization with *Pyroglyphis morganii* (Felt, 2009) (Figure 53.8). All jirds in the cage developed acute patchy hair loss and generalized pruritus. Direct examination, combing of fur, cellophane tape testing, and microscopic examination revealed that all jirds in the cage were infested with this mite. This species of mite had not previously been reported to infest mammals. Cage changes along with two doses of ivermectin (200µg per kg body weight) administered subcutaneously 14 days apart proved effective in eradicating the infestation. The fur grew back and pruritus subsided quickly following treatment of affected animals.

TICKS

Camel ticks (*Hyalomma drommedarii*) have been found feeding on wild-caught jirds.

Neoplasia

Several spontaneous neoplasms have been characterized previously in jirds (Rowe et al., 1974). Rowe found that 46% (11/24) of aged (>24 months) jirds developed spontaneous neoplasms. The incidence of neoplasia

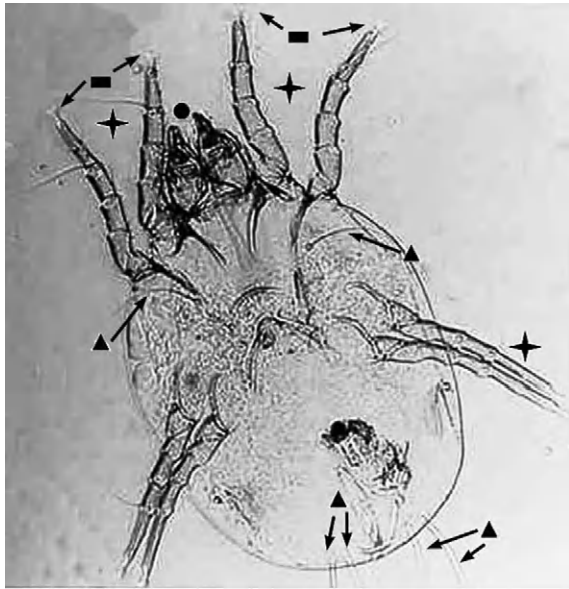


FIGURE 53.8 Magnification 4x. Pyroglyphid adult mite, *Pyroglyphis morgani*, infesting captive jirds with alopecia and pruritus. The following anatomical structures have been identified: ● = chelicerae; ■ = pulvillus of tarsus; + = legs; ■ = setae.

in NAMRU-3's colony is less than 5%. The difference in rates may be due to variation among subspecies, as Rowe housed both *Pachyuromys duprasi* and NAMRU-3 source *Pachyuromys duprasi natronensis*. Age may have also been a factor, as many of the jirds in Rowe's colony were of unknown age and may have been older than the NAMRU-3 colony. Further, genetics may have been a factor, as the NAMRU-3 colony originated from 24 founding animals and the majority of Rowe's animals were wild-caught.

Lymphoreticular Neoplasms

Lymphoreticular neoplasms, especially thymomas and B-cell lymphomas, are encountered most commonly. Thoracic B-cell lymphomas may present with acute dyspnea and metastasize to the spleen.

Trichofolliculoma

A recurrent dermal mass appeared on the muzzle and nasal planum of a captive-born 2.5-year-old male jird. The initial mass was raised, circular and approximately 3mm in diameter (Figure 53.9). It was surgically excised and the excision healed normally. Histopathology of the mass was most consistent with a diagnosis of trichofolliculoma and excision seemed complete. Approximately 8 months post-excision the mass recurred and within 3 weeks was more than triple its original size (1cm diameter) (Figure 53.10). Histologically, the recurrent mass was once again diagnosed as a trichofolliculoma. Attempts to identify a possible underlying infectious cause (such as papillomatosis) using electron microscopy were

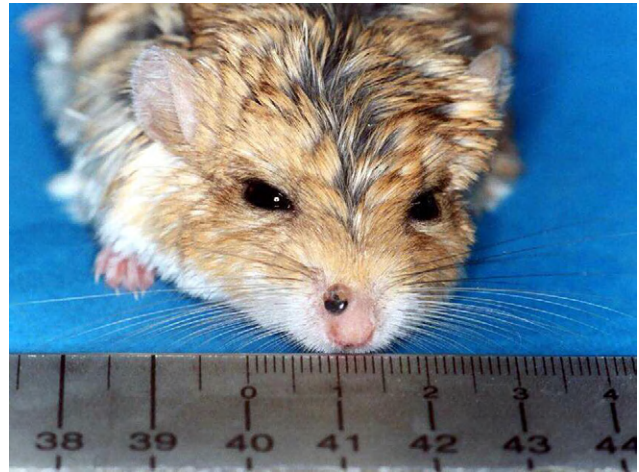


FIGURE 53.9 A 1½-year-old male jird with dermal mass prior to surgical removal.

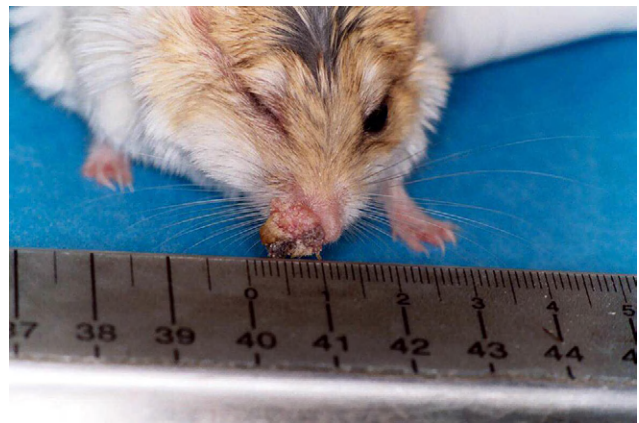


FIGURE 53.10 The same jird with a recurrent dermal mass 8 months later. Compared to the initial mass, this mass grew rapidly and had an irregular surface with raised areas and some thorn-like projections.

unsuccessful. Trichofolliculoma is the only neoplasm of the skin reported thus far in jirds.

Other Neoplasms

The only reported neoplasm affecting the digestive system is gastric adenocarcinoma. Neoplasms affecting the genital system include uterine leiomyosarcoma and prostatic adenocarcinoma. Endocrine neoplasms include pancreatic islet cell adenoma and both adrenal adenomas and adenocarcinomas (Rowe, 1974).

Miscellaneous Diseases

Dental Problems

Frequently, older jirds (≥ 2 years) develop excessively worn upper incisors and periodontitis. Caretakers may first note that jirds are thin or their appetite is



FIGURE 53.11 Chondrodysplastic 4-week-old jird (right) with normal-sized littermate (left). Besides the diminutive size the affected jirds tend to have dome-shaped skulls and at 5–8 months of age develop difficulty ambulating.

diminished. In addition to recessed upper incisors and periodontitis, some jirds will present with concurrent elongated lower incisors presumably due to loss of occlusive contact. The condition is not reversible but can sometimes be managed effectively for several weeks through teeth trimming. Affected animals may also benefit nutritionally from offering fruits, vegetables, meal worms, and crickets in addition to the rodent chow. For jirds that are more than 2 years old and are housed long term, we recommend conducting oral examinations and weighing animals monthly. If substantial weight loss (>10%) is noted, the animal should be evaluated for worn upper incisors or elongated lower incisors. Although the affected jird's condition may improve with teeth trimming, the prognosis is considered grave and euthanasia is recommended if the crowns of the upper incisors recess to the level of the gum.

Maxillary and mandibular abscesses have been reported to occur in pet jirds (Capello, 2003). If a decision is made to treat, surgical debridement is recommended. Both the maxillary and mandibular molars, the source of the abscess, are almost impossible to extract, so recurrence is likely. Antibiotic therapy should also be considered.

Dwarfism (Chondrodysplasia)

Spontaneous dwarfism has occurred in this species. As adults, such jirds only reached approximately 25% of the normal body weight (see Figure 53.11). It is thought that this disease is genetic with an autosomal recessive mode of inheritance, as all dwarf jirds were born to normal-sized parents and had normal-sized siblings. Attempts to mate dwarf jirds to further characterize the mode of inheritance have proven unsuccessful to date. Besides their diminutive size and possible infertility, affected jirds have visible skull and limb deformities, but are capable of surviving well into adulthood. They begin showing difficulty ambulating when they

reach 5–10 months of age and this usually warrants euthanasia. Certain anatomical sites such as the ribs, proximal tibia, distal radius, and distal femur seem to be affected by chondrodysplasia more than others.

ANESTHESIA/ANALGESIA, EUTHANASIA, AND NECROPSY

Chemical Restraint

The jird is effectively immobilized using ketamine (100mg/kg) given intramuscularly; this provides approximately 30 min of sedation. Alternatively a cocktail of ketamine (30mg/kg), xylazine (6mg/kg), and acepromazine (1mg/kg) administered intraperitoneally (Felt et al., 2009) provides approximately 5–10 min of sedation. Inhalant isoflurane (1–4%) works very well in this species and should be used when conducting invasive or longer procedures. The jird is highly susceptible to hypothermia. Body temperature should be closely monitored and exogenous heat should be provided as necessary.

Euthanasia

The jird can effectively and humanely be euthanized by several methods including: inhalant carbon dioxide, intraperitoneally delivered pentobarbital, cervical dislocation (under sedation), and exsanguination via cardiac or jugular venipuncture (under sedation).

USE IN RESEARCH

Introduction

The general characteristics of the jird that lend well to it being a research subject are its unique anatomy, small size, docility, fecundity, and ease at which it appears to adapt to captivity (Felt et al., 2008). Specifically with regard to cutaneous leishmania research, inoculations and subsequent lesion development in the tail of *P. duprasi* may result in less pain or discomfort than for other established models (e.g., foot pad infection of mice and hamsters).

Historical Uses

The jird has been used to study thermoregulation and circadian rhythms which indicated that the homeostatic control of body temperature is exerted separately from the circadian control (Refinetti, 1998).

Auditory studies of jirds include those focused on neuronal anatomy (Muller et al., 1991), frequency

representation in the auditory brainstem nuclei (Muller, 1990) and cochlea (Muller et al., 1991), and low-frequency auditory sensitivity (Plassmann and Kadel, 1991).

Less commonly, this species has been used to study phylogenetic taxonomy (Benazzou et al., 1984); rodent reproductive physiology (Eisenberg and Isaac, 1963; Héron-Royer, 1891; Lataste, 1882); and cyclic transmission of protozoal infections (Jakel, 1995).

More recently, infectious disease studies have used the jird, specifically to study *Leishmania major* and *L. tropica* (Hanafi et al., 2000, 2007).

Leishmaniasis

The jird's tail and foot pads have been established as effective sites for the development of cutaneous leishmaniasis and study of Old World *Leishmania* spp. (Hanafi et al., 2000, 2007).

The BALB/c mouse has been successfully used for study of *L. major* and the Syrian hamster for the study of *L. major*, *L. donovani*, and *L. infantum*; however, until recently there has not been a model for *L. tropica* (Bray, 1987; Bastien and Killick-Kendrick, 1992) which is widespread throughout eastern and northern Africa, the Middle East, and large parts of Asia (AlSamarai and AIObaidi, 2009; Hay, 2009; Katakura, 2009; Parvizi et al., 2010; Toz et al., 2009). The Old World anthroponotic cutaneous leishmaniasis caused by *L. tropica* was a significant public health problem during the First Gulf War (1990–1991). Prevention and treatment of this infection has been limited due to lack of knowledge regarding the biological interactions with its vectors and the lack of an animal model for such studies.

Leishmania amastigotes mature in the sandfly gut to promastigotes (infective stage) which then migrate via the esophagus and transfer to a receptive host during a blood meal. The infective stage infects blood cells, particularly macrophages, and develops into the amastigote stage, which grows, reproduces, and infects more cells near the site of infection. Sand flies feeding on an infected host ingest amastigote-laden macrophages and continue the cycle. A study conducted at the NAMRU-3, Cairo, Egypt explored the feasibility of using the tail of the jird for *Leishmania* propagation. This study demonstrated that the jird tail was susceptible to infection with *L. major* (Hanafi et al., 2000) but was not as efficient as BALB/c mice, the established animal model for *L. major*. Surprisingly, *L. tropica* was unable to propagate in the tail of the jird. The preferred usual site for infecting animals with *Leishmania* spp. is the dorsal surface of the foot (Laison and Shaw, 1987) although footpad inoculations were often used in earlier studies. A follow-up study at NAMRU-3 involving inoculation of the jird's hind foot with cultured *L. tropica* metacyclic promastigotes established the jird not only as an effective animal

model but also as a possible natural rodent reservoir for *L. tropica* (Hanafi et al., 2007).

BASIC EXPERIMENTAL METHODS

Many of the common handling and restraint techniques and bi methodologies used for comparably sized rodents may be applied to the jird. In general this species is considered quite docile and rarely bites even when provoked. Minimal physical restraint for non-invasive examinations and procedures is usually adequate. The size, shape, and vasculature of the jird tail pose unique challenges when considering it as a site for vascular access.

Physical Restraint

A single pair of latex or nitrile gloves is adequate protection for the hands of personnel during handling. The jird can readily be lifted from the cage by closing cupped hands over the animal and gently scooping it into the palm of one hand. This method is suitable for non-invasive examinations or procedures and for cage transfers.

Another method for physically restraining the jird is to hold the base of the tail with the handler's dominant hand, and then firmly scruff the animal over the shoulders with the thumb and forefinger of the non-dominant hand, rotating the non-dominant hand, so the jird is laying in the palm with ventrum upward and placing the tail between the pinky finger and palm for added control. This technique leaves the handler's dominant hand available for other manipulations.

Sampling Techniques

Blood Sampling

A number of anatomical sites have been evaluated as venipuncture sites in this species (Felt et al., 2009). The submandibular vessels are not visible even with hair removal and the lancet technique inconsistently yields small blood volume amounts (<0.05ml). The lateral saphenous vein, although visible and easily punctured with a needle, yields a small amount of blood (0.05ml). The tail venipuncture technique presents unique challenges due to the size, shape, and abundant adipose tissue of the tail, which collectively serve to obliterate vessel visibility. The orbital venous sinus, jugular vein, and cardiac venipuncture are all preferred bleeding anatomical sites.

Orbital Venous Sinus

This procedure should be performed only on anesthetized animals. The animal is placed in lateral recumbency.



FIGURE 53.12 “Blind” jugular venipuncture technique in a jird. A rubber band, placed behind the incisors, was used to extend and stabilize the head of the jird. The needle is inserted 2–4 mm medial to the shoulder at a 30–45° angle at the level of the clavicle.

Scruffing, although not required, seems to stabilize the head and results in protrusion of the ocular globe which may facilitate accessibility. A capillary tube is inserted into the medial canthus and directed medially and posteriorly to the globe until it reaches the center of the orbit. The capillary tube is gently rotated until the sinus is punctured and blood flows. Following blood collection, the capillary tube is removed, the eyelids closed, and gentle pressure applied for several seconds to ensure hemostasis. Antibiotic and/or lubricating eye ointment can be applied to the eye. This technique is suitable for obtaining blood volumes less than 0.1 ml.

Jugular Vein

Jirds should be anesthetized or sedated for this procedure, which is performed blindly and therefore, immobility and positioning are critical for success (Felt et al., 2009). The anesthetized jird is placed in dorsal recumbency. The ventral neck and chest are shaved and then cleaned with 70% isopropyl alcohol or alternate disinfectant. A rubber band is placed behind the incisors to stabilize and extend the head and neck rostrally (Figure 53.12). Using a 1-ml syringe with a 25-gauge needle, blood can be collected blindly by inserting the needle (23–25 g) with the bevel up at approximately a 30–45° angle at the level of the clavicle and 2–4 mm medial to the shoulder. The needle is advanced cranially 2–3 mm under the skin, the desired volume of blood withdrawn, and the needle removed. Direct pressure is applied using a cotton ball, gauze pad, or cotton swab for 30 seconds to aid hemostasis. Additionally, this technique can be used as a successful exsanguination method for euthanasia, yielding up to 3 ml of blood.

Cardiac Puncture

Blood can readily be collected directly from the heart using identical techniques (dorsal and lateral

recumbency) employed in the mouse and rat. This procedure should be performed as a terminal procedure on anesthetized animals only and can also be used to exsanguinate animals with yields up to 3 ml.

Compound Administration

Oral, intramuscular, subcutaneous, and intraperitoneal compound administrations can readily be performed in the jird using techniques identical to those used for the mouse and rat. Non-invasive intravenous administration is technically challenging in this species. The lateral saphenous vein can be used. It is important not to use a blood flash in the hub of the needle to verify patency, but rather look for signs of extravasation during injection. The tail, submandibular and jugular veins can all be accessed via cutdown procedure.

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Sand Rat

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OUTLINE

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INTRODUCTION

The focus of this chapter is to illustrate the use of a rodent, other than the mouse or rat, to serve as a model of an important human disease. Often, the idea of animal modeling suffers from a narrow focus on the species available for such work, and the goal here is to demonstrate the contributions of a lesser-known species. Thus, this chapter will provide limited information on basic species biology and husbandry and will instead concentrate on research uses.

Psammomys obesus is an animal model of the nutritionally induced type 2 diabetes-like model used in biological and medical research. *Psammomys* is a rodent belonging to the sub-family *Gerbillinae* living in the semi-desert regions of North Africa and the Middle East, from Morocco in the west, through Egypt and Israel, up to Arabia in the east (Osborn and Helmy, 1980).

Psammomys, first described by Cretzschmar in 1828, became renowned among the scientific community with the discovery that it was an excellent model for the study of human diabetes.

Diabetes in *Psammomys* was discovered by chance observation in desert rodents collected by the US Naval Medical Research Unit in Egypt in the 1960s. The animals were trapped on the sandy beaches of the Nile Delta and thought to be rats. They were trivially nicknamed “sand rats,” which is a misnomer because they are gerbils that belong to the family *Gerbillinae*. They should be referred to as *Psammomys*, according to the classification of Thomas (Pinshow, 1993; Thomas, 1902).

The *Psammomys* were sent to the laboratory of Schmidt-Nielsen at Duke University. Investigators there were the first to report that diabetes occurs in the majority of *Psammomys* maintained on a regular laboratory diet but not on a vegetable diet (Schmidt-Nielsen, 1964).

The diabetes ranged from mild hyperglycemia with hyperinsulinemia to hypoinsulinemia with ketoacidosis, which was a terminal stage with short survival (Haines et al., 1965; Miki et al., 1966). These early investigations were performed mostly on the first generation of Egyptian *Psammomys* because attempts to establish a multigeneration colony were not successful due to low reproductive capacity on the regular rodent diet. On this relatively high-energy diet, a large proportion of the animals gained excess weight with a characteristic superscapular hump and became insulin-resistant (Miki et al., 1967).

Psammomys attained additional interest when it was found to be an intermediate host of the parasite *Leishmania tropica* which is transmitted to humans by the sand fly, *Phlebotomus* sp. (Shafrir et al., 2006).

Cutaneous leishmaniasis in the Jordan Valley is maintained within the close association of *Psammomys* and sand fly *Phlebotomus papatasi*, which appear to be the exclusive host and vector species. The incidence of the disease was similar to the distribution of *Psammomys* colonies in the region, i.e., the plains of light stoneless soil. An infection rate of 93% was recorded in the very common *Psammomys*. All *Leishmania* isolates from *Psammomys* and from *Ph. papatasi* were identical to those from local human cases (Schlein et al., 1982).

Psammomys obesus is a unique polygenic animal model ideal for the study of obesity and type 2 diabetes. Unlike most other animal models used for obesity research, *Psammomys* displays a range of phenotypic characteristics, as might be expected for non-inbred progeny, when given free access to standard laboratory diet labeled as high-energy (HE) diet (Digestible energy – 2.93 kcal/g). Adult *Psammomys* have a wide range of body weight and body fat content that forms a continuous distribution.

The evolution of diabetes in *Psammomys* is gradual, as defined by Kalderon et al. (1986) and follows several defined stages: some animals remain normoglycemic and normoinsulinemic (stage A), others are hyperinsulinemic and normoglycemic (stage B), some develop overt insulin-resistant diabetes with hyperinsulinemia (stage C), and a small percentage develop consequential to the long-lasting hyperglycemia, severe decompensated diabetes, loss of beta cells and hypoinsulinemia (stage D). *Psammomys* are considered hyperglycemic when non-fasted blood glucose levels exceed 200 mg/dl, and considered hyperinsulinemic when non-fasted plasma insulin levels exceed 150 μ U/ml. Plasma insulin was measured by radioimmunoassay utilizing anti-human insulin antibodies and human insulin as standard. Dilutions of *Psammomys* serum and pancreatic insulin gave curves paralleling those of dilutions of human insulin. Cross reactivity with purified human insulin, used as standard, was 90–95% (Kalderon et al., 1986).

The speed of progression from stage A to the other stages depends on the amount of digestible energy in

the diet. On HE diet *Psammomys* progress from stage A to stage C within 7–28 days.

A genetic variability in diabetes susceptibility within the natural population has been observed. Several years ago a selection program was established in order to achieve divergent diabetes-prone (DP) and diabetes-resistant (DR) lines (Kalman et al., 1993; Ziv and Kalman, 2000). The DP line, 7–28 days following the transfer to HE diet, is characterized by hyperinsulinemia, and later by hyperglycemia.

Diet-induced “diabesity” (collusion of diabetes and obesity) in *Psammomys* involves attenuation of insulin action by inhibition of the tyrosine kinase essential for tyrosine phosphorylation of the insulin receptor substrate IRS-1 (Ikeda et al., 2001; Kanety et al., 1994; Shafrir, 1996), together with the increase in plasma triglyceride levels and augmentation of lipoprotein lipase activity (Chajek-Shaul et al., 1988). According to Coleman (Coleman, 1978) all these features lead to a metabolic state that is superimposed on a native “thrifty metabolism”, which might be seen as a peculiar natural adaptation to life in areas where only low-energy food is available.

SELECTION OF RELIABLE DIETS AND PSAMMOMYS LINES FOR DIABETES RESEARCH

In 1970 Adler established a *Psammomys* colony for medical research purposes at the Hebrew University Medical School in Jerusalem (Adler et al., 1976). The colony originated from ten pairs of *Psammomys* captured in the Dead Sea area. The colony was initially fed on the natural diet – the salt bush (*Atriplex halimus*). All animals fed this diet, which was supplied from the Dead Sea shore to the Jerusalem animal facility, maintained normoglycemia similar to their phenotype in the natural habitat. Animals from the colony served mainly for diabetes research, and were challenged by feeding them on regular laboratory rat diet, that for this species should be regarded as a diabetogenic diet.

In later stages it was decided to standardize the *Psammomys* diets, in order to enable diabetic research. For that purpose two diets were developed. The normoglycemic Mediterranean salt bush diet was replaced by an artificial low-energy (LE) *Psammomys* diet and the diabetic state was achieved by feeding the animals the HE diet. For composition and ingredients of both diets see Table 54.1. The major difference between both diets is in the digestible portion of the carbohydrates. The carbohydrate source of the LE diet derives mainly from alfalfa that has a high cellulose level, thus reducing carbohydrate availability to 59%. The carbohydrate source of the HE diet derives mainly from corn flour that contains a high starch level thus increasing carbohydrate availability to 77%.

TABLE 54.1 Composition, Energy, and Digestibility of *Psammomys* Diets

Components	HE diet		LE diet		Salt bush	
	%	Digestible portion	%	Digestible portion	%	Digestible portion
Protein	23.6	0.79	16.7	0.70	23.3	0.75
Fat	2.4	0.92	3.1	0.86	2.9	0.57
Carbohydrates	68.0	0.77	70.0	0.59	53.3	0.55
Ash	6.0		10.2		20.5	
Total digestibility		0.76		0.64		0.58
Total energy (kcal/g)	3.88		3.74		3.32	
Digestible energy (kcal/g)		2.93		2.38		1.92

HE diet major ingredients – ground wheat, ground corn, wheat middlings, dehulled soybean meal, corn gluten meal, soybean oil, calcium carbonate, brewers dried yeast, dicalcium phosphate, iodized salt. LE diet major ingredients – dehydrated alfalfa meal, barley, wheat bran, calcium carbonate, dicalcium phosphate, iodized salt.

**FIGURE 54.1** Evolution of Diabetes in *Psammomys* (DP & DR) on HE diet.

We used the HE diet to examine the diabetic potential of each individual, and to identify the diabetic and non-diabetic margins of the population. By using an assortative mating system based on a minimal inbreeding method (Baker, 1979) it was possible to separate the animals in the colony into two distinct lines differing phenotypically and genotypically: diabetes-prone (DP) and diabetes-resistant (DR) animals (Kalman et al., 1993). Details of the genotypic differences between the two lines are described later in this chapter and development of diabetes in the DP and DR lines is summarized in Figure 54.1. Animals to be mated were chosen according to phenotypic parameters (postprandial blood glucose levels of 200mg/dl and above for the DP breeders and 100mg/dl and lower for the DR breeders; plasma insulin levels of 100μU/ml and lower for the DR breeders). We isolated two lines that differ in their diabetic sensitivity without diluting their total genetic pool.

It should be noted that in addition to the composition of the HE diet, the physical characteristics (extruded versus pelleted) and the placement (on the bottom of the cage versus placing diet on the grid) influence both the rate of the development of hyperglycemia and the percentage of diabetic *Psammomys* in the colony. Extruded HE diet tends to be more diabetogenic than pelleted HE diet. Since the amount of diet that animals assume is similar on both diets, we assume that the difference in diabetogenicity is probably due

to the amount of energy the animal has to spend when chewing on the diet and to the fact that nutrient components become more available during the extrusion process. We have noticed that when *Psammomys* are fed the extruded HE diet, about 90% of DP animals become hyperglycemic within 14 days. When feeding *Psammomys* on pelleted HE diet, 80–90% of DP animals become hyperglycemic within 28 days.

The average life span of *Psammomys* is about 2 years (for DR animals fed HE diet and DP animals fed LE diet). When *Psammomys* from the DP line are transferred to the HE diet, they will become diabetic and survive on the HE diet for up to 3 months. They subsequently become hypoinsulinemic (stage D) and die within 10 days.

METABOLIC EFFICIENCY

In order to assess the difference between the two lines and to examine metabolic features related to the etiology of diabetes, we have compared blood glucose, plasma insulin, and plasma triglyceride levels in the two lines when fed on three different diets (*Atriplex halimus*, the Mediterranean saltbush, LE, and HE diets). The results following 14 days of *Psammomys* weaned and then fed for 14 days on the various diets are shown in Figures 54.2–54.5.

The marked difference between the two lines is evident by the development of hyperglycemia and hyperinsulinemia in the DP animals when fed the HE diet, while DR animals on both diets as well as DP animals on LE diet maintain normoglycemia and normoinsulinemia (summarized in Figure 54.6).

In our experiments animals reached stage C when the DP animals were fed the HE diet for 14 days. Our results indicated that when fed the HE diet, over 90% of the DP

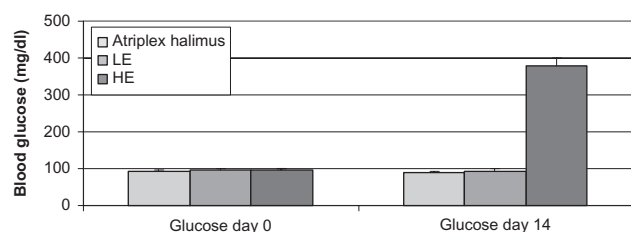


FIGURE 54.2 Blood glucose levels in *Psammomys* from the DP line fed on various diets for 14 days.

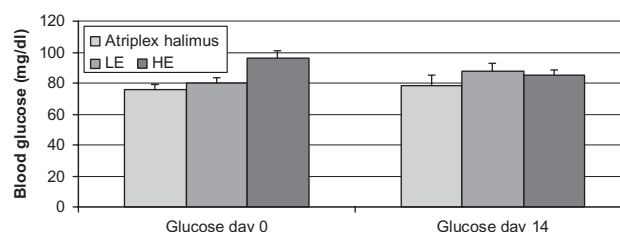


FIGURE 54.4 Blood glucose levels in *Psammomys* from the DR line fed various diets for 14 days.

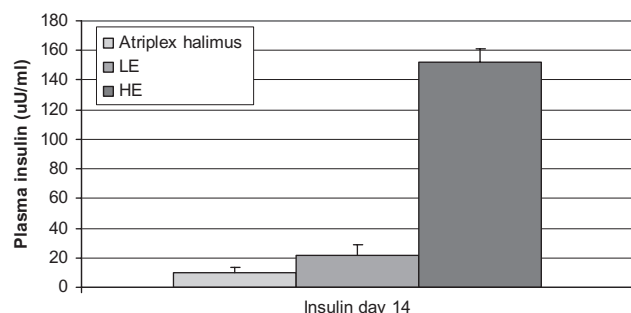


FIGURE 54.3 Plasma insulin levels in *Psammomys* from the DP line fed various diets for 14 days.

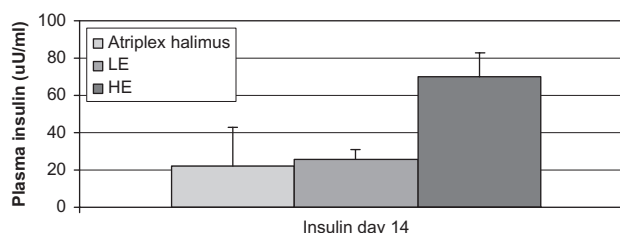


FIGURE 54.5 Plasma insulin levels in *Psammomys* from the DR line fed various diets for 14 days.

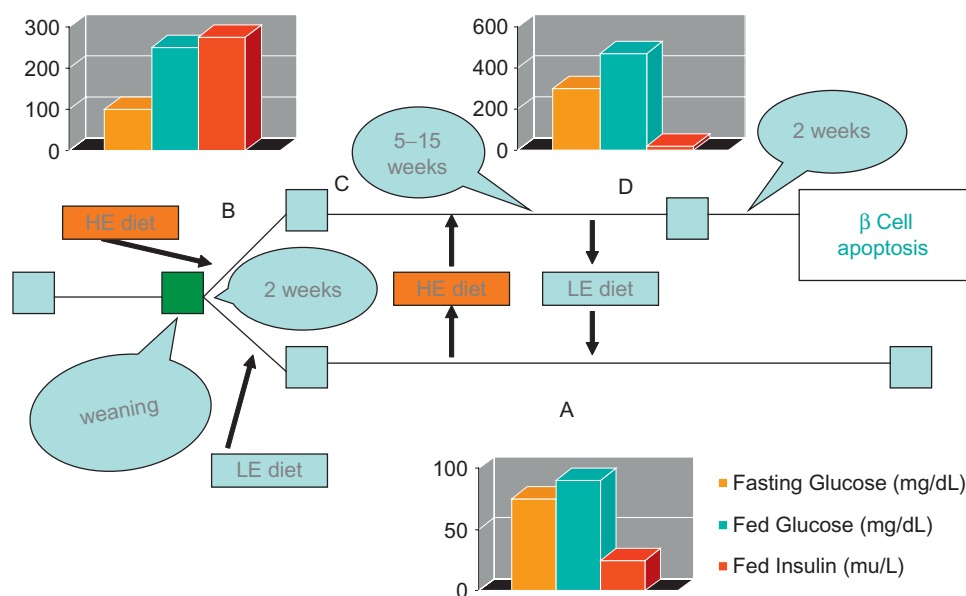


FIGURE 54.6 Development of diabetes in *Psammomys obesus*.

animals become diabetic within 7–28 days. In order to better understand the basis for the rapid evolution of diabetes in these animals, we have looked at several parameters including weight gain and obesity (Figure 54.7).

The different sensitivities to the development of diabetes between the DP and the DR lines may be caused by one or more factors: food intake, hepatic and

peripheral resistance, pancreas activity, or metabolic efficiency. To measure the metabolic efficiency, we followed animals during the period of their most rapid body growth after weaning (2.5–3.0g/day in males and 2.4–2.9g/day in females) and calculated metabolic efficiency as the relation between digestible energy intake and weight increment (Kalman et al., 1993).

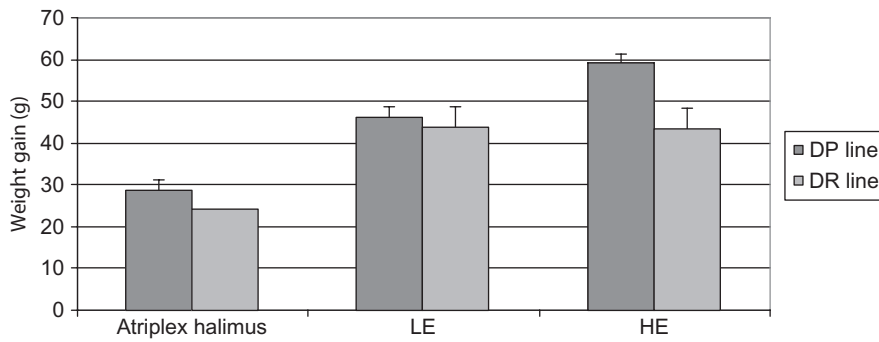


FIGURE 54.7 Weight gain in *Psammomys obesus* from the DP and DR lines on various diets for 14 days.

TABLE 54.2 Effect of HE and LE Diets on Diet and Energy Consumption and Energy Utilization by *Psammomys obesus* from the DP and DR Lines

Diet	DP line		DR line
	HE	LE	HE
Diet consumed in 14 days (g)	125.9 ± 7.0	122.8 ± 2.7	122.5 ± 7.4
Feces secreted in 14 days (g)	26.8 ± 1.9	52.1 ± 1.2	16.1 ± 1.1
Digestible energy consumed in 14 days (kcal)	376.5 ± 21.1	274.5 ± 7.1	402.1 ± 24.2
Energy consumed for growth of 1.0g weight (kcal/g)	6.3 ± 0.2	6.0 ± 0.2	9.4 ± 0.3
Number of animals	20	27	10

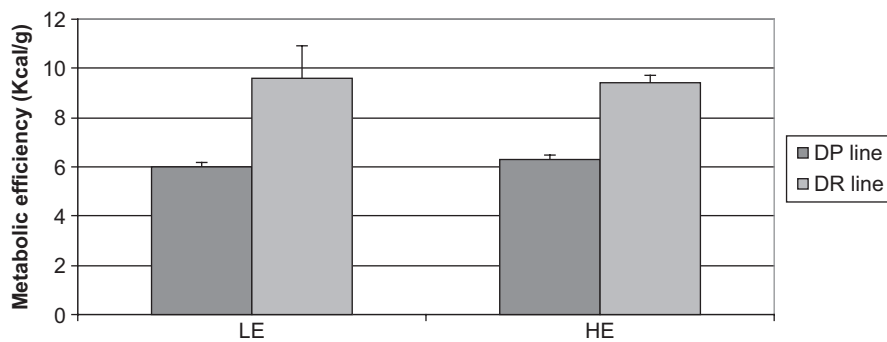


FIGURE 54.8 Metabolic efficiency of *Psammomys obesus* from the DP and DR lines on various diets for 14 days.

There is no hyperphagia in either DP or DR lines when fed HE or LE diets; hyperglycemia is related to energy availability in the diet. The quantity of feces was significantly greater in animals fed the LE diet than in animals fed the HE diet, in line with the dietary carbohydrate digestibility (Table 54.2). The metabolic efficiency in DP line *Psammomys* fed all diets was 6.0–6.6 kcal/g of weight increase, whereas in the DR line, metabolic efficiency was 9.0–9.6 kcal/g of weight increase (Figure 54.8).

The essence of thrifty metabolism represents high metabolic efficiency that enables existence in an environment characterized by constant availability of

LE diets. The artificial laboratory condition of an ad libitum accessible HE diet creates a continuous input of energy and leads to hyperinsulinemia and hyperglycemia.

In other studies performed on a branch of the Israeli colony in Australia (Barnett, 1995), hyperphagia was reported in diabetic *Psammomys* fed a standard rat maintenance diet, but the weight gain did not exceed 15% of body weight. Differences between DP and DR lines are the result not only of their dietary-induced diabetes but also of other zootechnical and reproductive characteristics as described in the next section (Kalman et al., 1996).

MORPHOLOGY, PHYSIOLOGY, BREEDING PERFORMANCE, AND HUSBANDRY

Breeding Performance, Husbandry, and Sources

Breeding performance data for the DP and the DR lines collected over a period of 3 years are summarized in Table 54.3.

TABLE 54.3 Breeding Efficiency of *Psammomys obesus* from the DP and DR Lines. DP Line Animals were Fed the HE Diet, DR Line Animals were Fed the LE Diet

Criteria	DP	DR
Total number of breeding pairs	247	65
Number of sterile pairs	34 (14%)	15 (23%)
Average interval between first two litters (weeks)	7.0	7.5
Average age of female at first birth (weeks)	19.7	20.6
Average mating time (weeks)	39.2	29.8
Number of females that littered at least once	213	50
Number of females that littered at least three times	178 (84%)	34 (68%)
Number of females that littered at least seven times	61 (29%)	8 (16%)
Average number of litters/female	4.7	3.9
Average number of newborn/litter	3.3	3.2
Average number of newborn at first litter	3.2	3.3
Average number of weaned pups/litter	2.2	2.3
Average number of weaned pups at first litter	1.9	2.4
Average total of newborn/female	15.8	12.4
Average total of weaned pups/female	10.5	9.0

Breeding efficiency in *Psammomys* is low compared to outbred rats (Dietrich et al., 1973; Lutsky et al., 1984; Kalman et al., 1996). Number of newborn per female per week is about 0.4. About 75% of the newborn reach the end of weaning. Breeding efficiency of the DP line is higher than of the DR line due to differences in percentages of non-productive breeding pairs (14 vs. 23%) and in total number of litters per female (4.7 vs. 3.9) which increases the total number of newborn per female (15.8 vs. 12.4). Average size of litters in both lines is very similar (3.2 and 3.3).

In the *Psammomys* colony, the DR animals are maintained on HE diet and the DP animals, on LE diet. These are the most suitable conditions for the survival and breeding of the two lines (see Table 54.4).

Psammomys are very docile and are handled very easily. Animals are housed in a room with a typical ventilation rate of 15 air changes per hour, and under a light:dark cycle of 12:12h. Polypropylene cages with wire mesh lids are suitable primary enclosures. A breeding ratio is 1:1 is recommended for reproduction. When the litter is born, the male is separated and the female is left with the litter for 19–26 days. After weaning, the breeding pair is reformed and the litter is transferred to stock cages. Aspen shavings provide adequate bedding and should be changed weekly.

At the time of writing, *Psammomys* are available from the original Hebrew University breeding colony. A branch of the Israeli *Psammomys* colony is also maintained in Australia (Barnett et al., 1994a, 1994, 1995). An additional colony of *Psammomys* is maintained at the Food and Drug Administration, Washington, DC, on which beta cell studies and assessment of diabetic lenses were performed (Chennault et al., 2002; Katzman et al., 2004).

TABLE 54.4 Diet Type and Placement for Breeding Pairs and Stock Animals in DR and DP *Psammomys obesus* Lines

Genetic Line		Diet type	Diet placement
DR line	Breeding pair (monogamy) without litter	HE diet (Harlan Teklad 2018SC)	On the grid
	Breeding pair (monogamy) with litter	HE diet (Harlan Teklad 2018SC)	On the grid and on bottom of the cage
	Stock animals up to 2.5 months of age	HE diet (Harlan Teklad 2018SC)	On the grid and on bottom of the cage
	Stock animals over 2.5 months of age	HE diet (Harlan Teklad 2018SC)	On the grid
DP line	Breeding pair (monogamy) without litter	LE diet (Harlan Teklad 3084S)	On the grid
	Breeding pair (monogamy) with litter	LE diet (Harlan Teklad 3084S) plus	On the grid
		GP diet (Harlan Teklad 2040S)	On the bottom of the cage
	Stock animals up to 2.5 months of age	LE diet (Harlan Teklad 3084S)	On the bottom of the cage
	Stock animals up to 2.5 months of age	LE diet (Harlan Teklad 3084S)	On the grid

Morphological, Physiological, and Biochemical Data

In order to study zootechnical parameters of the DP line animals, we used the pelleted HE diet that slows down the development of hyperglycemia and prevents the extreme diabetic state (stage D).

The morphological, physiological, and biochemical data collected from 151 *Psammomys* from the DP line fed pelleted HE diet, 74 males (M) and 77 females (F), were studied. Biochemical, metabolic, and nutritional balance data of male and female *Psammomys* at various ages are presented in Tables 54.5 (males) and 54.6 (females). Food consumption was 14–16 g/day in male *Psammomys* and 12.5–13 g/day in female *Psammomys*. Water consumption was about 25 ml/day per animal.

Psammomys were weaned at 19–26 days and the growth curves of *Psammomys* fed the HE diet are

presented in Figure 54.9. The results indicated that the period of most rapid growth lasts until 65 days of age (2.5 g/day in males and 2.4 g/day in females). Weight increment slowed until the age of 120 days (1.4 and 0.8 g/day, respectively) and was almost non-existent thereafter. Average weight at maximal growth was 260 g for males and 220 g for females.

Tables 54.7 and 54.8 present morphological and hematological data of male and female *Psammomys*. On the basis of these data Table 54.9 presents the relative size of organs at different ages. Heart and white suprascapular fat are the only organs in which relative weight increased with age. All other organs remained unchanged or reduced their relative weight with age. The adrenal gland was the only organ with a significant difference in relative weight between males and females. Relative size of the kidneys was higher than in other rodents while relative size for the heart was smaller.

TABLE 54.5 Male *Psammomys* from the DP Line, Fed on HE Pelleted Diet- Biochemical, Metabolic and Nutritional Balance Data During a Period of 14 Days at Different Ages

Age (days)	56–78	115–145	239–266	350–405
<i>n</i>	17	18	17	13
Blood glucose (mg/dl)	173 ± 18	190 ± 28	104 ± 17	139 ± 25
Plasma insulin (μU/ml)	174 ± 20	283 ± 96	207 ± 42	136 ± 58
Diet consumed per day (g)	14.1 ± 0.4	16.4 ± 0.9	15.6 ± 0.9	12.1 ± 1.1
Dry feces per day (g)	7.2 ± 0.3	8.1 ± 0.2	7.5 ± 0.4	6.9 ± 0.4
Water consumed per day (g)	25.6 ± 1.2	25.6 ± 1.5	26.9 ± 2.5	27.9 ± 2.7

Values are means ± SE.

TABLE 54.6 Female *Psammomys* from the DP Line, Fed on HE Pelleted Diet – Biochemical, Metabolic, and Nutritional Balance Data During a Period of 14 Days at Different Ages

Age (days)	59–82	111–140	233–266	357–405
<i>n</i>	17	18	19	10
Blood glucose (mg/dl)	162 ± 19	174 ± 25	69 ± 4	94 ± 27
Plasma insulin (μU/ml)	169 ± 22	238 ± 27	240 ± 63	114 ± 51
Diet consumed per day (g)	12.6 ± 1.1	12.6 ± 1.3	12.9 ± 0.4	12.4 ± 0.6
Dry feces per day (g)	6.8 ± 0.2	7.4 ± 0.3	6.8 ± 0.3	6.4 ± 0.3
Water consumed per day (g)	24.6 ± 1.9	26.8 ± 1.6	22.1 ± 1.2	24.6 ± 2.0

Values are means ± SE.

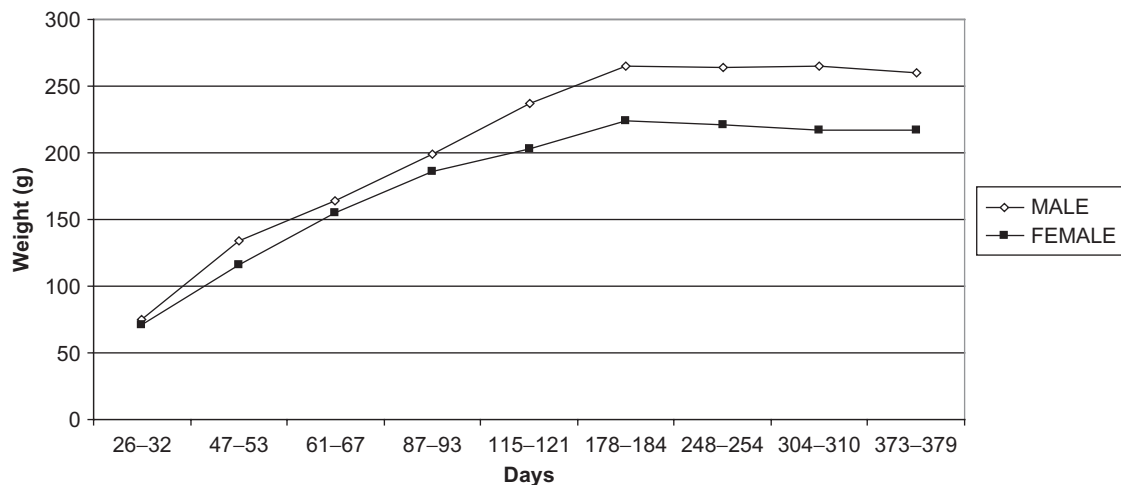


FIGURE 54.9 Growth curves of male and female *Psammomys obesus* from the DP line fed on pelleted HE diet.

TABLE 54.7 Male *Psammomys obesus* from the DP Line, Fed the HE Pelleted Diet – Body and Organ Weights at Different Ages

Age (days)	76–79	137–141	260–267	377–389
<i>n</i>	18	18	17	15
Weight (g)	196 ± 5 ^a	246 ± 5	262 ± 7	252 ± 7
Body length (cm)	17.6 ± 0.2	19.0 ± 0.1	19.8 ± 0.2	19.8 ± 0.3
Tail length (cm)	12.4 ± 0.1	12.7 ± 0.3	13.3 ± 0.2	13.9 ± 0.3
Epid. fat (g)	2.5 ± 0.1	4.3 ± 0.3	2.9 ± 0.2	2.2 ± 0.2
Testes (g)	1.0 ± 0.0	1.2 ± 0.0	1.2 ± 0.0	1.3 ± 0.1
Adrenals (mg)	136.2 ± 7.4	152.6 ± 5.7	142.5 ± 8.8	154.4 ± 7.0
Kidneys (g)	2.6 ± 0.1	2.9 ± 0.1	2.9 ± 0.1	2.9 ± 0.1
Spleen (mg)	158.4 ± 11.2	144.6 ± 9.6	172.1 ± 15.1	147.3 ± 11.6
Liver (g)	8.1 ± 0.3	8.4 ± 0.3	8.9 ± 0.6	7.9 ± 0.3
Lungs (mg)	589.1 ± 17.7	644.6 ± 14.5	699.2 ± 18.6	734.7 ± 20.9
Heart (mg)	433.9 ± 9.6	540.5 ± 11.8	582.8 ± 16.4	606.3 ± 18.7
Brain (g)	1.3 ± 0.0	1.5 ± 0.0	1.3 ± 0.0	1.4 ± 0.0
W.S.F. ^b (g)	1.7 ± 0.1	1.7 ± 0.4	4.1 ± 0.5	2.9 ± 0.4
B.I.F. ^c (mg)	651.2 ± 63.7	368.1 ± 96.5	555.5 ± 91.2	375.7 ± 75.0
Cataracts ^d	2	2	12	12
WBC *10 ⁹ /L	2.3 ± 0.3	3.7 ± 0.4	3.3 ± 0.5	3.6 ± 0.5
RBC *10 ¹² /L	7.1 ± 0.1	7.5 ± 0.1	7.0 ± 0.4	7.1 ± 0.2
Hgb (g/dl)	12.3 ± 0.1	13.0 ± 0.3	11.5 ± 0.7	12.1 ± 0.3
Hct (%)	38.3 ± 2.1	40.0 ± 2.0	40.6 ± 2.7	40.1 ± 1.0
PLT *10 ³ /μl	502.6 ± 18.3	517.9 ± 36.0	472.9 ± 36.6	517.6 ± 31.1

^aValues are means ± SE.^bWhite supescapular fat.^cBrown interscapular fat.^dNumber of positive animals.**TABLE 54.8** Female *Psammomys obesus* from the DP Line, Fed the HE Pelleted Diet – Body and Organ Weights at Different Ages

Age (days)	76–79	137–141	260–267	377–389
<i>n</i>	18	19	20	16
Weight (g)	186 ± 4 ^a	210 ± 5	216 ± 6	207 ± 7
Body length (cm)	17.4 ± 0.2	17.9 ± 0.9	18.8 ± 0.1	18.7 ± 0.3
Tail length (cm)	12.7 ± 0.2	12.7 ± 0.2	12.9 ± 0.2	12.6 ± 0.5
Ovaries (mg)	70.6 ± 5.5	75.3 ± 5.4	84.1 ± 6.9	68.0 ± 5.3
Adrenals (mg)	82.7 ± 3.1	77.8 ± 2.5	77.3 ± 3.2	75.1 ± 3.0
Kidneys (g)	2.4 ± 0.1	2.6 ± 0.1	2.4 ± 0.1	2.2 ± 0.1
Spleen (mg)	163.7 ± 8.9	148.1 ± 9.1	152.5 ± 8.7	124.8 ± 8.8
Liver (g)	7.5 ± 0.2	7.2 ± 0.3	6.7 ± 0.3	6.2 ± 0.3
Lungs (mg)	570.1 ± 14.1	596.5 ± 19.3	630.6 ± 14.8	621.5 ± 25.7

(Continued)

TABLE 54.8 (Continued)

Age (days)	76–79	137–141	260–267	377–389
Heart (mg)	419.1 ± 10.1	477.6 ± 14.3	519.3 ± 14.8	520.3 ± 21.9
Brain (g)	1.2 ± 0.0	1.6 ± 0.2	1.4 ± 0.0	1.3 ± 0.0
W.S.F. ^b (g)	2.1 ± 0.2	3.3 ± 0.4	4.4 ± 0.5	3.1 ± 0.3
B.I.F. ^c (mg)	609.7 ± 59.6	514.3 ± 84.6	527.9 ± 106.4	286.3 ± 62.6
Cataracts ^d	2	6	8	2
WBC *10 ⁹ /L	2.7 ± 0.4	3.0 ± 0.5	3.9 ± 0.6	4.2 ± 1.2
RBC *10 ¹² /L	6.8 ± 0.1	7.0 ± 0.1	6.7 ± 0.1	6.5 ± 0.1
Hgb (g/dl)	11.5 ± 0.2	11.8 ± 0.2	11.1 ± 0.2	10.5 ± 0.2
Hct (%)	37.2 ± 0.6	39.8 ± 0.8	39.6 ± 1.0	35.0 ± 0.7
PLT *10 ³ /μl	403.8 ± 18.3	427.6 ± 25.1	376.9 ± 25.0	456.1 ± 34.9

^aValues are means ± SEM.^bWhite suprascapular fat.^cBrown interscapular fat.^dNumber of positive animals.TABLE 54.9 Organ Weights^a of *Psammomys obesus* from the DP Line, Fed the HE Pelleted Diet Relative to 100 g of Body Weight

Age (days)	76–79		137–141		260–267		377–389	
Sex	M	F	M	F	M	F	M	F
n	18	18	18	19	17	20	15	16
Epididymal fat (g)	1.25 ± 0.06		1.75 ± 0.13		1.09 ± 0.06		0.87 ± 0.09	
Testes (g)	0.54 ± 0.02		0.49 ± 0.02		0.45 ± 0.02		0.51 ± 0.03	
Ovaries (mg)	37.8 ± 2.6		32.3 ± 3.0		38.8 ± 3.2		33.1 ± 2.7	
Adrenals (mg)	73.2 ± 4.1	45.0 ± 2.0	62.6 ± 2.8	37.3 ± 1.1	55.3 ± 4.0	35.8 ± 1.4	61.5 ± 2.8	36.5 ± 1.4
Kidneys (g)	1.35 ± 0.03	1.28 ± 0.02	1.18 ± 0.02	1.23 ± 0.02	1.09 ± 0.03	1.16 ± 0.03	1.13 ± 0.03	1.02 ± 0.08
Spleen (mg)	76.1 ± 5.7	88.6 ± 4.9	58.8 ± 3.7	71.3 ± 4.7	65.9 ± 5.6	70.1 ± 3.0	58.7 ± 4.8	59.8 ± 3.0
Liver (g)	4.06 ± 0.08	4.07 ± 0.11	3.39 ± 0.09	3.44 ± 0.08	3.36 ± 0.16	3.07 ± 0.08	3.14 ± 0.08	2.98 ± 0.08
Lungs (mg)	298.1 ± 8.2	308.2 ± 7.0	263.3 ± 5.3	285.6 ± 8.4	267.9 ± 6.1	292.9 ± 5.7	393.7 ± 11.0	304.3 ± 16.0
Heart (mg)	224.9 ± 5.7	226.4 ± 4.5	220.6 ± 3.7	228.9 ± 6.8	223.0 ± 4.6	241.1 ± 6.5	242.5 ± 9.6	251.3 ± 7.6
Brain (g)	0.68 ± 0.02	0.67 ± 0.02	0.61 ± 0.02	0.76 ± 0.1	0.51 ± 0.03	0.65 ± 0.02	0.54 ± 0.03	0.66 ± 0.03
W.S.F. ^b (g)	0.86 ± 0.06	1.13 ± 0.1	1.04 ± 0.19	1.53 ± 0.19	1.51 ± 0.17	1.96 ± 0.19	1.13 ± 0.12	1.46 ± 0.1
B.I.F. ^c (mg)	313.1 ± 32.0	329.8 ± 32.5	246.5 ± 40.8	270.2 ± 38.7	222.4 ± 37.8	259.7 ± 54.8	157.4 ± 34.4	150.7 ± 32.1

^aValues are means ± SE.^bWhite suprascapular fat.^cBrown interscapular fat.

THE BASIS OF DIABETES IN PSAMMOMYS

Diabetes does not occur in *Psammomys* on the LE diet which is similar in its caloric value to the native food of this animal. Although the metabolic efficiency of *Psammomys* is similar on both LE and HE diets, diabetes

develops on HE diet because of the relative excess nutrient availability resulting in enhanced insulin secretion (Nesher et al., 1999). On HE diet, the transition from the normoin-sulinemic-normoglycemic stage A to the hyperglycemic stage C is often rapid, and may occur within 1–2 weeks.

To examine whether deficiency of bioactive insulin is a possible cause of the hyperglycemia we administered

subcutaneous implants supplying exogenous bovine insulin to stage D, insulin-deficient *Psammomys*. In this stage *Psammomys* have elevated post-prandial glucose and triglyceride levels, and also have elevated fasting blood glucose level (as opposed to stage C *Psammomys*, where fasting blood glucose levels and post-prandial triglyceride levels are not elevated). As shown in Figure 54.10, the effect of insulin became evident by the decrease of fasting glucose levels in four *Psammomys* from 393 ± 55 mg/dl to 107 ± 21 mg/dl and fasting plasma triglyceride from 683 ± 23 mg/dl to 210 ± 86 mg/dl. Blood glucose levels in the fed state remained high (418 ± 24 mg/dl) (Ziv et al., 1996).

The lack of endogenous insulin causes death in diabetic *Psammomys* within 10 days. Addition of the exogenous bovine insulin prevented death, extending their life for up to 60 days, which is the period of activity of the exogenous insulin implants.

To further study the effect of insulin we administered insulin to non-diabetic stage A *Psammomys* and to normal albino rats (Sabra outbred strain, Hebrew University, Jerusalem). At the same time, we started feeding them the HE diet (Ziv et al., 1996). Administration of exogenous insulin did not produce hypoglycemia and failed to prevent the development of hyperglycemia in *Psammomys* (Figure 54.11A). *Psammomys* fed the LE diet and receiving exogenous insulin did not become hypoglycemic despite a marked elevation in circulating insulin levels (Figure 54.11B).

In contrast to *Psammomys*, albino rats fed the HE diet remained normoglycemic, but exogenous insulin caused a pronounced fall in their blood glucose levels. Albino rats fed the LE diet also maintained normoglycemia but

exogenous insulin caused severe hypoglycemia and the death of all animals within 24h (Figure 54.12A). Insulin levels in rats both on LE and HE diets increased markedly as a result of exogenous insulin treatment, which, however, did not result in fatal hypoglycemia in rats on the HE diet (Figure 54.12B).

From this experiment we concluded that it is not the deficiency of insulin that causes hyperglycemia in *Psammomys* fed the HE diet. Further, hyperinsulinemia induced by exogenous bioactive insulin did not result in hypoglycemia even in normoglycemic *Psammomys* fed the LE diet. These results indicated the possible existence of primary insulin resistance in *Psammomys*.

To better define the nature of the insulin resistance and to assess the effect of exogenous insulin on basal hepatic glucose production (HGP) and total glucose disposal in stage A *Psammomys* maintained on the LE diet, a hyperinsulinemic, euglycemic clamp was performed. The clamp was carried out on both DP and DR groups of *Psammomys* and albino rats which were matched for weight. Results are shown in Table 54.10. The basal fasting glucose and insulin levels were similar in all animals. The hyperinsulinemia resulting from the infusion of an identical amount of human insulin was higher in *Psammomys* than in rats by 44–74%, most probably due to low hepatic clearance (Ziv et al., 1986). The HGP in albino rats and in both groups of *Psammomys* was similar in magnitude. However, in albino rats the HGP became almost completely suppressed by exogenous insulin (Ziv et al., 1996).

Psammomys obesus developed a pronounced insulin resistance both at muscle and liver levels when maintained on a high-energy laboratory diet as compared

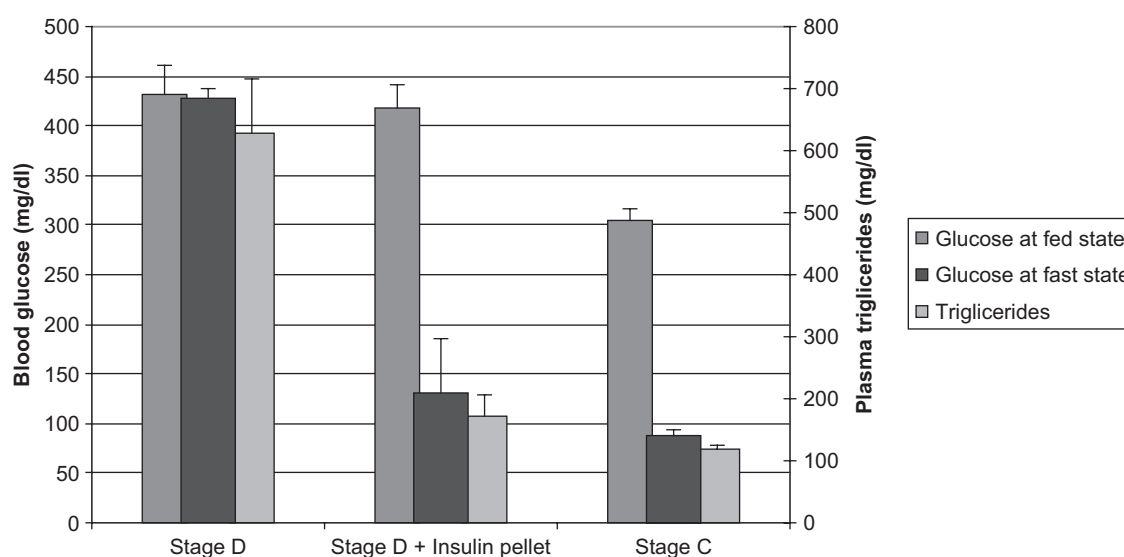


FIGURE 54.10 Blood glucose and plasma triglyceride levels in *Psammomys obesus* from the DP line at stages C and D diabetes, fed the HE diet, with and without administered insulin.

with the native dietary regimen. This is evident from decreased muscle glucose uptake and lack of restraint of hepatic gluconeogenesis in the face of hyperinsulinemia (Ziv et al., 1986). Insulin-induced receptor activation, a prerequisite for intracellular signal transduction is inhibited in the hyperinsulinemic-hyperglycemic stage C, indicating that the resistance is located at the insulin receptor site (Ikeda et al., 2001; Kanety et al., 1994; Ziv et al., 1996).

We have demonstrated that the insulin resistance in *Psammomys* may be an inherent characteristic even in the normoglycemic-normoinsulinemic stage A animals on the LE diet. The ability of *Psammomys* to withstand the hypoglycemic effect of insulin implants could not be explained by massively increased hepatic gluconeogenesis or glycogenolysis. However, because hepatic phosphoenolpyruvate carboxykinase (PEPCK), the rate-limiting gluconeogenic enzyme, was reduced only

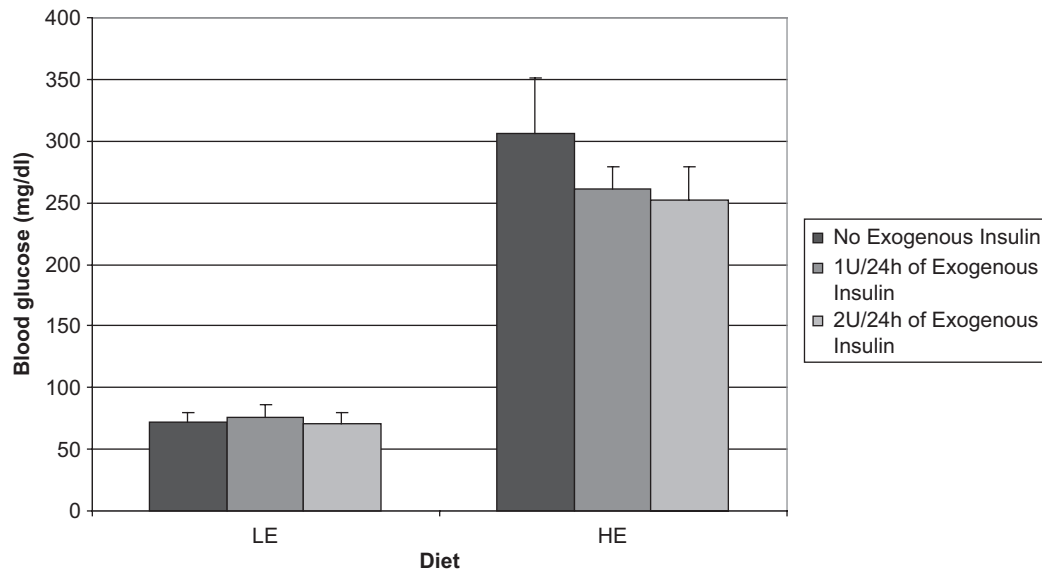


FIGURE 54.11A Effect of LE and HE diets and of exogenous insulin implants on blood glucose levels in *Psammomys obesus* from the DP line after 14 days of treatment.

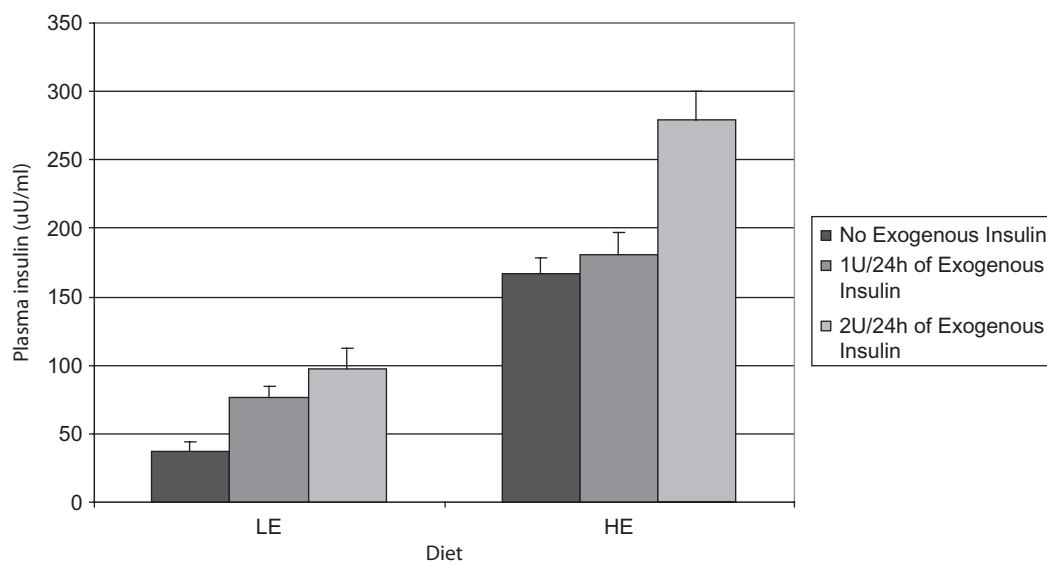


FIGURE 54.11B Effect of LE and HE diets and of exogenous insulin implants on plasma insulin levels in *Psammomys obesus* from the DP line after 14 days of treatment.

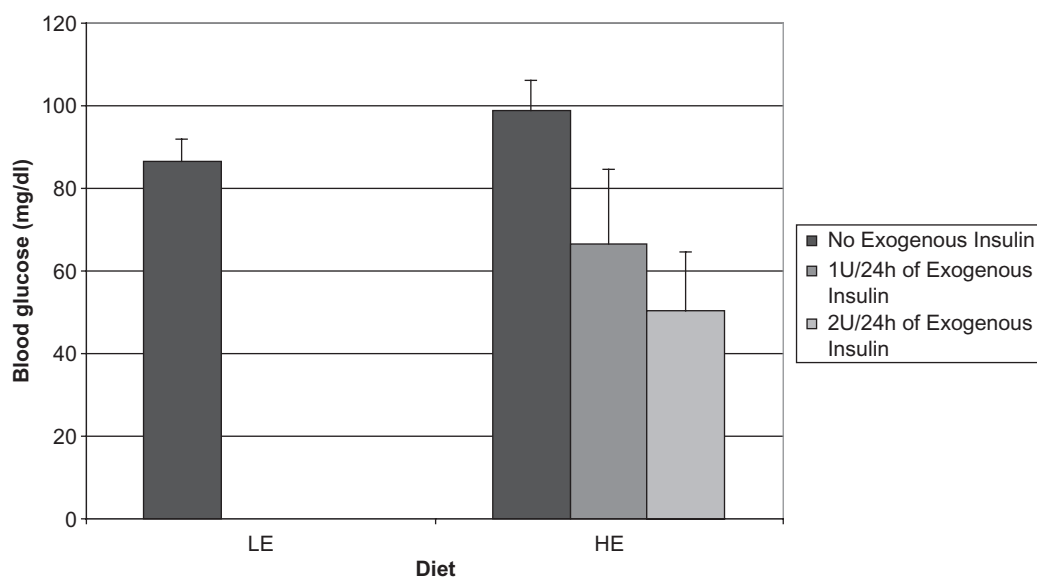


FIGURE 54.12A Effect of LE and HE diets and of exogenous insulin implants on blood glucose levels in albino rats after 14 days of treatment.

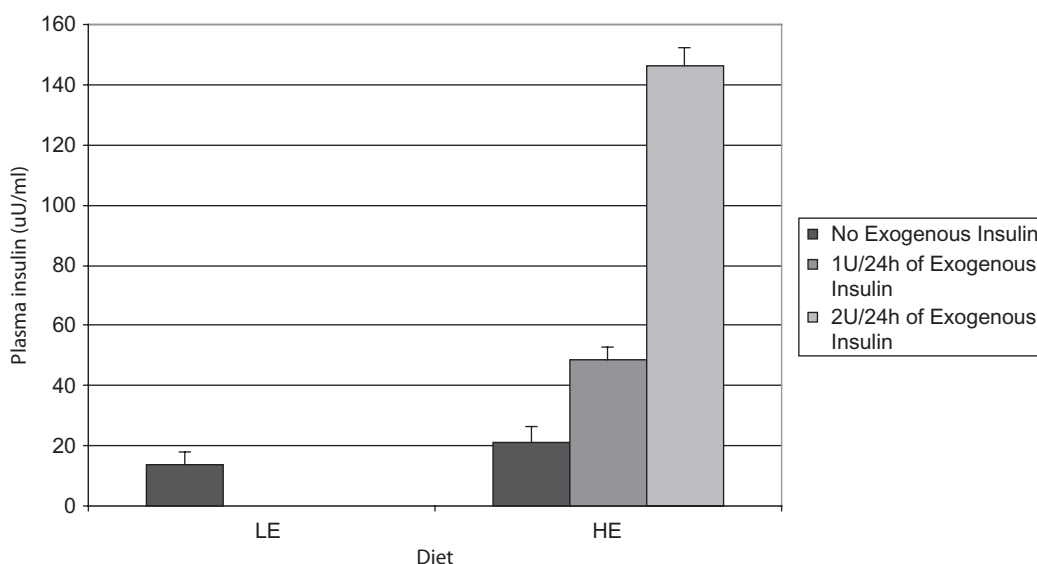


FIGURE 54.12B Effect of LE and HE diets and of exogenous insulin implants on plasma insulin levels in albino rats after 14 days of treatment.

in part by hyperinsulinaemia, this indicates that gluconeogenesis was proceeding and could compensate for the insulin-induced peripheral glucose uptake. It is also conceivable that the scarcity of hepatic and muscle insulin receptors in this animal species (Kanety et al., 1994) and their compromised function in the presence of hyperinsulinemia did not result in an excessive plasma glucose removal. In contrast, the similar hyperinsulinemia in albino rats resulted in reduced hepatic gluconeogenic capacity and enhanced glycogenolysis, which

however did not appear to be sufficient to compensate for the insulin-stimulated tissue glucose uptake. Thus, a profound hypoglycemia was evident, particularly when the animals were maintained on the LE diet.

The use of rats as reference animals in parallel with *Psammomys* does not imply that they are control animals. Rather, they reflect a response of a different species population that is well adjusted to consumption of the HE laboratory diet and characterized by marked sensitivity to insulin.

TABLE 54.10 Results of Hyperinsulinemic Euglycemic Clamp in *Psammomys* and Albino Rats

Albino rat (<i>n</i> = 14)	<i>Psammomys</i> DR line (<i>n</i> = 4)	<i>Psammomys</i> DP line (<i>n</i> = 16)	
95.4 ± 3.6	93.6 ± 5.4	88.2 ± 3.6	Clamp plasma glucose (mg/dl)
116.2 ± 13.4	167.2 ± 72.8	202.5 ± 18.4	Clamp plasma insulin (μU/ml)
11.0 ± 0.5	8.9 ± 1.7	10.0 ± 0.6	Basal HGP (mg min ⁻¹ kg ⁻¹)
0.7 ± 0.3 ^a	4.2 ± 0.2	3.8 ± 0.4	Insulin HGP (mg min ⁻¹ kg ⁻¹)
39.0 ± 1.9 ^a	11.8 ± 2.1	16.0 ± 1.9	Total glucose transport (mg min ⁻¹ kg ⁻¹)

Values are means ± SEM for the number of animals indicated in parentheses.

Body weight of *Psammomys* and albino rats ranged between 150 and 190 g. Fasting plasma glucose of *Psammomys* and albino rats was from 75.6 ± 3.6 to 79.2 ± 3.6 mg/dl and plasma insulin was from 4.2 ± 1.7 to 5.6 ± 1.7 μU/ml. No significant difference for all values between DP and DR *Psammomys*.

Hyperinsulinemia was induced by infusing human insulin.

^a <0.001 for difference between albino rats and both *Psammomys* groups.

Our premise of liver and muscle insulin resistance as characteristic of *Psammomys* species was confirmed by quantitative data on HGP and total glucose transport (TGT) in hyperinsulinemic-euglycemic clamp studies. Insulin infusion decreased the HGP and increased the TGT, again demonstrating the effectiveness of the exogenous insulin. However, HGP was only partly reduced, whereas in albino rats HGP was almost completely abolished (Kalman et al., 1996). Lack of complete suppression of HGP in *Psammomys* was evident even at a higher and longer-lasting level of hyperinsulinemia than in the rats, confirming our previous assumption that gluconeogenesis in *Psammomys* treated with insulin implants is sustained despite hyperinsulinemia.

We have noted a high proportion of circulating proinsulin was observed particularly in the hyperglycemic-hyperinsulinemic stage C *Psammomys* (Gadot, 1994). The hyperproinsulinemia was elicited most probably by the pancreatic beta-cell overtaxation as a result of protracted hyperglycemia. This finding raised the question, "to what extent is the availability of biologically active insulin reduced to account for the hyperglycemia or whether hyperglycemia is caused mainly by the peripheral and hepatic resistance to the available active insulin?" It could be postulated that in the presence of a high proportion of proinsulin or partially split insulin (known to have minimal bioactivity) in *Psammomys*, the HGP could not be totally suppressed in vivo and the peripheral glucose utilization could not be adequately promoted, thus resulting in hyperglycemia. However, the experiments with exogenous insulin, derived either from implants or from infusion during clamp studies, indicated that the hyperinsulinemia elicited by the nutritional overload at stages B or C is able to suppress the HGP at least in part. Full suppression, as observed in the rats, could not be achieved because of the particular resistance of the *Psammomys* species rather than to insufficient levels of bioactive insulin resulting from the

change in the insulin/proinsulin ratio. Similar observations (Shafir, 1988) on incomplete suppression of HGP by porcine insulin were also observed in another group of *Psammomys*. Lack of total HGP suppression in *Psammomys* was evident in these hyperinsulinemic clamp studies even at plasma levels of infused insulin exceeding 6000 pmol/L.

It has previously been shown that hyperinsulinemia is detrimental to the activity of insulin receptors, and the density of liver and muscle receptors in randomly chosen *Psammomys* is low (Kanety et al., 1994). The difference in hyperglycemic response between the two selected lines may be related to the insulin secretion response of pancreatic beta cells of the animals fed the HE diet, the number of available insulin receptors, or the rate of liver clearance of circulating insulin.

The findings of limited suppressibility of HGP by hyperinsulinemia in *Psammomys*, contribute to the understanding of the metabolic condition in the desert environment. With meager nutrition any glucose derived from non-glucose precursors would be primarily targeted to the vital glucose-dependent tissues and the released insulin should not be able to shut off the gluconeogenesis. HGP in the desert environment is probably of survival value both in terms of preferential glucose channeling to non-insulin-dependent nervous tissues and conversion of the available glucose to energy storage, e. g. hepatic lipogenesis. It is quite possible that the adaptation of both animals and humans to plentiful nutrition either in laboratory conditions or as a result of affluence of the western lifestyle have attenuated or selected out the "inborn" insulin resistance characteristics of populations living in nutritional scarcity. Absence of gluconeogenesis restraint in such populations together with rich nutrient intake would lead to enhancement of insulin secretion, excessive lipogenesis followed by insulin resistance and hyperglycemia. This, and eventual obesity with potentiation of insulin

resistance, is well exemplified in *Psammomys* because of the abundant hepatic lipogenic capacity and adipose tissue triglyceride uptake (Kalderon et al., 1983; Ziv et al., 2008).

Beta cell apoptosis and necrosis following prolonged insulin resistance with hyperinsulinemia does not last long enough to compensate for insulin resistance and the associated hyperglycemia in stage C diabetes of *Psammomys* (Bar-On et al., 1999; Donath et al., 1999). The insulin content of beta cells in native *Psammomys* is low compared with that of other animal models of diabetes like the ob/ob mouse or ZDF rat in which the hyperglycemia is effectively compensated. As reported by Leibowitz et al. (2001), the gene expression in *Psammomys* associated with PDX-1 transcription factor is very low or absent and limits beta cell secretory function. The secretion pressure presumably leads to apoptosis and beta cell demise.

The "spontaneous" diabetes in *Psammomys* occurs as a result of prolonged maintenance on HE diet which in stage D is associated with massive beta-cell degranulation, loss of insulin, apoptosis, and necrosis (Like and Miki, 1967). Joerns et al. (2002) followed in detail the consecutive beta-cell changes during the progression from stages A–D on HE diet, involving a gradual loss of beta-cell insulin, glucokinase and GLUT2 transporter immunoreactivities. Ultrastructurally, different signs of necrotic destruction of pancreatic beta cells such as the pyknosis of nuclei and a massive vacuolization in the cytoplasm were seen accompanied by swollen mitochondria and dilated cisternae of the Golgi complex and of the rough endoplasmic reticulum. When the pancreas was removed from animals at stage D, beta cells exhibited apoptosis and DNA fragmentation (Shafir et al., 1999). Similar observations were reported by Donath et al. (1999) and Nesher et al. (1999).

There is no direct evidence for the involvement of gluco- or lipotoxicity in the necrosis of beta cells in *Psammomys*. An attempt to prevent the possible effect of advanced glycation end products or of nitrous oxide by including the glycation inhibitor, aminoguanidine in the hyperglycemic incubation medium was not effective in protecting beta cells of *Psammomys* from apoptosis (Donath et al., 1999). In *Psammomys* the prompt damage of beta cell architecture is probably the result of exhaustion due to the insulin hypersecretion prior to the eventual glucotoxic effect.

Psammomys in stage C diabetes show increased plasma proinsulin levels, up to one half of the circulating immunoassayable total insulin (Gadot et al., 1994). The inordinate secretion pressure may cause a swift exocytosis of immature insulin granules escaping prior to the C peptide cleavage. This indicates that the compensation of the delayed glucose removal and suppression of the hepatic gluconeogenesis attributed

to insulin is not effective because proinsulin has only a minute fraction of insulin activity. Actually, a high level of circulating proinsulin does not mean that its secretion is similar to that of insulin since the half-life of proinsulin is much longer than that of insulin (Glauber et al., 1986) but in effect very little true insulin is released into the circulation. Deficient insulin secretion, with increased proportions of insulin precursor molecules, is a common feature of type 2 diabetes and could result both from inappropriate beta cell function and reduced beta cell mass. Bendayan et al. (1993) reported that beta cells of hyperglycemic *Psammomys* are in a chronic high-secretory mode with loss of secretory granules and impaired processing of proinsulin by the convertases PC1 and PC2 as compared with normoglycemic animals. Leibowitz et al. (2002) stated that inadequate response of the preproinsulin gene by glucose may contribute to the failure of *Psammomys* to cope with the increased demand for insulin associated with caloric excess, leading to depletion of insulin stores and diabetes.

The possible involvement of islet amyloid polypeptide (IAPP) in insulin depletion of diabetic *Psammomys* was also investigated. The immunoreactivity of pancreatic IAPP and insulin were considerably weaker in stage C diabetic *Psammomys* compared with normoinsulinemic group A. Plasma IAPP and insulin were significantly elevated suggesting co-excretion as a result of pancreatic beta cell overstimulation (Leckstrom et al., 1997).

It may be concluded that *Psammomys* represents a good model to address questions related to the role of insulin resistance and beta cell failure in nutritionally induced diabetes. Continued consumption of the HE diet thus imposes a gradually rising insulin resistance, resulting in depletion of pancreatic insulin stores, with increased proportion of insulin precursor molecules in the pancreas and the blood. Impaired processing of preproinsulin to the increased insulin needs is an important cause in diabetes progression (Leibowitz et al., 2002). Changes in beta cell mass do not correlate with pancreatic insulin stores and are unlikely to play a role in disease initiation and progression. The major culprit is the inappropriate insulin production with depletion of insulin stores as a consequence.

Psammomys of 1–12 months of age, and which are fed LE diet from weaning and transferred at different ages to HHHhhE diet, demonstrated that sensitivity to the development of diabetes increases from weaning to 5 months of age and decreases thereafter (Kalman et al., 1998; Ziv et al., 1999). At 5 months of age, the obesity factor measured as the proportion of epididymal fat weight to total body weight, is highest, while the proportion of other organs to total body weight remains unchanged (Figure 54.13). In animals older than 7–8 months, the potential to develop diabetes and obesity decreases.

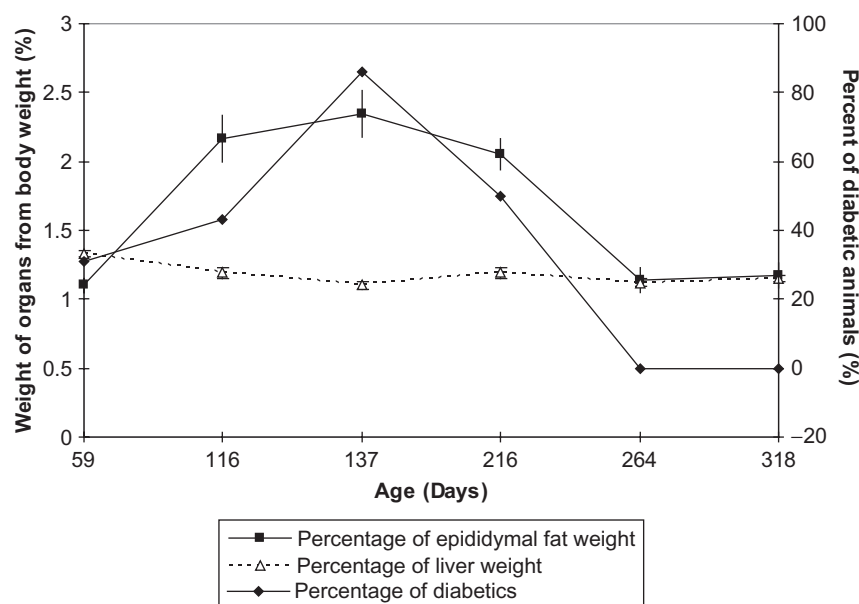


FIGURE 54.13 Changes in percentage of diabetic animals, epididymal fat and liver relative weight in male *Psammomys obesus* from the DP line at various ages after being switched to the HE diet for 5 weeks.

These changes are in correlation with the decrease in fertility of *Psammomys* from both DP and DR lines.

THE RELEVANCE OF PSAMMOMYS TO DIABETES RESEARCH

Psammomys represents an excellent model to test compounds attenuating diabetes in stages B or C since they are reversible to stage A diabetes following treatment. Stage D, in which the beta cells are already compromised, is not amenable to treatment. Vanadyl sulfate was effectively used to prevent the hyperglycemia and hyperinsulinemia of *Psammomys* maintained on the HE diet (Shafrir et al., 2001). Administration of 5mg/kg of vanadyl sulfate for 5 days resulted in a prolonged restoration of normoglycemia and normoinsulinemia and muscle GLUT4 transporter as well. Pretreatment with vanadyl sulfate significantly delayed the onset of hyperglycemia. Rosiglitazone administered to *Psammomys* on HE diet at 20mg/kg for 2 weeks also normalized the hyperglycemia and markedly reduced the hyperinsulinemia. The glucose metabolism in peripheral tissues was not the primary target of the beneficial effect of rosiglitazone but this thiazolidinedione prevented the damage to pancreatic beta cells and loss of insulin, thus enabling insulin secretion to compensate for the peripheral insulin resistance (Hefetz et al., 2006).

Among other beneficial treatments of *Psammomys*, administration of nicotine was shown to decrease the food intake and body weight in *Psammomys* via a non-leptin-dependent pathway (Sanigorski et al., 2002). A

sustained hypoglycemic effect was obtained by electroacupuncture (Shapira et al., 2000). Improvement was also obtained following treatment with an analog of glucagon-like peptide (GLP-1) acting like an incretin promoting the beta cell insulin secretion and availability (Uckaya et al., 2005). Novel peptides modulating a G-protein-coupled kinase had an anti-diabetic effect in *Psammomys* as well as in *db/db* mice and ZDF rats (Anis et al., 2004). An oral agent S15261 L-isomer of 2-([2-methoxy-2-[3-(trifluoromethyl)phenyl]ethyl]amino)ethyl-4-(2-([2-(9H-fluorenyl)acetyl]amino)ethyl)benzene preventing the beta cell lesion in the HE maintained *Psammomys* and restored the integrity of beta cell cytoarchitecture. Metabolic and anti-atherogenic effects were obtained by long-term administration of benfluorex in dyslipidemic insulin-resistant *Psammomys* (Marquie et al., 1997a, 1997b).

As already stated, *Psammomys obesus* exhibits muscle insulin resistance due to impaired insulin signaling pathway inhibition evident by reduced tyrosine kinase activity (Ikeda et al., 2001; Kanety et al., 1994; Shafrir et al., 1996). An overexpression of the enzyme protein kinase isoform epsilon (PKC ϵ) was found in *Psammomys* muscle (Ikeda et al., 2001), which prevented the tyrosine phosphorylation on the IRS-1 insulin receptor substrate by inhibiting phosphorylation of serine residues (Mack et al., 2008). Further, prevention of the detrimental serine phosphorylation on IRS-1 was achieved by using a competitive inhibitory peptide Kce-12 isolated from the catalytic domain of PKC (Niv et al., 2004), thereby abrogating the insulin signaling impediment and restoring normoglycemia in *Psammomys* maintained on the HE diet. Damage and loss of insulin in

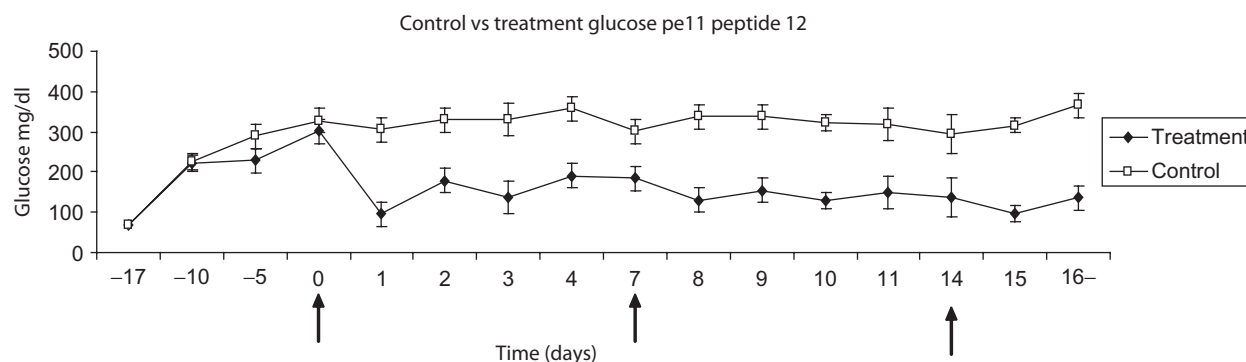


FIGURE 54.14 Blood glucose lowering by intraperitoneal injections of peptide Kce-12, indicated by arrows. Mean $\pm$ SE for groups of eight female *Psammomys obesus* maintained on the HE diet.

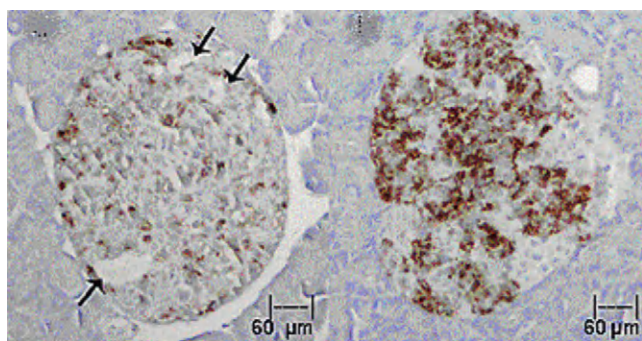


FIGURE 54.15 Beta cell insulin replenishment after treatment with Kce-12. Left: Islet cell from untreated diabetic *Psammomys obesus* on HE diet. Arrows indicate vacuoles. Right: Islet cell from treated diabetic *Psammomys obesus* on HE diet. Note the strong immunostaining of insulin and disappearance of vacuoles.

pancreatic beta cells on HE diet were also prevented by the inhibitory peptide (Mack et al., 2008) as presented in Figures 54.14 and 54.15. The anti-diabetic peptide Kce-12, and similar peptides copied from the catalytic region of the PKC molecule may lead to a functional therapeutical modality preventing the overnutrition-induced insulin resistance and diabetes.

THE GENETIC BASIS OF PSAMMOMYS DIABETES

The genetic predisposition of the DP line of *Psammomys* determines the development of “diabetes”, a collusion of diabetes and obesity (Shafir, 1996) in animals only when they are maintained on a HE diet, whereas the DR animals do not develop these untoward changes. The roles of genetic factors on one hand and dietary conditions on the other seem to be similar in humans and thus render development of type 2 diabetes in *Psammomys* an excellent model for the study of this multifactorial disease.

In order to better understand the genetic background of diabetes in *Psammomys*, a backcross generation (BC₁) was formed by crossing F₁ males with females of the DP line. The resulting progeny were assessed for blood glucose on high-energy (HE) diet. A clear bimodal distribution of blood glucose was observed; normoglycaemic and hyperglycemic at a 1:1 ratio (Hillel et al., 2005).

This distribution of progeny suggested that a major gene may be involved in the transition from normoglycemia to hyperglycemia, while the level of the blood glucose within each mode may be affected by more than one gene and by environmental factors. If this hypothesis is true, it implies that the selected DR line is homozygous for the dominant allele, the selected DP line is homozygous for the recessive allele, and that the F₁ is heterozygous for this single putative gene. In such a case, when F₁ individuals are backcrossed to the DP line, one should expect a bimodal distribution among the BC₁ progeny at a ratio of 1:1 between the resistant and the prone modes. In fact, we used the backcross to the DP line rather than to the DR line or to both lines since we hypothesized that the hyperglycemic animals are more likely to be homozygous recessive.

The mode of inheritance of nutritionally induced diabetes in DR *Psammomys* was investigated following the transfer from LE to HE diet. *Psammomys* selected for high or low blood glucose were used as two parental lines. At weeks 1–9 after weaning, a clear bimodal distribution, different from unimodal distribution, of blood glucose was observed, normoglycemic and hyperglycemic at a 1:1 ratio. This ratio is expected at the first backcross generation for traits controlled by a single dominant gene. From week 0 (prior to transfer to HE diet) until week 8, the hyperglycemic individuals were significantly heavier (4–17%) than the normoglycemic ones. The bimodal blood glucose distribution in BC₁ generation, with about equal frequencies in each mode, strongly suggests that a single major gene affects the transition from normo- to hyperglycemia. The wide

range of blood glucose values among the hyperglycemic individuals (180–500 mg/dl) indicates that several genes and environmental factors influence the extent of hyperglycemia. The DR allele appears to be dominant; the estimate for the dominance ratio is 0.97.

Our main conclusion is to challenge the accepted convention of polygenic development of insulin resistance and type 2 diabetes. We have shown that, in an established dietary regimen defined by us, a major gene comes into play. This gene may mask what might be a polygenic-based phenomenon in another environment. Thus, what may be polygenic in one environment with a neutralized major gene may be monogenic in another environment where that gene becomes expressed. Such an idea may have large implications to the human situation where individuals overconsume. We propose that a major monogenic factor initiates type 2 diabetes, which may be followed by, or associated with, nutritionally and/or environmentally evoked factors. This possibility may apply to the development of type 2 diabetes in animals and humans alike.

WORLDWIDE RESEARCH USING PSAMMOMYS

Diabetes in *Psammomys* originating from Algeria was described by Marquie et al. (1991a, 1991b). Approximately 40% of the animals developed the diabetes syndrome, with a few dying in ketosis. Other investigations in the Algerian *Psammomys* concerned the seasonal variations in the ultrastructure and production of testosterone-dependent proteins in the seminal vesicles and the role of glucose and insulin in the regulation of collagen synthesis in aortic smooth muscle cells (Aouichat-Bouguerra et al., 2001; Gernigon-Spychalowicz, 1992; Gernigon-Spychalowicz et al., 1997). A lack of physiologic changes in the activity of the hepatic enzyme glucose-6-phosphatase was established in the Algerian *Psammomys* (Koceir et al., 2003). An additional colony of *Psammomys* is maintained in Food and Drug Administration premises in Washington, DC, on which beta cell studies and assessment of diabetic lenses were performed (Chennault et al., 2002; Katzman et al., 2004).

A branch of the Israeli *Psammomys* colony has also been maintained by the Collier group in Australia (Barnett et al., 1994a, 1994b; Collier et al., 1997). It was observed that insulin resistance and hyperinsulinemia appear before weight gain, followed by adipose tissue accretion and diabetes. Tissue triglyceride deposition was driven by the ample hepatic lipogenesis, which continues unabated despite insulin resistance. With regard to the genetic characteristics it is of interest that the product of a hypothalamic gene termed 'BEACON' (Collier et al., 2000; Walder et al., 2002a)

was found to increase the food intake and body weight after intracerebroventricular injection. It also induced a two-fold increase in hypothalamic neuropeptide Y expression. The Australian investigators have discovered a TANIS gene assumed to be responsible for a link between type 2 diabetes and inflammation (Walder et al., 2002b). They also identified a mitochondrial intramembranal protease, known as presenilin-associated rhomboid-like protein (PSARL) to be associated with insulin resistance and possibly a new candidate gene related to development of type 2 diabetes (Walder et al., 2005).

Psammomys from the Israeli colony have also been maintained by Thora Bodvarsdottir and colleagues at the Novo-Nordisk Department of Pharmacology in Denmark. An important observation of this group was the development of the pancreas in the known propensity for agenesis in the presumed absence of the homeobox gene 1 (PDx-1) (Leibowitz et al., 2002). However, in spite of possible deficiency of PDx-1 the pancreas developed almost normally, similarly to *Muridae* species, and the sequence and expression of the Neurogenin-3 gene exhibited a highly conserved pattern (Vedtofte et al., 2007).

One of the recent developments in diabetology is the treatment of patients with incretin-like compounds activating insulin secretion by the intestinal glucagon-like peptide (GLP-1). Protection of this peptide and others like liraglutide requires inhibition of the intestinal enzyme dipeptidyl peptidase (DPP-4). Liraglutide administered to diabetic *Psammomys* significantly reduced the blood glucose often to normal levels in the majority of animals, whereas the inhibition of DPP-4 by vidagliptin neither reduced the blood glucose, nor affected the pancreatic insulin content. Thus, liraglutide decidedly improved the glycemic control of diabetes by improving the pancreatic insulin secretion while the vidagliptin was ineffective in *Psammomys* (Vedtofte et al., 2009).

COMPLICATIONS OF DIABETES IN PSAMMOMYS

Of the complications affecting long-term hyperglycemic animals, cataracts are the foremost (Hackel et al., 1965; Kohler and Knospe, 1980; Kuwabara and Okisaka, 1976), and are prominent after 2–4 months on the HE diet. Retinal damage is also evident due to degeneration of dopamine neurons (Larabi et al., 1991). With respect to cataracts, Gutman et al. (1976) found that the activity of the enzyme galactokinase is very low in *Psammomys* red blood cells and ocular tissues, with or without cataracts. This suggests that the formation of cataracts is due to consumption of the

calorie-rich diet and conversion of glucose and galactose to sorbitol or galactitol, followed by their accumulation in the presence of hyperglycemia. Treatment of *Psammomys* with acarbose resulted both in reduced glycemia and raised activity of aldose reductase with a significant prevention of cataract development (Cohen-Melamed et al., 1995). It was also found that treatment with lipoic acid reduced the hyperglycemia and increased the glutathione content in association with reduced incidence of cataract development (Borenshtein et al., 2001).

Microangiopathy characterized by thickening of the intima and deposition of glycosaminoglycans was seen in the Algerian *Psammomys* (Marquie et al., 1991b) and degeneration of the intervertebral discs with spondylosis was noted in the Jerusalem colony (Silberberg, 1988; Silberberg et al., 1979). Old *Psammomys* exhibited a tendency of hepatic malignancy (Ungar and Adler, 1978), and uterine neoplasms (Czernobilski et al., 1982). Various ontological deficiencies were found in *Psammomys* by Sichel et al. (1999), Perez et al. (2002), and Sohmer et al. (2004). Although *Psammomys* excrete highly concentrated urine (Jamison et al., 1979), various stages of nephropathy were observed in those with diabetes (Scherzer et al., 2000).

Neonatal malformations were observed in newborns of diabetic *Psammomys* females, the pregnancy of which is somewhat longer, 27 days versus 26 days (Patlas et al., 2006). Litter size average is reduced, and the newborns have smaller crown-rump lengths and lower birth weights. Neurodevelopmental parameters (e. g., first turnover, hair appearance, and eye opening) are delayed by 2–3 days. From the fourth week of life they become diabetic, even on the LE diet.

CONCLUDING REMARKS

Psammomys in its native habitat is a healthy animal with a metabolic-endocrine system that is adjusted to desert life on a low-caloric-density diet, thus enabling reasonable survival. *Psammomys* is not hyperphagic; however, when high HE nutrition becomes available, it is predisposed to diabetes, obesity, and beta cell overtaxation. This predisposition is due to feedback inhibition of the insulin-signaling pathway, involving serine phosphorylation and stoppage of tissue glucose transport but not lipogenesis. This ill adaptation to nutrient excess is an outstanding example of the "thrifty gene" effect as described by Wendorf and Goldfine (1991) and it therefore represents a suitable model for studying the mechanism of predisposition to insulin resistance and metabolic syndrome in human populations that evolve from scarcity to abundance in nutritional intake.

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Formulary

Sean Maguire and C. Terrance Hawk

O U T L I N E

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INTRODUCTION

We have endeavored to develop a formulary that includes drugs in common use within the laboratory animal community. This formulary is limited to the species relevant to this volume, with particular emphasis on rabbits, guinea pigs, hamsters, and degus. There is limited published information available for the other minor rodent species covered in this text. This formulary is not meant to be all-inclusive. There are many other excellent published sources that cover laboratory animals and other exotic species.

This formulary is divided into five major areas within each species. These areas include analgesics /sedatives,

anesthetics, anti-infectives, parasiticides, and a miscellaneous category. It must be noted that the vast majority of the drugs listed have not been approved by regulatory authorities for use in the species covered in this formulary. It is also important to note that few of the drugs have undergone formal studies to determine their efficacy, pharmacokinetics, or pharmacodynamics. Thus most drug dosages listed in this formulary are based on clinical observation and experience, or extrapolated from limited scientific data. Extrapolation to other species may be considered using allometric scaling (Hawk et al., 2005). Before any of the referenced doses are used in animals, it is the responsibility of the reader to study the source citation to understand how the listed dose was determined.

Abbreviations

bid	twice daily	prn	as needed
h	hour	q	every
IM	intramuscular	qid	four times daily
IN	intranasally	s	second
IP	intraperitoneal	SC	subcutaneous
IU	international unit	sid	once daily
IV	intravenous	tid	three times daily
kg	kilogram	U	unit
l	liter	µg	microgram
lb	pound	%	g/100 ml
mg	milligram		
ml	milliliter		
PO	per os		
ppm	parts per million		

Drugs are listed in alphabetical order by their generic name within each category. When multiple drugs are listed, the dose and route are listed in the same order as the drug names.

RABBIT (*ORYCTOLAGUS CUNICULUS*)

Analgesics and Sedatives

Generic name	Dose and route	Comments	Reference
Acepromazine	5 mg/kg IM		Flecknell, 1987
Acepromazine	2 mg/kg IM		Bauck, 1989
Acepromazine	1–2 mg/kg IM		Harkness and Wagner, 1983
Acepromazine	0.5–1 mg/kg IM	Dosage for vasodilation with minimal sedation	Morrissey et al., 2004
Acepromazine, codeine elixir	1 ml elixir/100 ml drinking water PO		Wixson, 1994
Acetaminophen	200–500 mg/kg PO		Gillett, 1994
Alfentanil	0.03–0.07 mg/kg IV	Intraoperative analgesia with 45 min duration	Wixson, 1994
Alphaxalone-alphadolone	9–12 mg/kg IM		Flecknell, 1987
Alphaxalone-alphadolone	6–9 mg/kg IV	Possible high mortality (Flecknell, 1987)	Green, 1982
Aminopyrine	50 mg/kg PO		Jenkins, 1987
Antipyrine	100 mg/kg IM, IP, or SC		Borchard et al., 1990
Antipyrine	500 mg/kg PO		Borchard et al., 1990
Antipyrine	100 mg/kg PO		Jenkins, 1987
Aspirin	100 mg/kg PO	Suggested q4h	Flecknell, 1991
Aspirin	400 mg/kg SC or PO sid		Harkness and Wagner, 1983
Aspirin	80–160 mg PO bid		Harvey, 1995
Aspirin	20 mg/kg PO sid		Marangos et al., 1994
Aspirin	10–100 mg/kg PO q8–24h		Morrissey et al., 2004
Buprenorphine	0.02–0.1 mg/kg IV or SC bid		Carpenter et al., 1995
Buprenorphine	0.02–0.0 mg/kg IM, IV, or SC q8–12h		Flecknell, 1985; Jenkins, 1987

(Continued)

Analgesics and Sedatives (Continued)

Generic name	Dose and route	Comments	Reference
Buprenorphine	0.01–0.05 mg/kg IV or SC q6–12h		Flecknell, 1991
Buprenorphine	0.01–0.05 mg/kg IM, IV, or SC q6–12h		Heard, 2009
Buprenorphine	0.025 mg/kg PO bid	Post nephrectomy	Rhody, 2006
Buprenorphine	0.5–0.75 mg/kg SC	Pre emptive analgesia	Sklenka et al., 2006
Buprenorphine	0.5 mg/kg per rectum bid	Analgesia	Huerkamp, 1995
Buprenorphine	0.01–0.05 mg/kg SC q6–8h	Relief from ileus, gastric stasis (Note: relief for severe pain, but repeated usage may cause ileus, GI hypomotility)	Reusch, 2005
Buprenorphine, acepromazine	0.5 mg/kg SC, 0.03 mg/kg SC	Sedation for immunizations	Halliday et al., 2004
Butorphanol	0.1–0.5 mg/kg IM, IV, or SC q4h	More sedative effect than buprenorphine, effective as preanaesthetic/preemptive analgesia	Carpenter et al., 1995
Butorphanol	0.1–0.5 mg/kg IV q4h		Flecknell, 1989
Butorphanol	0.5–2 mg/kg IM or SC qid		Harvey, 1995
Butorphanol	0.1–0.5 mg/kg SC	Analgesic for surgical thoracoscopic implantation of tumor	Kreuter et al., 2008
Butorphanol	0.1–0.5 mg/kg SC or IV q2–4h	Relief from ileus, gastric stasis	Reusch, 2005
Butorphanol, acepromazine	1 mg/kg IM, 1 mg/kg IM		Flecknell, 1996
Carprofen	1.5 mg/kg PO bid		Flecknell, 1996
Carprofen	3 mg/kg IV		Grint et al., 2006
Carprofen	4 mg/kg SC sid		Heard, 2009
Carprofen	1.5 mg/kg PO bid		Heard, 2009
Carprofen	1–2.2 mg/kg PO sid		Morrissey et al., 2004
Carprofen	2–4 mg/kg SC, IV, or PO sid	Relief from ileus, gastric stasis	Reusch, 2005
Chlorpromazine	1–20 mg/kg IM		Carpenter et al., 1995
Chlorpromazine	25 mg/kg IM	May produce myositis	Canadian Council on Animal Care, 1984
Diazepam	2 mg/kg IV		Flecknell, 1987
Diazepam	4 mg/kg IM or IP		Flecknell, 1987
Diazepam	5–10 mg/kg IM or IP		Green, 1982
Diazepam	5–10 mg/kg IM		Hrkness et al., 1983
Diazepam, xylazine	1 mg/kg IV, 3 mg/kg IV	Bolus for anesthetic induction	Hatch et al., 1988
Fentanyl	25 µg/h transdermal patch	Up to 72 hours, pharmacokinetic profile affected by hair removal method	Foley et al., 2001
Fentanyl/droperidol	0.17 ml/kg IM		Canadian Council on Animal Care, 1984
Fentanyl/droperidol	0.15–0.17 ml/kg IM		Lewis et al., 1972
Fentanyl/fluanisone	0.5 ml/kg IM		Jenkins, 1987
Flunixin	1–2 mg/kg IM or SC bid		Heard, 2009

(Continued)

Analgesics and Sedatives (Continued)

Generic name	Dose and route	Comments	Reference
Flunixin	1–3 mg/kg IM or SC sid or bid	Contraindicated with renal disease, does not appear to cause GI ulceration	Krempels et al., 2000
Flunixin	1.1 mg/kg IM or SC suggested bid	Use no more than 3 days (Carpenter, 2005)	Liles et al., 1992
Haloperidol	0.2–0.4 mg/kg IM bid		Iglauer et al., 1995
Hydromorphone	0.1–0.2 mg/kg IM, IV, or SC q6–8h		Johnston, 2005
Ibuprofen	10–20 mg/kg IV suggested q4h		Flecknell, 1991
Ibuprofen	2.0–7.5 mg/kg PO q12–24h		Morrissey et al., 2004
Indomethacin	12.5 mg/kg PO		Keller et al., 1988
Ketamine	30 mg/kg IM	Should be administered with other agents	Bauck, 1989
Ketamine	15–20 mg/kg IV	Should be administered with other agents (Hernandez-Divers, 2005)	Gillett, 1994
Ketamine	20–50 mg/kg IM	Should be administered with other agents, 60 min of sedation (Hernandez-Divers, 2005)	Gillett, 1994
Ketamine, acepromazine	25 mg/kg IM, 0.7 mg/kg IM	Anesthetic induction	Sklenka et al., 2006
Ketamine, diazepam	Diazepam 0.2–0.5 mg/kg IV, then Ketamine 10–15 mg/kg IV to effect	Sedation, use with isoflurane for anesthetic	Heard, 1993
Ketamine, diazepam	15 mg/kg IM, 0.3 mg/kg IM	Anesthesia, follow with isoflurane	Millis et al., 1992
Ketamine, diazepam	20–30 mg/kg IM then 0.5 mg/kg IV at 5–10 min	Anesthesia for dentistry	Hillyer, 1994
Ketamine, diazepam	20–30 mg/kg IM, 1–3 mg/kg IM		Hillyer, 1994
Ketamine, diazepam	20–40 mg/kg IM, 1–5 mg/kg IM		Heard, 2004
Ketamine, medetomidine	15 mg/kg IM, 0.250 mg/kg IM		Grint et al., 2006
Ketamine, medetomidine, buprenorphine	35 mg/kg IM, 0.5 mg/kg IM, 0.03 mg/kg IM	Anesthetic induction and pre surgical analgesia	Difilippo et al., 2004
Ketamine, midazolam	15 mg/kg IM, 3 mg/kg IM		Grint et al., 2006
Ketamine, midazolam	25 mg/kg IM, 5 mg/kg IM		Sireve et al., 2008
Ketamine, xylazine	0.75 ml (two parts 100 mg/ml ketamine: one part 20 mg/ml xylazine)/kg IM	Anesthetic induction	Kreuter et al., 2008
Ketamine, xylazine, buprenorphine	35 mg/kg IM, 5 mg/kg IM, 0.03 mg/kg IM	Anesthetic induction and pre surgical analgesia	Difilippo et al., 2004
Ketoprofen	3 mg/kg IM		Flecknell, 1996
Ketoprofen	3 mg/kg IM or SC sid		Heard, 2009
Ketoprofen	1 mg/kg IM		Perrin et al., 1990
Medetomidine	0.300–0.500 mg/kg	Reverse with atipamezole	Aeschbacher, 1995
Medetomidine	0.100–0.500 mg/kg IM or SC	Reverse with atipamezole	Flecknell, 1996
Medetomidine	0.250 mg/kg IM	Reverse with atipamezole	Ko et al., 1992
Medetomidine, Ketamine	0.35 mg/kg IM, 5–20 mg/kg IV 15 minutes after medetomidine	Anesthesia, surgical depth approximately 19 min	Hellebrekers et al., 1997

(Continued)

Analgesics and Sedatives (Continued)

Generic name	Dose and route	Comments	Reference
Medetomidine, Ketamine	0.1 mg/kg IV, 20 mg/kg IV 20 minutes after medetomidine	Anesthesia sufficient for cerebrospinal fluid spinal tap and cardiocentesis	Raekallio et al., 2002
Medetomidine Propofol	0.35 mg/kg IM then 3 mg/kg IV	Anesthesia; surgical depth approximately 11 min	Hellebrekers et al., 1997
Meloxicam	0.2 mg/kg PO sid	Used chronically for osteoarthritis, dental abscess, osteomyelitis, and postoperative pain, palatable (Hernandez-Divers, 2005)	Grint et al., 2006
Meloxicam	0.1–0.3 mg/kg PO or SC sid		Hawkins, 2006
Meloxicam	0.2 mg/kg IM or SC sid		Heard, 2009
Meloxicam	0.3 mg/kg PO sid		Heard, 2009
Meloxicam	0.1–0.5 mg/kg PO or SC q12–24h		Johnston, 2005
Meloxicam	1.5 mg/kg PO sid	Can be given up to 5 days, safe dose, efficacy not shown but suspected	Turner et al., 2006
Meloxicam	0.5 mg/kg PO bid for 10 days	Pain relief for retrobulbar abscess	Martinez-Jimenez et al., 2007
Meloxicam	0.12 mg/kg PO sid for 7 days	Post nephrectomy	Rhody, 2006
Meloxicam	0.1–0.6 mg/kg SC or PO bid	Relief from ileus, gastric stasis	Reusch, 2005
Meperidine	10–20 mg/kg IM or SC q2–3h	Analgesia	Flecknell, 1991
Meperidine	5–10 mg/kg SC, IP q2–3h	Analgesia	Flecknell, 1991
Meperidine	5–25 mg/kg SC, IM, IV	Analgesia	Gillett, 1994
Meperidine	0.2 mg/ml drinking water	Analgesia	Huerkamp, 1995
Meperidine	10 mg/kg IM or SC q2–3h	Analgesia	Jenkins, 1987
Midazolam	2 mg/kg IV		Flecknell, 1987
Midazolam	4 mg/kg IM or IP		Flecknell, 1987
Midazolam	0.5–2 mg/kg IM, IP, or IV		Flecknell, 1996
Midazolam, fentanyl/ fluanisone	1–2 mg/kg IV, 0.3 ml/kg IM	Infuse with 2–5 µg/kg/min IV fentanyl for long term anesthesia	Flecknell, 1996
Midazolam	2 mg/kg IM or IV		Morrissey et al., 2004
Midazolam	2 mg/kg IN		Robertson and Eberhart, 1994
Midazolam	1–2 mg/kg IM		Paul-Murphy, 2007
Meloxicam	0.12 mg/kg PO sid for 7 days	Post nephrectomy analgesia	Rhody, 2006
Morphine	5 mg/kg IM or SC q2–4h		Flecknell, 1985; Jenkins, 1987
Morphine	2–5 mg/kg IM or SC q2–4h		Flecknell, 1991
Nalbuphine	1–2 mg/kg IV q4–5h		Flecknell, 1989
Nalbuphine	1 mg/kg IP or SC		Flecknell, 1996
Nalbuphine	0.1 mg/kg IV		Flecknell, 1996
Naloxone	0.01–0.1 mg/kg IM, IV	Narcotic reversal	Hernandez-Divers, 2005
Oxymorphone	0.1–0.2 mg/kg IM or IV		Cantwell, 2001
Oxymorphone	0.05–0.2 mg/kg IM or SC q8–12h		Heard, 2009

(Continued)

Analgesics and Sedatives (Continued)

Generic name	Dose and route	Comments	Reference
Paracetamol (acetaminophen)	50 mg rectal suppository	Post procedure pain relief	Papaioannou et al., 2004
Paracetamol (acetaminophen)	25 mg PO bid	Post procedure pain relief	Papaioannou et al., 2004
Paracetamol (acetaminophen)	10–20 mg/kg IM or SC q4h	Post procedure pain relief	Flecknell, 1985; Jenkins, 1987
Pentazocine	5 mg/kg IV q2–4h		Flecknell, 1991
Pethidine	10 mg/kg IM or SC q2–3h		Flecknell, 1987
Piroxicam	0.3 mg/kg PO q48h		Flecknell, 1996
Piroxicam	0.2 mg/kg PO tid	Analgesia, NSAID	More et al., 1989
Promazine	1–2 mg/kg IM, IV	Preanesthetic	Gillett, 1994
Propofol	2–3 mg/kg IV for induction after premedication, maintain with approximately 1 mg/kg IV q15 min		Hernandez-Divers 2005
Tramadol	10 mg/kg PO sid		Johnston, 2005
Tramadol	> 11 mg/kg PO q4–6h	Variable absorption orally, 11 mg/kg did not reach plasma concentrations needed for analgesia based on humans	Souza et al., 2008
Xylazine	1–3 mg/kg IM		Flecknell, 1987
Xylazine	1–5 mg/kg IM or SC		Armeno et al., 2002
Xylazine	3–5 mg/kg IM		Harkness and Wagner, 1983
Xylazine	5 mg/kg IM		Hughes, 1981
Yohimbine	0.2–1.0 mg/kg IM, IV	Xylazine reversal	Hernandez-Divers, 2005

Anesthetics

Generic name	Dose and route	Comments	Reference
Chloralose	80–100 mg/kg IV	1 % solution	Green, 1982
Diazepam	2 mg/kg IV	Surgical anesthesia	González-Gil et al., 2004
Fentanyl, medetomidine	8 µg/kg IV, 0.300 mg/kg IV		Flecknell, 1996
Fentanyl/droperidol	0.4 ml/kg SC		González-Gil et al., 2003
Fentanyl/droperidol	0.1–0.5 ml/kg IM		Green, 1982
Fentanyl/fluanisone, diazepam	0.3 ml/kg IM, 1–2 mg/kg IP or IV		Flecknell, 1996
Fentanyl/fluanisone, midazolam	0.3 ml/kg IM, 2 mg/kg IM or SC		Armeno et al., 2002
Fentanyl/fluanisone, midazolam	0.3 ml/kg IM, 1–2 mg/kg IP or IV		Flecknell, 1996
Fentanyl/fluanisone, midazolam	0.3 ml/kg IM, 2 mg/kg IV	For long-term anesthesia follow with fentanyl 2–5 µg/kg/min IV	Flecknell, 1996
Ketamine	44 mg/kg IM		Weisbroth and Fudens, 1972
Ketamine	50 mg/kg IM	For immobilization	Flecknell, 1987
Ketamine	25 mg/kg IN	Light surgical anesthesia	Robertson and Eberhart, 1994

(Continued)

Anesthetics (Continued)

Generic name	Dose and route	Comments	Reference
Ketamine	50 mg/kg IM	Anesthetic induction	Pekar et al., 2004
Ketamine, acepromazine	75 mg/kg IM, 5 mg/kg IM		Flecknell, 1987
Ketamine, acepromazine	50 mg/kg IM, 1 mg/kg IM		Flecknell, 1996
Ketamine, xylazine	10 mg/kg IV, 3 mg/kg IV	Surgical anesthesia	González-Gil et al., 2004
Ketamine, diazepam	30–40 mg/kg IM, 2–5 mg/kg IM		Hernandez-Divers, 2005
Ketamine, diazepam	25 mg/kg IM, 5 mg/kg IM		Flecknell, 1987
Ketamine, diazepam	10 mg/kg IV, 2 mg/kg IV		González-Gil et al., 2003
Ketamine, diazepam	20–30 mg/kg IM, 1–3 mg/kg IM	Used with isoflurane	Carpenter et al., 1995
Ketamine, medetomidine	25 mg/kg IM, 0.50 mg/kg IM		Flecknell, 1996
Ketamine, medetomidine	5 mg/kg IV, 0.35 mg/kg IM	Use with supplemental oxygen	Hellebrekers et al., 1996
Ketamine, medetomidine, diazepam	20 mg/kg IM, 0.30 mg/kg IM, 0.75–1.5 mg/kg IM		Ko et al., 1997
Ketamine, midazolam	25 mg/kg IM, 2 mg/kg IM		Morrissey et al., 2004
Ketamine, midazolam	30 mg/kg IM, 0.20 mg/kg IM		Vachon et al., 1999
Ketamine, midazolam	25 mg/kg IM, 5 mg/kg IM	Anesthetic induction	Papaioannou et al., 2004
Ketamine, midazolam, xylazine	30 mg/kg IM, 0.2 mg/kg IM, 3 mg/kg IM		Vachon et al., 1999
Ketamine, xylazine	20–40 mg/kg IM or SC, 3–5 mg/kg IM or SC		Arnemo et al., 2002
Ketamine, xylazine	10 mg/kg IV, 3 mg/kg IV		Flecknell, 1987
Ketamine, xylazine	35 mg/kg IM, 5 mg/kg IM		Flecknell, 1996
Ketamine, xylazine	50 mg/kg IM, 10 mg/kg IM		Lipman et al., 1987
Ketamine, xylazine	10 mg/kg IN, 3 mg/kg IN		Robertson and Eberhart, 1994
Ketamine, xylazine	35 mg/kg IM, 4.2 mg/kg IM	Anesthetic induction	Styer et al., 2005
Ketamine, xylazine	10 mg/kg IV, 1 mg/kg IV	Anesthetic maintenance	Styer et al., 2005
Ketamine, xylazine	0.3 ml (two parts 100 mg/ml ketamine: one part 20 mg/ml xylazine) prn by monitoring	Anesthetic maintenance	Kreuter et al., 2008
Ketamine, xylazine	50 mg/kg IM, 10 mg/kg IM	Anesthetic induction	Smith et al., 2004
Ketamine, xylazine, acepromazine	35 mg/kg IM, 5 mg/kg IM, 1 mg/kg IM or SC		Flecknell, 1996
Ketamine, xylazine, acepromazine	35 mg/kg IM, 5 mg/kg IM, 0.75 mg/kg IM		Marini et al., 1989
Ketamine, xylazine, butorphanol	35 mg/kg IM, 5 mg/kg IM, 0.1 mg/kg IM		Marini et al., 1992
Medetomidine, midazolam, propofol	0.250 mg/kg IM, 0.500 mg/kg IM, 2 mg/kg IV		Ko et al., 1992
Medetomidine, propofol	0.35 mg/kg IM, 3 mg/kg IV	Anesthesia, surgical depth for approximately 11 min, reverse medetomidine with atipamezole	Hellebrekers et al., 1996
Medetomidine, propofol	0.25 mg/kg IM, 4 mg/kg IV		Ko et al., 1992

(Continued)

Anesthetics (Continued)

Generic name	Dose and route	Comments	Reference
Methohexital (methohexitone)	10 mg/kg IV		Flecknell, 1987
Midazolam, hydromorphone, glycopyrrolate	0.5 mg/kg IM, 0.1 mg/kg IM, 0.01 mg/kg IM	Anesthetic induction	Martinez-Jimenez et al., 2007
Pentobarbital	28 mg/kg IP or IV		Croft, 1964
Pentobarbital	20–45 mg/kg IP or IV	Marginal anesthesia (Hernandez-Divers 2005),	Morrissey et al., 2004
Pentobarbitone	30 mg/kg IV	Surgical anesthesia	González-Gil et al., 2005; González-Gil et al., 2004
Propofol	7.5–15 mg/kg IV	Can be titrated to effect, slow administration. May cause apnea (Heard, 2004)	Adam et al., 1990
Propofol	2–15 mg/kg IV	Can be titrated to effect, slow administration. May cause apnea (Heard, 2004)	Morrissey et al., 2004
Propofol	1.5 mg/kg IV bolus	Supplement with 0.2–0.6 mg/kg/min continuous infusion	Blake et al., 1988
Propofol, buprenorphine	1 mg/kg IV, 5 mg total SC	Animals weighed between 3.2 and 4.4 kg	Sireve et al., 2008
Thiopental	28 mg/kg IV or IP		Croft, 1964
Thiopental	15–30 mg/kg IV to effect		Wixson, 1994
Thiopentone	20 mg/kg IV	Surgical anesthesia	González-Gil et al., 2005; González-Gil et al., 2004
Tiletamine/zolazepam	10–25 mg/kg IM		Fowler, 1978
Tiletamine/zolazepam	10 mg/kg IN	No nephrotoxicity	Robertson and Eberhart, 1994
Tiletamine/zolazepam	Not recommended		Schobert, 1987
Tiletamine/zolazepam		Not recommended by injection; nephrotoxicity	Doerning et al., 1990; Doerning et al., 1992
Tiletamine/zolazepam, medetomidine	2.5–5 mg/kg IM or SC, 0.50 mg/kg IM or SC		Armeno et al., 2002
Tiletamine/zolazepam, xylazine	15 mg/kg IM or SC, 5 mg/kg IM or SC		Armeno et al., 2002
Tiletamine/zolazepam, xylazine	15 mg/kg IM, 5 mg/kg IM		Popilskis et al., 1991
Tiletamine/zolazepam, xylazine	20 mg/kg IM, 3 mg/kg IM		Vachon et al., 1999
Urethane	1000 mg/kg IV or IP		Flecknell, 1987

Anti-Infectives

Generic name	Dose and route	Comments	Reference
Amikacin	10 mg/kg IM or SC q8–12h		Hernandez-Divers, 2005 et al., 2005
Amikacin	2–5 mg/kg IM or SC q8–12h		Harkness and Wagner, 1995

(Continued)

Anti-Infectives (Continued)

Generic name	Dose and route	Comments	Reference
Amikacin	5–10 mg/kg IM, IV, or SC divided q8–24h		Morrissey et al., 2004
Amikacin	1.25 g/20 g methylmethacrylate	Place in bone after surgical debridement of jaw abscess	Bennett, 1999
Amoxicillin	Not recommended	Toxic	Morrissey et al., 2004
Amoxicillin/clavulanate	Not recommended	Toxic	Morrissey et al., 2004
Amphotericin B	15 mg/kg IV, three times per week for 3 weeks	Liposomal form	Clemons et al., 2002
Amphotericin B	5 mg/kg IV sid	Liposomal form; sid	Petratis et al., 2002
Ampicillin	10–25 mg/kg IM sid for 5–7 days		Bowman and Lang, 1986
Ampicillin	10–25 mg/kg IM tid		Raphael, 1981
Ceftriaxone	40 mg/kg IM bid for 2 days		Korting et al., 1987
Cephalexin	15–20 mg/kg PO bid		Flecknell, 1987
Cephalexin	15 mg/kg SC bid		Flecknell, 1996
Cephalexin	11–22 mg/kg PO tid		Morrissey et al., 2004
Cephaloridine	10–25 mg/kg IM or SC sid for 5 days		Bowman and Lang, 1986
Chloramphenicol palmitate	50 mg/kg PO bid		Bauck, 1989
Chloramphenicol palmitate	30–50 mg/kg PO bid for 5–7 days		Hernandez-Divers, 2005
Chloramphenicol palmitate	50 mg/kg PO sid for 5–7 days		Harkness and Wagner, 1983
Chloramphenicol palmitate	750 mg/pint drinking water PO	Per pint drinking water	Raphael, 1981
Chloramphenicol succinate	50 mg/kg SC bid		Bauck, 1989
Chloramphenicol succinate	30 mg/kg IM or IV tid for 5–7 days		Carpenter et al., 1995
Chloramphenicol succinate	15 mg/kg IM bid		Flecknell, 1996
Chloramphenicol succinate	30 mg/kg IM sid for 5–7 days		Harkness and Wagner, 1983
Chloramphenicol succinate	50 mg/kg SC, IM, or IV tid		Russell et al., 1981
Chlortetracycline	50 mg/kg PO bid		Morrissey et al., 2004
Ciprofloxacin	40 mg/kg IM bid for 14 days		Bayer et al., 1985
Ciprofloxacin	12–20 mg/kg PO		Göbel, 1996
Ciprofloxacin	10 mg/kg PO bid for 21 days		Martinez-Jimenez et al., 2007
Ciprofloxacin	10–20 mg/kg PO q12–24h		Morrissey et al., 2004
Ciprofloxacin	40 mg/kg IM tid for 28 days		Norden and Shinnors, 1985
Ciprofloxacin	50 mg/kg IM tid for 4 days		Strunk et al., 1985
Ciprofloxacin ophthalmic	One drop topical q8–12h	Nasal pasteurellosis, maintains therapeutic levels in tear film for at least 6 h after application with tears draining into nasal sinus	Green et al., 1996

(Continued)

Anti-Infectives (Continued)

Generic name	Dose and route	Comments	Reference
Ciprofloxacin ophthalmic	Two drops 0.3% topical q1h for 7–14h	Ocular penetration injuries, good penetration into aqueous and vitreous humor	Öztürk et al., 2000
Clotrimazole	1.0% topical	Available as commercial 1% solution	Harvey, 1995
Difloxacin	5 mg/kg PO or SC		Fernández-varón et al., 2007
Dimetridazole	0.025% solution	Prepare with 45 g active ingredient per 50 gallons drinking water	Williams, 1979
Doxycycline	2.5 mg/kg PO bid		Hernandez-Divers, 2005
Enrofloxacin	5 mg/kg PO bid		Broome et al., 1991
Enrofloxacin	5–10 mg/kg PO		Dorrestein, 1992
Enrofloxacin	100 mg/L drinking water PO		Dorrestein, 1992
Enrofloxacin	5–15 mg/kg IM, PO or SC bid	Limit injections as may cause muscle necrosis, sterile abscesses (Hernandez-Divers, 2005)	Morrissey et al., 2004
Enrofloxacin	10 mg/kg IM	Limit injections as may cause muscle necrosis, sterile abscesses (Hernandez-Divers, 2005)	Rhody, 2006
Enrofloxacin	5 mg/kg PO bid		Rhody, 2006
Enrofloxacin	5.25 mg/kg PO bid for 10d	Post nephrectomy	Rhody, 2006
Fluconazole	80 mg/kg PO sid		Park et al., 1995
Fluconazole	25–43 mg/kg IV slow bid	Systemic fungal disease	Louie et al., 1998
Furazolidone	400 mg/pint drinking water PO		Russell et al., 1981
Furazolidone	2.5 g PO	Prepare in 100lb feed	Russell et al., 1981
Gentamicin	5–8 mg/kg SC sid		Bauk, 1989
Gentamicin	2.5 mg/kg IM or SC tid		Carpenter et al., 1995
Gentamicin	2 mg/kg IM or SC tid		Harvey, 1995
Gentamicin	4 mg/kg IM sid		Russell et al., 1981
Gentamicin	4 mg/kg IM or SC sid	Use with caution or avoid use	Morrissey et al., 2004
Griseofulvin	12.5–25 mg/kg PO bid for 30–45 days	Advanced cases of dermatophytosis, decrease dose by 50% with ultramicrosize forms which has better absorption	Hernandez-Divers, 2005
Griseofulvin	12.5 mg/kg PO bid		Morrissey et al., 2004
Ketoconazole	10–40 mg/kg PO sid for 14 days	Dermatophytosis	Harkness and Wagner, 1995
Marbofloxacin	5 mg/kg PO sid for 10 days		Carpenter et al., 2009
Marbofloxacin	2 mg/kg PO or SC sid		Grint et al., 2006
Metronidazole	20 mg/kg PO bid		Carpenter et al., 1995
Minocycline	6 mg/kg IV tid		Nicolau et al., 1993

(Continued)

Anti-Infectives (Continued)

Generic name	Dose and route	Comments	Reference
Moxifloxacin	40 mg/kg IV q12–24h (suggested frequency)	Bacterial meningitis	Østergaard et al., 1998
Neomycin	30 mg/kg PO bid for 5 days		Carpenter et al., 1995
Neomycin	0.2–0.8 mg/ml in drinking water PO	Prepare in drinking water	Flecknell, 1996
Nitrofurazone	11 mg/kg PO sid		Melby et al., 1976
Nitrofurazone	100 mg/ml drinking water PO		Schuchman, 1977
Ofloxacin	20 mg/kg SC tid	Urogenital, skin, respiratory infections	Marangos et al., 1997
Oxytetracycline	0.4–1 g in drinking water PO		Bowman and Lang, 1986
Oxytetracycline	15 mg/kg IM tid for 7 days		Carpenter et al., 1995
Oxytetracycline	15 mg/kg IM or SC		Flecknell, 1987
Oxytetracycline	30–100 mg/kg PO	In divided doses per day	Bowman and Lang, 1986
Penicillin	60 000 U/kg sid for 10 days; as procaine		Jaslow et al., 1981; Welch et al., 1987
Penicillin	40 000–60 000 IU/kg IM bid × 57 d	Rabbit syphilis (<i>Treponema</i>)	Fish and Besch-Williford, 1992
Penicillin G, benzathine	42 000–60 000 IU/kg IM q48		Gillett, 1994
Penicillin G, benzathine	42 000 or 84 000 IU/kg once per week for 3 weeks		Cunliffe-Beamer et al., 1981
Penicillin G, procaine	40 000 IU/kg IM sid × 5–7 d	Rabbit syphilis	Hernandez-Divers, 2005
Polymyxin B	3 mg PO	300–400-g animal	Farrar, Jr. and Kent, 1965
Rifampin, azithromycin	40 mg/kg PO bid, 50 mg/kg PO sid	<i>Staphylococcus</i> osteomyelitis	Shirliff, et al., 1999
Rifampin, clarithromycin	40 mg/kg PO bid, 80 mg/kg PO bid	<i>Staphylococcus</i> osteomyelitis	Shirliff, et al., 1999
Silver sulfadiazine cream	0.01% topical	Skin infection	Donnelly, 2005
Streptomycin	10 mg/kg IM q4h		Canadian Council on Animal Care, 1984
Sulfadimethoxine	12.5 mg/kg PO bid		Carpenter et al., 1995
Sulfadimethoxine	75–100 mg/kg PO sid for 7 days		Rossof, 1974
Sulfamethazine	900–1350 mg	Prepare in one liter drinking water	Canadian Council on Animal Care (CCAC), 1984
Sulfaquinoxaline	0.05% in drinking water PO		Patton, 1979
Sulfaquinoxaline	6 mg/lb sid for 5–7 days		Russell et al., 1981
Tetracycline	20 mg/kg PO bid		Bauck, 1989
Tetracycline	50 mg/kg PO q8–12h		Morrissey et al., 2004
Tetracycline	30–100 mg/kg PO	Give in divided doses	Bowman and Lang, 1986
Tilmicosin	25 mg/kg SC	Give on hours 0, 72. Use cautiously, may cause anemia, leucopenia and death (Hernandez-Divers, 2005)	Geertsema et al., 2008
Tilmicosin	25 mg/kg SC	Give once	McKay et al., 1996

(Continued)

Anti-Infectives (Continued)

Generic name	Dose and route	Comments	Reference
Trimethoprim sulphadoxine	48 mg/kg SC	Coliobacillosis, May cause tissue necrosis SC (Hernandez-Divers, 2005)	Reusch, 2005
Trimethoprim/sulfadiazine	30 mg/kg SC sid	May cause tissue necrosis SC (Hernandez-Divers, 2005)	Bauck, 1989
Trimethoprim/sulfadiazine	0.2 ml/kg of 240 mg/ml solution bid		Flecknell, 1987
Trimethoprim-sulfamethoxazole	30 mg/kg PO bid		Reusch, 2005
Trimethoprim-sulfamethoxazole	15 mg/kg PO bid	Dosed on sulfa concentration	Bauck, 1989
Tylosin	10 mg/kg IM, PO, or SC bid		Hernandez-Divers, 2005
Tylosin	10 mg/kg PO or SC bid		Morrissey et al., 2004
Vancomycin	50 mg/kg IV tid		Nicolau et al., 1993

Parasiticides

Generic name	Dose and route	Comments	Reference
Amprolium	1 ml of 9.6% solution /7kg sid for 5 days		Hernandez-Divers, 2005
Amprolium	5 ml of 9.6% solution/gallon drinking water PO for 5 days		Hernandez-Divers, 2005
Carbaryl powder	5% powder topical	Can be diluted 1:1 with talc	Harkness and Wagner, 1983
Fenbendazole	50 ppm mixed in food PO for 5 days		Duwel and Brech, 1981
Fenbendazole	20 mg/kg PO sid for 5 days		McKellar, 1989
Fenbendazole	10–20 mg/kg PO repeated in 10–14 days	Pinworm, <i>Passalurus ambiguus</i>	Reusch, 2005
Fenbendazole	10–20 mg/kg PO	Repeat in 14 days	Morrissey et al., 2004
Fipronil	Not recommended	Not recommended by manufacturer (see MSDS)	
Fipronil	Not recommended	Toxic	Morrissey et al., 2004
Imidacloprid	Topical	Cat dose, once each month	Morrissey et al., 2004
Imidacloprid, moxidectin	10 mg/kg topical, 12 mg/kg topical	Apply as a spot-on	Hansen et al., 2005
Ivermectin	200–500 µg/kg SC or PO		Harvey, 1995
Ivermectin	400 µg/kg SC		McKellar et al., 1992
Ivermectin	200–400 µg/kg SC or PO		Messonnier, 1994
Ivermectin	200–400 µg/kg SC	Repeat in 10–14 days	Carpenter et al., 1995; Morrissey et al., 2004
Lasalocid	120 ppm in feed	Coccidiosis	Harkness and Wagner, 1995
Lime sulfur	2.50% topical	Dip every 7 days	Morrissey et al., 2004
Lufenuron	30 mg/kg PO q30d		Morrissey et al., 2004
Malathion	0.50% topical	Sponged two times each week	Russell et al., 1981
Mebendazole	10 mg/kg PO sid for 5 days		McKellar, 1989

(Continued)

Parasiticides (Continued)

Generic name	Dose and route	Comments	Reference
Metronidazole	20 mg/kg PO bid for 10 days		Carpenter et al., 1995
Nicarbazin	125 ppm PO	Mixed in food for 30 days	Sorribas et al., 1992
Niclosamide	150 mg/kg PO		Williams, 1976
Phenothiazine	1 g PO	Place in 50 g molasses-treated feed	Siegmund, 1979
Piperazine	100 mg/ml drinking water PO		Hagan and Gorham, 1976
Piperazine	500 mg/kg PO sid for 2 days	As adipate	Morrissey et al., 2004
Piperazine	0.5 mg/kg PO	As adipate; sid for 2 days	Brooks, 1979
Piperazine	200 mg/kg PO	As citrate: repeat in 14 days (Morrissey et al., 2004)	Harkness and Wagner, 1983
Praziquantel	5 mg/kg SC		McKellar, 1989
Praziquantel	10 mg/kg PO		McKellar, 1989
Praziquantel	5–10 mg/kg PO	Cestodes, trematodes	Reusch, 2005
Praziquantel	5–10 mg/kg IM, PO or SC	Repeat in 10 days	Carpenter et al., 1995
Pyrantel pamoate	5–10 mg/kg PO	Repeat in 14 days	Morrissey et al., 2004
Pyrethrin	0.50% topical	Dust	Russell et al., 1981
Selamectin	6 mg/kg topical		Morrissey et al., 2004
Selamectin	6 or 18 mg/kg topical	Apply once or twice 28 days apart	McTier et al., 2003
Sulfadimethoxine	50 mg/kg PO sid, then 25 mg/kg PO sid for 10–20 days	Coccidiosis	Morrissey et al., 2004
Sulfamerazine	100 mg/kg PO or 0.05–0.15% in drinking water	Coccidiosis	Gillett, 1994
Sulfamethazine	100 mg/kg PO sid 0.77 g/L drinking water 0.5–1.0% in feed	Coccidiosis	Gillett, 1994
Sulfamethoxine	50 mg/kg PO on day 1, then 25 mg/kg PO sid × 10–20 d		Brown, 1996
Thiabendazole	50 mg/kg PO sid, repeat in 3 weeks		Hernandez-Divers, 2005
Thiabendazole	100 mg/kg PO sid for 5 days		McKellar, 1989
Thiabendazole	50–100 mg/kg PO sid for 5 days		Morrissey et al., 2004
Thiabendazole	25 mg/kg PO		Williams, 1976
Thiabendazole	50 mg/kg PO repeated 10–14 d	Pinworm, <i>Passalurus ambiguus</i>	Reusch, 2005
Toltrazuril	25 ppm in drinking water (or 25 mg/kg PO) q24h × 2 days, repeat after 5 days	Coccidiosis	Harcourt Brown, 2002
Trimethoprim-sulfamethoxazole	30 mg/kg PO bid for 10 d	Intestinal coccidiosis	Reusch, 2005

Miscellaneous

Generic name	Dose and route	Comments	Reference
Atimpacezole	0.2 mg/kg IV	Medetomidine reversal	Flecknell, 1996
Atimpacezole	1 mg/kg IV or SC	Medetomidine reversal	Flecknell, 1996

(Continued)

Miscellaneous (Continued)

Generic name	Dose and route	Comments	Reference
Atimpacezole	1.25 mg/kg IM	Medetomidine reversal	Ward, 2006
Atracurium	7.5 µg/kg/min IV		Gómez-Iglesias et al., 1991
Atropine	0.20 mg/kg IM, IV, or SC		Green, 1982
Atropine	0.1–0.5 mg/kg IM or SC		Morrissey et al., 2004
Atropine	1–3 mg/kg IM or SC	30 min prior to surgery	Green, 1982
Atropine	10 mg/kg SC	Every 20 min for organophosphate overdose	Harkness and Wagner, 1983
Bretylum	10 mg/kg IV		Borchard et al., 1990
Calcium EDTA	27 mg/kg SC q6–12h		Morrissey et al., 2004
Calcium gluconate	5–10 ml PO	As 10% solution	Raphael, 1981
Calcium gluconate	3–5 ml IV	As 10% solution	Raphael, 1981
Cholestyramine	2 g/20 ml drinking water PO for 21 days	As active ingredient	Lipman et al., 1989
Cholestyramine	2 g/20 ml water PO sid	Bacterial enteritis to bind toxins	Reusch, 2005
Cimetidine	5–10 mg/kg IM, IV, PO, or SC q8–12h		Morrissey et al., 2004
Cisapride	0.5 mg/kg SC bid or tid		Paul-Murphy and Ramer, 1998
Cisapride	0.5 mg/kg PO q8–12h	Prokinetic for ileus. Gastric stasis (withdrawn from US, Canada, western Europe)	Reusch, 2005
Cyclosporine	10 mg/kg IV	Daily, at rate of 2 mg/kg/min IV	Petratiene et al., 2001
Dexamethasone	0.5–2 mg/kg IM or IV		Paul-Murphy and Ramer, 1998
Dextrose	2 ml/kg	Of 50% solution	Williams, 1976
Digoxin	0.005–0.01 mg/kg PO q24–48h		Morrissey et al., 2004
Digoxin	0.005 mg/kg q12–24h PO	Positive inotrope, myocardial failure, congestive heart failure	Pariaut, 2009
Diltiazem	0.5 mg/kg PO q8–24h	Calcium channel blocker, supraventricular tachycardia	Pariaut, 2009
Dimethicone	20–40 mg/kg PO qid	Treatment of ileus with gas	Reusch, 2005
Diphenhydramine	2 mg/kg PO or SC q8–12h		Morrissey et al., 2004
Doxapram	2–5 mg/kg IV	Respiratory stimulant	Flecknell, 1987
Doxapram	2 mg/kg SC or IV q15 minutes	Respiratory stimulant	Paul-Murphy, 2007
Enalapril	0.5 mg/kg PO q12–24h	ACE inhibitor, treatment for congestive heart failure	Pariaut, 2009
Epinephrine	0.2 mg/kg IV or 0.2–0.4 mg/kg IT	Cardiac arrest	Morrissey et al., 2004
Furosemide	2–5 mg/kg IM, IV, PO, or SC bid		Paul-Murphy and Ramer, 1998
Furosemide	1–3 mg/kg PO q8–24h	Treatment for congestive heart failure	Pariaut, 2009
Furosemide	1–4 mg/kg SC or IV prn	Treatment for congestive heart failure	Pariaut, 2009
Furosemide	1–4 mg/kg IM q6–8h	Diuretic	Paul-Murphy, 2007
Gallamine	1 mg/kg IV		Flecknell, 1996

(Continued)

Miscellaneous (Continued)

Generic name	Dose and route	Comments	Reference
Gallamine	0.2–0.3 mg/kg IV		Lumb et al., 1984
Glycopyrrolate	0.011 mg/kg IV	Use as part of pre-anesthetic regimen rather than atropine	Aeschbacher, 1995
Glycopyrrolate	0.1 mg/kg IM or SC	Use as part of pre-anesthetic regimen rather than atropine	Flecknell, 1996
Glycopyrrolate	0.01 mg/kg IV	Use as part of pre-anesthetic regimen rather than atropine	Flecknell, 1996
Glycopyrrolate	5 mg IM total	Animals between 3.2–4.4 kg	Sireve et al., 2008
Heparin	5 mg/kg IV		Melby et al., 1976
Human chorionic gonadotropin	20–35 IU IV	Ovulation	Hafez, 1970
Hydroxyzine	2 mg/kg PO q8–12h	Antihistamine, antipyretic	Morrissey et al., 2004
Iron dextran	4–6 mg/kg IM, one dose		Morrissey et al., 2004
Lidocaine	1–2 mg/kg IV prn	Sodium channel blocker, ventricular tachycardia	Pariaut, 2009
Loperamide	0.1 mg/kg PO tid for 3 days, then sid for 2 days		Banerjee et al., 1987
Loperomide	0.1 mg/kg PO tid for 3 days then sid	Treatment for diarrhea from bacterial enteritis	Reusch, 2005
Meclizine	2–12 mg/kg IM or SC q4–6h	Treatment for torticollis with rolling	Paul-Murphy, 2007
Meclizine	2–12 mg/kg PO sid	Treatment for torticollis with rolling	Paul-Murphy and Ramer, 1998
Meprobamate	50–150 mg/kg IM		Melby et al., 1976
Methylprednisone	5 mg/kg IV sid		Petratiene et al., 2001
Metoclopramide	0.2–0.5 mg/kg PO or SC q6–8h	Stimulates gastrointestinal motility, use for gastric stasis, trichobezoars	Hillyer, 1994, Messonnier, 1996
Metoclopramide	0.5 mg/kg PO or SC q8–24h	Stimulates gastrointestinal motility, use for gastric stasis, trichobezoars	Morrissey et al., 2004
Metoclopramide	0.34 mg/kg SC or PO	For inappetence	Fisher, 2006
Metoclopramide	0.5 mg/kg PO or SC	Prokinetic for ileus, gastric stasis	Reusch, 2005
Metocurine	0.01–0.015 mg/kg		Lumb et al., 1984
Midazolam	0.25–0.50 mg/kg IV or IM	Anticonvulsant	Paul-Murphy, 2007
Oxytocin	1–2 U IM or SC		Melby et al., 1976
Oxytocin	0.2–3 units/kg IM, IV, IP, or SC		Schuchman, 1977
Pancuronium	0.1 mg/kg IV		Flecknell, 1996
Pancuronium	0.008 mg/kg IV		Lumb et al., 1984
Pimobendan	0.1–0.3 mg/kg q12–24h	Positive inotrope, myocardial, congestive heart failure	Pariaut, 2009
Potassium citrate	33 mg/kg PO tid		Morrissey et al., 2004
Prednisone	0.5–2 mg/kg PO bid		Morrissey et al., 2004

(Continued)

Miscellaneous (Continued)

Generic name	Dose and route	Comments	Reference
Ranitidine	2–5 mg/kg PO q12–24h	Prokinetic for ileus, gastric stasis	Reusch, 2005
Simethicone	65–130 mg/animal q1h × 2–3 treatments	May reduce abdominal discomfort associated with excess gas	Krempels et al., 2002
Stanozolol	1–2 mg PO once	Stimulates appetite after surgery or illness	Harkness and Wagner, 1995
Streptokinase	4000 U/kg/h IV		Agnelli et al., 1985
Succinylcholine	0.5 mg/kg IV		Green, 1982
Sucralfate	25 mg/kg PO q8–12h	Treatment of esophagitis	Morrissey et al., 2004; Reusch, 2005
Sulfasalazine	1/8–1/4 crushed 400-mg tablet/animal q8–24h	May reduce inflammation of intestinal mucosa	Krempels et al., 2000
Suxamethonium	0.5 mg/kg IV		Flecknell, 1996
Tubocurarine	0.4 mg/kg IV		Flecknell, 1996
Tubocurarine	0.09–0.15 mg/kg IV		Lumb et al., 1984
Vitamin K	1–10 mg/kg IM prn		Morrissey et al., 2004

GUINEA PIG (*CAVIA PORCELLUS*)

Analgesics and Sedatives

Generic name	Dose and route	Comments	Reference
Acepromazine	0.5–1 mg/kg IM		Morrissey et al., 2004; Johnson-Delaney, 2008
Acepromazine	0.5 mg/kg IM	Pre surgical induction	Martinho, 2006
Alphaxalone-alphadolone	40 mg/kg IM		Flecknell, 1987
Aminopyrine	130 mg/kg PO		Jenkins, 1987
Aspirin	270 mg/kg IP sid		Canadian Council on Animal Care, 1984
Aspirin	86 mg/kg PO suggested q4h		Flecknell, 1987
Aspirin	87 mg/kg PO suggested sid, bid		Flecknell, 1998
Aspirin	50–100 mg/kg PO q4h		Morrissey et al., 2004; Johnson-Delaney, 2008
Buprenorphine	0.05 mg/kg SC q8–12h		Flecknell, 1985; Jenkins, 1987
Buprenorphine	0.05 mg/kg SC q6–12h		Flecknell, 1991
Buprenorphine	0.03–0.05 mg/kg IM or SC		Mans, 2007
Butorphanol	2 mg/kg IM q4h		Heard, 2009
Butorphanol	0.4–2 mg/kg SC q2–4h		Johnson-Delaney, 2008
Butorphanol	0.4–2 mg/kg SC q4h		Morrissey et al., 2004
Butorphanol	0.2 mg/kg SC	Intraoperative analgesia	Kaiser et al., 2007
Butorphanol	0.1 mg/kg IM	Pre surgical induction	Martinho, 2006
Carprofen	5 mg/kg SC bid		Heard, 2009

(Continued)

Analgesics and Sedatives (Continued)

Generic name	Dose and route	Comments	Reference
Carprofen	4 mg/kg IM or SC sid		Heard, 2009
Carprofen	4 mg/kg IM, IV, or SC q12–24h		Johnson-Delaney, 2008
Carprofen	1–2 mg/kg PO q12–24h		Morrissey et al., 2004
Chlorpromazine	0.5 mg/kg IM		Canadian Council on Animal Care, 1984
Chlorpromazine	25 mg/kg SC		Johnson-Delaney, 2008
Chlorpromazine	0.2 mg/kg SC		Lumb et al., 1984
Diazepam	5 mg/kg IP		Flecknell, 1987
Diazepam	2.5 mg/kg IM or IP		Green, 1982
Diazepam	0.5–3 mg/kg IM		Morrissey et al., 2004
Diclofenac	2.1 mg/kg PO		Albengres et al., 1988
Fentanyl/droperidol	0.5–1 ml/kg IM		Canadian Council on Animal Care, 1984
Fentanyl/droperidol	0.08–0.66 ml/kg		Hughes, 1981
Fentanyl/droperidol	0.22–0.88 ml/kg IM		Johnson-Delaney, 2008
Fentanyl/droperidol	0.22 ml/kg IM		Sander, 1992
Fentanyl/fluanisone	0.65–1 ml/kg SC		Arnemo et al., 2002
Fentanyl/fluanisone	1 ml/kg		Flecknell, 1987; Jenkins, 1987
Fentanyl/fluanisone	0.5–1 ml/kg IM		Johnson-Delaney, 2008
Fentanyl/fluanisone, midazolam, water	8 ml/kg IM or IP	1 part fentanyl: 1 part midazolam: 2 parts water	Johnson-Delaney, 2008
Flunixin	1–2 mg/kg SC suggested sid, bid		Flecknell, 1998
Flunixin	2.5 mg/kg SC q12–24h		Heard, 2009
Flunixin	2.5–5 mg/kg SC q12–24h		Morrissey et al., 2004
Ibuprofen	10 mg/kg IM suggested q4h		Flecknell, 1991
Indomethacin	8.8 mg/kg PO		Albengres et al., 1988
Ketamine	25–30 mg/kg IM		Bauck, 1989
Ketamine	22–64 mg/kg IP		Sander, 1992
Ketamine, medetomidine	40 mg/kg IM or SC, 0.50 mg/kg		Arnemo et al., 2002
Ketoprofen	1 mg/kg IM or SC q12–24h		Morrissey et al., 2004; Johnson-Delaney, 2008
Medetomidine	0.500 mg/kg SC		Cantwell, 2001
Medetomidine	0.300 mg/kg		Morrissey et al., 2004
Meloxicam	0.1–0.3 mg/kg PO or SC		Hawkins, 2006
Meloxicam	0.2 mg/kg PO or SC sid		Johnson-Delaney, 2008
Meperidine	10–20 mg/kg IM or SC q2–3h		Flecknell, 1991
Meperidine	20 mg/kg IM or SC q2–3h		Jenkins, 1987
Methadone	3–6 mg/kg SC		Jenkins, 1987
Midazolam	1–2 mg/kg IM		Morrissey et al., 2004

(Continued)

Analgesics and Sedatives (Continued)

Generic name	Dose and route	Comments	Reference
Morphine	10 mg/kg IM or SC q2–4h		Flecknell, 1985; Jenkins, 1987
Morphine	2–5 mg/kg IM or SC q4h		Flecknell, 1991
Nalbuphine	1–2 mg/kg IM q3h		Johnson-Delaney, 2008
Nalbuphine	1–2 mg/kg IM, IP, or IV		Flecknell, 1996
Nalbuphine	1 mg/kg IP or SC		Flecknell, 1996
Naproxen	14.9 mg/kg PO		Albengres et al., 1988
Oxymorphone	0.2–0.5 mg/kg IM or SC		Heard, 2009
Pethidine	20 mg/kg IM or SC q2–3h		Flecknell, 1987
Phenylbutazone	40 mg/kg PO		Wilhelmi, 1974
Piroxicam	5.7 mg/kg PO		Albengres et al., 1988
Piroxicam	5.7–6 mg/kg PO q12–24h		Johnson-Delaney, 2008
Xylazine	5 mg/kg IP		Arnemo et al., 2002
Xylazine	3–5 mg/kg IM		Harkness and Wagner, 1983

Anesthetics

Generic name	Dose and route	Comments	Reference
Alphaxalone-alphadolone	40 mg/kg IP		Flecknell, 1987
Alphaxalone-alphadolone	10–20 mg/kg IV		Green, 1982
Alphaxalone-alphadolone	40 mg/kg IM or IP		Johnson-Delaney, 2008
Alphaxalone-alphadolone	4.5 mg/kg IV over 30 s	Anesthetic induction	Schwenke and Cragg, 2004
Alphaxalone-alphadolone	9.75 mg/kg/h IV	Anesthetic maintenance	Schwenke and Cragg, 2004
Buprenorphine	0.05 mg/kg IM	Post operative pain relief for cannulation surgery	Schwenke and Cragg, 2004
Chloral hydrate	200–300 mg 10% solution/kg IP		Green, 1982
Fentanyl/droperidol	0.5–1 ml/kg IM		Canadian Council on Animal Care, 1984
Fentanyl/droperidol	0.66–0.88 ml/kg SC		Hughes, 1981
Fentanyl/fluanisone	0.5 ml/kg IM	Addition of diazepam is advised	Cooper, 1984
Fentanyl/fluanisone, diazepam	1 ml/kg IM or SC, 2.5 mg/kg IM or IP		Arnemo et al., 2002
Fentanyl/fluanisone, diazepam	1 ml/kg IM, 2.5 mg/kg IP		Flecknell, 1996
Ketamine	44 mg/kg IM	Atropine or glycopyrrolate recommended in addition	Weisbroth and Fudens, 1972
Ketamine	100–200 mg/kg IM	For immobilization	Flecknell, 1987
Ketamine	25 mg/kg IV	Anesthetic induction	Martinho, 2006
Ketamine, acepromazine	125 mg/kg IM, 5 mg/kg IM		Flecknell, 1987
Ketamine, diazepam	100 mg/kg IM, 5 mg/kg IM		Flecknell, 1987
Ketamine, diazepam	20–30 mg/kg IM, 1–2 mg/kg IM		Morrissey et al., 2004

(Continued)

Anesthetics (Continued)

Generic name	Dose and route	Comments	Reference
Ketamine, medetomidine	40 mg/kg IP, 0.50 mg/kg	Reverse medetomidine with atipamezole	Flecknell, 1996
Ketamine, midazolam	5–10 mg/kg IM, 0.5–1 mg/kg IM		Morrissey et al., 2004
Ketamine, xylazine	25–40 mg/kg IM, 5 mg/kg SC		Arnemo et al., 2002
Ketamine, xylazine	40 mg/kg IM, 5 mg/kg SC		Flecknell, 1987
Ketamine, xylazine	50 mg/kg IP, 5 mg/kg IP		Strother and Stokes, 1989
Ketamine, xylazine	5.5 mg/kg IV, 1.4 mg/kg IV	Anesthetic induction	Schwenke and Cragg, 2004
Ketamine, xylazine	87 mg/kg IP, 13 mg/kg IP	Use for restraint, mildly painful procedures	Radde et al., 1996
Ketamine, xylazine	14.6 mg/kg/h IV, 3.7 mg/kg/h IV	Anesthetic maintenance	Schwenke and Cragg, 2004
Methohexital (methohexitone)	31 mg/kg IP		Flecknell, 1987
Pentobarbital	28 mg/kg IP		Croft, 1964
Pentobarbital	30–45 mg/kg IP		Morrissey et al., 2004
Pentobarbital, xylazine	45 mg/kg IP, 7 mg/kg IM	Give pentobarbital first. Supplement with pentobarbital 3.25–6.5 mg/kg IP or IV as needed	Rhodes et al., 2001
Pentobarbitone	10 mg/kg IV over 2 minutes	Anesthetic induction	Schwenke and Cragg, 2004
Pentobarbitone	10 mg/kg IV over 2 minutes	Anesthetic induction	Schwenke and Cragg, 2004
Pentobarbitone	22 mg/kg/h IV	Maintenance infusion	Schwenke and Cragg, 2004
Pentobarbitone, fentanyl/droperidol mixture	8.3 mg/kg/h IV, 0.15 mg/kg SC q1–1.5h	Anesthetic maintenance	Schwenke and Cragg, 2004
Proparacaine hydrochloride (0.5%)	Single drop OU	Corneal anesthesia	Coster et al., 2008
Propofol	10 mg IV	To effect	Johnson-Delaney, 2008
Tiletamine/zolazepam	10–30 mg/kg IM		Fowler, 1978
Tiletamine/zolazepam	25–50 mg/kg IP	Suitable for short periods of chemical restraint, not for painful procedures	Radde et al., 1996
Tiletamine/zolazepam, butorphanol, xylazine	60 mg/kg IP, 0.10 mg/kg IM, 5 mg/kg IP		Jacobson, 2001
Tiletamine/zolazepam, detomidine	40 mg/kg IM, 5 mg/kg IM		Buchanan et al., 1999
Tiletamine/zolazepam, xylazine	40 mg/kg IM, 5 mg/kg IM		Buchanan et al., 1999
Urethane	1500 mg/kg IV or IP		Flecknell, 1987
Yohimbine	1 mg/kg IP	Xylazine reversal	Strother and Stokes, 1989

Anti-Infectives

Generic name	Dose and route	Comments	Reference
Amikacin	10–20 mg/kg IM, IV, or SC divided q8–12h		Johnson-Delaney, 2008
Amikacin	10–15 mg/kg IM, IV, or SC divided q8–24h		Morrissey et al., 2004

(Continued)

Anti-Infectives (Continued)

Generic name	Dose and route	Comments	Reference
Amoxicillin	Not recommended	Toxic	Flecknell, 1987
Amoxicillin/clavulanate	Not recommended	Toxic	Morrissey et al., 2004
Ampicillin	6 mg/kg SC tid for 5 days	Use with extreme caution (Hernandez-Divers, 2005)	Young et al., 1987
Ampicillin	Not recommended	Possible enterocolitis	Bartlett et al., 1978
Azithromycin	15 mg/kg PO sid		Johnson-Delaney, 2008
Cefazolin	100 mg/kg IM bid		Fritz et al., 1987
Ceftiofur	1 mg/kg IM sid		Johnson-Delaney, 2008
Ceftiofur	1 mg/kg IM	For treatment of pneumonia	Donnelly and Brown, 2004
Cephalexin	15 mg/kg SC sid		Flecknell, 1996
Cephalexin	50 mg/kg PO divided q12–24h	Use with extreme caution (Hernandez-Divers, 2005)	Morrissey et al., 2004
Cephalexin	50 mg/kg IM sid for 14 days		Richardson, 1992
Cephaloridine	15–25 mg/kg SC sid		Bauck, 1989
Cephaloridine	12.5 mg/kg IM sid for 14 days		Dixon, 1986
Cephaloridine	12.5 mg/kg IM q 8–24h for 5–14 days		Donnelly and Brown, 2004
Cephaloridine	10–25 mg/kg IM sid for 5–7 days		Harkness and Wagner, 1977
Cephaloridine	10–25 mg/kg IM q8–24h		Johnson-Delaney, 2008
Chloramphenicol palmitate	50 mg/kg PO bid		Bauck, 1989
Chloramphenicol palmitate	50 mg/kg PO bid		Donnelly and Brown, 2004
Chloramphenicol palmitate	50 mg/kg PO bid; or 1 mg/ml in water		Johnson-Delaney, 2008
Chloramphenicol palmitate	50 mg/kg PO tid		Russell et al., 1981
Chloramphenicol succinate	50 mg/kg SC bid		Bauck, 1989
Chloramphenicol succinate	30–50 mg/kg SC, IM, bid		Donnelly and Brown, 2004
Chloramphenicol succinate	20 mg/kg IM bid		Flecknell, 1987
Chloramphenicol succinate	30–50 mg/kg PO bid		Johnson-Delaney, 2008
Ciprofloxacin	10–20 mg/kg PO bid		Donnelly and Brown, 2004
Ciprofloxacin	10–15 mg/kg PO bid		Johnson-Delaney, 2008
Ciprofloxacin	5–15 mg/kg PO q12–24h		Morrissey et al., 2004
Clindamycin	Not recommended	Possible enterocolitis	Bartlett, 1979
Doxycycline	2.5–5 mg/kg PO bid		Johnson-Delaney, 2008
Doxycycline	2.5 mg/kg PO bid		Morrissey et al., 2004
Doxycycline	75 mg/kg SC every 7 days	Long acting formulation	Johnson-Delaney, 2008
Enrofloxacin	5–10 mg/kg PO or IM bid		Donnelly and Brown, 2004
Enrofloxacin	5–10 mg/kg PO		Dorrestein, 1992
Enrofloxacin	100 mg/L drinking water PO		Dorrestein, 1992
Enrofloxacin	5–10 mg/kg SC bid		Flecknell, 1996
Enrofloxacin	2.5–5 mg/kg IM, PO, or SC bid		Göbel, 1996

(Continued)

Anti-Infectives (Continued)

Generic name	Dose and route	Comments	Reference
Enrofloxacin	5–15 mg/kg IM, PO. or SC bid		Morrissey et al., 2004
Erythromycin	Not recommended	Possible enterocolitis	Bartlett et al., 1978
Furazolidone	5.5 mg/ml	Prepare in drinking water	Burgmann and Percy, 1993
Gentamicin	5–8 mg/kg SC sid		Bauck, 1989
Gentamicin	6 mg/kg SC sid		Campbell et al., 1996
Gentamicin	2 mg/kg SC tid		Campbell et al., 1996
Gentamicin	5–8 mg/kg IM or SC Divided q8–24h		Morrissey et al., 2004
Gentamicin	6 mg/kg SC sid	Use cautiously	Donnelly and Brown, 2004
Griseofulvin	75 mg/kg PO sid for 14 days		Harkness and Wagner, 1983
Griseofulvin	15–25 mg/kg PO sid		Harvey, 1995; Morrissey et al., 2004
Griseofulvin	1.50% topical bid for 14 days		Post and Saunders, 1979
Itraconazole	5 mg/kg IM sid		Johnson-Delaney, 2008
Ketoconazole	10–40 mg/kg PO sid for 14 days		Johnson-Delaney, 2008
Marbofloxacin	3–5 mg/kg PO or SC sid		Johnson-Delaney, 2008
Metronidazole	1–40 mg/kg PO sid		Donnelly and Brown, 2004
Metronidazole	20–25 mg/kg PO bid		Johnson-Delaney, 2008
Metronidazole	20 mg/kg PO bid		Morrissey et al., 2004
Metronidazole	20 mg/kg PO or SC sid		Richardson, 1992
Neomycin	12–16 mg/kg PO bid		Donnelly and Brown, 2004
Neomycin	30 mg/kg SC		Flecknell, 1987
Neomycin	10 mg/kg PO sid		Flecknell, 1987; McKellar, 1989
Neomycin	15 mg/kg PO bid		Morrissey et al., 2004
Neomycin	5 mg/kg PO bid		Richardson, 1992
Nitrofurazone	50 mg/kg PO sid for 3 days		Johnson-Delaney, 2008
Oxytetracycline	5 mg/kg IM tid		Johnson-Delaney, 2008
Oxytetracycline	5 mg/kg IM bid		Siegmund, 1979
Penicillin	Not recommended	Possible enterocolitis	Bartlett, 1979
Succinylsulfathiazole	0.10% in drinking water PO	Prepare in drinking water	Williams, 1976
Sulfadimethoxine	1–15 mg/kg PO bid		Donnelly and Brown, 2004
Sulfadimethoxine	10–15 mg/kg PO bid		Johnson-Delaney, 2008
Sulfamerazine	40 ml of 12.5% solution/gallon drinking water PO		Russell et al., 1981
Sulfamerazine	1 mg/ml in drinking water PO	Prepare in drinking water	Johnson-Delaney, 2008
Sulfamethazine	1 mg/ml in drinking water PO		Donnelly and Brown, 2004
Sulfamethazine	166–517 mg/L drinking water PO		Canadian Council on Animal Care, 1984
Sulfamethazine	1–5 mg/ml drinking water PO		Johnson-Delaney, 2008

(Continued)

Anti-Infectives (Continued)

Generic name	Dose and route	Comments	Reference
Sulfaquinoxaline	0.25–1.0 g sid for 30 days	Daily for 30 days; prepare in one liter drinking water	Schuchman, 1977
Sulfaquinoxaline	1 mg/ml Prepare in drinking water	Up to 60 days	Johnson-Delaney, 2008
Terbinafine	10–30 mg/kg PO sid for 4–6 weeks	Bronchodilator	Johnson-Delaney, 2008
Tetracycline	20 mg/kg PO bid		Bauck, 1989
Tetracycline	112–350 mg/l drinking water PO		Canadian Council on Animal Care, 1984
Tetracycline	10 mg/kg PO q8–12h; use with caution		Morrissey et al., 2004
Tetracycline	5 mg/kg IM bid		Richardson, 1992
Tetracycline	50 mg/kg PO divided into three doses	Divide into three doses	Richardson, 1992
Tobramycin	30 mg/kg SC bid		Johnson-Delaney, 2008
Tobramycin	30 mg/kg IV sid		Kapsunik et al., 1988
Trimethoprim/sulfadiazine	30 mg/kg SC sid	May cause tissue necrosis, irritation (Harkness and Wagner, 1995)	Bauck, 1989
Trimethoprim/sulfadiazine	0.5 ml/kg of 240 mg/ml solution SC		Flecknell, 1987
Trimethoprim-sulfamethoxazole	15 mg/kg PO bid	Dosed on sulfa concentration	Bauck, 1989
Tylosin	10 mg/kg PO or SC bid	Use with extreme caution	Morrissey et al., 2004

Parasiticides

Generic name	Dose and route	Comments	Reference
Amitraz	0.3% topical	Treat every 7 days	Morrissey et al., 2004
Carbaryl	5% powder topical	Dust lightly once each week	Johnson-Delaney, 2008
Enilconazole	0.2% solution topical	Dip every 7 days	Johnson-Delaney, 2008
Fenbendazole	20–50 mg/kg PO sid for 5 days		Johnson-Delaney, 2008
Fenbendazole	20 mg/kg PO sid for 5 days		Morrissey et al., 2004
Fipronil	Topical	Apply days 0, 10	Beck and Pantchev, 2007b
Imidacloprid, moxidectin	100 mg/kg topical, 10 mg/kg topical	Applied as 0.1 ml commercial topical solution to base of neck	Beck et al., 2007a
Ivermectin	500 µg/kg SC		McKellar et al., 1992
Ivermectin	200–400 µg/kg SC	Give every 10 days for 2–3 treatments	Harvey, 1995
Ivermectin	400 µg/kg SC	Once; repeat in 14 days	Clyde, 1996
Lime sulfur	2.5% topical	Dip every 7 days	Morrissey et al., 2004
Lime sulfur	1:40 topical	Prepare in dimethyl sulfoxide, sponge solution over body for 6 weeks	Russell et al., 1981
Lindane	1% topical	Use as dip	Zajac et al., 1980
Malathion	0.5% topical	Use as dip	Russell et al., 1981
Metronidazole	25 mg/kg PO bid		Morrissey et al., 2004

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Parasiticides (Continued)

Generic name	Dose and route	Comments	Reference
Metronidazole	20 mg/kg PO or SC sid		Richardson, 1992
Piperazine	4–7 mg/ml in drinking water PO	As adipate, 3–10 days (Morrisey et al., 2004)	Williams, 1976
Piperazine	2–5 mg/ml PO	As citrate	Johnson-Delaney, 2008
Piperazine	10 mg/ml PO	As citrate; in drinking water – for 7 days, off 7 days, on 7 days	Morrisey et al., 2004
Praziquantel	5–10 mg/kg IM, PO, or SC	Repeat in 10 days	Morrisey et al., 2004; Johnson-Delaney, 2008
Selamectin	6 mg/kg topical		Morrisey et al., 2004; Johnson-Delaney, 2008
Sulfadimethoxine	25–50 mg/kg PO sid for 10 days		Morrisey et al., 2004
Thiabendazole	100 mg/kg PO sid for 5 days		Morrisey et al., 2004; Johnson-Delaney, 2008

Miscellaneous

Generic name	Dose and route	Comments	Reference
Aluminum hydroxide	0.5–1 ml PO prn	Amphojel®	Johnson-Delaney, 2008
Aminophylline	50 mg/kg IM as needed q4–8h	bronchodilator	Johnson-Delaney, 2008
Atimpacezole	1 mg/kg IM or SC	Reverses medetomidine	Johnson-Delaney, 2008
Atropine	0.02–0.05 mg/kg IM, IV, or SC		Canadian Council on Animal Care, 1984
Atropine	0.1–0.2 mg/kg IM or SC		Morrisey et al., 2004
Atropine	10 mg/kg SC	Organophosphate poisoning; may cause cardiovascular irregularities	Johnson-Delaney, 2008
Betamethasone	0.1 mg/kg SC		Johnson-Delaney, 2008
Betamethasone	0.1–0.2 ml IM or SC		Richardson, 1992
Bismuth subsalicylate	0.2 ml q6–8h per adult		Johnson-Delaney, 2008
Calcium carbonate	4 mg/kg PO		Johnson-Delaney, 2008
Calcium EDTA	30 mg/kg SC bid		Morrisey et al., 2004
Calcium gluconate	100 mg/kg IM	Use in dystocia; follow with oxytocin	Johnson-Delaney, 2008
Chlorpheniramine	5 mg/kg SC sid	Antihistamine	Johnson-Delaney, 2008
Cholestyramine	100 mg/ml PO	In water, binds clostridial endo/enterotoxins	Johnson-Delaney, 2008
Cimetidine	5–10 mg/kg IM, IV, PO, or SC q6–12h		Morrisey et al., 2004; Johnson-Delaney, 2008
Cisapride	0.5 mg/kg PO q8–12h	Motility enhancer	Morrisey et al., 2004
Cyclophosphamide	300 mg/kg IP sid		Johnson-Delaney, 2008
Dexamethasone	0.5–2 mg/kg PO or SC bid		Johnson-Delaney, 2008
Dexamethasone	0.5–2 mg/kg IM, IV, or SC		Morrisey et al., 2004
Dexamethasone	0.1 ml SC		Richardson, 1992
Diphenhydantoin	25–50 mg/kg PO bid		Johnson-Delaney, 2008

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Miscellaneous (Continued)

Generic name	Dose and route	Comments	Reference
Diphenhydramine	12.5 mg/kg IP		Johnson-Delaney, 2008
Diphenhydramine	5 mg/kg SC		Melby et al., 1976; Johnson-Delaney, 2008
Diphenhydramine	24 mg/kg PO prn		Johnson-Delaney, 2008
Dopamine	0.8 mg/kg IV		Johnson-Delaney, 2008
Doxapram	5 mg/kg IV	Respiratory stimulant	Flecknell, 1987
Doxapram	10–15 mg/kg IM or SC	Respiratory stimulant	Richardson, 1992
Epinephrine	0.003 mg/kg IV prn		Morrissey et al., 2004
Furosemide	5–10 mg/kg SC, IM, or PO bid	Diuretic, pulmonary congestion, edema, ascites	Johnson-Delaney, 2008
Furosemide	2–5 mg/kg PO or SC bid	Diuretic, pulmonary congestion, edema, ascites	Morrissey et al., 2004
Gallamine	0.1–0.2 mg/kg IV		Flecknell, 1996
Glycopyrrolate	0.01–0.02 mg/kg IM or SC	Useful part of pre-anesthetic instead of atropine	Mason, 1997
Gonadotropin-releasing hormone	25 µg/kg	Route not stated	Mayer, 2003
Heparin	5 mg/kg IV		Melby et al., 1976
Human chorionic gonadotropin	1000 units IM	Repeat in 7 days	Harvey, 1995
Hydralazine	1 mg/kg IV	Prn, antihistamine, pruritis	Johnson-Delaney, 2008
Isoproterenol	0.05 mg/kg IM	Prn, bronchodilator	Johnson-Delaney, 2008
Kaolin	0.2 ml PO q6–8h	As Kaopectate V	Richardson, 1992
Leuprolide	0.2–0.3 mg/kg IM	Depot formulation q28d	Johnson-Delaney, 2008
Loperamide	0.1 mg/kg PO tid for 3 days, then sid for 2 days	Enteropathy, antidiarrheal. Give in at least 1 ml of water	Johnson-Delaney, 2008
Magnesium hydroxide, simethicone	0.5–1 ml PO prn	Diovol Plus®	Johnson-Delaney, 2008
Meprobamate	100 mg/kg IM		Melby et al., 1976
Methylprednisolone	2 mg/kg IM	Every 30 days	Bauck, 1989
Metoclopramide	0.5 mg/kg SC tid, prn		Johnson-Delaney, 2008
Metoclopramide	0.2–1 mg/kg IM, PO, or SC bid		Morrissey et al., 2004
Nalorphine	2–5 mg/kg IV		Johnson-Delaney, 2008
Naloxone	0.01–0.1 mg/kg IP or IV		Flecknell, 1987
Oxytocin	0.2–0.3 U/kg	Repeat as needed	Harrenstien, 1994
Oxytocin	1–2 IU IM	Total dose	Richardson, 1992
Oxytocin	1.5 IU/kg IM	Uterine inertia	Martinho, 2006
Pancuronium	0.06 mg/kg IV		Flecknell, 1996
Prednisone	0.5–2.2 mg/kg IM, PO, or SC		Morrissey et al., 2004
Sucalfate	50 mg/kg PO		Johnson-Delaney, 2008
Sucalfate	25–50 mg/kg PO		Morrissey et al., 2004
Tubocurarine	0.1–0.2 mg/kg IV		Flecknell, 1996

(Continued)

Miscellaneous (Continued)

Generic name	Dose and route	Comments	Reference
Vitamin A	500–5000 IU/kg IM		Morrissey et al., 2004; Johnson-Delaney, 2008
Vitamin B complex	0.02–0.2 ml/kg		Johnson-Delaney, 2008
Vitamin C	200 mg/L drinking water PO		Harkness and Wagner, 1977
Vitamin C	50 mg PO		Williams, 1976
Vitamin C	10 mg/kg IM	Followed by oral supplementation	Bauck, 1989
Vitamin C	10–30 mg/kg IM or PO sid	Includes other parenteral routes	Harrenstien, 1994
Vitamin D	200–400 IU/kg IM or SC		Johnson-Delaney, 2008
Vitamin E	5–10 mg/kg IM sid		Johnson-Delaney, 2008
Vitamin K	1–10 mg/kg IM prn		Morrissey et al., 2004

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Generic name	Dose and route	Comments	Reference
Acepromazine	0.5–1 mg/kg IM		Morrissey et al., 2004
Aminopyrine	130 mg/kg PO		Jenkins, 1987
Aspirin	240 mg/kg IP sid		Canadian Council on Animal Care, 1984
Aspirin	100 mg/kg PO suggested sid, bid		Flecknell, 1998
Aspirin	240 mg/kg PO sid		Morrissey et al., 2004
Buprenorphine	0.5 mg/kg SC tid		Flecknell, 1987
Buprenorphine	0.01–0.05 mg/kg IV or SC q8–12h		Flecknell, 1998
Buprenorphine	0.1–0.25 mg/kg PO q8–12h		Flecknell, 1998
Buprenorphine	0.1 mg/kg SC q6–12h		Heard, 2009
Buprenorphine	0.05 mg/kg SC q8–12h		Jenkins, 1987
Buprenorphine	0.1–0.5 mg/kg SC tid		Morrissey et al., 2004
Butorphanol	1–5 mg/kg SC q4h		Heard, 2009
Carprofen	5 mg/kg SC sid		Flecknell, 1998
Chlorpromazine	0.5 mg/kg IM		Melby et al., 1976
Diazepam	5 mg/kg IP		Canadian Council on Animal Care, 1984)
Diazepam	3–5 mg/kg IM		Morrissey et al., 2004
Diclofenac	10 mg/kg PO sid, bid		Flecknell, 1998
Fentanyl/fluanisone	0.5 ml/kg IM or SC		Armeno et al., 2002
Fentanyl/fluanisone	1 ml/kg		Jenkins, 1987
Flunixin	2.5 mg/kg IM or SC bid		Flecknell, 1998
Flunixin	2.5 mg/kg SC bid		Heard, 2009; Morrissey et al., 2004
Ibuprofen	15 mg/kg PO suggested sid, bid		Flecknell, 1998
Ketamine	60 mg/kg IM		Bauck, 1989
Ketoprofen	5 mg/kg SC suggested sid, bid		Flecknell, 1998

(Continued)

Analgesics and Sedatives (Continued)

Generic name	Dose and route	Comments	Reference
Medetomidine	0.1 mg/kg		Cantwell, 2001
Medetomidine	0.1 mg/kg		Morrissey et al., 2004
Meloxicam	1–2 mg/kg IM, PO, or SC		Heard, 2009
Meperidine	10–20 mg/kg SC q2–3h		Heard, 2009
Meperidine	20 mg/kg IM or SC q2–3h		Jenkins, 1987
Methadone	3–6 mg/kg SC		Jenkins, 1987
Midazolam	1–2 mg/kg IM		Morrissey et al., 2004
Morphine	10 mg/kg SC q2–4h		Jenkins, 1987
Morphine	2–5 mg/kg SC q2–4h		Morrissey et al., 2004
Nalbuphine	1–2 mg/kg IM q3h		Flecknell, 1998
Nalbuphine	2 mg/kg SC		Flecknell, 1996
Oxymorphone	0.2–0.5 mg/kg SC q6–12h		Heard, 2009
Oxymorphone	0.15 mg/kg IM		Trim et al., 1987
Pentazocine	10 mg/kg SC q3–4h		Flecknell, 1998
Pentobarbital	50–90 mg/kg IP	Anesthesia for blood transfusion	Elliott et al., 2005
Piroxicam	3 mg/kg PO suggested sid, bid		Flecknell, 1998
Tiletamine/zolazepam, xylazine	20 mg/kg IP, 10 mg/kg IP		Forsythe et al., 1992
Xylazine	10 mg/kg IP		Arnemo et al., 2002
Xylazine	4 mg/kg IM		Bauck, 1989

Anesthetics

Generic name	Dose and route	Comments	Reference
Alphaxalone-alphadolone	150 mg/kg IP		Flecknell, 1987
Alphaxalone-alphadolone	14 mg/kg IV	Anesthetic induction	Green et al., 1978
Alphaxalone-alphadolone	4–6 mg/kg IV q15 minutes	Anesthetic maintenance	Green et al., 1978
Atipamezole	1 mg/kg SC	Reverses medetomidine	Flecknell, 1996
Chloral hydrate	270–360 mg/kg IP		Hughes, 1981
Fentanyl/droperidol	Not recommended		Thayer et al., 1972
Fentanyl/droperidol, diazepam	1 ml/kg IP, 5 mg/kg IP		Green, 1982
Fentanyl/fluanisone, diazepam	1 ml/kg IM or SC, 2.5 mg/kg IP		Arnemo et al., 2002
Ketamine	100–300 mg/kg IP		Strittmatter, 1972
Ketamine	200 mg/kg IP	For immobilization	Flecknell, 1987
Ketamine, acepromazine	150 mg/kg IM, 5 mg/kg IM		Flecknell, 1987
Ketamine, diazepam	70 mg/kg IP, 2 mg/kg IP		Flecknell, 1996
Ketamine, medetomidine	100 mg/kg IP, 0.25 mg/kg IP		Flecknell, 1996

(Continued)

Anesthetics (Continued)

Generic name	Dose and route	Comments	Reference
Ketamine, medetomidine	75 mg/kg IM or IP, 1 mg/kg IM or IP		Morrissey et al., 2004
Ketamine, xylazine	50–150 mg/kg IM or SC, 10 mg/kg IM or SC		Armeno et al., 2002
Ketamine, xylazine	50–200 mg/kg IP, 10 mg/kg IP		Curl and Peters, 1983
Ketamine, xylazine	200 mg/kg IP, 10 mg/kg IP		Flecknell, 1987
Pentobarbital	50–90 mg/kg IP		Morrissey et al., 2004
Pentobarbital	90 mg/kg IP	Supplement with 12 mg/kg as necessary	Whitney, 1963
Pentobarbital, ketamine	35–60 mg/kg IP, 35–60 mg/kg IP	Anesthesia for terminal IV heparin	Portilla-De-Buen et al., 2004
Tiletamine/zolazepam	Not recommended		Silverman et al., 1983
Tiletamine/zolazepam, xylazine	30 mg/kg IP, 10 mg/kg IP		Forsythe et al., 1992

Anti-Infectives

Generic name	Dose and route	Comments	Reference
Amikacin	10 mg/kg IM or SC bid		Morrissey et al., 2004
Amoxicillin	Not recommended	Toxic	Flecknell, 1987
Amoxicillin/clavulanate	Not recommended	Toxic	Morrissey et al., 2004
Ampicillin	Not recommended	Toxic	Flecknell, 1987
Cephaloridine	30 mg/kg IM bid	Use with caution (Ness, 2005)	Flecknell, 1987
Cephaloridine	10 mg/kg IM bid	Use with caution (Ness, 2005)	Holmes, 1984
Cephaloridine	10–25 mg/kg IM or SC sid	Use with caution (Ness, 2005)	Anderson, 1994
Cephaloridine	Not recommended	Possible enterocolitis	Bartlett et al., 1978
Chloramphenicol palmitate	50 mg/kg PO tid		Bauck, 1989
Chloramphenicol palmitate	200 mg/kg PO tid		Russell et al., 1981
Chloramphenicol succinate	50 mg/kg SC bid		Bauck, 1989
Chloramphenicol succinate	30 mg/kg IM bid		Flecknell, 1987
Chloramphenicol succinate	30 mg/kg SC bid		Flecknell, 1996
Chlortetracycline	20 mg/kg PO bid		Morrissey et al., 2004
Ciprofloxacin	10–20 mg/kg PO bid		Capello, 2001
Ciprofloxacin	10 mg/kg PO q12–24h		Harvey, 1995
Ciprofloxacin	10 mg/kg PO bid		Morrissey et al., 2004
Dimetridazole	500 mg/L drinking water PO	Prepare in 1 L drinking water	La Regina et al., 1980
Doxycycline	2.5 mg/kg PO bid		Morrissey et al., 2004
Enrofloxacin	5–10 mg/kg PO		Dorrestein, 1992

(Continued)

Anti-Infectives (Continued)

Generic name	Dose and route	Comments	Reference
Enrofloxacin	100 mg/L drinking water PO		Dorrestein, 1992
Enrofloxacin	10 mg/kg SC bid		Flecknell, 1996
Enrofloxacin	2.5–5 mg/kg IM, PO, or SC bid		Göbel, 1996
Enrofloxacin	5–10 mg/kg IM, PO, or SC bid		Morrissey et al., 2004
Enrofloxacin	0.1 mg/ml in drinking water PO	Prepare in drinking water	Tynes, 1998
Erythromycin	Not recommended	Possible enterocolitis, toxic	Bartlett et al., 1978
Furazolidone	30 mg/kg PO sid		Burgmann and Percy, 1993
Gentamicin	5–8 mg/kg SC sid		Bauck, 1989
Gentamicin	5–8 mg/kg IM or SC divided q8–24h		Morrissey et al., 2004
Gentamicin	5 mg/kg IM sid		Russell et al., 1981
Griseofulvin	25–50 mg/kg PO sid		Capello, 2001
Griseofulvin	25 mg/kg PO sid		Morrissey et al., 2004
Ketoconazole	10 mg/kg PO sid		Morrissey et al., 2004
Metronidazole	20 mg/kg PO bid		Morrissey et al., 2004
Neomycin	10 mg/kg PO sid		Flecknell, 1987
Neomycin	125 mg/L drinking water PO		La Regina et al., 1980
Neomycin	30 mg/kg PO bid		Morrissey et al., 2004
Neomycin	0.5 mg/ml in drinking water PO	Prepare in drinking water	Anderson, 1994
Oxytetracycline	20 mg/kg SC sid		McKellar, 1989
Sulfamethazine	665–800 mg PO	Prepare in one liter drinking water	Canadian Council on Animal Care, 1984
Tetracycline	20 mg/kg PO bid		Bauck, 1989
Tetracycline	400 mg/L drinking water PO		La Regina et al., 1980
Tetracycline	10–20 mg/kg PO q8–12h		Morrissey et al., 2004
Trimethoprim/sulfadiazine	30 mg/kg SC sid		Bauck, 1989
Trimethoprim/sulfamethoxazole	15 mg/kg PO bid	Dosed on sulfa concentration	Bauck, 1989
Tylosin	100 mg/kg PO sid	Use with caution (Ness, 2005)	Flecknell, 1987
Tylosin	10 mg/kg IM or SC sid	Use with caution (Ness, 2005)	McKellar, 1989
Tylosin	100 mg/kg PO sid for 21 days	Use with caution (Ness, 2005)	Russell et al., 1981
Tylosin	2–8 mg/kg PO or SC bid	Use with caution	Morrissey et al., 2004
Vancomycin	20 mg/kg PO	By gavage	Boss et al., 1994

Parasiticides

Generic name	Dose and route	Comments	Reference
Amitraz	1.4 ml topical	Treat every 7 days	Morrissey et al., 2004
Amitraz	Topical	Use half the strength of the dilution directions; sponge on or dip every 2 weeks; may be lethal	Harvey, 1995

(Continued)

Parasiticides (Continued)

Generic name	Dose and route	Comments	Reference
Fenbendazole	20 mg/kg PO sid for 5 days		Morrissey et al., 2004
Fipronil	7.5 mg/kg topical q30–60d		Morrissey et al., 2004
Ivermectin	200–400 µg/kg SC	Repeat in 10–14 days	Morrissey et al., 2004
Lime sulfur	2.5% topical	Dip every 7 days	Morrissey et al., 2004
Metronidazole	70 mg/kg PO tid		Morrissey et al., 2004
Niclosamide	500 mg PO	Mix in 150-g food	Burke, 1979
Niclosamide	100 mg/kg PO	Once; repeat in 2 weeks	Russell et al., 1981
Piperazine	3–5 mg/ml drinking water PO	As adipate; 7 days on, 7 days off	Russell et al., 1981; Morrissey et al., 2004
Piperazine	10 mg/ml PO	As citrate: in drinking water – for 7 days, off 5 days, on 7 days	Unay and Davis, 1980
Piperazine	10 mg/ml PO	As citrate; give 2 ml	Unay and Davis, 1980
Praziquantel	6–10 mg/kg PO or SC	Repeat in 10 days	Morrissey et al., 2004
Pyriminyl pamoate	0.8 mg/L in drinking water PO for 30 days		Frost and Van Hoosier, 1977
Selamectin	6 or 18 mg/kg topical		Fisher et al., 2007
Sulfadimethoxine	25–50 mg/kg PO sid for 10–14 days		Morrissey et al., 2004
Thiabendazole	100 mg/kg PO sid for 5 days		Morrissey et al., 2004

Miscellaneous

Generic name	Dose and route	Comments	Reference
Atropine	0.02–0.05 mg/kg IM, IV, or SC		Canadian Council on Animal Care, 1984
Atropine	0.1–0.4 mg/kg IM or SC		Morrissey et al., 2004
Cimetidine	5–10 mg/kg IM, IV, PO or SC q6–12h		Morrissey et al., 2004
Cisapride	0.1–0.5 mg/kg PO bid		Morrissey et al., 2004
Dexamethasone	0.5–2 mg/kg IM, IV, or SC		Morrissey et al., 2004
Digoxin	0.05–0.1 mg/kg PO		Morrissey et al., 2004
Diphenhydramine	1–2 mg/kg PO or SC		Morrissey et al., 2004
Doxapram	5–10 mg/kg IV		Flecknell, 1987
Furosemide	2–10 mg/kg PO or SC bid		Morrissey et al., 2004
Glycopyrrolate	0.01–0.02 mg/kg SC		Morrissey et al., 2004
Meprobamate	100 mg/kg IM		Melby et al., 1976
Metoclopramide	0.2–1 mg/kg IM, PO, or SC bid		Morrissey et al., 2004
Naloxone	0.01–0.1 mg/kg IP or IV		Flecknell, 1987
Oxytocin	0.2–3 IU/kg IM, IV, or SC		Morrissey et al., 2004
Prednisone	0.5–2.2 mg/kg IM, PO, or SC		Morrissey et al., 2004
Sucralfate	25–50 mg/kg PO		Morrissey et al., 2004

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Miscellaneous (Continued)

Generic name	Dose and route	Comments	Reference
Thyroxine	0.1 mg	Suspend in 10 ml water. Place 1 ml of suspension, place into 50 ml drinking water	Harvey, 1995
Vitamin A	500–5000 IU/kg IM		Morrissey et al., 2004
Vitamin K	1–10 mg/kg IM prn		Morrissey et al., 2004

DEGU (OCTODON DEGUS)**Analgesics and Sedatives**

Generic name	Dose and route	Comments	Reference
Acepromazine	0.5–1 mg/kg IM	Pre anesthetic dose	Johnson-Delaney, 1996
Aspirin	100–200 mg/kg PO q6–8h		Johnson, 2002
Aspirin	50–100 mg/kg PO q4h prn		Johnson-Delaney, 1996
Butorphanol	0.2 mg/kg IM		Johnson-Delaney, 1996
Butorphanol	0.2 mg/kg SC		Powers et al., 2008
Diazepam	1–2 mg/kg IM or IP		Johnson-Delaney, 1996
Flunixin	2.5 mg/kg SC q12–24h		Johnson-Delaney, 1996
Meperidine	10–20 mg/kg IM or SC qid		Johnson, 2002
Meperidine	10–20 mg/kg IM or SC q3–4h		Johnson-Delaney, 1996

Anesthetics

Generic name	Dose and route	Comments	Reference
Ketamine	44 mg/kg IP		Johnson, 2002
Ketamine	40 mg/kg IM		Johnson-Delaney, 1996
Ketamine, acepromazine	40 mg/kg IM, 0.5 mg/kg IM		Johnson-Delaney, 1996
Ketamine, diazepam	20–40 mg/kg IM, 1–2 mg/kg IM		Johnson-Delaney, 1996
Ketamine, xylazine	35–40 mg/kg IM, 4–8 mg/kg IM		Johnson-Delaney, 1996
Ketamine, xylazine	90 mg/kg IM, 10 mg/kg IM		Suckow et al., 1996
Pentobarbital	30 mg/kg IV		Johnson-Delaney, 1996
Pentobarbital	35–40 mg/kg IP		Johnson-Delaney, 1996
Thiopental	40 mg/kg IV		Johnson-Delaney, 1996
Yohimbine	0.5–1 mg/kg IV		Johnson-Delaney, 1996

Anti-Infectives

Generic name	Dose and route	Comments	Reference
Amikacin	2 mg/kg IM, IV, or SC tid		Johnson, 2002
Amikacin	2–5 mg/kg IM or SC q8–12h		Johnson-Delaney, 1996

(Continued)

Anti-Infectives (Continued)

Generic name	Dose and route	Comments	Reference
Amoxicillin	Not recommended	Toxic	Johnson-Delaney, 1996
Ampicillin	Not recommended	Toxic	Johnson-Delaney, 1996
Cephaloridine	10–25 mg/kg IM or SC q8–24h		Johnson-Delaney, 1996
Chloramphenicol palmitate	30–50 mg/kg PO bid		Johnson, 2002
Chloramphenicol palmitate	10 mg/30 ml drinking water PO		Johnson, 2002
Chloramphenicol palmitate	50 mg/kg PO bid		Johnson-Delaney, 1996
Chloramphenicol succinate	30–50 mg/kg IM or SC bid		Johnson-Delaney, 1996
Chlortetracycline	50 mg/kg PO bid		Johnson-Delaney, 1996
Doxycycline	2.5 mg/kg PO bid		Johnson-Delaney, 1996
Enrofloxacin	5–10 mg/kg IM, PO, or SC bid		Johnson, 2002
Enrofloxacin	4.4 mg/kg SC sid for 7 days		Powers et al., 2008
Enrofloxacin	2.5–5 mg/kg IM, PO or SC bid	Limit injections	Johnson-Delaney, 1996
Gentamicin	2–5 mg/kg IM or SC q8–12h		Johnson-Delaney, 1996
Gentamicin	2 mg/kg IV		Johnson-Delaney, 1996
Griseofulvin	25 mg/kg PO sid for 28–40 days	Not for use in pregnant animals	Johnson-Delaney, 1996
Metronidazole	10–40 mg/kg PO sid		Johnson-Delaney, 1996
Neomycin	15 mg/kg PO bid		Johnson, 2002
Neomycin	15 mg/kg PO sid		Johnson-Delaney, 1996
Oxytetracycline	50 mg/kg PO bid		Johnson, 2002
Oxytetracycline	1 mg/ml in drinking water PO	Prepare in drinking water	Johnson, 2002
Penicillin	Not recommended	Toxic	Johnson-Delaney, 1996
Sulfadimethoxine	10–15 mg/kg PO bid		Johnson-Delaney, 1996
Sulfamerazine	1 mg/ml in drinking water PO	Prepare in drinking water	Johnson-Delaney, 1996
Sulfamethazine	1–5 mg/ml drinking water PO		Johnson-Delaney, 1996
Tetracycline	50 mg/kg PO bid		Johnson-Delaney, 1996
Tetracycline	0.3–2 mg/ml in drinking water PO	Prepare in drinking water	Johnson, 2002
Trimethoprim/sulfadiazine	30 mg/kg IM or SC bid		Johnson-Delaney, 1996

Paraciticides

Generic Name	Dose and route	Comments	Reference
Carbaryl	5% powder topical	Dust lightly once each week for 3 weeks	Johnson-Delaney, 1996
Fenbendazole	20 mg/kg PO sid for 5 days		Johnson-Delaney, 1996
Ivermectin	200–400 µg/kg SC or PO	Repeat in 7–14 days	Johnson-Delaney, 1996
Piperazine	2–5 mg/ml in drinking water PO	As citrate, 7 days on, 7 days off, repeat	Johnson-Delaney, 1996
Praziquantel	5–10 mg/kg IM, PO, or SC	Repeat in 10 days	Johnson-Delaney, 1996
Thiabendazole	50–100 mg/kg PO sid for 5 days		Johnson-Delaney, 1996

Miscellaneous

Generic name	Dose and route	Comments	Reference
Atropine	0.05 mg/kg IM or SC		Johnson-Delaney, 1996
Calcium EDTA	30 mg/kg SC bid		Johnson-Delaney, 1996
Dexamethasone	0.5–2 mg/kg IM, IP, IV, or SC		Johnson-Delaney, 1996
Doxapram	2–5 mg/kg IV		Johnson-Delaney, 1996
Furosemide	5–10 mg/kg bid, PO, SC, IV		Johnson-Delaney, 1996
Glycopyrrolate	0.01–0.02 mg/kg SC	For control of mucus secretions	Johnson-Delaney, 1996
Magnesium hydroxide, simethicone	1 ml PO prn	Diovol Plus®	Johnson-Delaney, 1996
Metoclopramide	0.5 mg/kg IM or SC tid	Motility enhancer	Johnson-Delaney, 1996
Oxytocin	0.2–3 IU/kg IM, IV, or SC		Johnson, 2002
Oxytocin	0.5–1 IU IM, IV, or SC	Total dose per animal	Johnson-Delaney, 1996
Prednisone	0.5–2 mg/kg PO		Johnson-Delaney, 1996
Vitamin B ₁	0.02–0.2 ml/kg		Johnson, 2002
Vitamin B ₁	1 mg/kg PO	Prepare in food	Johnson-Delaney, 1996
Vitamin K	1–20 mg/kg prn	Route not indicated, listed as IM in Johnson, 2002	Johnson-Delaney, 1996

OTHER SPECIES

Analgesics and Sedatives

Species Name	Generic name	Dose and route	Comments	Reference
Patagonian cavy (<i>Dolichotis patagonum</i>)	Buprenorphine	0.02 mg/kg IV		Joyner et al., 2004
Patagonian cavy (<i>Dolichotis patagonum</i>)	Butorphanol	2 mg/kg IM	Pre-medication for anesthesia	Joyner et al., 2004

Anesthetics

Species Name	Generic name	Dose and route	Comments	Reference
Cotton rat (<i>Sigmodon hispidus</i>)	Ketamine, acepromazine	25 mg/kg IM, 2.5 mg/kg IM		Prince, 1994
Brush mice (<i>Peromyscus boylii</i>), California Meadow voles (<i>Microtus californicus</i>), Chipmunks (<i>Tamias spp.</i>), Deer mice (<i>Peromyscus maniculatus</i>), Dusky footed wood rats (<i>Neotoma fuscipes</i>), Kangaroo rats (<i>Dipodomys californicus</i>), Pinon mice (<i>Peromyscus truei</i>)	Ketamine, medetomidine	50 mg/kg IP, 1 mg/kg IP	For examination and skin biopsy, response to deep toe pinch not eliminated, add 0.5% proparacaine hydrochloride ophthalmic drops for retro-orbital bleeding	Hahn et al., 2005
Djungarian hamster (<i>Phodopus campbelli</i>)	Ketamine, xylazine	40 mg/kg IP, 4 mg/kg IP	Surgical plane of anesthesia in 3-day-old pups, 30 minutes, no recovery tested	Vella et al., 2004
Cotton rat (<i>Sigmodon hispidus</i>)	Ketamine, xylazine, acepromazine	22 mg/kg, 10 mg/kg, 5 mg/kg	Route not indicated	Niewiesk and Prince, 2002

Anti-Infectives

Species Name	Generic name	Dose and route	Comments	Reference
Patagonian cavy (<i>Dolichotis patagonum</i>)	Trimethoprim-sulfamethoxazole	30 mg/kg PO bid	Post surgery	Joyner et al., 2004

Miscellaneous

Species Name	Generic name	Dose and route	Comments	Reference
Brush mice (<i>Peromyscus boylii</i>), California Meadow voles (<i>Microtus californicus</i>), Chipmunks (<i>Tamias</i> spp.), Deer mice (<i>Peromyscus maniculatus</i>), Dusky footed wood rats (<i>Neotoma fuscipes</i>), Kangaroo rats (<i>Dipodomys californicus</i>), Pinon mice (<i>Peromyscus truei</i>)	Atimpacezole	5 mg/kg IP	Reversal of medetomidine	Hahn et al., 2005

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Normative Values

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OUTLINE

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The purpose of this chapter is to provide supplemental normative values and references for a number of the species which are covered in detail earlier in this book. Basic hematology and many other values are provided in the individual chapters for these species. In providing the tables below, we have used discretion to select values which are most likely to be useful for the laboratory animal veterinarian or research scientist. However, for many species, some values may not be widely applicable to contemporary housing and husbandry conditions. When possible, peer-reviewed publications were used as the source of the values unless such references were not found. When values for some animals were unavailable in the published literature direct communications from individuals utilizing those species or various websites

were used as the source of information. In those cases, reference values may be presented here which have limited documentation or are anecdotal in source, such as those values found in an unreferenced website.

The normative values presented here, like all published values, may have been influenced by the laboratory test, animal source, animal genotype, age, gender, blood sampling method, use of anesthesia, or feed or housing conditions of the animals tested. Some normative values may have been derived from feral or newly confined animals. For this reason, it is always recommended that control tests be run concurrently with tests for animals which are suspected to be ill, prior to making final diagnostic conclusions or selecting treatment regimens.

RABBITS (*ORYCTOLAGUS CUNICULUS*)

	Value	Reference
ADULT WEIGHT		
Male	4.5 kg	Suckow et al., 2002
Female	4.5 kg	Suckow et al., 2002
LIFE SPAN		
Usual	5–6 years	Carpenter et al., 2001
Maximum Reported	15 years	Harkness and Wagner, 1995
Surface area (cm ²)	$9.5 \times \text{BW (in g)}^{2/3}$	Harkness and Wagner, 1995
Chromosome number	44	
Water consumption	120 ml/kg/day	
Food consumption	33–60 g pelleted diet/kg BW	Quesenberry and Carpenter, 2004
Body temperature	38.5–39.5°C	Suckow et al., 2002
Dental formula	2(I 2/1, C 0/0, PM 3/2, M 2–3/3) = 26–28	Suckow et al., 2002
Vitamin C requirement	High levels can lead to renal disease	Suckow et al., 2002
Digits	5 front, 4 rear	Richardson, 2000
GI transit time	4–5 hours	Suckow et al., 2002
PUBERTY		
Male	6–7 months	Quesenberry and Carpenter, 2004
Female	4–9 months	Hrapkiewicz and Medina, 2007
Breeding season	Spring (longer days, warmer temperatures)	Quesenberry and Carpenter, 2004
Gestation	31–32 days	Brewer, 2006
Litter size	7–9 per litter	Suckow et al., 2002
Birth weight	65 g	Brewer, 2006
Eyes open	10 days	Brewer, 2006
Weaning	8 weeks	Brewer, 2006
Number of mammary glands	8 or 10	
Heart rate	200–300 beats/min	Suckow et al., 2002
BLOOD PRESSURE		
Systolic	90–130 mmHg	Suckow et al., 2002
Diastolic	80–90 mmHg	Suckow et al., 2002
Respiration frequency	32–60/min	Suckow et al., 2002
Tidal volume	4–6 ml/kg body weight	Suckow et al., 2002
Minute volume	0.6 liter/min	Suckow et al., 2002
Stroke volume	0.49 ± 0.16 ml	Suckow et al., 2002

GUINEA PIGS (CAVIA PORCELLUS)

	Value	Reference
ADULT WEIGHT		
Male	900–1000 g	Harkness et al., 2002
Female	700–900 g	Harkness et al., 2002
LIFE SPAN		
Usual	4–5 years	Harkness and Wagner, 1995
Maximum reported	6–7 years	Harkness et al., 2002
Surface area (cm ²)	200–680 g— $9.2 \times (\text{BW in g})^{2/3}$ 700–830 g— $10.1 \times (\text{BW in g})^{2/3}$	Liu, 1988
Chromosome number	64	Makino, 1948
Water consumption	10 ml/100 g BW/day	Harkness et al., 2002
Food consumption	6 g/100 g BW/day	Harkness et al., 2002
Body temperature	37.2–39.8°C	Harkness et al., 2002
Dental formula	2(I 1/1 C 0/0 PM 1/1 M 3/3) = 20	
Vitamin C requirement	10 mg/kg/day, pregnant 30 mg/kg/day	Quesenberry and Carpenter, 2004
Digits	4 front, 3 rear	Harkness and Wagner, 1995
GI transit time	13–30 hours	Harkness and Wagner, 1995
PUBERTY		
Male	3–4 months	Harkness et al., 2002
Female	2–3 months	Harkness et al., 2002
Breeding season	Non-seasonally polyestrous	Quesenberry and Carpenter, 2004
Gestation	68.8 days	Goy et al., 1957
Litter size	2–5	Harkness and Wagner, 1995
Birth weight	45–115 g (pups less than 60 g rarely survive)	Harkness et al., 2002
Eyes open		
Weaning	21 days	Harkness et al., 2002
Heart rate	230–380 beats/minute	Harkness et al., 2002
BLOOD PRESSURE		
Systolic	80–94 mmHg	Harkness et al., 2002
Diastolic	55–58 mmHg	Harkness et al., 2002
Cardiac output		
Respiration frequency	42–104/min	Harkness and Wagner, 1995
Tidal volume	2.3–5.3 ml	Harkness and Wagner, 1995
Plasma		
pH	7.17–7.53	Harkness et al., 2002
CO ₂	18–26 mM/L	Harkness et al., 2002
CO ₂ pressure	21–59 mmHg	Harkness et al., 2002

HAMSTERS

There are over 20 hamster species worldwide; however the majority used in research are Syrian and

Chinese. Focus in this section will be on these two species, as well as the European and Djungarian, for which some aspects of normative biology have been investigated.

Syrian (*Mesocricetus auratus*)

	Value	Reference
ADULT WEIGHT		
Male	110–140 g	Hankenson and Van Hoosier, 2002
Female	110–140 g; females larger than males	Hankenson and Van Hoosier, 2002
LIFE SPAN		
Usual	1.5–2 years	
Maximum reported	3 years; female may be markedly shorter	Hankenson and Van Hoosier, 2002; Bernfield et al., 1986
Chromosome number	44	
Water consumption	30 ml/day	
Food consumption	10–15 g/day	
Body temperature	36.2–37.5°C	
Dental formula	2(I 1/1 C 0/0 PM 0/0 M 3/3) = 16; erupted at birth	
PUBERTY		
Male	6–8 weeks (85–110 g)	
Female	8–12 weeks (95–120 g)	
Gestation	15–18 days	Field, 1999
Litter size	4–12	
Birth weight	2–3 g	
Eyes open	15 days	
Weaning	21 days (35–40 g)	Bernfield et al., 1986
Heart rate	280–412 beats/min	Bernfield et al., 1986
BLOOD PRESSURE		
Systolic	150 mmHg	Johnson-Delaney, 1996
Diastolic	100 mmHg	Johnson-Delaney, 1996
Respiration frequency	33–127/min (average = 74/min)	Bernfield et al., 1986
Tidal volume	0.6–1.4 ml	Field, 1999
Plasma		
pH	7.45–7.51	Field, 1999

Chinese (*Cricetulus griseus*)

	Value	Reference
ADULT WEIGHT		
Male	39.3–45.7 g	Hankenson and Van Hoosier, 2002
Female	39.3–45.7 g	Hankenson and Van Hoosier, 2002
LIFESPAN		
Usual	2.5–3.0 years	
Chromosome number	22	
Water consumption	11.4 ml/100 g BW/day (M); 12.9 ml/100 g BW/day (F)	Thompson, 1971
PUBERTY		
Male/female	8–12 weeks	Yerganian, 1958
Gestation	20.5 days	Yerganian, 1958
Litter size	4.5–5.2	Festing, 1972
Birth weight	1.5–2.5 g	
Weaning	21–25 days	Yerganian, 1958

European (*Cricetus cricetus*)

	Value	Reference
ADULT WEIGHT		
Male	300–400 g	Anonymous, 2010a
Female	300–400 g	Anonymous, 2010a
LIFE SPAN		
Usual	2.6 years (M); 2.8 years (F)	
Chromosome number	22	
Water consumption	5 ml/100 g BW	
Food consumption	2.9 g/BW (summer); 1.8 g/100 g BW (winter)	
PUBERTY		
Male	60 days	Mohr et al., 1973
Female	80–90 days	Mohr et al., 1973
Breeding season	Summer	
Gestation	18–21 days	
Litter size	6–9	Reznick-Schuller et al., 1974
Weaning	25 days (75 g)	

Djungarian (*Phodopus* spp.)

	Value	Reference
ADULT WEIGHT		
Male	18–25 g	Hankenson and Van Hoosier, 2002
Female	18–25 g	Hankenson and Van Hoosier, 2002
LIFE SPAN		
Usual	0.75–1.25 years	
Maximum reported	2 years	Lawrie and Megahy, 1991
Chromosome number	28	
Breeding season	Photoperiod critical	
Gestation	18 days	
Litter size	1–9	Pogosianz, 1975
Weaning	21 days	

GERBILS (*MERIONES UNGUICULATUS*)

	Value	Reference
ADULT WEIGHT		
Male	65–100 g	Harkness and Wagner, 1995
Female	55–85 g	Harkness and Wagner, 1995
LIFE SPAN		
Usual	3–4 years	Harkness and Wagner, 1995
Surface area (cm ²)	$10.5 \times (\text{BW in g})^{2/3}$	Harkness and Wagner, 1995
Chromosome number	44	
Water consumption	4–7 ml/100 g/day	Harkness and Wagner, 1995
Food consumption	5–8 g/100 g/day	Harkness and Wagner, 1995
Body temperature	37.0–38.5°C	Harkness and Wagner, 1995
Dental formula	$2(I\ 1/1\ C\ 0/0\ P\ 0/0\ M\ 3/3) = 16$	Quesenberry and Carpenter, 2004
Digits	5 front, 4 rear	
GI transit time	5.8 hours	Kobayashi et al., 2009
PUBERTY		
Male	10–12 weeks	Hafez, 1970
Female	9–12 weeks	Hafez, 1970
Breeding season	Non-seasonal polyestrus	Hafez, 1970
Gestation	24–26 days	Hafez, 1970
Litter size	4.5 (1–12)	Marston and Chang, 1965
Birth weight	2.5–3.5 g	Hafez, 1970
Eyes open	16–20 days	Hafez, 1970

(Continued)

GERBILS (*MERIONES UNGUICULATUS*) (Continued)

	Value	Reference
Weaning	21 days	Hafez, 1970
Heart rate	Average 360 beats/min (range 260–600)	Harkness and Wagner, 1995
BLOOD PRESSURE		
Mean	78–100 mmHg	Laas, 1984
Respiration frequency	Average 90/min (range 70–120)	Harkness and Wagner, 1995
Plasma		
pH	7.249–7.405	Laas, 1984
CO ₂ pressure	30–44 mmHg	Laas, 1984

CHINCHILLAS (*CHINCHILLA LANIGER*)

	Value	Reference
ADULT WEIGHT		
Male	400–500 g	Quesenberry and Carpenter, 2004
Female	400–600 g	Quesenberry and Carpenter, 2004
LIFE SPAN		
Usual	10 years	Quesenberry and Carpenter, 2004
Maximum reported	20 years	Quesenberry and Carpenter, 2004
Chromosome number	64	Hrapkiewicz and Medina, 2007
Food consumption	21 g/day	Hrapkiewicz and Medina, 2007
Body temperature	37–38°C	Quesenberry and Carpenter, 2004
Dental formula	2(I 1/1 C 0/0 PM 1/ 1 M 3/3) = 20	Quesenberry and Carpenter, 2004
Digits	4 front, 3 rear	Kraft, 1987
PUBERTY		
Male	8 months	Quesenberry and Carpenter, 2004
Female	8 months	Quesenberry and Carpenter, 2004
Breeding season	Seasonally polyestrus (November–May)	Quesenberry and Carpenter, 2004
Gestation	105–118 days	Quesenberry and Carpenter, 2004
Litter size	1–6 (2 is usual)	Quesenberry and Carpenter, 2004
Birth weight	30–50 g	Quesenberry and Carpenter, 2004
Eyes open	At birth	Quesenberry and Carpenter, 2004
Weaning	6–8 weeks	Quesenberry and Carpenter, 2004
Heart rate	100–150 beats/min	Quesenberry and Carpenter, 2004
Respiration frequency	45–65/min	Hrapkiewicz and Medina, 2007

OTHER RODENTS

Degu (*Octodon degus*)

	Value	Reference
ADULT WEIGHT		
Male	180–250 g	
	170–300 g	
Female	180–250 g	Lester et al., 2005; Donnelly and Quimby, 2002; Cadillac et al., 2003; Najecki and Tate, 1999
	170–300 g	
	250 g for breeders	
LIFE SPAN		
Usual	3–5 years	Lester et al., 2005; Edwards, 2009
	7–10 years	
Maximum reported	9 years	Lee, 2004
Chromosome number	58	Woods and Boraker, 1975
Water consumption	10–40 ml/day	Edwards, 2009
Food consumption	57.8–94.2 g/kg BW/day	Edwards, 2009
Body temperature	36.0–37.9°C	Woods and Boraker, 1975
Dental formula	2(I 1/1 C 0/0 PM 1/1 M 3/3) = 20	Woods and Boraker, 1975
Digits	5 front, 5 rear	Woods and Boraker, 1975
GI transit time	5.1–5.2 hours	Edwards, 2009
PUBERTY		
Male	6 months	Altmann et al., 1994; Weir, 1970
Female	6 months	Altmann et al., 1994; Weir, 1970
Breeding season	Seasonal induced ovulators	
Gestation	84–90 days	Weir, 1970; Rojas et al., 1982
	90 days	
Litter size	1–10, average 5–7	Mabry et al., 1988; Reynolds and Wright, 1979
Birth weight	14 g	Reynolds and Wright, 1979
Eyes open	At birth	Mabry et al., 1988
Weaning	14–28 days	Donnelly and Quimby, 2002; Edwards, 2009
	4–6 weeks	
Heart rate	274 beats/min	Long, 2007
Respiration frequency	123/min	Long, 2007; Tomasco et al., 2010
	196–264/min	
Tidal volume	0.14–0.22 ml/g	Tomasco et al., 2010

Naked Mole rat (*Heterocephalus glaber*)

	Value	Reference
ADULT WEIGHT		
Male	29–38.8 g	Jarvis and Sherman, 2002
Female	35–80 g	Jarvis and Bennett, 1991
LIFE SPAN		
Usual	22 years	O'Connor et al., (2002)
Maximum Reported	28	O'Connor et al., (2002)
Chromosome number	60	Deuve et al., 2008
Water consumption	Acquired from food	Urison and Buffenstein, 1994
Food consumption	5–8 g/40 g BW	Goldman et al., 1999
Body temperature	30.0–32.0°C	Johansen et al., 1976
Dental formula	2(I 1/1 C 0/0 M 0/0 M 3/3) = 16	Hamilton, 1928
Digits	5 front, 5 rear	Mabry et al., 1988
PUBERTY		
Male	1 year	O'Connor et al., 2002
Female	1 year	O'Connor et al., 2002
Breeding season	Year round	Jarvis and Sherman, 2002
Gestation	76–84 days	Wood and Mendez, 2010; Maree and Faulkes, 2008
Litter size	1–24 pups, average of 10–19; 5.1–17.5	Wood and Mendez, 2010; Jarvis and Sherman, 2002
Birth weight	1–2 g	Jarvis, 1991
Weaning	5 weeks	Jarvis and Sherman, 2002
Number of mammary glands	9–15, 11–12	Sherman et al., 1999; Segelken, 1999
Heart rate	60–100 beats/min	Anonymous, 1998
BLOOD PRESSURE		
Systolic	140–155 mmHg	Anonymous, 1998
Diastolic	100–145 mmHg	Anonymous, 1998

Deer mouse (*Peromyscus maniculatus*)

	Value	Reference
ADULT WEIGHT		
Male	20.15–20.85 g	Hayssen, 2001
Female	20.15–20.85 g	Hayssen, 2001
LIFE SPAN		
Usual	5.5 years (<i>P. leucopus</i>)	Burger and Gochfeld, 1992
Maximum reported	8 years	Linzey, 1998
Chromosome number	48	Arakaki et al., 1970

(Continued)

Deer mouse (*Peromyscus maniculatus*) (Continued)

	Value	Reference
Water consumption	2.65 g	French, 1962
Food consumption	2.54 g	French, 1962
Body temperature	36.6°C	Nowak and Paradico, 1983
Dental formula	2(I 1/1 C 0/0 PM 0/0 M 3/3) = 16	Bunker, 2001
Digits	4 front, 5 rear	Cabrera, 2010
PUBERTY		
Male	7–8 weeks	Hygnstrom et al., 2010
Female	35–49 days	Nowak and Paradico, 1983; King, 1968
Breeding season	Year round indoors Spring through fall in cooler climates	Hygnstrom et al., 2010
Gestation	22.4–25.5 days	Kirkland and Layne, 1989
Litter size	4–4.3 1–11; average 4–6	Botten et al., 2001; Forrester, 1975; Baker, 1983
Birth weight	2 g	Bunker, 2001
Eyes open	15 days	Bunker, 2001
Weaning	25–35 days	Bunker, 2001
Heart rate	650–900 beats/min	Rosenmann and Morrison, 1974
BLOOD PRESSURE		
Systolic	Males 114–162 mmHg Females 103–123 mmHg	Turney and Lockwood, 1986

Dormice (*Graphiurus* spp.)

	Value	Reference
ADULT WEIGHT		
Male	18–20 g	West, 2010
Female	18–20 g	West, 2010
LIFE SPAN		
Usual	3 years	West, 2010
Maximum reported	4 years	West, 2010
Dental formula	2(I 1/1 C 0/0 PM 0-1/0-1 M 3/3) = 16-20	West, 2010
PUBERTY		
Female	6–8 weeks	West, 2010
Breeding Season	Year round in captivity	West, 2010
Gestation	Approximately 4 weeks	West, 2010
Litter size	4 Average	West, 2010
Weaning	4–6 weeks	West, 2010
Heart rate	180–200 beats/min	West, 2010

Kangaroo rat (*Dipodomys* spp.)

	Value	Reference
ADULT WEIGHT		
Male	40–50 g	Lancaster, 2000
Female	40–50 g	Lancaster, 2000
LIFE SPAN		
Usual	2–5 years	Anonymous, 2010b
Maximum Reported	9.8 years	Lancaster, 2000
Water consumption	Liquid water not required on seed diet	Anonymous, 2010b; Soholt, 1975
Food consumption	2–5 g/day (temperature-dependent)	Soholt, 1975
Body temperature	37°C	Wunder, 1974
Dental formula	2(I 1/1 C 0/0 PM 1/1 M 3/3) = 20	Vaughan et al., 1999
Digits	4 rear	West, 2010
PUBERTY		
	60–84 days	West, 2010
Breeding season	January–August	West, 2010
Gestation	28–32 days	West, 2010; Eisenberg and Issac, 1963
Litter size	1–6; average of 3	West, 2010; Eisenberg and Issac, 1963
Birth weight	3–8 g	West, 2010
Eyes open	2 weeks	Allen, 2010
Weaning	21–29 days	West, 2010; Eisenberg and Issac, 1963
	15–25 days	
Heart rate	417 beats/min	Soholt et al., 1973
Respiration frequency	90–125/min	Gardner, 2010
Tidal volume	150–170 ml	Gardner, 2010

Cotton rat (*Sigmodon hispidus*)

	Value	Reference
ADULT WEIGHT		
	70–200 g	Niewiesk and Prince
	80–310 g	Faith et al., 1997
LIFE SPAN		
Usual	23 months	Jimenz, 1969
Maximum reported	3 years	Jimenz, 1969
Surface area (cm ²)	$47.66 + 2.476 \times \text{BW (g)} - 0.006 \times \text{BW (g)}^2$	Ohwada and Katahira, 1993
Chromosome number	52	Prince, 1994
Water consumption	16.6–16.8 ml/day	Savabieasfahani et al., 1998
Food consumption	11.24–11.32 g/day	Savabieasfahani et al., 1998

(Continued)

Cotton rat (*Sigmodon hispidus*) (Continued)

	Value	Reference
Body temperature	36.4–36.6°C	Kirksey et al., 1975
Dental formula	2(I 1/1 C 0/0 PM 0/0 M 3/3) = 16	Faith et al., 1997
Digits	5 front, 5 rear	Hygnstrom et al., 2010
PUBERTY		
	40–60 days	Nowak, 1999
Male	8 weeks	Jackson, 1997
Female	30–50 days	Evans, 1981
Breeding season	Year round in captivity	Faith et al., 1997
Gestation	27 days	Donnelly and Quimby, 2002
Litter size	1–12 mean 5–7	Donnelly and Quimby, 2002
Birth weight	6.5–8 g	Donnelly and Quimby, 2002
Eyes open	24 hours	Donnelly and Quimby, 2002
Weaning	5 weeks	Faith et al., 1987
Respiration frequency	256–300/min	Eichelberger et al., 2004
Tidal volume	0.8 ml/g BW	Trias et al., 2009

Sand rat (*Psammomys obesus*)

	Value	Reference
ADULT WEIGHT		
Male	190–260 g	Adler et al., 1985; Kalman et al., 1996
Female	157–200 g	Adler et al., 1985; Kalman et al., 1996
LIFE SPAN		
Usual	3 years	Biagi and Myers, 2004
Maximum reported	3.2 years	Weigl, 2005
Chromosome number	46	Mostafa et al., 2006
Water consumption	22–28 ml/day	Kalman et al., 1996
Food consumption	Various custom diets, including 12–16 g/day of pellets 6.4 g of <i>Altriplexhalimus</i> (salt bush) or 7.0 g of Alfalfa pellets	Adler et al., 1985; Kalman et al., 1996; Sanigorski et al., 2002; Cope, 2010
Body temperature	37.3–38.8°C	Ben-Porat et al., 1976
Dental formula	2(I 1/1 C 0/0 PM 0/0 M 3/3) = 16	Ulmansky et al., 1984
PUBERTY		
Male	28–56 days	Windmill et al., 2007
Female	100 days	Anonymous, 2010c
Breeding season	January–April in wild	Cope, 2010

(Continued)

Sand rat (*Psammomys obesus*) (Continued)

	Value	Reference
Gestation	26 days	Patlas et al., 2006
Litter size	3	Kalman et al., 1996; Patlas et al., 2006
Birth weight	7.2 g	Patlas et al., 2006
Eyes open	1 week	Biagi and Myers, 2004
Weaning	19–26 days	Kalman et al., 1996
Heart rate	Average 310 beats/min 276–292 beats/min	Horowitz and Meiri, 1993; Hilzenrat et al., 1996
BLOOD PRESSURE		
Mean	79–88.4 mmHg	Hilzenrat et al., 1996
Cardiac output	84–100 ml/min	Hilzenrat et al., 1996
PLASMA		
Blood pH	7.362–7.430	Frenkel et al., 1972
CO ₂ pressure	27.6–33.4 mmHg	Frenkel et al., 1972

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