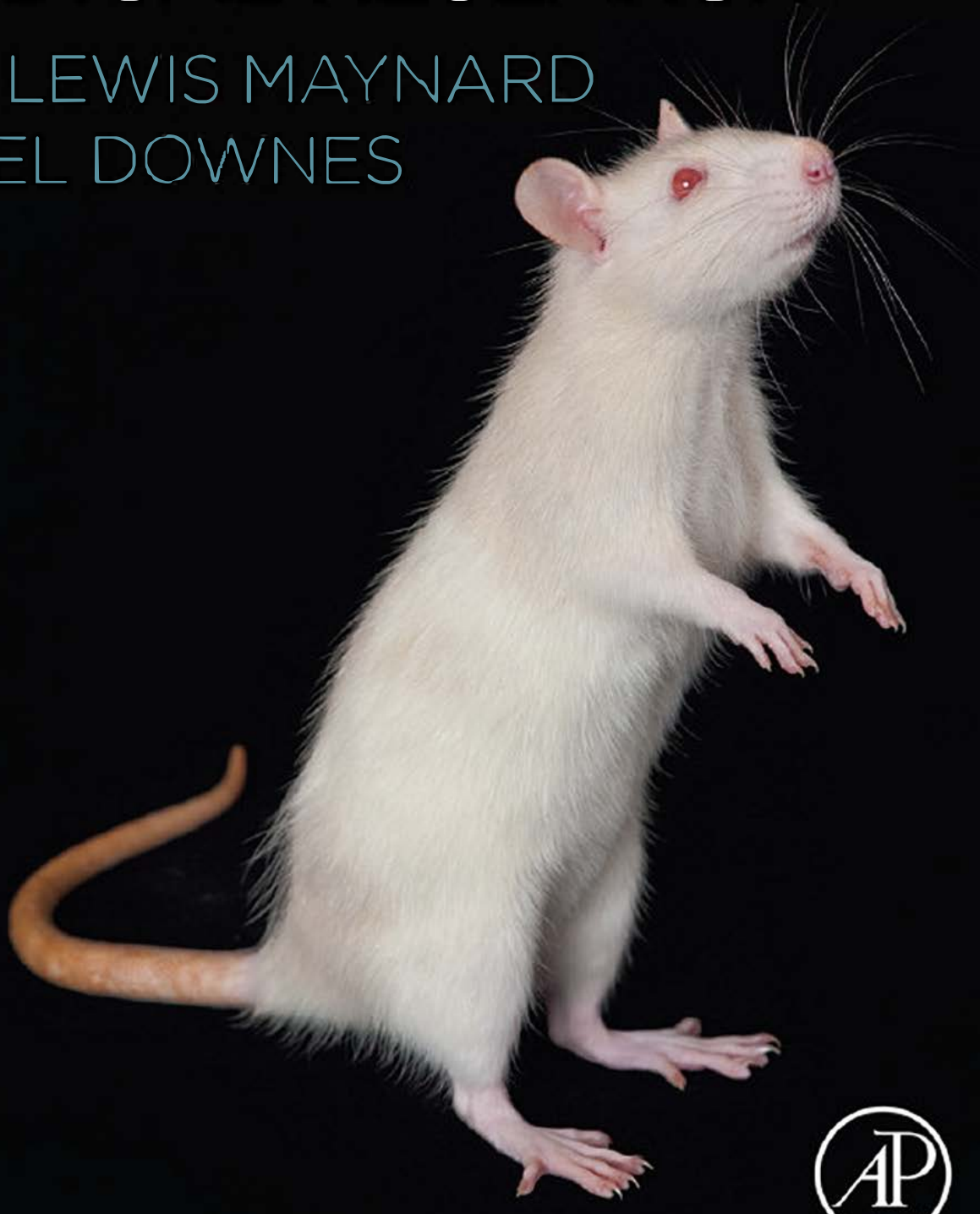
ANATOMY AND HISTOLOGY OF THE LABORATORY RAT IN TOXICOLOGY AND BIOMEDICAL RESEARCH

ROBERT LEWIS MAYNARD
AND NOEL DOWNES

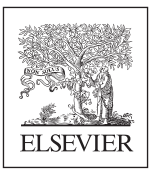


Anatomy and Histology of the Laboratory Rat in Toxicology and Biomedical Research

Anatomy and Histology of the Laboratory Rat in Toxicology and Biomedical Research

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Dedication

This book is dedicated to Helen Graham Quiller Rowett MA FRSA (1915–2012)

Helen Rowett was an outstanding teacher. After a most distinguished undergraduate career at Girton College, Cambridge, Helen Rowett spent her life teaching zoology, becoming senior lecturer at Plymouth Polytechnic.

Her interests were wide ranging: she was a notable illustrator of books, an excellent water-colourist and engraver, an expert on stage lighting for the theatre and had a deep knowledge of natural history. Her guides to dissection played a part in the education of many thousands of students.

To her memory this book is respectfully dedicated.

R.L. Maynard
N. Downes

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Preface

Many groups of students and research workers need to acquire a working knowledge of mammalian anatomy and histology. Such knowledge can be acquired from books though this a very poor second best to acquiring the information from the animal itself. Dissection and the preparation and examination of histological sections remain the best methods. Knowledge of anatomy is necessary for all those who use histological techniques: the taking of specimens for histological work presumes some knowledge of anatomy. But not all those who work with small mammals receive training in mammalian anatomy and histology. In the past this was less of a problem: zoology was grounded in comparative anatomy and morphology; dissection formed a part of every school, college and undergraduate course in biology and zoology. This is less the case today. In addition, not all those who work with small mammals, for example, in toxicology laboratories, have a zoological background.

In this book we have tried to provide an introduction to the anatomy and histology of one small mammal, the rat. The rat, and its relative the mouse, are the most widely used experimental animals and many research workers and trainees need to be familiar with these species. We have assumed that the reader wishes to learn by practical experience: our approach is based on dissection and on the preparation of histological sections. Our earlier book, 'Histological Techniques: An introduction for beginners in toxicology' deals with techniques in some detail. In our descriptions of dissections we have stressed both anatomical points and the techniques applied in post-mortem examination of experimental animals.

We have tried to relate the anatomy of the rat to that of man: the reader with a purist's interest in rat anatomy may ignore these attempts. We think that a number of readers will be seeking information on the rat with a view to using it to model the responses of man in a range of experimental settings; for these readers the comparisons may be more important.

Anyone who tries to produce a short account of the anatomy of the rat will meet, at the outset, a serious difficulty. This is that there is no modern 'Gray's Anatomy of the rat,' at least we have not found one in the English language. There are, of course, a number of well known works on the anatomy of the rat. Two will be mentioned here as they have been of great help to us. The first is Greene's Anatomy of The Rat (1935). This invaluable work provides an extraordinarily detailed atlas of rat anatomy but comparatively little text. It is an entirely descriptive work that includes no discussion of, for example, the functions of muscles or muscle groups though the attachments, nerve and blood supplies of individual muscles are described in detail. An atlas of rat osteology is provided but here, too, the text is brief and no attempt to relate the skeletal structure of the rat to mammals in general is made. These were not the objectives of the author. On the other hand, the atlas of blood vessels and of the peripheral nervous system is as detailed as anyone could wish. Hebel and Stromberg's Anatomy of the Laboratory Rat is rather different. In comparison with Greene's book a higher ratio of text to illustrations is provided and a good deal of histology is included. But, again, the authors provide neither discussion of mammalian anatomy in general nor many comparisons with man. The authors assumed that the reader had had some training in mammalian anatomy. Older books, including Hunt's little book A Laboratory Manual of the Anatomy Of The Rat (1925) and Donaldson's The Rat: Data and Reference Tables (1924) are now difficult to find. Rowett's 'The Rat as a Small Mammal' is an excellent introductory account and has provided many of the illustrations for the present work: see the separate note on illustrations that follows the Preface. Krinke's admirable book, The Laboratory Rat (2000) provides a detailed illustrated account of some aspects of the anatomy of the rat, and much else, but is not aimed at the beginner who may not have had formal training in anatomy, zoology or, indeed, biology. Suckow, Weisbroth and Franklin's excellent book The Laboratory Rat (2005) provides a short but very helpful account of the anatomy of the rat. Sharp and Villano's The Laboratory Rat (2012) is rather similar in scope to the last two works named here, though rather shorter.

We are very aware that we have not written a reference work on the anatomy and histology of the rat. We have provided a Bibliography to guide readers to the literature of our subject. Our aim has been to provide an account of these subjects which can act as a guide for the

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reader who is learning the subject. We think that students taking Masters Degrees in toxicology should find the book particularly helpful. We also hope that those working in other areas including zoology, anatomy, physiology, experimental pathology and immunology might also find this book interesting. Readers who find errors, we do not doubt that there may be some, are invited to contact us through our publishers.

Writing this book has been enjoyable. We hope the reader will find it helpful in learning about mammalian anatomy and histology by the best of all methods: making and examining his or her own dissections and histological preparations.

We should like to thank our wives for their patience whilst we were writing this book. We should also like to thank Rafael Teixeira, Pat Gonzalez and Sreejith Viswanathan of our publishers and Rob Duckwall for his work on the illustrations.

R.L. Maynard and N. Downes

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The Illustrations and How This Book Developed

Fifty years ago, the dissection of the rat formed an essential part of the UK Advanced Level zoology syllabus. The Advanced Level examinations (A Levels) were taken at the age of eighteen and an A Level in zoology was standard for students going on to medical, dental and veterinary schools. Those studying A level zoology at that time were fortunate in having for their guidance the works of Helen Rowett who produced a series of dissection guides, including one on the rat, and a further, more detailed, account of the rat: The Rat as a Small Mammal. These excellent books were very familiar to me. The third edition of The Rat as a Small Mammal appeared in 1974.

The use of dissection as a method of learning topographical anatomy has fallen into decline since Helen Rowett's day. Few students now dissect a range of animal types, including the rat, in school classes and, remarkably, the time devoted to dissection in university courses on zoology has also decreased.

In 1992, I formed the view that an updated and somewhat amended edition of Helen Rowett's book, 'The Rat as a Small Mammal' might provide a useful text for beginners in toxicology. I contacted Helen Rowett, then aged 77, who was enthusiastic about the idea and suggested that I took over the work. I contacted her publisher but failed to convince them of the need for a new edition. The decline of dissection had, I suppose, made the economic prospects of a new edition rather bleak. Nothing more happened for some time.

A few years ago the present authors published 'Histological Techniques: An introduction for Beginners in Toxicology.' Whilst working on that book the idea for the present book arose. On reviewing the idea it was decided to write a completely new book. We realised that the problem of producing illustrations for the new book would be to a large extent solved if we could acquire permission to use material from Helen Rowett's work.

This has been made possible by Maryla (a niece of Helen Rowett) and Edward Green, her husband, who are the executors of Miss Rowett's estate. They kindly gave permission for us to use Helen Rowett's drawings in our book.

In recognition of this generous act we have dedicated our book to the memory of Miss Rowett.

R.L. Maynard

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Chapter 1

The Rat's Place in Nature

This short chapter provides some zoological background to our study of the rat; for a more detailed account of topics dealt with in this chapter, the reader is advised to consult Cox and Hautier (2015).

Ignoring for a moment the rat's teeth and jaw muscles, we can say that the rat is a rather unspecialised small mammal. The rat's small size, undemanding requirements as regards diet and husbandry, rapid reproduction and an unspecialised physiology lead the toxicologist to choose it as a model for man. All mammals are characterised by mammary glands that are functional only in females, though Vaughan et al. (2000) reported a species of bat in which the males also lactate, and a number of other anatomical and physiological features. The following list is modified from Vaughan et al. (2000) and derived from a longer list provided by McKenna and Bell (1997) (Table 1.1).

A number of these features will be described in the chapters dealing with organ systems.

Mammals are a class of vertebrates, the other classes are: fish, amphibians, reptiles and birds. Vertebrates themselves fall within the phylum Chordata, the chordates, which are characterised by the presence of a notochord at some stage of their life history. In addition to the mammals, the chordates include a small group of rather odd creatures that include the sea-squirts (tunicates) as examples of the Urochordata; the very odd acorn worms as examples of the Hemichordata, and the little lancelet (amphioxus or, more correctly, *Branchiostoma lanceolatum*) as one of the two genera of Cephalochordates. The vertebrates (named for the vertebrae that protect their spinal cords) or craniates (named for the crania that protect their brains) form a subphylum of the chordates. This subphylum can be divided into the small group of agnathans (fishes without jaws, including the lampreys and the hagfish) and a large group the gnathostomes (vertebrates with jaws). The gnathostomes include all fish (other than lampreys and hagfish), amphibians, reptiles, birds and mammals. It is widely known that when a new group arises in an evolutionary sequence, anatomical features of its parent group are retained, but often adapted for a different function. Tracing such evolutionary

TABLE 1.1 Diagnostic Characteristics of Mammals

1. Jaw joint lies between mandible and squamous temporal bone; bones that formed the jaw joint in reptiles relegated to bones of the middle ear and external ear canal
2. Stapes small relative to size of skull
3. Atlas intercentrum and neural arches fused to form a ring of bone
4. Epiphyses present on long bones and girdles
5. Heart completely divided into four chambers
6. Heart has an atrioventricular node and Purkinje fibres
7. Single aortic trunk, invariably on the left
8. Pulmonary artery with three semilunar valves
9. Mature erythrocytes lack nuclei
10. Endothermic
11. Central nervous system covered by three meningeal layers: dura, arachnoid and pia mater
12. Cerebellum folded
13. Superficial muscles of the face expanded and differentiated
14. Muscular diaphragm present
15. Hair, sweat glands, sebaceous glands present
16. Mammary glands present
17. Kidney tubules characterised by a loop of Henle

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pathways has its own fascination, for example, the little bones of the mammalian middle ear (the malleus, incus and stapes) originated as breathing aids supporting the gill arches of primitive fish, then evolved into feeding aids as part of the jaws and of the jaw joint, and eventually ended up as hearing aids in the middle ear of mammals (Romer, 1970).

THE ORIGIN OF MAMMALS

The surprisingly long history of mammals can be traced back to an early group of reptiles that appeared in the Permian period of the Palaeozoic Era. The annex to this chapter defines the sequence of geological time. The Permian was a time of reptile diversification. The group of reptiles that eventually led to mammals were the therapsids (Therapsida), of which the South African genus Cynognathus has been studied in detail. This early reptile was about the size of a large dog, a fast-moving carnivore with a well differentiated dentition. Most reptiles have an undifferentiated dentition, but Cynognathus had well developed canines (Cynognathus means dog jawed) and a series of cheek teeth that could chew meat. Cynognathus also had a respectable secondary palate to the roof of its mouth, a feature seen only in crocodiles among the reptiles. In other reptiles, the bony roof of the mouth is formed from other bones (see Chapter 5: The Skull) and the posterior nares lie well forward. The evolution of the secondary palate allowed the posterior nares to move back so that the air stream entering the nose was separated from the food entering the mouth. This was an important step as it allowed breathing and chewing to continue simultaneously, permitting the continuous supply of oxygen needed for the higher metabolic rate required by endothermic physiology. Despite these and other developments, perhaps including the appearance of hair, Cynognathus remained an egg laying reptile. The therapsids flourished in the Triassic period before becoming extinct, having lasted for 40 million or so years. There seems to be little doubt that they disappeared as a result of the competition provided by the so called Ruling Reptiles. These were flourishing and diversifying at the same time, and though perhaps not as physiologically advanced as the therapsids, they were clearly more successful. When the therapsids disappeared, they left behind them the fore-runners of the mammals, which were eclipsed by the dominant Ruling Reptiles for around 80 million years. How can we explain the survival of the fore-runners of the mammals?

In the 1960s, comparatively little was known of the history of the fore-runners of the mammals, but a lot more is now known, and some forms have been described in detail. We know that the early mammals were small carnivorous creatures of mouse–rat size eating insects and perhaps small reptiles. It seems likely that the priceless advantage of endothermy allowed them to operate by night, and that their secretive, hidden existence in the undergrowth and woods provided the selective pressures that drove the evolution of improved olfactory and auditory senses, and an increasingly nimble brain. Small, smart, quick moving and nocturnal, the mammals evolved from egg laying to placental nourishing of their unborn offspring. The development of lactation and an extended period of care for their young also developed. All this provided an alternative way of life to that of the Ruling Reptiles, and when the Ruling Reptiles failed in the late Cretaceous period, mammals were still there, ready to diversify explosively. The Age of Mammals began.

WHEN DID REPTILES BECOME MAMMALS?

Much emphasis has been placed on the jaw joint, which underwent marked changes as reptiles advanced towards mammals. In primitive reptiles, the muscles responsible for mouth closure (the abductor muscles of the jaw) ran between the brain case, the lateral parts of which were not well ossified, and the covering dermal bones. Dermal bones are bones of the exoskeleton that ossify in membrane rather than in cartilage. Today, this primitive arrangement is seen in turtles and tortoises, but as reptiles evolved, it is thought that gaps appeared between the dermal bones, allowing expansion of the temporalis muscle that lay beneath the dermal bones. The word ‘apse’ originally described an arched recess such as those seen in churches, and has come to be used to mean a window. In the present context, animals without ‘windows’ between the dermal bones covering the lateral aspects of their skulls are defined as anapsids. Animals with two gaps are the diapsids and those with but one gap (though that gap was once thought to represent the fusion of two earlier gaps) are synapsids.

The Ruling Reptiles of the Mesozoic and the birds arose from the diapsida, whereas mammals evolved from the synapsid reptiles such as Cynognathus. The reptiles that evolved into mammals also underwent a change in the arrangement of the articulation of the lower jaw. In reptiles, the lower jaw comprises no less than seven bones on each side, of which only the dentary, a dermal bone, survives in the lower jaw of mammals. The reptilian jaw joint lies between bones derived from the first branchial arch, the articular bone of the lower jaw and the quadrate bone of the upper jaw. In Cynognathus and similar synapsids, a second joint appeared between the surangular bone of the lower jaw and the squamous temporal bone of the skull. This proved to be a diversion, and it was only somewhat later that the articulation between the dentary and the squamous temporal bone that defines the mammalian joint appeared.

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The 'old joint' remains in mammals as the malleus and incus of the middle ear. The articular bone has evolved into the malleus and the quadrate bone into the incus, which has established an articulation with the stapes, a bone that had long been in existence as a derivative of the second branchial arch. All this was worked out in the late 19th century and is one of the triumphs of comparative vertebrate anatomy (Goodrich, 1930; Vaughan et al., 2000). See Chapter 23, The Ear, for further discussion.

Mammalian characteristics developed within this group over a period of 30–40 million years (Benton, 2005). Despite the rapidly improving fossil record, it is impossible to define the point at which these creatures took the step from reptiles to mammals. In any case identifying that point would depend on the definition of what constitutes a mammal. If only fossil evidence were available, the definition would be based on details of skeletal structure, for example of the jaw joint, but there is more to being a mammal than that.

An Example of an Early Mammal

Morganucodon was a small mammal of the late Triassic named after the quarry in Glamorgan, South Wales, where it was discovered (Morganuc was the Doomsday book term for Glamorgan). Since the discovery of a tooth by Kühne (1949) almost complete specimens have been discovered in other countries. The full name is *Morganucodon watsoni*: Prof. D. M. S. Watson, a leading palaeontologist, communicated the discovery to the Zoological Society of London and Kühne named the fossil in his honour. Reconstructions of Morganucodon suggest it was rather like a large shrew, weighing about 20–30 g, about the size of the average laboratory mouse. The forelimbs seem to have sprawled laterally, though the hind limbs were drawn in under the body. It may well have been furry (reconstructions show it as such), with mammary glands, and both deciduous and permanent teeth, the form of which indicates it may have been an insectivore (Jenkins and Parrington, 1976). Egg laying may have been retained long after the Triassic period of mammalian evolution so we are not sure whether it was an egg layer or not (Vaughan et al., 2000). What is certain is that it had no lumbar ribs, which suggests it had a diaphragm: a defining characteristic of mammals.

Later evolutionary changes led to separation of the marsupials from the more advanced mammals. Benton (2005) puts the origin of placental mammals at about 125 million years ago in the early Cretaceous period and provides details of small furry animal called *Eomaia*, first reported by Ji et al. (2002). Not only was the entire skeleton well preserved but the presence of fur was also clearly shown.

EVOLUTION OF RODENTS

These developments occurred from the Permian to the Cretaceous and predate the appearance of rodents, which appeared in the early Tertiary. The earliest rodents appear to have been sciuromorphs (see below). *Paramys*, an early Eocene mammal, is recognised as the earliest rodent yet discovered. Rats and mice appeared in the later Eocene and radiated explosively in the Miocene. The rapidity of their evolution was noted by Benton (2005).

Rodents as a Group

Defining rodents as group is not too difficult, but classifying rodents within that group presents almost insuperable difficulties. Rodents are characterised by the following features

1. One pair of incisors in the upper and lower jaws. These teeth bear enamel only on their anterior surfaces, have open roots, and grow continuously.
2. A wide gap or diastema separates the incisors from the cheek teeth. The canines are invariably absent.
3. The paraconid (see Chapter 5: The Skull) is lost.
4. The orbital cavity lies just dorsal to the cheek teeth.
5. The ramus of the zygoma lies anterior to the first cheek tooth.
6. The glenoid fossa of the temporomandibular joint is a trough that runs anterior–posterior and allows the lower jaw to move forwards and backwards.

The rodents include more than 2000 species: some are well known, others are known from just a few specimens, and some described as extant may well be extinct. New species are described fairly often. Some sort of subclassification is clearly necessary, but this has proved to be difficult. The account provided by Simpson (1945) represents a landmark in mammalian classification and is worth looking out. Simpson's style is engaging and often very amusing; his criticism of work he regarded as unsatisfactory is scathing.

Box: Taxonomy and Classification

Taxonomy is a specialised field of biological endeavour that involves the naming and classifying of living things. Few areas of zoology have produced fiercer and more intemperate debates than taxonomy: see Simpson (1961) and Mayr (1969) for accounts of classical and more modern methods of animal taxonomy. Both favoured what could be considered the classical approach to taxonomy, which clearly separated the determination of phylogenies from the construction of classifications. Mayr was particularly insistent on this point. These debates have been characterised

(*Continued*)

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(Continued)

by a level of asperity usually reserved for points of theological dogma and political theory. The root of this controversy lies in the reaction to the work of Hennig (see the exchanges between Mayr, 1974 and Hennig, 1975) who proposed the method known as 'cladistics' (Hennig, 1966, for the English translation of his seminal work published in 1950). Ridley's (1986) short and accessible account 'Evolution and Classification' provides an account of the controversy. The cladistic approach overturned the older approach and required new names, new ideas and new methods. Today most zoologists adopt a cladistic approach: see Hildebrand and Goslow (2001) for a short introduction to this subject. The revision of Simpson's classification by McKenna and Bell (1997) provides a classification of mammals which is based on cladistic principles. What Simpson would have made of the revision can scarcely be imagined.

Initially four groups of rodents were identified because the lagomorphs (rabbits, hares) were included with the rodents, the other groups being the sciuromorphs, the myomorphs and the hystricomorphs. These groups were separated on the grounds of the differing arrangements of their masseter muscles, the development of which is a mammalian innovation (Simpson, 1945).

This division lasted well, despite a proposal that a division into two groups based on the conformation of the mandible was more appropriate. This alternative division was based on the position of the angle of the mandible with respect to a vertical plane through the lower incisor and its root within the mandible. If the angle lay in this plane, the specimen was described as falling within the Sciurognathi, if it fell outside the plane, the specimen was of the Hystricognathi. This approach has been widely adopted in recent years.

While discussing these approaches Vaughan et al. (2000) adopted a simpler approach, with the Rodentia defined as an order with 29 families, including the Muridae (rats and mice in broad terms). Within the Muridae, 17 subfamilies were listed, these included the Murinae (Old World rats and mice), which included 529 species. The genus *Rattus* lies within the Murinae. For those who prefer an alternative approach, that of Anderson and Jones (1984) is given below:

Order	Rodentia
Suborder	Sciurognathi
Infra-order	Myomorpha
Superfamily	Muroidea
Family	Muridae
Subfamily	Murinae
Genus	*Rattus*



FIGURE 1.1 It may seem trite to observe that the laboratory rat's place in nature is in a cage, but the presence of artificial lighting cycles and environmental barriers does affect the physiology of the animal compared to its wild cousins.

The genus *Rattus* was described in detail by Nowak and Paradiso (1983) where 4 subgenera and 78 species were listed. The subgenus *Rattus* (same name as the genus) included 36 species of which 1 is *Rattus norvegicus*, the brown rat. The common laboratory rat is a member of an albino strain of *R. norvegicus*. The black rat is *Rattus rattus* (Fig. 1.1).

CONCLUSION

The rat is a small mammal that evolved in the early part of the Tertiary era and is a member of a group, the rodents, which have enjoyed great success. This success is due to their rather unspecialised anatomy and physiology, a capacity for dealing with a wide range of food, their remarkable fecundity, and an incompletely explained power of speciation that confers a capacity to occupy a wide range of evolutionary niches.

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ANNEX GEOLOGICAL PERIODS

Era	Period	Epoch	Million Years Since Beginning of Interval
Cenozoic			
	Quaternary		
		Holocene	0.01
		Pleistocene	1.8
	Tertiary		
		Pliocene	5
		Miocene	24
		Oligocene	34
		Eocene	55
		Palaeocene	65
Mesozoic			
	Cretaceous		144
	Jurassic		200
	Triassic		251
Palaeozoic			
	Permian		290
	Carboniferous		354
	Devonian		417
	Silurian		443
	Ordovician		495
	Cambrian		545
Precambrian			4560

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Chapter 2

Introduction to Anatomical Terminology

THE USE OF WORDS

Anatomy involves a good deal of description, and if descriptions expressed in words are to convey a precise and unmistakable meaning, then those words have to be carefully defined. For example, if we say that the abdominal aorta of the rat lies ventral to the vertebral column, we must know what the word ventral means. Here there is no difficulty; ventral is an adjective derived from the Latin noun venter meaning belly. The undersurface of the abdomen is described as the belly and the abdominal aorta of the rat runs on the same side of the vertebral column as the belly. The terms ventral, and its opposite, dorsal – meaning towards the back, maintain their meaning irrespective of whether the rat is standing on its four feet or lying on its back, because they refer not to the position of the parts in space, but to their relationship to one another. But if we said that the vertebral column was superior to, or above the abdominal aorta, we would need to define the position of the animal in space as superior and inferior refer to relative positions in three dimensional space, so the meaning of the term is only clear when we first define the position of the body in space.

Human anatomists have defined an 'anatomical position' that is clearly explained in all textbooks of human anatomy. It comprises a front view of the human body, feet together and toes pointing forwards, arms held at the sides of the body and palms facing to the front. This reference system allows the thumb to be defined as lying lateral to the little finger that is further away from the midline of the body. A casual glance at the back of your hand might suggest that your little finger lies lateral to your thumb, but in anatomical terms it always lies medial to the thumb. Although this system is universally accepted, it is not beyond criticism and has been described as 'absurd' and attributed to 'the centuries old folly of the human anatomist. . .' (Wood-Jones, 1946).

The standard position for describing a quadruped like the rat is defined by looking at the nose of the rat while it stands on its four feet. The anterior end of the rat is towards you, its posterior end is away from you. In this position, there is no doubt that the vertebral column lies both dorsal and superior to the abdominal aorta.

Transferring human terminology to the rat can cause difficulties. For example, there are two mesenteric arteries, both branches of the abdominal aorta. In man, they are named superior and inferior, in the rat, they are named anterior and posterior. Care needs to be taken in describing parts of the body that are not examined in the anatomical position. For example, when describing the lower surface of the skull, it is very easy to say when the skull is turned upside down for examination that some structure points upwards, when in fact it actually points downwards in the living animal. It may seem obvious, but when the anatomist speaks of left and right, he means the animal's left and right not the observer's. All descriptions should refer to the animal or part placed in the anatomical position.

REFERENCE PLANES

It is often useful to be able to describe the position of a structure with respect to a defined reference plane. The dictionary definition of a plane, in the sense in which we are using the word, is '[a] surface such that the straight line joining any points in it lies wholly in it; [an] imaginary surface of this kind used for stating position etc' (Fowler and Fowler, 1969). If we consider the body of a rat as being roughly cylindrical in shape and we view it in the anatomical position, we can define a series of planes (Fig. 2.1).

The vertical plane which divides the animal into left and right halves, effectively a vertical longitudinal section, is known in human anatomy as the sagittal plane. This plane is so named because it runs in the line of the sagittal suture of the skull. The word sagittal means like an arrow: the sagittal suture of the baby's skull looks rather like an arrow, with the arrow head formed by the diamond shaped gap between the frontal and parietal bones and the tail of the arrow by the lambdoid suture (shaped like the Greek letter lambda: λ) between the parietal bones and the occipital bone. Planes parallel to the sagittal plane are para-sagittal planes.

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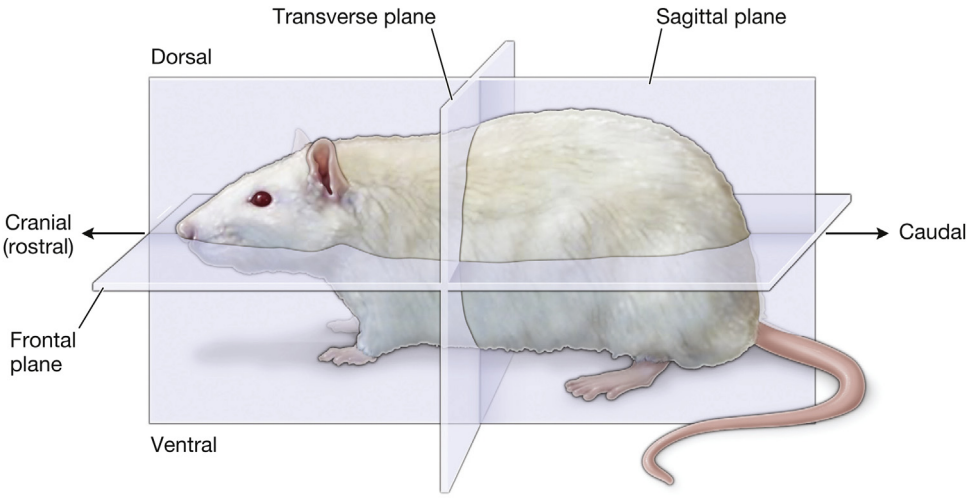


FIGURE 2.1 Anatomical planes used in describing the anatomy of the rat.

The horizontal longitudinal plane which divides the body into upper and lower halves is called the frontal plane in textbooks of veterinary anatomy. Running at 90 degrees to the sagittal plane, this is the equivalent of the coronal plane of human anatomy, so named because it runs in the line of the coronal suture on the top of the human skull. In the rat coronal sections through the head are parallel to transverse or cross sections, taken through the body. This difference occurs because the upper surface of the rat's skull lies in line with the vertebral column, while that of man lies at 90 degrees to the vertebral column.

A section taken across the main axis of the body of a rat at 90 degrees to the vertebral column, is a transverse or cross section. Such a section of the head of the rat is the same as a coronal section.

These three planes, median or sagittal, horizontal and transverse are the three fundamental planes to which structures are mapped.

SPECIFIC TERMINOLOGY

Proximal and Distal

The proximal end of a limb is that end nearest the body; the distal end is the other end of the limb. These terms are also used when describing structures such as ducts: the proximal end of the duct of a gland is the end nearest the secretory tissue; the distal end is where the duct opens into some cavity. Similarly, the distal airways of the lung are those furthest from the larynx, and the proximal end of the vagus nerve is where it emerges from the brain stem, the distal end is where it terminates. Physiologists speak of material being transported along nerves, away from the bodies of neurones, from proximal to distal: this is centrifugal (away from the centre) transport, the converse is centripetal transport.

Craniad and Caudad

Craniad means towards the head and caudad towards the tail. For example, the ureter runs caudad from the kidney to the urinary bladder; the carotid artery runs craniad from its origin. These terms are not widely used in the United Kingdom. Cephalad is sometimes used as an alternative to craniad.

Superficial and Deep

These terms are used to describe relationships between structures. For example, the dermis lies deep to (beneath) the epidermis, while the epidermis is superficial to (above) the dermis.

Superior and Inferior

Anatomists use these terms to mean above and below. In human anatomy, they refer to the body in the anatomical position: the heart lies superior to the diaphragm. The possibilities for confusion when these terms are transferred to the rat have already been mentioned. In the rat, the heart lies anterior to the diaphragm.

THE ORIGINS OF ANATOMICAL NAMES FOR STRUCTURES

Learning a large number of names for structures or features of structures is one of the burdens of every student of anatomy. The burden is increased by many of the names being derived from Latin or Greek, and so being intrinsically meaningless to those who have little or no knowledge of these languages.

The anatomical term pterygoid process, is derived from Greek and means 'like a wing'. Pteryx means wing and the suffix 'oid' means like. But the pterygoid process

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of the sphenoid bone, even in man, does not look much like a wing. The sphenoid bone has two other processes, which do look like wings: the greater and lesser wings of the sphenoid bone. The official Latin term for the greater wing is *Ala major ossis sphenoidalis*, 'Ala' being the Latin word for wing. So the Greek and Latin words for wing are both used when describing features of the sphenoid bone. Sphenoid itself means wedge shaped and is derived from the Greek.

Another example – the temporal bone of the skull. To an English speaker with some knowledge of Latin, temporal suggests time (tempus fugit: time flies), and it is said that the name derives from the greying of the hair of the scalp over the bone that occurs over time. The German word for the bone is Schlafenbein, from schlafe meaning sleep, as it is the part of the head that rests on the pillow. Perhaps so, but the German word schläfe means temporal. Part of the temporal bone is described as the petrous temporal bone. Petrous means hard, like a stone, as in petrology the study of rocks, or petroleum meaning oil (oleum) derived from rocks. Although the petrous temporal bone is certainly very dense and hard, the adjacent venous sinuses that are described as the superior and inferior petrosal sinuses, are not at all hard, but merely run close to the petrous temporal bone.

A point, or rather a small area, on the lateral surface of the human skull is referred to as the pterion. Again this suggests wings and refers to the winged helmet of Hermes an enthusiastic messenger of Greek mythology.

EPONYMS

Anatomy used to be littered with names derived from the names of those who first described structures, and although modern anatomists eschew eponyms, some still remain. Many such terms were swept away in the early 20th century, and few would now refer to the nerve of the pterygoid canal as the Vidian nerve (Vidius 1500–69, also referred to as Guido Guidi for some reason unknown to us, was Professor of Medicine in Pisa). We must accept this removal of eponyms though many linger on and some are used in this book.

Many students use widely accepted terms to describe anatomical structures without knowing what the words mean. This is no great drawback, as the words are purely labels and may well mean very little. Anatomists prefer to know what the words they use actually mean, and derive some pleasure from their knowledge. The word mamillary, as used in the mamillary body of the brain, is derived from mamilla meaning nipple, not from mamma meaning breast. In this book, a large number of anatomical terms are used. We have not provided information on the origin of every term as it arises, but for many we have done so. It is hoped that this will help the reader and provide him or her with some mild amusement. There are, no doubt, some terms that we have not explained, and the reader might need to look elsewhere for specific information. A large medical dictionary will give the origins of most anatomical terms. For the enthusiast, the little book by Ffrangcon Roberts (Medical Terms: Their Origin and Construction, 1959) is invaluable. Perhaps we may end this paragraph with a quotation given by Ffrangon Roberts: 'The meaning of anything is usually another word for the same thing'. Charles Chaplin, *Limelight*.

We have mentioned that there are 'official names' for anatomical structures. Those appropriate for veterinary anatomy are defined in the nomina anatomica veterinaria. (www.wava-amav.org/Downloads/nav_2012.pdf)

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Chapter 3

Introduction to the Skeleton: Bone, Cartilage and Joints

It seems hardly necessary to explain that the bones and joints of the rat comprise the skeleton of the animal (Fig. 3.1). Of course we should add that cartilage plays an important part, as do the ligaments that form parts of the joints between bones. The skeleton is usually divided into the axial skeleton comprising the skull, vertebral column, ribs, sternum and hyoid bone, and the appendicular skeleton comprising the pectoral and pelvic girdles and the bones of the limbs. In addition, the male rat has a baculum that braces the penis: the os penis.

BONES

Long Bones

The femur, humerus and other long bones of the limbs are characterised by a secondary centre of ossification forming an epiphysis, which occurs at either one or both ends of the bones. The bones of the digits (phalanges) are short in man, and although very short in the rat are still classified as long bones as each has an epiphysis. Long bones typically have a medullary cavity: a central space that contains fat or 'yellow bone marrow' in the adult. The bone comprises a shell of compact bone with cancellous bone filling the ends of the bone and around the periphery of the medullary cavity. The articular surfaces of the bones are covered by articular cartilage.

Short Bones

In the short bones such as the carpal and tarsal bones, development proceeds from a single central centre. These bone have no medullary cavity: the entire bone is made of cancellous bone, with a shell of compact bone.

Flat Bones

As the name suggests these bones form flat or curved plates; the bones of the roof of the skull and the ribs are examples of flat bones. Two outer layers of compact bone enclose a layer of cancellous bone that contains bone marrow. In skull bones, this space (though filled with cancellous bone and red marrow) is called the diploe. In addition, some of the bones of the skull contain spaces filled with air: these are for example the sinuses of the maxilla and the frontal bone. In the rat, the sinuses are small but present. The sinuses are lined by muco-periosteum, a special form of periosteum that secretes mucus. We shall return to these when we consider the respiratory system.

Irregular Bones

This is a remainder category for bones that do not fit neatly into the above three groups. The vertebrae and the sphenoid bone of the skull provide examples.

The skeleton gives the body its characteristic form and prevents the collapse that would occur in animals living on land if it were not present. The skeleton also provides a series of movable levers that allow one part of the body to move with relation to another and the body to move in relation to the ground. Muscles are attached to, and move these levers. Furthermore the skeleton provides protection for organs such as the brain within the skull, the spinal cord within the vertebral column and the organs of the thorax and upper abdomen within the thoracic cavity. Lastly, the bones provide a safe location for the bone marrow and a store of calcium and phosphorus.

THE STRUCTURE OF BONE

Bone is a highly organised and dense connective tissue. Like all connective tissue, it comprises cells and intercellular material produced by those cells. This intercellular matrix makes up the majority of the tissue and is itself made up of collagen fibres (mainly Type 1 collagen but with the addition of other subgroups) disposed in a gel of mucopolysaccharides and glycoproteins.

What makes bone, dentine and the cement substance of the sockets of the teeth unusual is the fact that the

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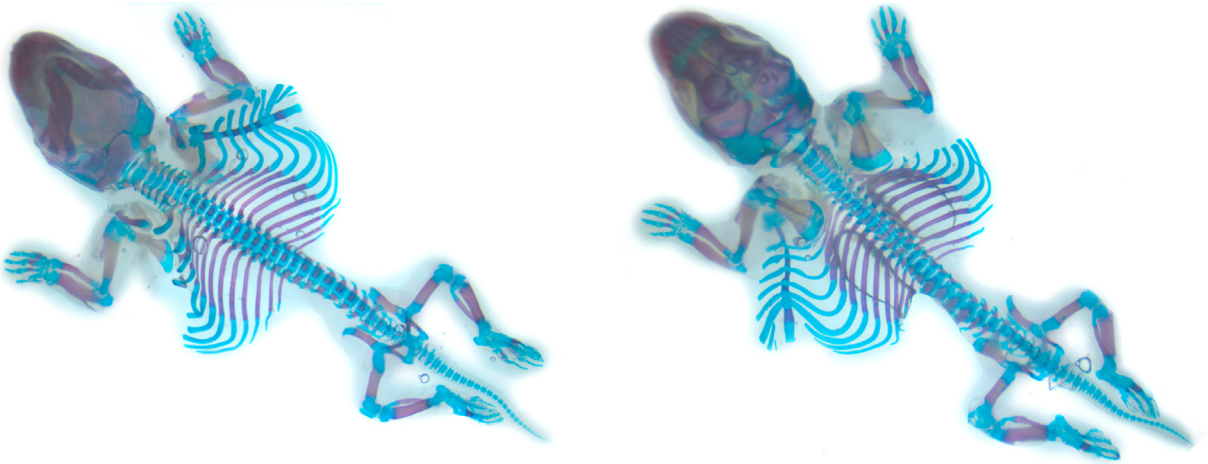


FIGURE 3.1 Dorsal and ventral views of the rat skeleton at day 21 of age. The double staining highlights the magenta stained bone (alizarin red) and turquoise cartilage (alcian blue). As the animals matures much of the cartilage will be replaced by bone.

matrix is mineralised. Enamel is also mineralised but in a rather different way and the cells that produce enamel are derived from the neural crest rather than, as in the cases of bone and dentine, from mesenchyme. The mineralisation of bone is characterised by the deposition of crystals of calcium salts (calcium apatite, phosphate and carbonate) in association with the collagen fibres. The crystals are embedded amongst the fibres, indeed the fibres may play a part in causing crystallisation to occur. The collagen forms the organic component of the matrix; the calcium salts provide the inorganic component. If the organic component is removed, the bone become brittle (the bones of a prepared skeleton or the individual bones used in teaching osteology are in this state); if the inorganic material is removed, the bone becomes as flexible as rubber. Living bone is a remarkable material: hard, slightly flexible, not brittle, and capable of self-repair. Though we shall return to this point later it is worth noting now that in contrast to cartilage, bone requires a blood supply. If a bone is broken or cut, it bleeds.

Terms Used to Describe the Parts of a Long Bone

Osteology has its own terminology, the shaft of a long bone is the diaphysis, the ends of the bone are the epiphyses, and the region where the diaphysis runs into the epiphysis is the metaphysis. The epiphyses are covered with articular cartilage: the remainder of the bone is covered by periosteum. All these terms will be explained again when we consider the development of bones.

THE HISTOLOGY OF BONE

Before looking in more detail at the histology of bone we shall examine some histological sections. We shall return to these again and again in this chapter, at the moment only the key points will be considered. The sections shown in the figures are of decalcified bone: bone from which the inorganic material has been removed either by treatment with dilute acid or chelating agents. Sections of undecalcified bone can be cut on specially strong microtomes or prepared by grinding pieces of bone to thin sheets. In most toxicological work, bone is decalcified before sectioning, and herein lies an issue when we are looking at the toxicity of substances towards the skeleton. Agents toxic to the skeleton such as retinoids cause bone mass and geometry changes typified by cortical bone loss that presents microscopically as a reduction in bone diameter, with an associated increase in osteoclast activity (Kneissel et al., 2005). While this is not always easy to quantify, and requires consistent sectioning, it is at least detectable histologically. By contrast the loss of calcification that may be associated with chelating agents is often not apparent under the microscope, and alternative methodologies will be required to assess the toxicity of such agents to the skeleton.

The sections have been stained with haematoxylin and eosin (H&E) (Fig. 3.2).

The first and most obvious thing to notice is that the bone is hollow. The central cavity, the medullary cavity, contains fat and bone marrow. Round this is the wall or outer shell of the bone. At low magnification the wall appears to be solid, it is described as being made of compact bone, and is covered by periosteum. The periosteum is an essential component of bone and is made up of connective tissue containing collagen fibres and some elastic fibres with blood vessels and nerve fibres also present. Blood vessels run from the periosteum into the bone, and bone that has lost its periosteum cannot survive for long. The cells of the periosteum are fibroblasts that are not easy to see, but the spindle shaped nuclei can be made

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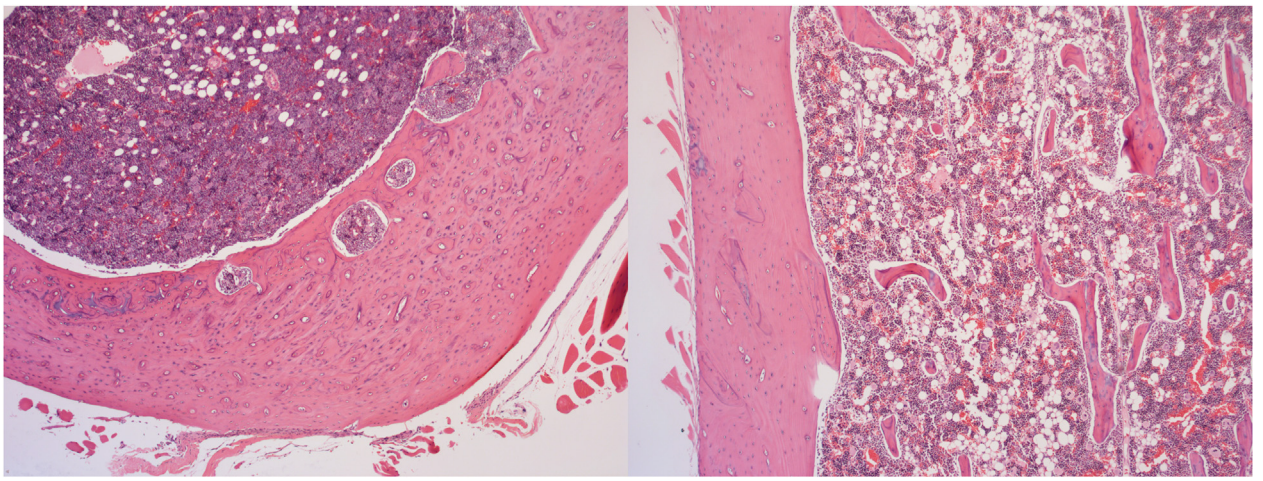


FIGURE 3.2 Sections of bone. Transverse section and longitudinal sections taken at a moderate magnification showing trabeculae and marrow.

out at high magnification. The innermost layer of the periosteum is particularly important. Here, though difficult to distinguish from fibroblasts, are the osteoprogenitor cells and osteoblasts. In the growing bone, these cells lay down matrix in which they become enclosed and turn into osteocytes. Periosteum is firmly fixed to bone by bundles of collagen fibres running into the bone: Sharpey's fibres. These are particularly well developed where a tendon inserts into bone, often via a region of fibrocartilage (see below). If we look at the inner surface of the wall of the bone, we find a thin layer of cellular connective tissue: the endosteum. It is very much thinner than the periosteum, and sometimes only a few rather flat nuclei reveal its presence.

The osteocytes are to be found in rows and clumps in compact bone. Now we have a problem: the standard textbooks of histology, Fawcett (1994), Ham (1974) and Weiss (1988), all focus on human bone. This is not surprising as these are textbooks of human histology, but the structure of rat bone is subtly different from human bone. Human compact bone is made up of well-defined, multiwalled, tubular structures called osteons or, to be accurate, secondary osteons (also known as Haversian systems). Each Haversian system comprises a central canal surrounded by layers (or laminae or lamellae) of bone. The laminae are made up of matrix, but the essential point is that the collagen fibres of the laminae vary in direction from lamina to lamina. This variation causes the laminae to stand out when the section of bone is examined under polarised light. The osteocytes lie in rows between the lamellae. The Haversian systems are formed during the process of remodelling of bone: indeed they are a product of remodelling and we shall consider this in some detail later in this chapter. The rat lacks Haversian systems. The rat is not alone in this: many mammals lack Haversian systems, or have a very different pattern of distribution of Haversian systems from that found in man. In man, the wall of the shaft is made up of a few circumferential layers, lamellae, at the outside and inside, and most of the remainder of the compact bone of the wall is made up of Haversian systems. Blood vessels reach the Haversian canals (the canals at the centres of the Haversian systems) via another system of canals running into the bone from the periosteum. These are Volkmann's canals. In rat bone, there are certainly blood vessels that are the equivalent of those of Volkmann's canals of human bone.

The reader who is interested in the comparative histology of bone is encouraged to consult the monograph prepared by Foote, 'A contribution to the comparative histology of the femur', published in 1916. This remarkable work is now available via the internet: Biodiversity Heritage Library: Smithsonian Contributions to Knowledge. The monograph runs to 242 pages and deals with the appearance of many hundred cross sections of femurs from amphibians, reptiles, birds and mammals. It is an extraordinary work and has been quoted by many who write on bone histology. A glance at the plates provided shows that the structure of bone varies across mammals. Haversian systems are dominant in man, but not in many other mammals. To take an example, in the pig, the majority of the wall of the shaft is made up of well-defined circumferential lamellae (laminae) with blood vessels running around the bone between the lamellae and branching so as to divide the lamella into a system of blocks that looks very like a brick wall. Only two areas of the bone show Haversian systems: these are at the posterior of the shaft and form two perfectly clear blocks of tissue. In the rat (*Mus rattus*, the black rat not the laboratory rat), the outer part of the wall comprises well-defined circumferential lamellae, but the inner part if broken into blocks in which the osteocytes nuclei appear to be randomly arranged. It is not possible at the magnification provided to see any Haversian systems.

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If you feel that a century and counting represents a rather distant perspective, and would prefer a somewhat more contemporaneous account, you will be out of luck. There are remarkably few such accounts, and the subject of the rat musculoskeletal system has been largely neglected, to the extent that we were unable to find a comprehensive review of rat bone in the literature. Of the papers that do address this area, Martiniakova et al. (2005, 2009) have provided short accounts, Bagi et al. (2011) have provided a valuable account of bone in laboratory animals and Hillier and Bell (2007) focussed on distinguishing human from animal bone, a subject of forensic importance. Jowsey (1966) has provided a valuable account of human and animal osteons, pointing out that short-lived animals, such as the rat, showed little development of secondary osteons.

Currey (2003) has provided an interesting account of the comparative histology of bone. He stressed the distinction between lamellar bone and woven bone. This is a complex issue as both compact and cancellous bone can be made up of either, or both, woven and lamellar bone. In lamellar bone, the collagen fibres are neatly arranged in parallel arrays, in woven bone, they are not. Woven bone is produced whenever bone is growing rapidly. Typically this is in the embryo, in calluses that form when bone is repaired, and close to lesions such as bone tumours. Woven bone persists in many mammals, including the rat, but is to a large extent replaced in human compact bone by Haversian systems. Why this should be the case is uncertain. Currey has explored this problem in detail and his review should be consulted for further details. Why Haversian systems develop at all is a difficult question. Currey put forward a series of suggestions, focussing on the repair of microcracks in bone as a plausible explanation. In a detailed review of Haversian systems, Enlow (1962) argued that the Haversian systems appear at points of stress in the bone, for example where tendons are inserted into bone. Much remains to be explained. A last point: the non-Haversian bone discussed earlier is also described as fibrolamellar or plexiform bone: see Hillier and Bell (2007) for a good description of this type of bone. To the best of our knowledge, these interspecies differences do not affect the risk assessment of the toxicants against the skeletal system.

The longitudinal section shows that the epiphyses of the bone are filled by a spongy network of bone: this is cancellous bone. Cancellous bone is also found on the inner surface of the walls of the shaft of the bone where it is continuous with the compact bone of the walls of the shaft. It has the same composition as compact bone: osteocytes and mineralised matrix, but unlike compact bone it is arranged as a mass of spicules or trabeculae. At first glance these seem to run in all directions, but this is not the case as the trabeculae are arranged so as to optimise the mechanical properties of the bone. In the neck of the human femur for example the trabeculae are arranged in a distinct pattern that braces the head and neck of the bone so as to carry the weight of the body.

THE DEVELOPMENT OF BONE

Before delving into the development of bone, two golden rules should be noted:

Bone, unlike cartilage, can grow only at a surface: that is by appositional growth. Cartilage, as will be seen later, can grow by interstitial growth as well as by appositional growth.

Bone cannot grow without a blood supply.

Bone development (ossification) is usually divided into intramembranous (IM) and endo-cartilaginous (EC) ossification. In the former, bone appears amidst mesenchyme, in the latter, bone appears in and replaces a cartilaginous model of the embryonic bone.

Intramembranous Ossification

Flat bones, like those of the roof of the skull and the blade of the scapula, ossify in membrane: no cartilage is involved. Mesenchymal cells transform into osteoblasts and these cells begin to lay down the organic components of the bone matrix. As mineralisation of the matrix progresses the osteoblasts are trapped amidst their own products and become osteocytes. Bone only grows at a surface: osteoblasts lay down more bone on that which has already appeared and spicules of bone are produced. Between the spicules there are gaps filled with mesenchyme and, later, with fat and bone marrow cells. The bone that is formed is woven bone, see above. It is not difficult to imagine how a cancellous structure could be converted into a solid one. Ham (1974) described the osteoblasts as applying ‘plaster’, bone matrix, to the inner surfaces of the spaces within the cancellous system. This leads to the formation of osteons. Osteons? We have used the word, we shall now define it. An osteon is a tube of bone with a narrow lumen and a relatively thick wall. Its wall is made up of laminae of bone, at least that is the case in man. In the first osteons formed, the primary osteons, the bone of the walls is woven bone; only when secondary osteons are formed is lamellar bone deposited. Secondary osteons (Haversian systems) can be distinguished from primary osteons by their cement line. This is a line of deeply staining matrix at the periphery of the osteons. The lamellae of secondary osteons are quite separate from the lamella adjacent to it; in primary osteons, the lamella of the osteons blend with adjacent lamellae.

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Compact bone is produced at the outer surfaces of the developing bone, it is here that the formation of primary osteons occurs. The trabeculae of the cancellous bone are avascular as the distance from the outside of a trabecula (one trabecula, two or more trabeculae) to the inside is small. Growth of such bones involves the deposition of more bone under the periosteum (bone grows at a surface) and the removal of bone at the endosteal surface. As the bone grows further expansion of the cancellous filling occurs. The clavicle and mandible both ossify in membrane, but these bones are unusual in that secondary cartilage develops (see section on cartilage) at the ends of the clavicle and at the head of the mandible. A further anomaly with regards to bones that ossify in membrane and form synovial joints is that the articular cartilage of the joints is fibrocartilage rather than hyaline cartilage.

Endo-Chondral Ossification

A bone such as the femur first appears in embryo as a cartilaginous template or model of the early embryonic bone. The hyaline cartilage of the model forms in mesenchyme. Towards the middle of the shaft, cells of the periosteum differentiate into osteoblasts and begin to lay down bone: a collar forms around the model at this point. The cartilage model grows by interstitial growth, and the cells at the centre of the shaft begin to degenerate and swell. Calcification, very different from the mineralisation of bone, occurs in the cartilage matrix, and at the same time a bud of periosteum invades the wall of the model carrying blood vessels and osteoblasts deep into the degenerating cartilage. Osteoblasts, discovering pieces of degenerating cartilage, begin to lay down bone on the surface of those pieces creating a cancellous network of spicules of bone. Initially, this cancellous bone fills the centre part of the shaft before a medullary cavity appears and the cancellous bone is relegated towards the walls of the shaft. While this is going on, the collar has been widened and thickened by the deposition of more bone under the periosteum. To prevent overthickening of the wall of the shaft, bone is removed from the endosteal surface by osteoclast activity, which we shall describe shortly.

In essence, we now have all we need: a means of building the walls of the shaft, a mechanism for producing cancellous bone in the shaft, and a capacity for growth that will extend up and down the bone. Of course, we might imagine that a lot of remodelling would be needed, and so it is. Remodelling of human bone is relatively easily explained, with secondary osteons being formed by the drilling of channels though the bone by osteoclasts, which are subsequently filled by osteoblasts. In the rat, where few secondary osteons form, it is less easy to see how remodelling proceeds. But proceed it does, and the bone laid down in embryo is replaced and replaced again and again as the bone grows and takes on its adult shape. The remodelling must be due to osteoclast/osteoblast activity. In long bones that have only one epiphysis, the phalanges of man and the femur of the mouse for example (see Cole et al., 2013, for a study of the development of the mouse femur), the process described earlier accounts for ossification to the end of the bone that lacks an epiphysis. Now for another problem of terminology. Although the term epiphysis means the end of a bone, it is often taken to mean the end of a bone that has a secondary centre of ossification. We shall now consider the development of an epiphysis with a secondary centre of ossification.

At either or both ends of long bones a secondary centre of ossification appears long after the appearance of the primary centre at the middle of the bone. In man, the secondary centres largely appear at around the time of birth. The process is the same as at the primary centre with bone being deposited under the periosteum. A periosteal bud appears and drives into the cartilage that is degenerating in the middle of the bone and cancellous bone is formed, leaving compact bone as the wall of the bone. The periosteum is lost from the articular surfaces of the developing bone leaving hyaline cartilage that is not replaced by bone. The cancellous bone advances to beneath the articular cartilage and compact bone forms to support the cartilage.

The bone has been developing from the primary centre in the shaft for sometime, and the developing cancellous bone of the shaft advances towards the cartilage of the epiphysis. The cartilage of the epiphysis (long before the secondary centre of ossification appears) grows by interstitial and appositional growth. At the boundary, where the advancing front of cancellous bone meets the cartilage, important things happen. The cartilage cells of the epiphysis multiply and generate matrix, the cartilage cells near the boundary form up into columns (lying in the same direction as the long axis of the bone), and the cells nearest the boundary swell and degenerate leaving tunnels in the cartilaginous matrix that coalesce to form larger tunnels. These tunnels are invaded by osteoblasts from the diaphyseal side of the boundary. Hence, the boundary is a site of intense activity as cancellous bone of the diaphysis is advancing, cartilage is being destroyed, but more cartilage is forming in the epiphysis which is retreating from the boundary. The bone is lengthening, and the lengthening is taking place across the boundary. The boundary is described as the epiphyseal plate and is critical for bone growth. Damage to the plate prevents further growth.

In man, this process continues until growth stops, when the advancing cancellous bone advances triumphantly into the epiphysis and makes contact with the cancellous bone that has developed there. The plate

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closes, and a line visible on only X-ray marks the position of the epiphyseal plate. In the rat things are different, and the epiphyseal plates remain open throughout life, although clearly activity subsides as rats do not continue to grow throughout their lives.

Remodelling Revisited

In man, remodelling involves the formation of secondary osteons or Haversian systems. The bone that is being remodelled is drilled by osteoclasts and the cavities and small tunnels produced filled by osteoblasts layering bone on their inner surfaces. As Ham (1974) commented, the osteoclasts act rather like a dentist clearing out a cavity before filling it, with the osteoblasts doing the filling. The tunnels seldom run in perfectly straight lines: see Robling and Stout (1999) for an account of the drifting osteons. In the rat, remodelling seems to occur in a less organised way than in man. Remodelling, whether or not by the formation of Haversian systems, is a continuous process that continues throughout life. The relationship between changes in bone architecture, in line with the demands put upon the bone, is described as Wolff's Law. Space does not permit an examination of this subject, the interested reader is referred to Frost (1994).

Blood Supply of Bone

We have seen that blood vessels are carried into the developing dosphysis by the periosteal bud vessels to provide the nutrient artery that supplies the interior of the shaft of the bone. Smaller vessels enter from the periosteum: see Volkmann's canals, above. The epiphysis is supplied by vessels that enter as a collar on the epiphyseal side of the epiphyseal plate. In the femur, some vessels enter the head of the bone via the teres ligament (see Chapter 4 on bones of the skeleton).

The Cells of Bone

The osteoblast, the osteocyte and the osteoclast are the major cells of bone. To these, we should add the rather shadowy osteoprogenitor cell. In addition, there are of course the cells of tissues associated with bone: those of blood vessels, nerves and lymphatic vessels. Here we shall deal, briefly, with the light microscopic appearance of the major bone cells. We shall refer to findings of electron microscopy, but will not attempt to summarise the welter of recent work on the physiology and molecular biology of these cells. Suffice it to say that this work has shown that bone cells produce, and are controlled by, a battery of local hormones and transmitters. The molecular biology and cellular physiology of the bone cells is clearly as complicated as those of any other cells of the body.

Detailed accounts can be found in: Hall (2015), a book of about 1000 pages dealing with many aspects of bone and cartilage; a series of reviews published in Nature in 2003: see Boyle et al. (2003) on osteoclasts, Harada and Rodan (2003) on the control of osteoblast activity; Florencio-Silva et al. (2015); Bonewald (2011) and Franz-Odendaal et al. (2006).

The Osteocyte

Osteocytes are small ovoid cells that occur both in cancellous bone and compact bone. They have long cellular processes that ramify in the canaliculi of the matrix of bone and ovoid nuclei that stain rather palely in H&E sections. EM examination reveals plentiful rough endoplasmic reticulum and a well-developed Golgi apparatus (Fig. 3.3). The cell processes are described as filopodia, and like the cell bodies do not entirely fill the spaces, but are bathed in tissue fluid that pervades both the canaliculi and the lacunae. Lengthy sections of filopodial membrane are approximated and gap junctions connect one cell with the next, so that ions and small molecules can pass from cell to cell. The canaliculi can be stained by Schmorl's thionin phosphomolybdic acid method and radiate outwards from the individual osteocytes.

Quekett (1852) produced an excellent description: 'The canaliculi nearest to each Haversian system open into it whilst those more distant from the same canal anastomose with the canaliculi of the next lamina; those of the outer row of bone cells do not anastomose with the canaliculi of neighbouring laminae, but nearly all bend back and join those of the preceding lamina'. It would be difficult to produce a better description. In H&E stained sections, the matrix adjacent to each osteocyte stains a deeper pink than more distant matrix. This reflects its

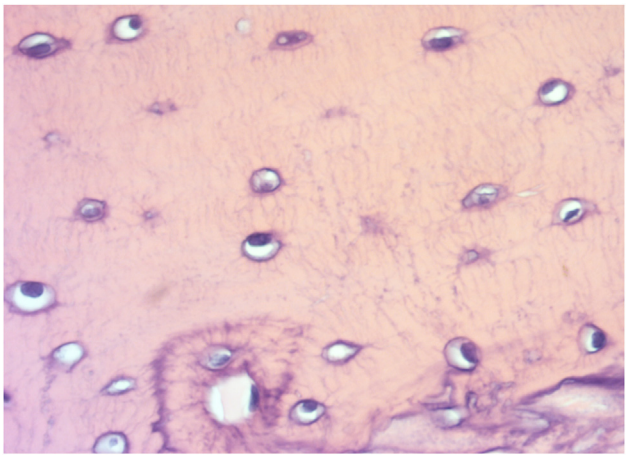


FIGURE 3.3 High power TS of bone—canaliculi just about visible.

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higher organic to inorganic ratio: recently deposited matrix has this characteristic. In diseases, where mineralisation is impaired one can see large areas of deeply stained matrix, this material is osteoid (note that 'osteoid' is used as a noun). It was thought that osteocytes were responsible for day to day turnover of minerals in bone. This is no longer accepted, and the osteoclast, aided rather surprisingly by the osteoblast, is now regarded as the key to this process.

The Osteoblast

Osteoblasts are best seen in bone that is forming, where the osteoblasts form up as a series of epithelioid cells (look like epithelium but are not true epithelium) on the surface of the forming bone tissue, where they are actively producing the organic components of the bone matrix. The cells are cuboidal or low columnar in shape with large darkly staining nuclei. EM examination shows cells rich in rough endoplasmic reticulum with a large Golgi apparatus located between the nucleus and the base of the cell. At light microscopy the position of the Golgi apparatus can sometime be seen as a poorly stained area, something that can be seen more easily in plasma cells. Small areas of pink stained material can sometimes be seen in the cytoplasm of osteoblasts: this is thought to represent procollagen. Osteoblasts secrete a range of proteins: Type 1 collagen, osteocalcin, osteonectin and osteopontin. In addition, a battery of locally active chemical factors including osteoclast stimulating factor are produced. Osteoblasts play an interesting role in bone resorption: osteoclasts cannot function without osteoblasts, at least not without the messenger molecules secreted by osteoblasts.

The Osteoclast

The osteoclast is a multinucleated giant cell about 150 μm in diameter that can be identified at light microscopy on the surfaces of spicules of cancellous bone, usually lying in, or on, a hollow in the surface of the bone known as Howship's lacuna. Osteoclasts belong to the monocytes-macrophage lineage and are derived from bone marrow cells. A great deal is known of the molecular biology of these cells (Boyle et al., 2003). EM examination shows that the membrane of the osteoclast in contact with the bone surface is 'ruffled'; Fawcett (1994) reported that cinematography showed that this surface was in constant motion. At its edges, the cell is sealed to the bone and beneath the cell, in the fluid stirred by the cell processes, reabsorption of bone occurs.

The cytoplasm contains many lysosomes that are thought to contain the enzymes and acid that is released into the absorption space, a space that is filled with a mixture of enzymes (including collagenase) and inorganic acid that result in both organic and inorganic components of bone matrix being attacked. Osteoclast activity is suppressed by calcitonin, and activated by parathyroid hormone, whose activity is mediated by osteoblasts producing osteoclast stimulating factor. Multinucleated giant cells are comparatively rare as physiological entities, in pathological scenarios they are seen in foreign body reactions and in tubercular foci; physiologically only the osteoclasts and the platelet producing megakaryocytes are normally multinucleated. What advantage is conferred upon the osteoclasts by being multinucleated is obscure.

Osteoprogenitor Cells

We have described these cells as rather shadowy, indeed they cannot be distinguished, at light microscopy, from resting osteoblasts. These elongated cells have pale nuclei and cytoplasm and lie against the surface of quiescent bone. They arise from mesenchymal stem cells in the bone marrow and can differentiate into osteoblasts or chondrocytes depending on the stimuli they are exposed to, giving rise to either bone or cartilage. These cells are important for bone formation and maintenance and can be found in the deepest layer of the periosteum and as the endosteum inside bones.

Other Components of Bone

Cartilage is considered below. Bone marrow is considered in Chapter 9.

CARTILAGE

Cartilage has a long evolutionary history. It occurs in invertebrates (Cole and Hall, 2004) and fish (Benjamin, 1990) in a bewildering variety of forms. Bone, on the other hand is found only in vertebrates, the calcified tissues of invertebrates, as found for example in echinoderms, is not bone: the major mineral is calcium carbonate rather than calcium phosphate. Three histological types of cartilage occur in the rat and in man: hyaline cartilage, fibrocartilage and elastic cartilage. Before considering these in a little more detail, we should note some features that all types of cartilage have in common. Cartilage, like all connective tissue, comprises cells (the chondrocytes), and matrix containing connective tissue fibres and a supporting material that in cartilage forms a stiff gel.

Romer (1963) argued that cartilage was an embryological adaptation, and that bone preceded it in the evolution of vertebrates. Those interested in following up this argument should consult Donoghue et al. (2006) and Wagner and Aspenberg (2011). Cartilage is certainly an admirable

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material as far as the embryo is concerned. Bones formed as cartilaginous models can grow, change shape and eventually ossify; which had they been bone from the start would have been much more difficult. One reason for this, and a key difference between cartilage and bone, is that cartilage can grow by the mechanism of both appositional and interstitial growth, bone grows only by the former.

In early bone development, the perichondrium that surround the developing bones appears to serve as a reservoir of mesenchymal stem cells that can develop into osteoblasts, adipocytes and chondroblasts. In the more mature animal, the perichondrium is a layer of dense irregular connective tissue consisting of an outer fibrous layer containing fibroblasts, and an inner chondrogenic layer. It can be found around the perimeter of elastic cartilage and hyaline cartilage, but does not cover articular surfaces, nor is it seen in fibrocartilage.

Cartilage grows by the multiplication of chondroblasts in the chondrogenic layer and by the cells embedded in the cartilage that synthesise and release matrix before eventually becoming trapped as this matrix surrounds them, at which time they become recognisable as chondrocytes. Osteocytes can exchange material with the matrix, at least they are thought to do so, but once the bone matrix has been mineralised it cannot be expanded from within. We have noted that cartilage lacks a blood supply. This is true in the adult but in large masses of developing cartilage, in man, a system of cartilage canals carry blood vessels into the tissue, but these disappear in time and are not seen in mature cartilage (Blumer et al., 2005).

In mature cartilage, the chondrocytes in the matrix lie in lacunae or spaces, where they may divide to form little nests of two or four chondrocytes all lying within the same lacuna. No canaliculi connect the lacunae. Chondrocyte nuclei are small and the basophilic cytoplasm is rich in rough endoplasmic reticulum. Lipid droplets can occasionally be seen, and glycogen demonstrated with special stains. At the EM level, the rough endoplasmic reticulum is obvious, as is the well-developed Golgi apparatus, and the chondrocytes can be seen to fill the lacunae; the space seen by light microscopic examination of wax sections is purely artefact.

The matrix comprises sulphated mucopolysaccharides (chondroitin sulphate and keratan sulphate) and water. Some of the water is rather tightly bound to the negatively charged groups of the mucopolysaccharides. Water makes up 70%–80% of the weight of cartilage.

As a tissue cartilage presents difficulties for the histologist as penetration of fixatives into the matrix is slow, with the result that chondrocytes are often poorly preserved in conventional sections. Empty lacunae, due to loss of chondrocytes during processing, are not uncommon. Thin, plastic sections provide better material for studying chondrocytes than wax sections.

Hyaline Cartilage

Hyaline cartilage is named for its glassy appearance, and with a few exceptions occurs at the articular surfaces of most bones (see section on fibrocartilage for exceptions), the ventral parts of the ribs, the cartilages of the trachea, the first few bronchi in the rat and in some laryngeal cartilages (Fig. 3.4).

The matrix of hyaline cartilage contains fibres made up of Type II collagen. They are thinner than the more familiar fibres made of Type I collagen, and cannot be seen in conventional histological sections: indeed the matrix looks remarkably amorphous. These fibres can be stained with Picrosirius red, and when examined under polarised light the fibres stand out clearly (Schmitz et al., 2010). Silver staining (Hwang et al., 1990) and immunohistochemical methods can also be applied (Dodge and Poole, 1989). Poole's review of articular cartilage (1997) provides much useful information. In addition to Type II collagen, small amounts of Types VI, IX, X and XI are also found in the matrix. The high concentration of mucopolysaccharides in the matrix causes it to stain metachromatically with dyes such as thionin or toluidine blue: matrix adjacent to lacunae stains particularly deeply.

Articular cartilage is a type of hyaline cartilage that differs from 'ordinary' hyaline cartilage in the arrangement of the chondrocytes and the collagen fibres of the matrix. The chondrocytes near the surface are flattened, those deeper in the tissue are of more conventional shape; in the deepest layers, the chondrocytes are stacked in columns and the matrix is calcified (Poole, 1997). The collagen fibres run vertically towards the surface and then turn

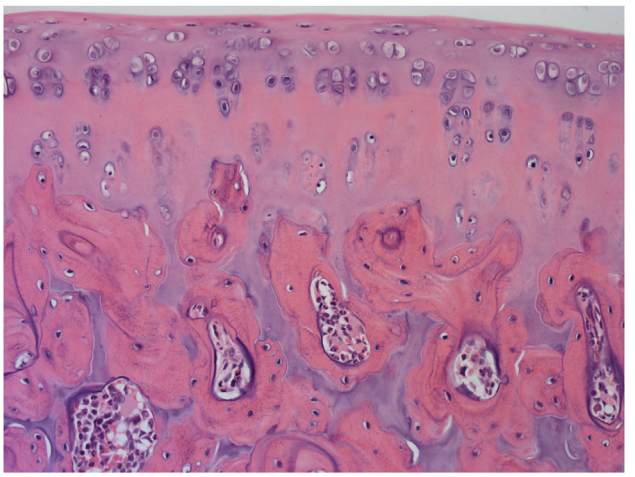


FIGURE 3.4 Hyaline cartilage from the head of the rat femur. Note the chondrocytes sitting in their lacunae.

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back forming a series of arches: the arrangement of the fibres reflects a response to the mechanical pressures put on the tissue.

Fibrocartilage

Fibrocartilage contains large bundles of collagen fibres made up of Type I collagen. These bundles run linearly through the tissue separated by a cartilage matrix containing chondrocytes. Fibrocartilage provides the tough material of the intervertebral discs; the intraarticular cartilages of the knee, wrist and temporo-mandibular joints; the articular cartilage of the temporo-mandibular joint and of the joint between the clavicle and the sternum. It also appears at the insertion of tendons and ligaments into bone. Benjamin and Ralphs (1998) have provided a detailed review of this tissue that provides references to earlier studies and those of Benjamin's group. Badi (1972) noted that calcification and ossification could occur in the fibrocartilaginous attachment of the patellar ligament of the rat. For information on the staining of fibrocartilage, see Flint et al. (1975): these authors point out the differential staining of collagen under compression as compared with that of collagen under tension.

Elastic Cartilage

The matrix of elastic cartilage is dominated by large elastic fibres that can be stained with any conventional stain for elastic fibres. Fawcett (1994) pointed out that the elastic fibres are not formed by the chondrocytes, but by mesenchymal cells during the development of the tissue. Elastic cartilage, unlike fibrocartilage, is characterised by a perichondrium. Elastic cartilage can be found in the external ear of the rat, in the auditory tube, in the epiglottis and in some of the smaller laryngeal cartilages.

JOINTS

The joints or arthroses (one arthrosis, two or more arthroses) connect one bone to another, and often allow one bone to move in relation to another. Several methods for classifying joints have been proposed, and a number of terms have been used to describe individual types of joint. The classification set out in Table 3.1 is a morphological one based on the nature of the material that connects one bone to the other within the joint. In the case of synovial joints that material is synovial fluid.

There are two types joint found in the rat that are likely to be of interest to the toxicologist. The secondary

TABLE 3.1 Classification of Joints

Classification	Types	Description and Examples
Fibrous: fibrous tissue, including fibrocartilage connects bones	Suture	Found between the bones of the roof of the skull: these disappear with age in man and rat and are replaced by bone: a synostosis. Fibrocartilage is present at the sutures
	Syndesmosis	Bones joined by ligament, dense connective tissue: the inferior radius-ulna joint is a classic example in man. Limited movement occurs at these joints. If the joint is of the peg and socket type (tooth insertion into jaw bone) the joint is a gomphosis; if the joint comprises an edge of bone inserted into a groove (e.g. the joint between the vomer and palatine bones) the joint is a schindylesis
Cartilaginous: an intervening plate of cartilage lies between the participating bones	Primary	The plate is made of hyaline cartilage: for example between the epiphysis and metaphysis of a growing bone. No movement is permitted. In man, these epiphyseal joints become synostoses with age; in the rat the cartilaginous joints persist. The only other example is the union between the first rib and the sternum
	Secondary	The plate is made of fibrocartilage. The articular surfaces of the participating bones are covered either with hyaline or fibrocartilage. Examples, in man: the pubic symphysis, the manubrio-sternal joint and the joints between the vertebral bodies where the intervertebral disc provides the plate. Little movement permitted
Synovial: a synovial cavity containing synovial fluid connects the participating bones		The ends of the participating bones are usually covered by articular cartilage. When the participating bones have ossified in membrane (rather than in cartilage) the ends of the bones may be covered with fibrocartilage: temporo-mandibular joint, joint between clavicle and sternum for example in man. Large amount of movement permitted. Subclassified according to shape of articular surfaces for example: flat (plane), hinge, ball and socket, saddle, etc.

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cartilaginous joints that join the vertebral bodies, and the synovial joint such as seen in the rat knee and wrist, which have been used as experimental models of arthropathies.

These animal models were used initially to study the basic mechanisms of joint disease, but may be seen occasionally by the toxicologist when the efficacy of a new agent is being tested. Arthritis has been induced by various stimuli. To date the most commonly seen models in toxicity studies rely on the generation of auto-immunity to cartilage components or disturbing immune responses with adjuvants. Models using agents such as bacteria and viruses to trigger immune responses may also be seen, but recently well-focused transgenic models have become the model of choice.

These models develop experimental arthritis with some of the histopathological features seen in man, but as with any model all have some short-coming or another, and as yet we do not have a single model that fully represents the human disease.

Characteristic histopathological features of these models may include some or all of the following; immune complexes in the articular cartilage, inflammatory cell changes with infiltration by macrophages, lymphocytes and plasma cells. The end stage lesion is usually typified by fibrosis and synovial hyperplasia, sometimes with frank erosion of the articular surface. There are many reviews on the subjects and given the nature of the subject the reader is advised to consult the most recent available. At the time of writing, we would suggest the reviews of Gregory et al. (2012) and Asquith et al. (2009) for translational and traditional models, respectively.

Key Features of Synovial Joints, Rat Knee Joint Taken as an Example

Articular Cartilage

The ends of the bones are covered with hyaline cartilage. As described earlier the chondrocytes towards the surface tend to be single, oval on shape with their long axes lying parallel with the surface. Deeper in the tissue the chondrocytes are large, often multiple within their lacunae, and rounded in shape. The deepest layer of the articular cartilage may be calcified and blood vessels can sometimes be detected in this layer. Calcified cartilage is separated from the more superficial layers by a deep-staining band that stains blue-purple in H&E sections. The junction between the articular cartilage and the underlying bone is wavy with short tongues of bone extending into the cartilage. The surface of the cartilage is damaged by wear and tear in man and, of course, by disease of the joint or as a result of the introduction of irritants in animal models of arthritis. In man, little pieces of articular cartilage may break away and become loose in the joint: these are described as 'joint mice'. Wang et al. (2009) reported a study that correlated Magnetic Resonance Imaging (MRI) appearances of the rat knee joint with its histology. These authors reported that the articular cartilage had a 'curvy or dipped' surface.

The Joint Capsule

The joint capsule is attached at the epiphyseal line of each bone connecting the periosteum of one bone with that of the other bone participating in the joint. It may move during development so as to either include more of the diaphysis within the capsule or to expose part of the epiphysis. It comprises dense connective tissue that may be thickened in places to form ligaments. As one might expect the capsule is thicker at the sides of a hinge joint than at the surface involved in bending of the joint.

Accessory Ligaments

Ligaments that hold the bones of the joint together, but which are not attached to the joint capsule, are described as accessory ligaments of the joint. The two cruciate ligaments of the knee are intra-articular accessory ligaments. These ligaments, like all intra-articular surfaces NOT involved in weight bearing are covered with synovium. The teres ligament of the hip joint is also covered with synovium.

Synovium

All nonarticular surfaces within a synovial joint are covered with synovium. The synovium, or synovial membrane, is an unusual tissue, and one that has provoked a good deal of controversy among histologists. To the histologist, the word 'membrane' suggests a surface covered by an epithelium, as in the mucous membrane of the gut. But this is not the case for the synovium, which is a layer of rather loose, and certainly very vascular connective tissue, that at its surface shows an incomplete layer of cells, some of which function as macrophages, and others that function as secretory cells producing the glycoproteins of the synovial fluid. Between the surface cells, the underlying connective tissue is exposed to the synovial fluid. EM studies of the synovium began with Lever and Ford (1958). Those interested in the details of the structure of the synovium, indeed in any aspects of the structure of synovial joints, are referred to Ghadially's classic monograph: Fine Structure of Synovial Joints (1983). Other accounts have been provided by Barland et al. (1962), Iwanaga et al. (2000) especially valuable for the scanning electron-micrographs of the synovial surface (Smith et al., 2003; De Sousa et al., 2014). Graabaek's (1982) account of the rat synovium is important in that it is one of the few ultrastructural studies that focuses on this mammal. Graabaek defined two cell types: A and S, the former

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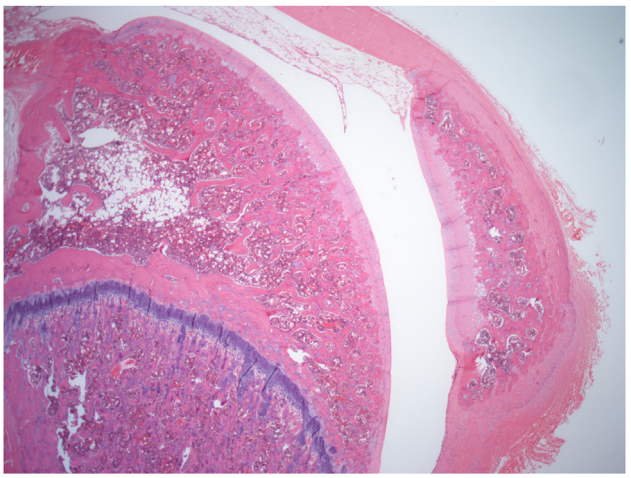


FIGURE 3.5 Section though the knee joint of the rat. Note that even in this older animal there remains cartilage at the epiphysis.

being macrophage-like, the latter being secretory. These correspond to the A and B cell types described by others (see Ghadially, 1983 for a thorough discussion of terminology) (Fig. 3.5).

Intraarticular Discs

The knee joint contains a medial and a lateral meniscus (semilunar cartilage), which are attached to the tibia. The parts of these cartilages that come into contact with the articular surfaces of the femur and tibia are not covered with synovium. The menisci receive their blood supply from their outer edges (Day et al., 1985). A detailed description of the menisci in man has been provided by Pauli et al. (2011). The exact function of the menisci is not easy to define: they appear to improve the congruity of the articulating surfaces of the tibia and femur, but play no part in weight bearing. Indeed, if a meniscus is caught and nipped between the femoral and tibial condyles it may be damaged, and it appears that removal of a damaged meniscus makes little difference to the functioning of the knee joint.

Fat Pads

Fat pads are found within some synovial joints, usually tucked away in the corners of the cavity of the joint. What they do is obscure: Last (1973) suggested that they help to spread the synovial fluid, this seems unlikely as they are covered with synovium and not, presumably, in direct contact with the synovial fluid.

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Chapter 4

Vertebrae, Ribs, Sternum, Pectoral and Pelvic Girdles, and Bones of the Limbs

THE VERTEBRAL COLUMN OR SPINE

The vertebral column of the rat is a flexible rod comprising a series of individual vertebrae attached to one another by synovial joints between the articular processes, fibrocartilagenous joints between the bodies of the vertebrae and the intervertebral discs, and ligaments between the processes of the vertebrae and between their neural arches. Muscles also connect the vertebrae to one another. The intervertebral discs comprise a firm outer ring of fibrocartilage that encloses a rather soft centre. In man, the centre contains cells (physaliphorous cells) scattered in a semi-fluid matrix of ground substance. This soft centre is described as the nucleus pulposus, which can herniate and press on a spinal nerve: the so called 'slipped disc'.

The rat has the typical mammalian vertebral column, with none of the specialisations of the cervical vertebrae seen in jumping (richochetal) rodents like the jerboa, or in edentates and sirenians. The vertebrae of the rat are divided into:

Cervical: seven in number, without ribs but with foramina in their composite transverse processes through which pass the vertebral arteries.
Thoracic: 13 in number, all articulating with ribs.
Lumbar: six in number, without ribs.
Sacral: four in number, transverse processes fused; the anterior two sacral vertebrae articulating with the pelvis.
Caudal: about 30 in number, no ribs, chevron bones and haemal arches associated with at least some of the vertebrae.

We have used the term transverse process: this is shorthand for a process that projects laterally from a vertebra. In morphological terms, it is more complicated than that: see below.

Development of the Vertebrae

Vertebrae develop from the somites which are blocks of mesodermal tissue that form on either side of the midline in the developing embryo. The medial part of the somite, the sclerotome, gives rise to bone. The essential point to recall about somites is that they define the segmentation of the embryo and induce the segmental development of the spinal cord, spinal nerves and the segmental arrangement of the skeletal muscle system. So far, so good. But when we turn to the vertebrae, we immediately notice that they are not arranged segmentally, but intersegmentally, as though they had moved up or down half a segment during development and have come to rest overlapping the original segments. The picture is very clear in fish: the segmental muscles of the body wall are each attached to two vertebrae, and the spinal nerves supplying the segmental muscles leave the vertebral column between contiguous vertebrae. The standard explanation as to how this comes about is that each block of sclerotome splits: the anterior half moves forwards and fuses with the posterior half of the next block which has moved backwards. The two half blocks fuse to form a new block, and this forms one intersegmental vertebra. The mesenchyme of the new blocks expands, surrounds the notochord, and throws out two dorsal processes, which surround and fuse dorsal to the spinal cord. Ribs develop from the blocks of mesenchyme while they are still cartilaginous, but later separate from them, allowing causing the ribs to articulate with the vertebrae. Of course, not all vertebrae in the rat develop ribs. All this development takes place in cartilage. When ossification begins, centres of ossification appear in the half arches that pass to each side of the spinal cord and fuse dorsally, and in the mass of cartilage that has formed around the notochord. The notochord disappears within this mass but contributes to the unossified intervertebral discs that separate one vertebra from the next. The ossified arches fuse with the centrum of the vertebra to form the body of the vertebra. This means that the vertebral body is not identical with the original centrum: its dorso-lateral parts are formed from the neural arches and a neurocentral suture defines the line of fusion.

The neural arches give rise to a number of processes. A dorsal (neural or spinous) process develops, and a

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lateral (transverse) process extends outwards from each side of the arch, while paired articular processes develop on both the anterior and posterior surfaces of the arch. The parts of the arch between the articular processes and the body of the vertebra are called the pedicles of the vertebrae; the parts dorsal to the articular processes are the laminae. The pedicles are cylindrical in section and notched on both surfaces. The anterior notches and the deeper posterior notches are contiguous, forming a series of intervertebral foramina though which run the spinal nerves.

The terminology of the processes is complicated. Richard Owen, in the mid-19th century (see Owen, 1866), introduced terminology that is still in use. Each process was described by a combination of a part derived from the term 'physis', meaning growth, and a descriptive adjective. For example, zygapophysis was used to describe the articular processes that yoked together two vertebrae: zygon = yoke, as in zygoma or zygomatic process in the skull. An anterior articular process was a prezygapophysis; a posterior articular process was a postzygapophysis. The hypapophysis (not to be confused with hypophysis, the pituitary gland, though the philological derivations are similar) is a ventral midline process arising from the body of a vertebra: not found in the rat, but well developed in some mammals for example the hare. The diapophysis is the transverse process proper. Of all Owen's many pophyses the parapophysis and the pleurapophysis cause most confusion. The pleurapophysis is a rib, or that part of a rib that persists despite the fact that no true rib appears, and which then fuses with the diapophysis to form a composite transverse process. The rib, pleurapophysis, also articulates with the parapophysis: developed from that part of the base of the neural arch that fused with the centrum. The parapophysis is reduced to a facet in most mammals.

The view that all vertebrae develop from two-half sclerotomes has not gone unchallenged. In 1979, Wake wrote:

> *Most illustrations in textbooks are in error in showing blocks of tissue that separate, migrate and regroup to form definitive vertebrae, but it is too early to go so far as to deny that vertebrae are formed from components of adjacent somites. This remains an area of controversy.*

Recent textbooks of human embryology (Larsen, 1993) show the primitive segmental blocks of tissue dividing unequally, with a larger caudal than cranial 'half'. This is not the only area of controversy with regard to the evolution and development of the vertebrae. For many years 'Evolution of the Vertebral Column' (Gadow, 1933, published posthumously) was regarded as the definitive text, and his derivation of each vertebra from arcualia (little chips of cartilage arranged along the spinal cord in agnathans) was widely accepted. Romer (1970) pointed out, with some regret, that at least as far as tetrapods were concerned, this theory was no longer tenable. The evolution of the vertebrae, and in particular of the centra, is fiendishly complex: Romer (1970), Wake (1979), Zhang (2009) and Fleming et al. (2015) provide accessible accounts. The enthusiastic reader who is interested in terminology will enjoy Cave's account (1975) of the mammalian pleurapophysis: an account as elegant as it is erudite.

The Cervical Vertebrae

The cervical vertebrae are divided into the atlas and axis and, with the exception of C7, see below, the typical cervical vertebrae. Cervical vertebrae are known by their numbers: C1 is the atlas, C2 is the axis and so on to C7. We shall consider, first, a typical cervical vertebra.

Typical Cervical Vertebrae

Fig. 4.1 shows the major features of the bone. Rowett has distinguished the centrum from the lateral parts of the body of the vertebra by two lines that represent the neurocentral sutures (see above). The neural arch enclosing the neural or vertebral canal is obvious, as are the pre and postzygapophyses. Romer (1970) introduced a handy way of remembering the orientation of the articular facets of these processes: up and in at the front, down and out at

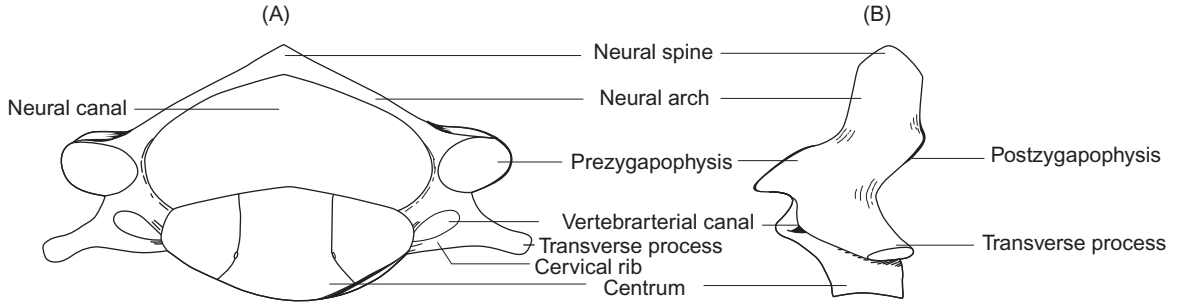


FIGURE 4.1 (A) Third cervical vertebra (anterior aspect). (B) Third cervical vertebra (lateral aspect).

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the back, like the members of a parade. With the exception of C7, cervical vertebrae are characterised by the foramen transversarium (labelled the vertebrarterial canal in the diagram) in the composite transverse process. The foramen lies between the diapophysis and the pleurapophysis, between the true transverse process and the remnant of a cervical rib. Note how the notches on the pedicles form the intervertebral foramina when two vertebrae are approximated. The spinal nerves leave the vertebral canal ventral to the articular processes, the zygapophyses, dorsal to the vertebral artery that runs through the vertebrarterial canal. The dorsal tubercles that spring from the neural arches are short in all cervical vertebrae, with the exception of C7. The vertebral artery enters its canal at C6 (Hunt, 1924). C6 is also a little unusual, in that it carries a ventral process arising from the base of each transverse process. This is described by Hebel and Stromberg (1976) as the 'lamina ventralis cervicalis C6': these authors described it as homologous with a cervical rib: see Cave (1975) for a critical discussion of this point.

The Atlas, C1

The atlas has a foramen transversarium through which the vertebral artery emerges on the anterior surface of the bone. In man, it turns across the bone into the vertebral canal, but in the rat runs through a foramen in the bone, the atlantal foramen, to reach the vertebral canal. The dorsal spine of the atlas in man is very small, but in the rat, it comprises a substantial plate that fits into a notch on the anterior edge of an equivalent plate arising from the axis. The atlas of the rat bears a short forked spine on its ventral arch.

Fig. 4.2 shows the atlas as a ring of bone without a body or, rather, a centrum. The ventral part of the ring is said to develop from the hypochordal bow (a bow of fibrous tissue ventral to the notochord). The original centrum has moved and fused with the centrum of the axis to form the dens or odontoid process of that vertebra (dens, odontoid and dental: all to do with or like teeth). The dens protrudes through the ring of the atlas and articulates with the dorsal surface of the ventral arch. The spinal cord runs dorsal to the dens and is separated from it by a transverse ligament attached to two tubercles on the inner, medial and surfaces of the ring of the atlas.

The loss of the centrum causes complications. Neither the articular processes of the atlas (both anterior and posterior) or the anterior articular processes of the axis are homologous with the articular processes of other cervical vertebrae, but with the tiny joints that connect one cervical vertebra to the next, and which are found in the region of the neurocentral sutures. These are the joints of Luschka in human anatomy (Remya et al., 2012). This rather technical point explains why the first and second cervical nerves leave the vertebral canal dorsal to the anterior articular process of the atlas, and dorsal to the anterior articular process of the axis. In all other cervical vertebrae, indeed in all other vertebrae, the spinal nerves leave ventral to the articular processes. Spinal nerve C1 emerges between the occipital bone and the atlas, and spinal nerve C8 emerges between C7 and thoracic vertebra 1 (T1), with the result that there are eight cervical nerves, but only seven cervical vertebrae.

The Axis, C2

As noted above, the centrum of the axis is extended by the displaced centrum of the atlas, and the anterior

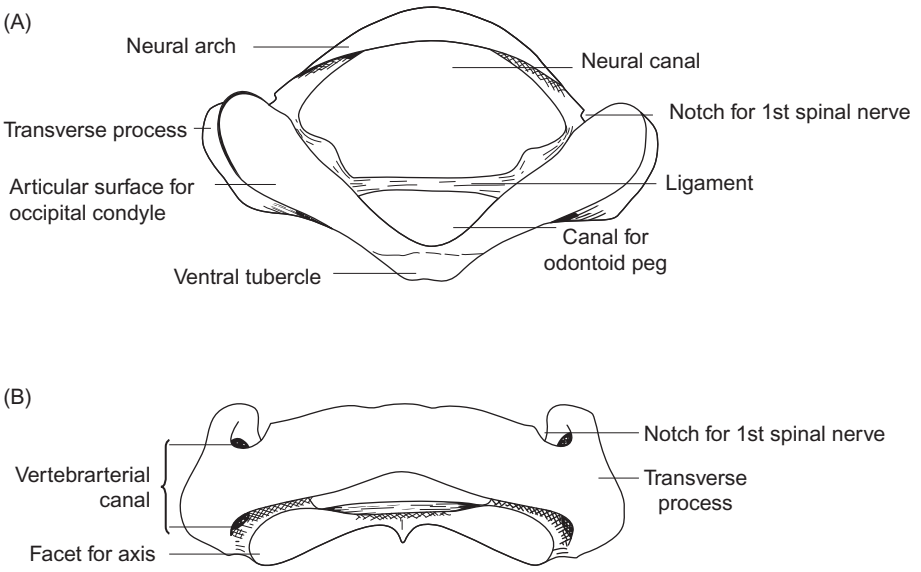


FIGURE 4.2 (A) Atlas (anterior aspect). (B) Atlas (dorsal aspect).

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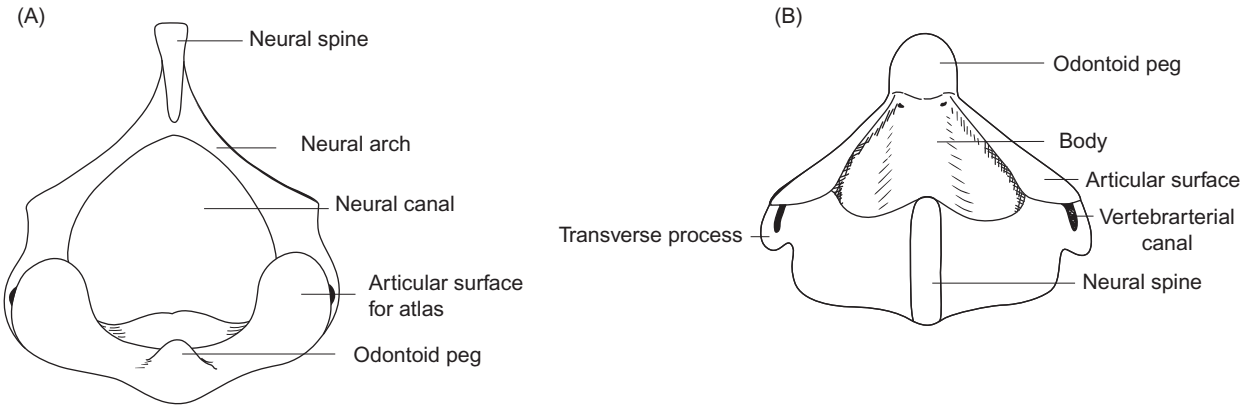


FIGURE 4.3 (A) Axis (anterior aspect). (B) Axis (dorsal aspect).

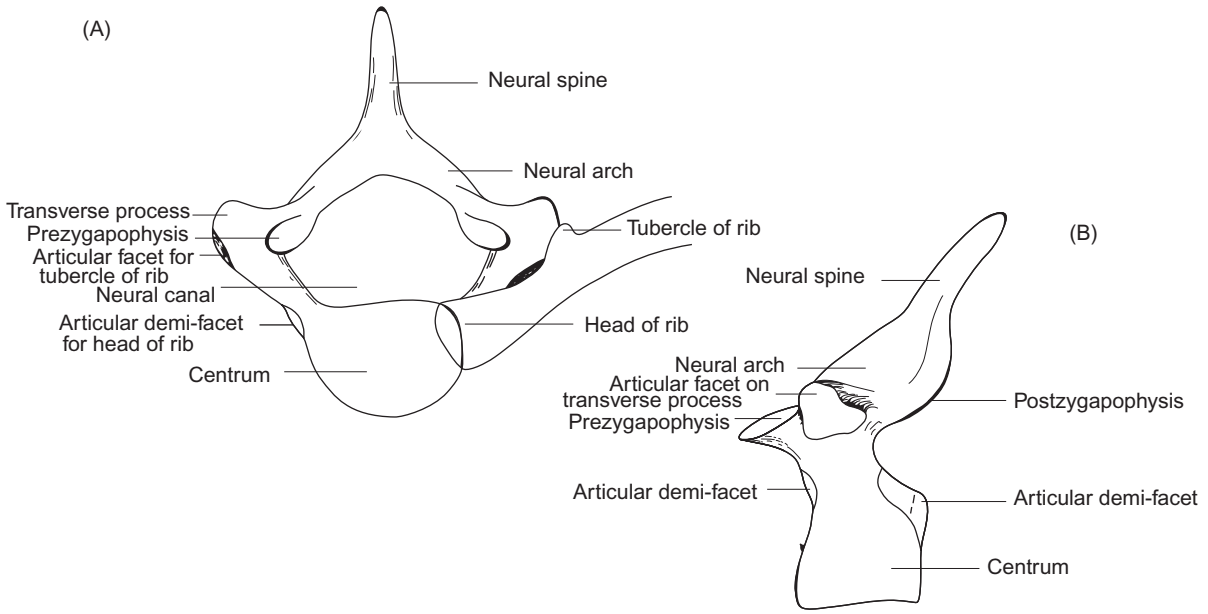


FIGURE 4.4 (A) Seventh thoracic vertebra (anterior aspect). (B) Seventh thoracic vertebra (lateral aspect).

articular processes are not homologous with the posterior articular processes. The dorsal spine of the axis is large and flattened in the sagittal plane, extending forwards to meet the posterior edge of the dorsal spine of the atlas and back over the third cervical vertebra. The combined dorsal spines of C1 and C2 are more prominent that those of any other cervical vertebrae (Fig. 4.3).

The Thoracic Vertebrae

The 13 thoracic vertebrae (T1−T13) Fig. 4.4, are defined by their articulations with ribs. T2 is unusual as it bears a long dorsal spine that is further extended by a pointed cartilage, which may ossify in adulthood (Johnson et al., 1999). The majority of the thoracic vertebrae articulate with both the head (capitulum) and tubercle of a rib. The head articulates with a pair of demi-facets: one at the antero-lateral part of the body of the vertebra with the same number as the rib, and one at the postero-lateral part of the body of the vertebra immediately anterior. Thus rib 3 articulates with T3 and T2; rib 1 articulates with T1 and C7 and so forth, but towards the posterior end of the thoracic vertebrae, the facets may be single and appear only on the vertebra with the same number as the rib in question. As mentioned above, the articulation of the head is with the parapophysis of Owen's terminology. The tubercles of the ribs articulate with transverse processes, that is, with the diapophyses. Spinal nerves emerge via the

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intervertebral foramina formed by the notches of the pedicles, emerging ventral to the articular processes. The posterior thoracic vertebrae may bear further 'pophyses': the accessory and mamillary processes (see below).

Lumbar Vertebrae

The rat has six lumbar vertebrae (man has five). There are no ribs attached to them, though the remnants of ribs are incorporated into the transverse processes (L3 is typical, see Fig. 4.5). All the usual processes are present, with the dorsal spine forming a flat plate that runs the length of the vertebra in the sagittal plane. We mentioned in the last section the accessory and mamillary processes. Much confusion attends these processes, and a variety of names have been applied to them. The following table sets out the terminology.

Standard zoological terminology, after Owen	Terminology of human anatomy as applied to lumbar vertebrae	Terminology of human anatomy as applied to the last few thoracic vertebrae that may bear these processes
Anapophysis	Accessory process	Inferior tubercle of the transverse process
Metapophysis	Mamillary process	Superior tubercle of the transverse process

The accessory process is easy to find in the rat, forming a small, sharp spike that projects posteriorly and lies beneath the posterior zygapophysis. The mamillary process is less obvious, but appears as a thickening on the lateral aspect of the anterior zygapophysis.

Sacral Vertebrae, the Sacrum

The word sacrum has the same origin as sacred: it was believed that the body could be resurrected from the sacrum. The rat has four sacral vertebrae, man has five. In man, these are fused to form a rather solid triangular sacrum. In the rat, all the transverse processes of the sacral vertebrae are linked together and form a series of linked plates. The lateral surface of the fused transverse processes of S1 and S2 articulate with the ilium of pelvis. S3 and S4 are not involved in this articulation. Rowett shows (Fig. 4.6) the transverse processes of S3 and S4 linked by a narrow bar of bone, and Hebel and Stromberg (1976) show the connection as rather less attenuated. Foramina allow the posterior and anterior primary rami of the sacral nerves to escape from the sacrum. The spinal cord of the rat terminates at L3; only the anterior and posterior roots of the remaining spinal nerves occupy the vertebral canal at lower levels. Interestingly the dorsal, or posterior, root ganglia are faithful to their position in the more anterior parts of the vertebral column and are found at the intervertebral foramina.

Caudal Vertebrae

Unlike man the rat has a fine series of about 30 caudal or coccygeal vertebrae, though the latter term seems hardly appropriate. Even if one could mistake a human coccyx for the beak of a cuckoo (the origin of coccyx: kokkux meaning cuckoo), one could hardly make this mistake about a rat's tail. The caudal vertebrae begin by looking rather like other vertebrae but soon assume a simpler shape (Fig. 4.7), and have small chevron bones wedged ventrally between the vertebrae. Equally small haemal arches attached to these bones enclose, rather incompletely, the major blood vessels of the tail. The chevron bones are the equivalent of intercentra; bones that play an

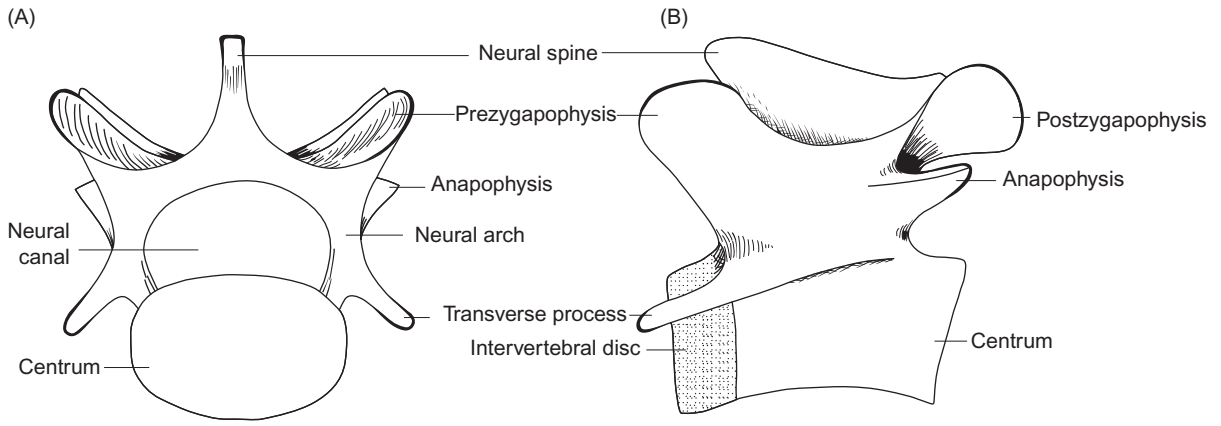


FIGURE 4.5 (A) Third lumbar vertebra (anterior aspect). (B) Third lumbar vertebra (lateral aspect).

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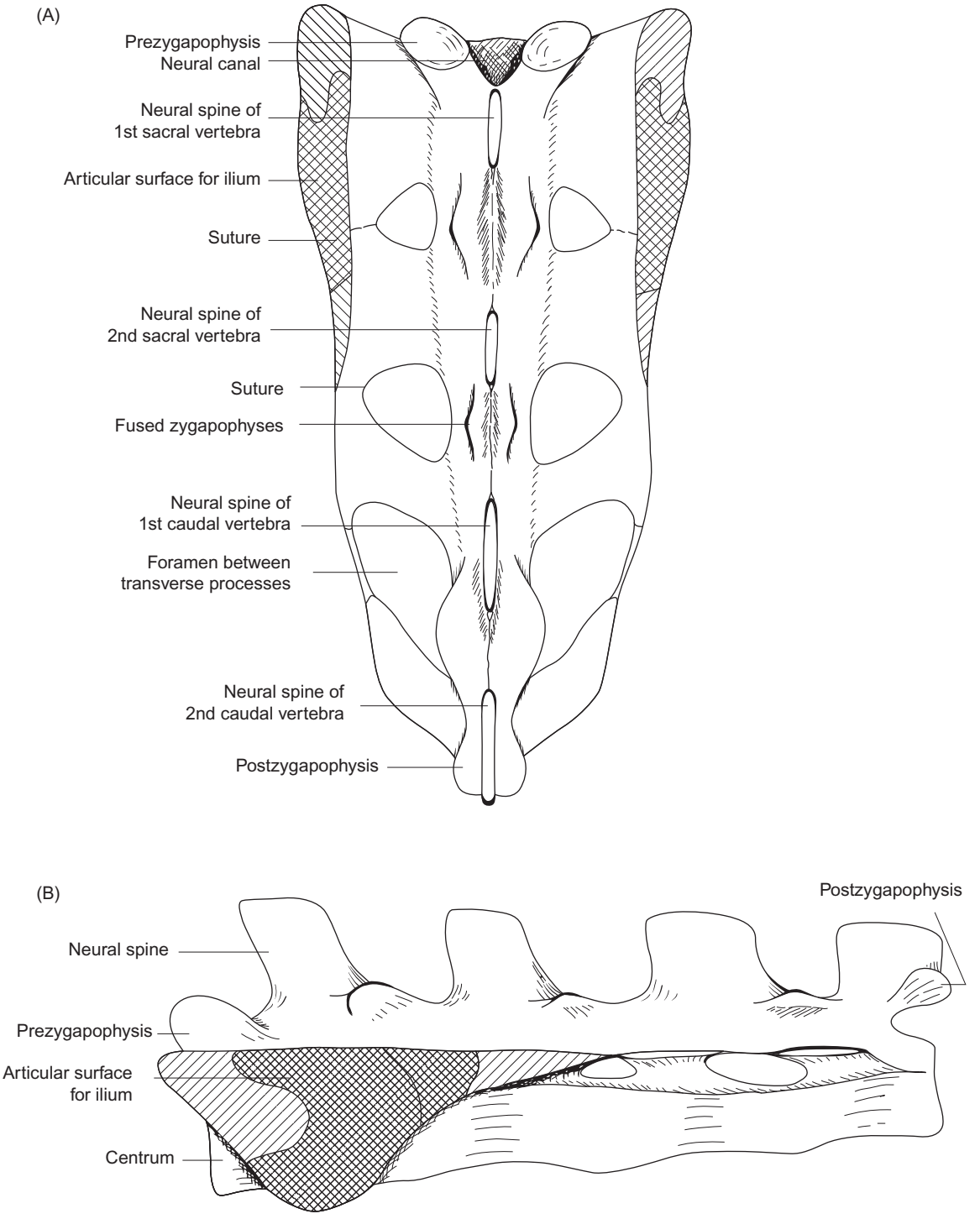


FIGURE 4.6 (A) Sacrum (dorsal aspect). (B) Sacrum (lateral aspect).

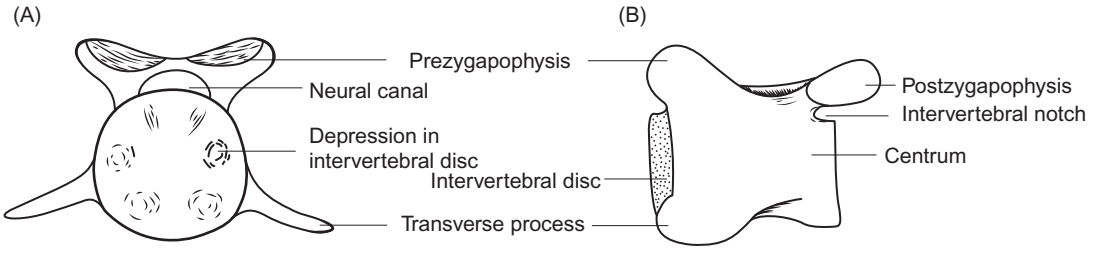


FIGURE 4.7 (A) Third free caudal vertebra (anterior aspect). (B) Third free caudal vertebra (lateral aspect).

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important part in forming the centra of the vertebrae in lower vertebrates, but in mammals are only seen in the tail.

STERNUM AND RIBS

Like all mammals the rat has a sternum, which with the ribs and the thoracic vertebrae forms the framework of the thorax, and protects the thoracic viscera.

The sternum develops from the fusion of a pair of parallel cartilages that develop in association with the ventral ends of the ribs. The origin of the sternum from rib cartilages is revealed by the division of the sternum into a series of seven fused sternebrae (Fig. 4.8A). The anterior member of the series is the manubrium sternum (manubrium: handle of the dagger shaped sternum of man) and the 7th is the xiphisternum, which remains as a cartilaginous structure in rat, though it may ossify in man. The manubrium articulates with the clavicles and the first ribs. Ribs 1−7 articulate directly with the sternum in the rat, and ribs 8−10 (the so called false ribs) articulate with the sternum via the cartilage of the each adjacent anterior rib (Fig. 4.8B), whereas ribs 11−13 (the so called floating ribs) do not reach the sternum at all.

Each rib has an ossified dorsal portion and a cartilaginous ventral part. They arch around the thorax with a rather sharp dorsal bend described as the angle of the rib. The part between the head and the tubercle is the neck of the rib. In addition to being curved the ribs are twisted: from the 5th to the 12th the broadest aspects of the ribs face posteriorly and laterally.

The thoracic cavity is rather conical in shape with a narrow anterior aperture, and a much large posterior aperture. Each aperture can be thought of as an angled ring (a ring which is not circular but longer from ventral to dorsal than from side to side) made up of vertebrae, ribs and sternum. Man has a broad thorax, the rat's thorax is narrow from side to side.

THE SHOULDER OR PECTORAL GIRDLE

The pectoral or shoulder girdle of the rat comprises the scapulae and clavicles. The scapula is a flattened triangular bone that lies against the chest wall and articulates with the clavicle, rising above the level of the vertebral column and providing the point of articulation for the humerus. The scapula moves on the chest wall with the movements of the forelimb. The lateral surface of the scapula bears a sharp ridge: the spine of the scapula. A variety of names have been given to the edges or borders of the scapula: the terminology of Hebel and Stromberg (1976) is adopted here. Three borders: cranial (indented or concave), dorsal (convex and rising, caudally, above the vertebral column) and caudal (nearly straight).

The mammalian pectoral girdle contains remnants of earlier pectoral girdles. In early fish, the girdle comprised exoskeletal (dermal) bones that linked the girdle with the occipital bone of the skull, and endoskeletal bones that formed in cartilage, providing the articulation with the first segment of the pectoral fin. There were two endoskeletal components, the scapulae (dorsal) and the coracoids (ventral), and rather more exoskeletal components. The cleithrum, which could be subdivided into several parts, and the clavicle, met ventrally in the midline forming an arch. In mammals, most of the exoskeletal components have disappeared and the coracoid, no longer a separate bone, is much reduced and fused with the scapula. Despite these changes, it is the scapula along with the coracoid (depending on species) that provides the articulation with the humerus, and the clavicles that provide the ventral arch. In some mammals, an interclavicle appears ventrally. In the rat, a few nodules of cartilage represent remnants of lost bones (Fig. 4.9 shows the procoracoid, a bone found in early reptiles, as represented by two nodules), one at each end of the clavicle; note also the nodule representing the omosternum associated with the manubrium.

The Scapula

The scapula bears two marked processes: the acromion and, rather less obvious, the coracoid. The acromion springs like the head of a golf club from the spine of the scapula, extends anterior to the glenoid fossa and carries a small subsidiary process, the metacromion. The scapula provides the glenoid fossa (glenoid = socket): both the scapula and the coracoid process contribute to the articular surface.

The muscles of the scapula arise from the anterior and posterior spinous fossae on either side of the spine, and from the deep surface of the bone. The deep surface is ridged by the attachments of subscapularis and serratus anterior. The scapula ossifies from two major centres, one for the scapula and one for the coracoid process. In the rat, the borders of the scapula long remain cartilaginous.

The Clavicle

The clavicle of the rat is a very small bone, connected by synovial joints to the manubrium of the sternum, and the acromion of the scapula. In many mammals, particularly those that use their forelimbs only for running, and not for manipulation of objects, the clavicle is reduced or lost. In these species, the forelimb is attached to the rest of the body only by muscles. In man the ossification of the clavicle is unusual: it begins in membrane (see Chapter 3: Introduction to the Skeleton: Bone, Cartilage and Joints), and then develops a pair of cartilaginous

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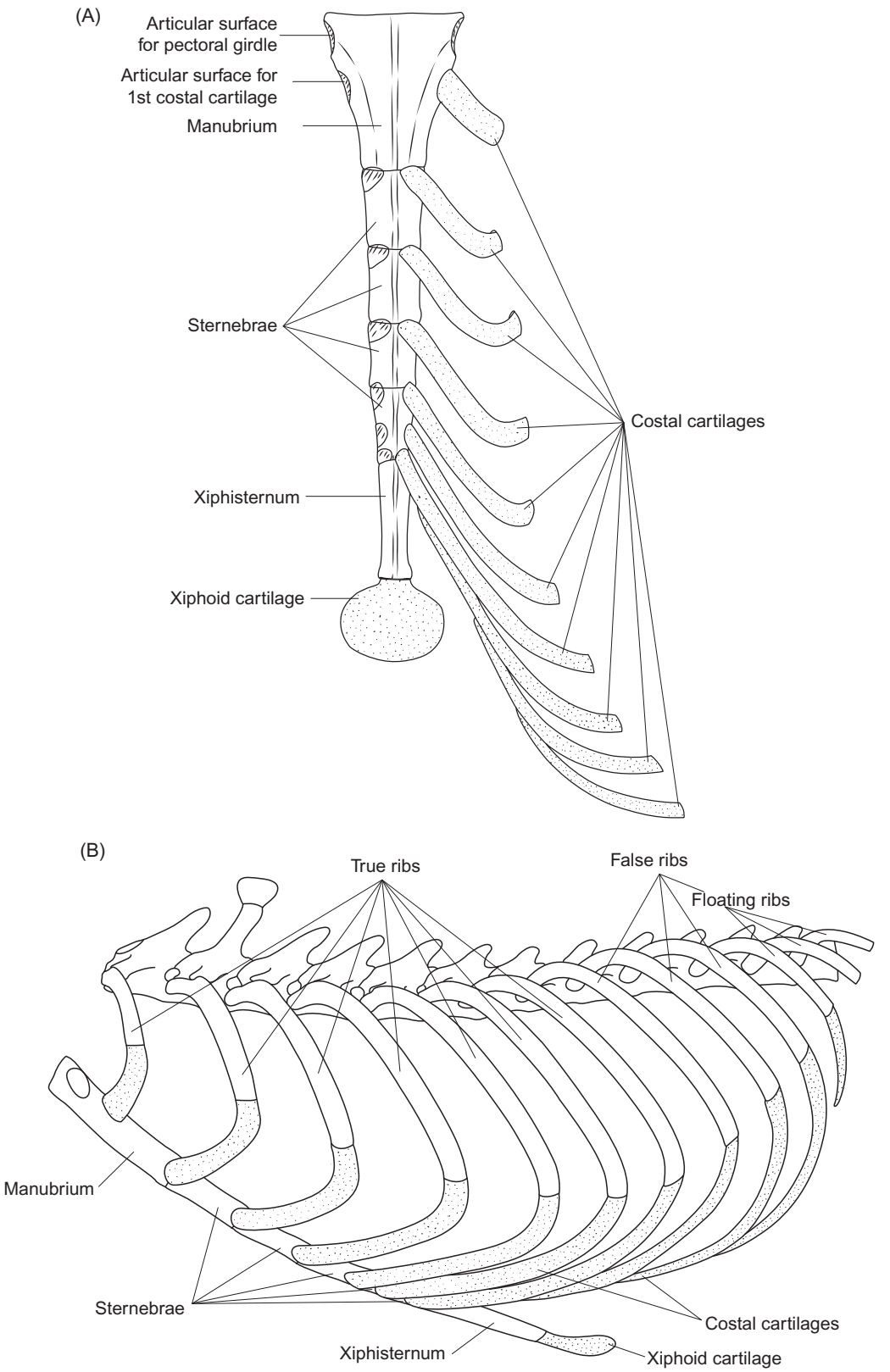


FIGURE 4.8 (A) Sternum and left costal cartilages. (B) Thoracic cage.

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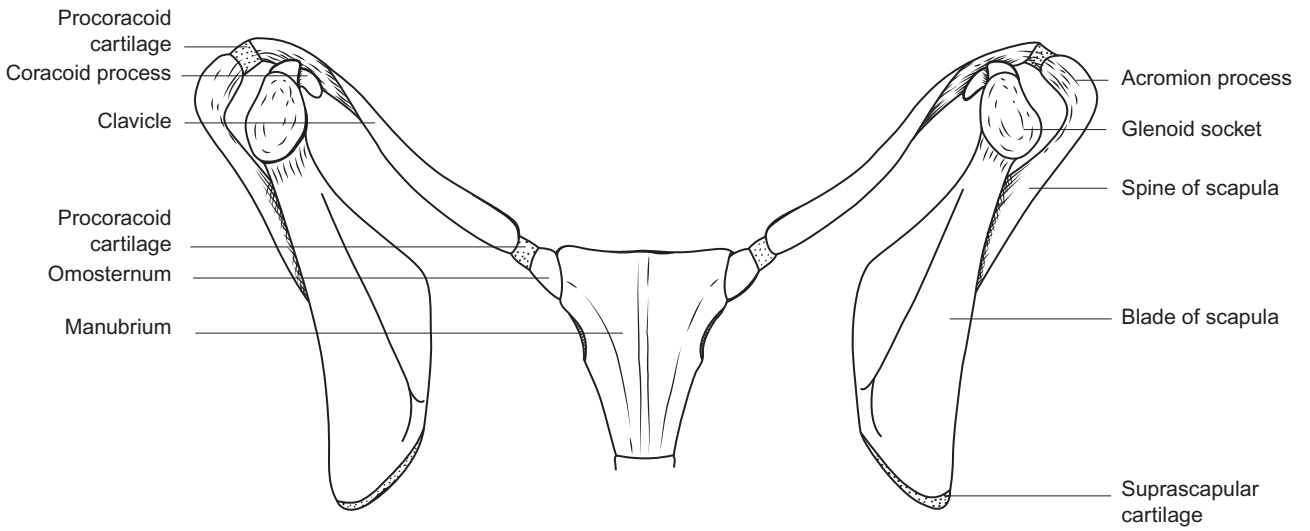


FIGURE 4.9 Pectoral girdle (ventral aspect).

centres that ossify. The clavicle contains bone marrow, but lacks a medullary cavity: at least this is the condition of the bone in man.

THE PELVIC GIRDLE

Unlike the pectoral girdle, the pelvic girdle is firmly attached to the vertebral column at the sacrum. The pelvis comprises two bones, the os coxae or innominate bones.

The innominate bone comprises three fused bones: the ilium, ischium and pubis see Fig. 4.10. Ischium is derived from the Greek word for hip joint and pubis refers to the genitalia: pudic (Latin) meaning modest or chaste. The innominate bone is described as triradiate in shape: the ilium and pubis point forwards, the ischium points back.

The ilium is a long narrow bone in the rat, but in man it is a broad curved bone with a superior edge, the iliac crest, which sweeps around the flank; in the rat, the bone runs directly forwards. The deep greater sciatic notch that is so obvious in the human bone is much less marked in the rat. It is hardly a notch at all, more a lengthy indentation that runs from a sharp upper edge (the posterior, inferior iliac spine: this is human anatomy terminology and not really applicable to the rat) down the posterior border of the ischium to the ischial spine, which forms a sharp ridge on the bone. At the medial or deep surface of its dorsal edge is the articulating surface for the sacrum. This surface is small, narrow, rather wider posteriorly than anteriorly, and occupies only the caudal part of the surface anterior to the greater sciatic notch. Despite only two sacral vertebrae being fused with the ilium in the rat, the bearing surface of the ilium is described, as in man, as the auricular surface, though it looks very unlike a rat or human ear.

The ischium of the rat is relatively broad and the pubis narrow. The large obturator foramen that is enclosed by the ischium and pubis, is almost completely closed (obturated) by fascia and muscle in the living animal. The pubis bears the articulating surface of the pubic symphysis: a small area on the rather narrow anterior edge of the bone. Both the ischium and pubis are divided into superior and inferior rami.

The three parts of the innominate bone radiate out from the acetabulum, which is the circular cavity that articulates with the head of the femur (acetabulum means vinegar cup: 'acet' as in acetic acid). Although proportionally smaller in the rat than in man, the acetabulum is similar in shape, being circular but for a notch that appears in its posterior border (lower border in man). The ilium, ischium and pubis meet in the acetabulum, and although the suture lines cannot easily be seen in the depths of the acetabulum, they are visible on the medial or deep surface of the bone. The articular surface is an incomplete ring of smooth dense bone, covered with hyaline cartilage. The edges of the ring are extended by the labrum, a ring of fibrocartilage that grips the head of the femur. A short ligament, the terres ligament, which runs from the depths of the acetabulum to the head of the femur, is said to be important for holding the head of the femur into the acetabulum. This is not its major function, muscles and large extra-articular ligaments do that. What it does do is to convey blood vessels to the developing head of the femur. This is shown by the work of Norman et al. (1998) on experimental necrosis of the femoral head in the rat. Perumal et al. (2016) provide a detailed review of the role of this ligament in man.

Rowett described a cotyloid bone as forming part of the wall of the acetabulum. Although difficult to make

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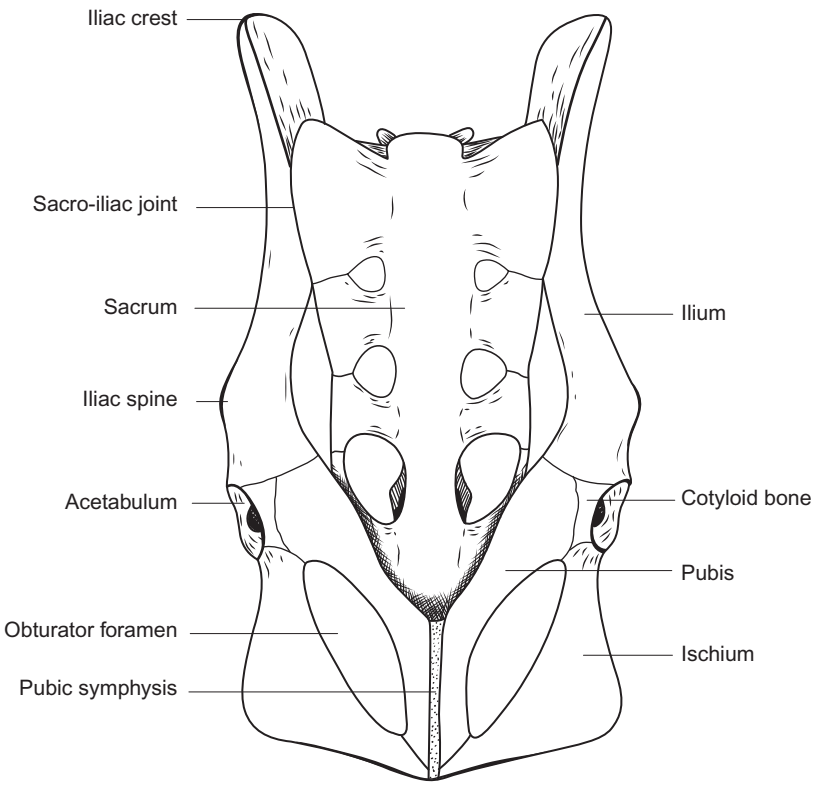


FIGURE 4.10 Pelvic girdle (ventral aspect).

out in the adult bone, it is apparent in the developing rat. Described in man as the os acetabulum, again it usually disappears in the adult. A detailed description of this unimportant little bone and several other little nodules is given in that treasury of human anatomy, *Quain's Elements of Anatomy* (Bryce, 1915).

BONES OF THE LIMBS

Forelimb

The forelimb is made up from the humerus, radius, ulna, the carpal and metacarpal bones, and the phalanges. It is very mobile in man, but less so in the rat, where the forelimbs are primarily for locomotion, although rats are surprisingly good at manipulating small objects with their forepaws (Fig. 4.11 shows the bones of the forelimb).

Humerus

The humerus is a classic long bone (see Chapter 3: Introduction to the Skeleton: Bone, Cartilage and Joints), with an extensor surface that faces posteriorly, and a flexor surface that faces anteriorly. Most of the rounded head of the upper end of the humerus can be seen in this view (Fig. 4.11). Note that the smooth, curved surface of the head continues for a little way down the posterior aspect of the bone, and lower down the shaft the deltoid crest or tubercle (a tubercle is a protrusion from the surface of the bone) stands out on the lateral side as it sweeps round the lateral margin of the bone and down onto the flexor surface. Returning to the upper end of the bone, there are two tubercles. The lesser tubercle lies on the medial side, the greater tubercle on the lateral side, projecting a little above the level of the head with the lesser tubercle somewhat lower. From the flexor surface both tubercles are visible and between them lies the biciptal groove, which is occupied by the tendon of the long head of biceps muscle.

The lower end of the humerus is complicated. On the extensor surface, the olecranon fossa is easy to recognise. In man, it receives the olecranon process of the ulna when the elbow joint is fully extended, but in some rodents the olecranon process continues far beyond the semilunar fossa (see below), and cannot be received by the olecranon fossa. Distal to the fossa, lies the easily recognised saddle shaped trochlea (trochlea = pulley, and it looks like one), which articulates with the semilunar notch of the ulna, forming the hinge of the elbow joint. To the medial side of the trochlea lies a groove for the ulnar nerve, which passes from the extensor compartment of the upper arm to the flexor compartment of the forearm. At this point, and to the medial side, is the medial epicondyle. This is the nerve you injure when you knock your 'funny bone'. You can feel this nerve by pressing your fingers into the groove in the inner surface of the back of your elbow; pressure

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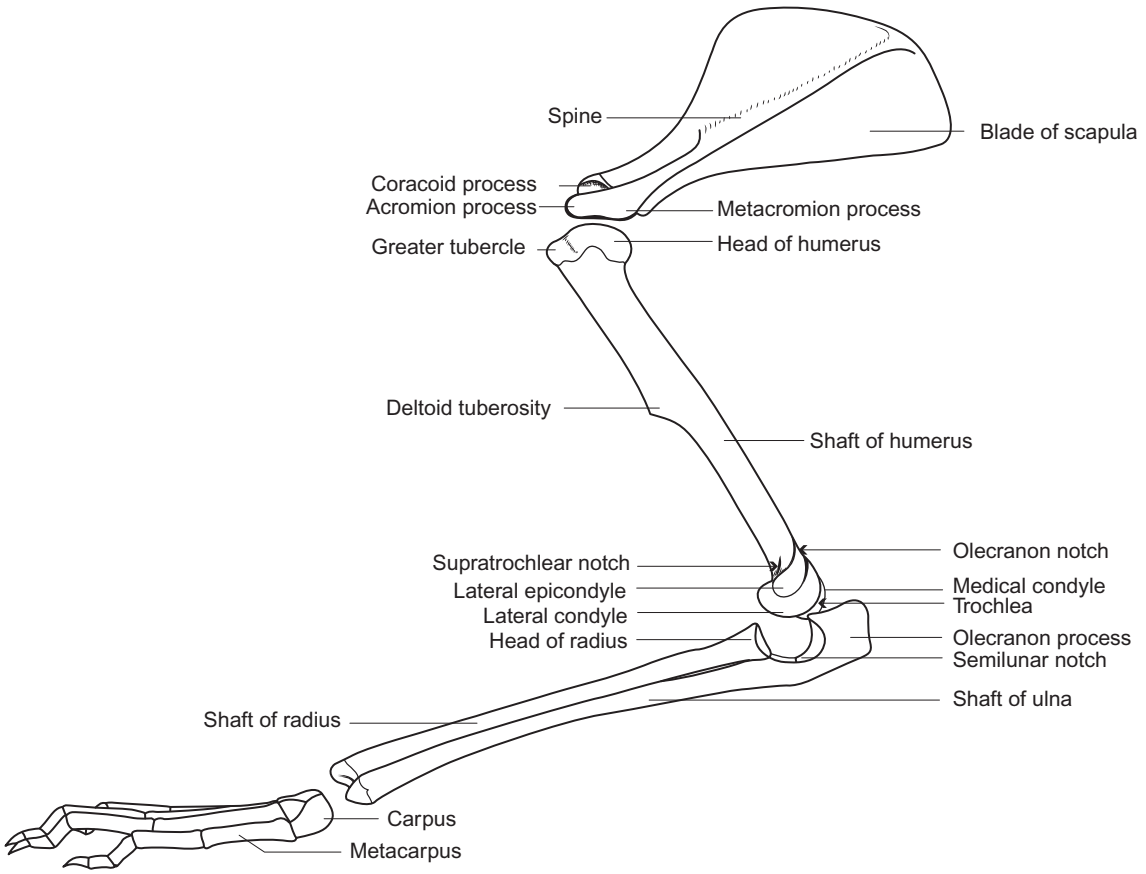


FIGURE 4.11 Left forelimb and scapula, partially disarticulated (lateral aspect).

produces tingling. The sharp projection medial to the nerve is the medial epicondyle. On the other side of the trochlea lies the lateral epicondyle. The lateral part of the trochlea is enlarged and rounded and called the lateral condyle or capitulum. In man, the capitulum is a rather obvious structure that stands to the lateral side of the trochlea.

On the flexor surface the supratrochlear (coronoid fossa in man) is to be found. In man, this fossa receives the coronoid process of the ulna when the elbow is fully flexed. In some rodents, the thin wall of bone between the fossae disappears and a foramen is produced. Lateral to the coronoid fossa, in man, is a smaller radial fossa which receives the edge of the head of the radius during full flexion of the elbow.

There is a major difference between the human humerus and the rat humerus. In man the bone is twisted and the lower end lies in a plane very different plane from that of the head, but this is not the case in the rat, or in most other mammals.

Radius and Ulna

The reader will know that in man the radius rotates around the ulna thus allowing pronation and supination of the wrist (Chapter 6: The Musculature of the Rat). The head of the human radius forms a rather flat round disc. It is dished on the upper surface that rotates against the capitulum of the humerus. The side of the disc turns on the smooth articular surface at the lateral side of the coronoid process of the ulna, which is only separated by a narrow ridge from the rest of the articular surface of the semilunar notch of the ulna. The joint space between the side of the head of the radius and the side of the coronoid process is confluent with that between the articular surface of the semilunar notch and the trochlea of the humerus. The head of the radius is held firmly against the side of the coronoid process of the ulna by the annular (ring shaped) ligament.

The anatomy of these bones is a little different in the rat. It is generally considered that the radius and ulna of the rat are held firmly together, and that the radius does not rotate on the ulna. The radius sits on the anterior surface of the ulna, with the result that the forearm is permanently pronated, thus bringing the palm or ventral surface of the forepaw into contact with the ground (Young, 1957). If this is so, we can discard the idea that the head of the radius rotates on the capitulum of the humerus. With the radius fixed in this position, we might expect the upper end of the radius to come into contact with the flexor surface of the lower end of the humerus when the

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elbow is fully flexed. Indeed this is what Hunt (1924) says: he speaks of a radial fossa: 'to receive the radius during flexion of the arm'. But is the radius firmly, immovably, fixed to the ulna? Whishaw (2003) had no doubts about this: 'Primates pronate and supinate the hand by rotating the radius over the ulna, but these bones are fused in the rat preventing this movement'. Bonnan et al. (2016) reported that the rat radius did rotate on the capitulum of the humerus. Other authors (Young, 1957) report limited movement between the radius and ulna. Hunt (1924) also speaks of a flattened bearing surfaces between the side of the head of the radius and the side of the coronoid process of the ulna. If this is the case, then it is difficult to see how the bones could rotate one against the other, as this would require at least one bearing surface to form part of a circle. More work on this appears to be needed.

Ulna

The ulna is a long thin bone with a small distal head that bears the styloid process, and an expanded proximal end. The proximal end terminates in the olecranon process and bears the semilunar notch on its upper surface. In man, the head of the ulna does not articulate with any of the bones of the carpus. In the rat, the ulna may articulate via its styloid process with the triquetrum and possibly the pisiform bone (see below for a discussion of this point). The radius and ulna are connected more or less throughout their length by an interosseus ligament, which contributes to the origins of some muscles of the forearm.

Radius

The radius is shorter than the ulna. The distal end of the radius is expanded and bears a styloid process on its lateral border, away from the articulation with the ulna. There is room for confusion here: in human anatomy, the styloid process is on the lateral border of the bone when the arm is placed in the anatomical position (see Chapter 2: Introduction to Anatomical Terminology, for a discussion of standard anatomical terminology and positions). Along with the triangular articular disc, the expanded distal end of the radius forms a major part of the wrist joint articulating with the scapho-lunate and triquetrum and almost completely relieves the ulna of the task of articulating with carpal bones (Fig. 4.12).

The Carpus

The carpus is made up of the carpal bones and lies between the radius and ulna and the metacarpals, which articulate with the first segments of the digits. Even in as large a mammal as man the carpal bones are small; in the rat they are tiny nodules. The reader may be relieved to know that the carpal bones of the rat seem not to have been described with the loving detail lavished on their human counterparts. In the bad old days of anatomical teaching, recognition and detailed knowledge of the carpal bones was required. Indeed it was said that a medical student should be able to produce any specified carpal bone from a collection in his waistcoat pocket by touch alone. Telling one carpal bone from another was bad enough, orientating it (telling one surface from another) and 'siding it' (distinguishing the left bone from the right) was for virtuosi only. Of course such exercises were largely futile, and toxicologists are not likely to have any interest in the carpal bones of the rat, although the rat wrist joint has been used as a model for various types of arthritis and repetitive strain injury (Driban et al., 2011).

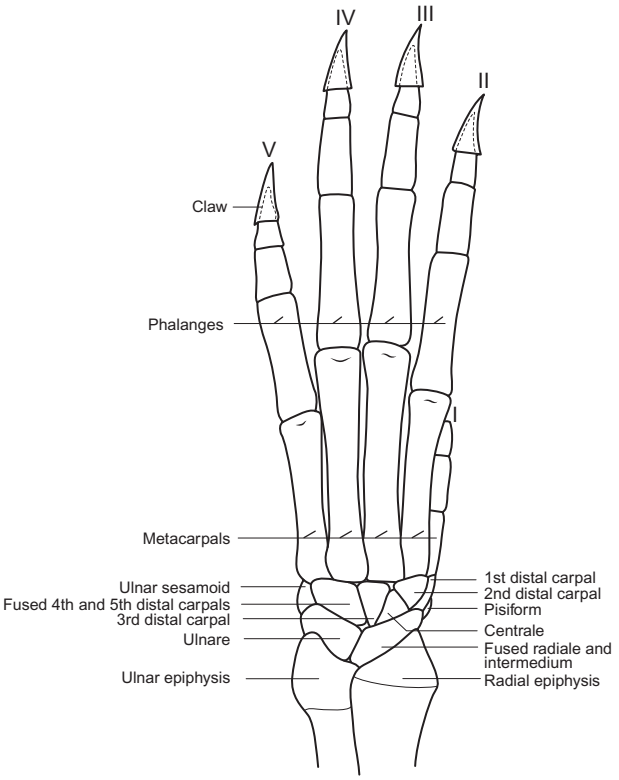


FIGURE 4.12 Left manus (upper surface).

Early tetrapods had 12 carpal bones (Romer, 1970) arranged in 3 rows and named accordingly.

Proximal Row

From the radial side (the side defined by the radius: lateral in human anatomy using the standard anatomical position: see Chapter 2: Introduction to Anatomical Terminology) radiale, intermedium and ulnare. The proximal row in the rat comprises the scapho-lunate (fused scaphoid and lunate) and the triquetrum (or triquetral bone). It might be useful to note that though scaphoid is an adjective (meaning boat-shaped), as is lunate,

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(meaning shaped like a half-moon) the word is also used as a noun: the scaphoid bone or the scaphoid. The pisiform sits on the palmar surface of the triquetral bone and is a sesamoid bone (see below).

Middle Row

Initially four bones in the early tetrapod, the central carpal bones or carpal centralia are numbered 1−4 from the radial side. The centralia have been much reduced in mammals: from 4 to 2 or 1. In the rat, one of the centralia has survived as the centrale, but in man they have gone completely: the last one fused with the scaphoid bone.

Distal Row

Initially five bones, the carpaliare are also numbered from the radial side, and have also been reduced in mammals. The equivalents are 1: trapezium, 2: trapezoid, 3: capitate and 4 + 5: hamate. Only an enthusiast will wish to know that the hamate is named for the little hook (hamulus) it bears, and that it is also known as the unciform bone (uncus = hook). The rat differs from man in having a falciform bone, a narrow sesamoid bone that curves across the wrist from the radial side and encloses the flexor tendons on their way to the digits. This bone, sometimes called the prepollux, has evolved in the common mole as a digging aid and provides the 'thumb' for the giant panda.

Some carpal bones have been lost during evolution, some have fused one with another, and a few new bones (not strictly carpal bones but sesamoid bones) have appeared. Sesamoid bones, so named because they reminded somebody of sesame seeds, are formed in tendons: the patella is the largest sesamoid in man. The forepaw of the rat is well provided with sesamoid bones. In addition to the pisiform, falciform and ulnar sesamoids (ulnar sesamoid: a large sesamoid to the ulnar side of the carpus), there are two on the flexor surface of each metacarpophalangeal joint, and one at the flexor surface of the joints between the distal phalanges and those immediately proximal to them.

The Metacarpals

Five bones, all but the first are rather long bones, with the third and fourth longer than the second, which is longer than the fifth. Hunt (1924) points out that the first is so short that it might be mistaken for a carpal bone.

The Phalanges

Each digit has three phalanges, except the thumb which has two. Each terminal phalanx carries a claw, that of the thumb being flattened. The first digit lacks its proximal phalanx. At least this is the accepted view, though some have argued that it is the first metacarpal that has disappeared. It is odd that, in man at least, what we call the first metacarpal has its epiphysis (it only has one) at its proximal end (like phalanges) and not at its distal end like other metacarpals.

The Hindlimb

The bones of the hindlimb are the femur or thigh bone, the fused tibia and fibula of the second segment, the tarsus, metatarsus and phalanges (Fig. 4.13). To these we should add the sesamoid bones: the patella (kneecap), the pair of fabelli found behind the knee in association with the gastrocnemius muscle and the sesamoids found in association with the tendons on the ventral aspect of the foot or pes.

The Femur

The femur, like the humerus, is a classic long bone, instantly recognisable by a head attached to a short neck at the proximal end of the bone and a distal end expanded by a large epiphysis. The shaft of the femur is slightly curved and the anterior surface slightly convex.

The head forms a roughly hemispherical expansion at the end of the neck, which is proportionally shorter in the rat than in man. The head and neck form one arm of a Y, the other arm is formed by the greater trochanter, a large mass of bone on the lateral side. Medial to the greater trochanter, between the trochanter and the neck, lies a deep pit: the trochanteric fossa. On the posterior aspect of the bone the greater trochanter can be seen to be connected by the intertrochanteric crest to the lesser trochanter, below which lies the third trochanter.

The distal end of the femur articulates with the head of the tibia via a large epiphysis: a conspicuous epiphysis as Rowett put it. This epiphysis does not fuse with the rest of the bone until old age, if it does at all, and can be displaced and lost from a specimen of bone. If the epiphysis has been lost, the distal end of the femur terminates in four little pyramids, two anterior and two posterior, which fit into the proximal side of the epiphysis in the living bone. Two large, rounded condyles sweep round the epiphysis from the posterior to the anterior surface. To either side lies an epicondyle, between which there is a deep intercondylar fossa that is partly occupied in life by the cruciate ligaments which arise from the anterior and posterior ends of the intercondylar groove on the head of the tibia and run to the medial aspects of the femoral condyles. The ligaments cross, hence the name cruciate.

Above and between the articular surfaces of the condyle, on the anterior aspect of the bone, lies the articular surface for the patella. The rat patella differs from the human patella as the anterior surface continues upwards

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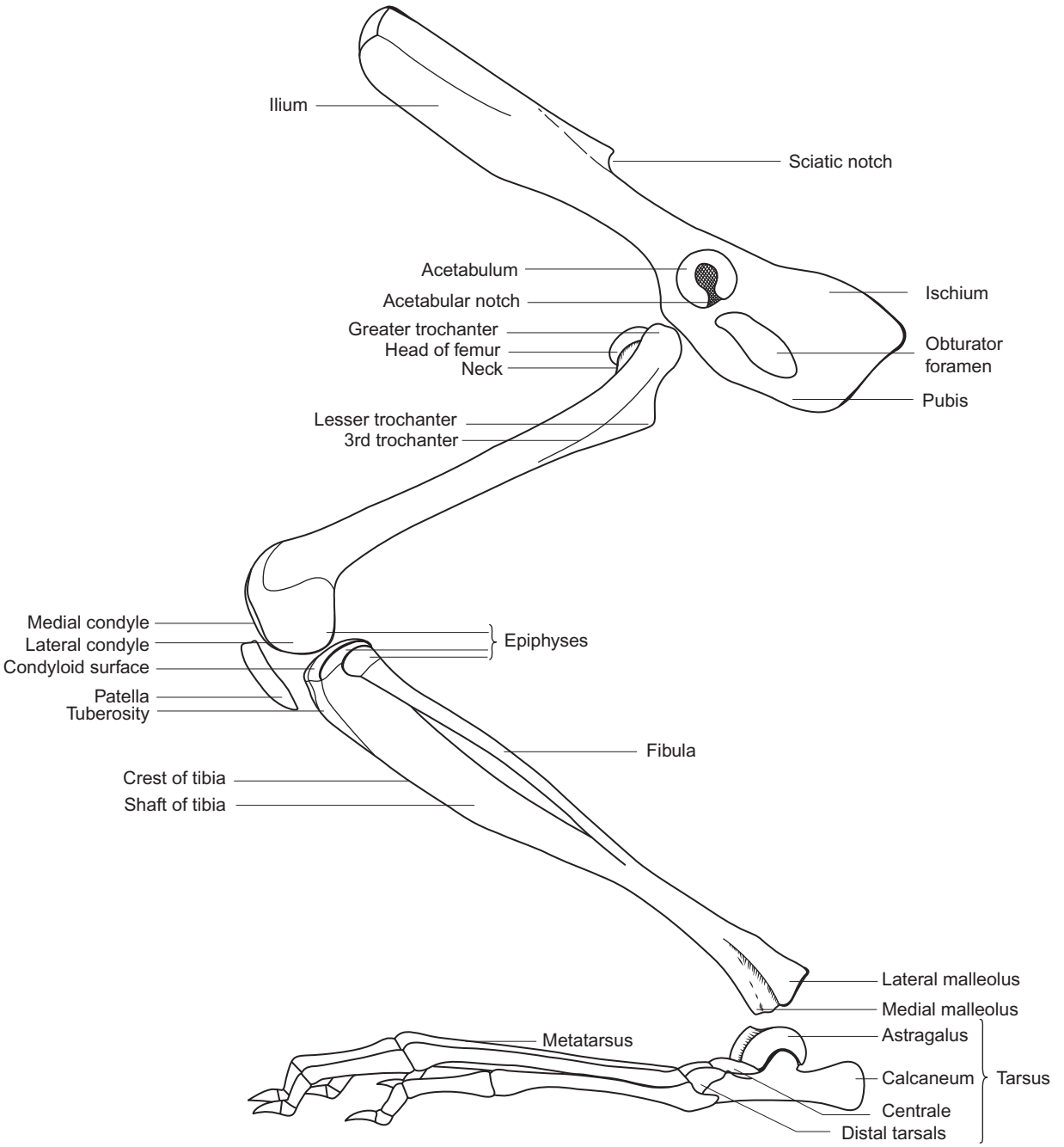


FIGURE 4.13 Left hindlimb and innominate bone, partially disarticulated (lateral aspect).

as a cartilaginous extension contributing to the anterior wall of the suprapatellar bursa (Hildebrand et al., 1991). In man there are a number of bursae around the knee joint that provide a cushion between points of pressure. Housemaid's knee is caused by inflammation of the prepatellar bursa. The knee joint contains two crescentic cartilages: the menisci (one meniscus, two or more menisci). Composed of fibrocartilage (see Chapter 3: Introduction to the Skeleton: Bone, Cartilage and Joints), these are wedge-shaped in cross section with the thin ends of the wedges pointing inwards. It is said that they improve the fit of the femoral condyles to the tibial condyles. The menisci are well developed in the rat, and 'pyramid shaped ossicles' (presumably small areas of ossification) occur within the fibrocartilage along with cushions of fat (Hildebrand et al., 1991).

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The femur can be correctly orientated by recalling that the head lies on the medial side of the bone, and that the proximal part of the lateral margin bears a large crest, similar to the one of the humerus. In the resting position, that is crouching, the proximal end of the femur is closer to the ground than the distal end. The flexor surface is ventral; the extensor surface is dorsal.

The Tibia and Fibula

The tibia is equivalent to the radius of the forelimb, and is the weight bearing member of the second segment of the hindlimb. In the rat, the fibula is much reduced compared with man fusing with the tibia in its lower part, but breaking free in its most distal part to provide the lateral malleolus of the ankle joint. Hunt (1924) described the combination of tibia and fibula as resembling an archer's bow with the fibula providing the bowstring.

The articular surface at the proximal end of the tibia comprises two concave or dished areas: the upper surfaces of the medial and lateral condyles, and between them the intercondylar groove or fossa. The menisci are anchored anteriorly and posteriorly to the tibial surface in the groove. The tibia bears an anterior crest that separates its lateral and medial surfaces. The proximal anterior surface of the tibia is prominent, forming the tibial tuberosity, to which is attached the patellar ligament. The proximal end of the fibula articulates with the tibia at a facet beneath the overhang of the lateral tibial condyle. The joint is synovial in man, which according to Flower (1885) is typical of mammals. Hunt (1924) says nothing of this articulation but states, 'The slender fibula expands dorsally, where it is attached by a ligament to the external condyle of the tibia'. Flower points out that the fibula 'often ankyloses [fuses] with the tibia at one or both ends'.

In man, the distal ends of the tibia and fibula form a mortice that grips the talus and forms the ankle joint. The tibia carries the weight of the body and the fibula provides only the lateral side of the mortice. Neither Greene (1935), Hebel and Stromberg (1976) nor Hunt (1924) provide any details of the ankle joint in the rat, but Hunt described the distal end of the tibia as containing a pair of longitudinal groves, which presumably fit the ridges at the sides of the articular surface of the talus. De Araujo et al. (2012) provide some information, they used the rat for comparison with caviomorph rodents, and Vickerton et al. (2014) provide illustrations that show the distal end of the fibula resting on what appears to be a lateral extension of the talus. In this study, Finite Element Analysis, used to identify regions of strain in the tibia-fibula, showed the joint between the medial aspect of the lateral malleolus (of the fibula) and the lateral aspect of the talus appeared to be a region of considerable strain.

The Tarsus

The tarsus and carpus are similar in terms of their primitive structure. As in the carpus the original pattern was one of three rows (Fig. 4.14):

Proximal Row

From the tibial side: tibiale, intermedium and fibulare

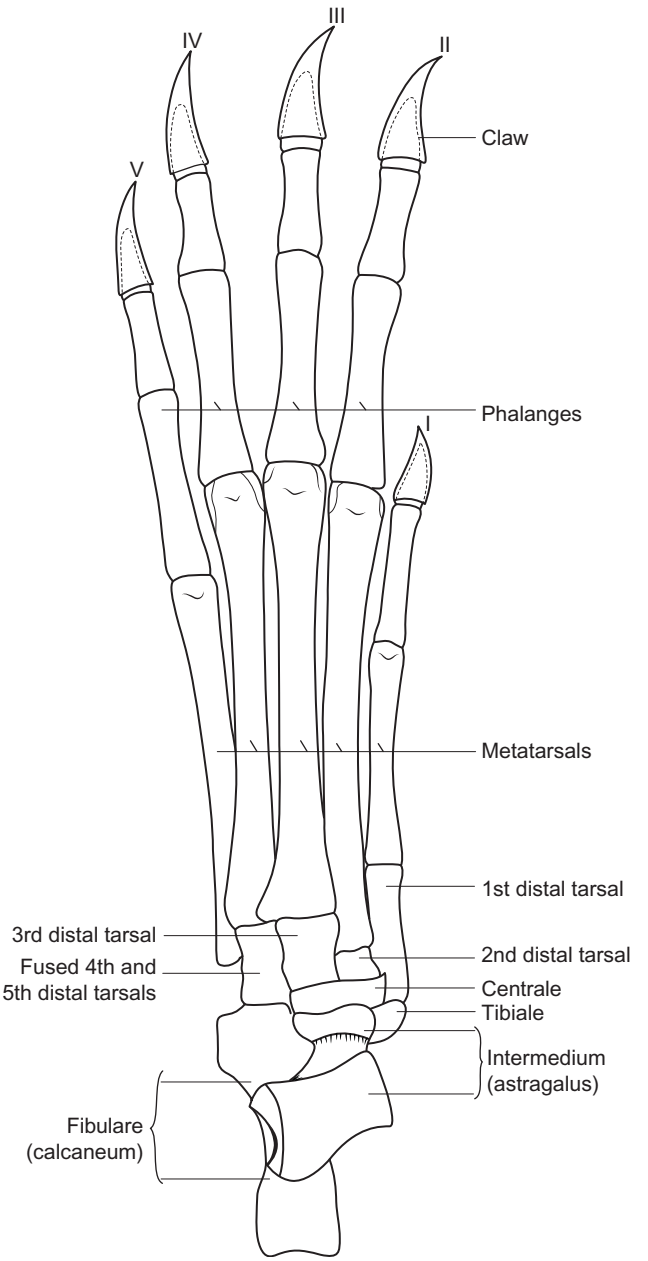


FIGURE 4.14 Left pes (upper surface).

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Middle Row

As in the carpus the tarsal centralia originally comprised four bones numbered 1–4 from the tibial side, but have been reduced to one bone in mammals (Romer, 1970).

Distal Row

There were originally five tarsalia, numbered 1–5 from the tibial side, but the fifth of the tarsalia has tended to disappear. In the carpus, we described the fifth and fourth carpalia as fusing, it is possible that this occurs with the equivalent tarsalia (Greene, 1935). Romer (1970) noted that no equivalent of the pisiform bone appeared in the pes, and argued that the talus (astragulus: the terminology is confusing) represented a fusion of the tibiale with the intermedium and a 'central element'. This may well be the case in mammals in general, but the rat has a well-developed tibiale, a bone without an equivalent in the carpus. Fortunately, there is a detailed study of all this. Lewis (1964) explored the homologies of the mammalian tarsal bones. The following conclusions are taken from his summary:

No evidence to support the 'common view' that the talus represents fusion of the tibiale and the intermedium was found.

A free tibiale exists in monotremes and rodents, but not apparently in other mammals. The insertion of the tibialis posterior muscle into the tibiale of the rat reflects the homology of this bone with the tibial part of the navicular in other mammals.

The mammalian navicular (presumably except in monotremes and rodents) is homologous with the centrale plus the tibiale; the talus with the intermedium, the calcaneus (or calcaneum) with the fibulare and the cuboid with tarsalia 4 and 5.

Lewis considered that Greene (1935) was in error regarding the insertion of the tibialis posterior muscle into the tarsal bones. It inserts into the tibial navicular only, and not into the fibular navicular and medial cuneiform. The terms fibular and tibial navicular refer, respectively, to the tibial and fibular sides of the navicular bone.

The talus sits on the calcaneus and is supported by a large flange, the sustentaculum tali, that springs from the medial, tibial, side of the calcaneus. It is important to remember that the bones of the tarsus are held together by synovial joints and by a series of strong ligaments.

The Metatarsus

The rat metatarsus is unspecialised, all the bones are long (the first and fifth are shorter than the others). It is interesting to note that in some rodents that specialise in jumping, for example, the jerboa, the metatarsals are fused to form a single cannon bone.

The Phalanges

Each digit has three phalanges with the exception of the first, the big toe or hallux, which has two.

Sesamoid Bones

As in the forepaw, the hindpaw of the rat is well endowed with sesamoid bones.

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Chapter 5

The Skull

INTRODUCTION

The mammalian skull evolved from the skull of early reptiles, which in turn evolved from the skull of labyrinthodont amphibians (see Chapter 1: The Rat's Place in Nature for an overview of mammalian evolution). During this long history, and longer if we include the preamphibian phase, the skull has continued to discharge its essential functions of protecting the brain, the organs of the special senses, and providing a base for the jaws and sites for the attachment of muscles that move the jaws. Over time the structure has evolved, generally the number of bones making up the skull has decreased, and the articulation of the jaws has changed. Very few 'new' bones have appeared. These evolutionary changes and the sequence of evolutionary events are to some extent reflected in the embryological development of the mammalian skull.

The evolution of the skull, indeed of the head itself, has attracted the attention of comparative anatomists from the time of Goethe, who in addition to being the first to describe the premaxilla, argued that the skull developed from a series of modified cervical vertebrae (see Kuratani, 2005 for early references in this field). In part this was correct, the occipital bone at the back of the skull does develop from somites in series with those giving rise to the cervical vertebrae. But further forwards the picture is less clear, so the theory of a vertebral origin for the whole skull has been discarded. Huxley (1857–1859) played a part in this process, his demolition of the 'from-vertebrae' theory is one the classics of scientific polemic. It is accepted that segmentation as defined by the appearance of somites, is a characteristic of vertebrates, but whether the head is segmented was a burning question in the late 19th century. Balfour (1885) argued for segmentation and Goodrich (1930), Gegenbaur and Gaup (see Kuratani, 2005) and de Beer (1937) followed him. De Beer's monumental work, 'The Development of the Vertebrate Skull', is a landmark in the field of comparative anatomy. Few nonspecialists would try to read de Beer's great work, but he provided an admirable summary in the chapters on the skull and on the head and neck in his 'Vertebrate Zoology' (1951).

More recently authors have devoted books to the evolution and development of the skull. Moore (1981) provided an admirable account of the mammalian skull, and Hanken and Hall's three volume work, The Skull (1993), is an authoritative account of modern views and a deliberate updating of de Beer's classic work. Kuratani (2005) has provided a readable account that summarises current thinking and identifies problems yet to be solved. There are many such problems. In this chapter we cannot hope to explore this specialised subject in any depth. A brief overview of the evolution of the mammalian skull is followed by an equally brief and general account of the development of the mammalian skull, and then by a description of the rat skull. The reader may wonder why space has been devoted to evolution and development, but we think that the rat skull cannot be properly understood without some exploration of these subjects.

To the anatomist, the skull comprises the braincase and the lower jaw. The cranium is taken to mean the skull minus the lower jaw and the calvaria the cranium minus the bones of the face. Calvaria sounds as though it should be the plural form of calvarium, but this is not the case. Hooton (1947), who was a classicist before he became an anthropologist, defined the calvarium as the skull minus lower jaw, and the calvaria as the skull minus lower jaw and facial bones.

The part of the skull that develops from the original cartilaginous braincase and from the dermal bones that roof the skull, is defined as the neurocranium. The parts that develop from the visceral arch cartilages and the dermal bones that clad and in part replace these bones, are defined as the viscerocranium.

It would be convenient if all vertebrates had evolved from fish with skulls composed entirely of cartilage, as is the case in the chondrichthyes (the class represented today by the sharks and dogfish). In these animals, the skull comprises a cartilaginous braincase surrounding the brain, cartilaginous capsules for the organs of the special sense, and a cartilaginous upper and lower jaw. The upper jaw is attached to the braincase and the lower jaw articulates with the posterior end of the upper jaw. Unfortunately for us, the cartilaginous fish evolved from bony predecessors

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that had more complicated skulls. But the skull of cartilaginous fish reappears, though only in part, in the development of the mammalian skull. We shall return to this later.

Romer (1970) provided an admirable summary of the evolution of the mammalian skull. This account is based on his and uses some of his illustrations, but we shall ignore the details of the evolution of the fish skull, passing instead from early amphibians to early reptiles, and thence to mammals. Even with many omissions the story is a complicated one.

Before we can begin, we need to understand that the bones forming the skull arise from a number of sources. These are:

1. Cartilages that evolved and still develop in embryo as a sort of trough that supports the brain.
2. Cartilages that evolved and still develop in embryo as capsules surrounding the organs of the special senses (the olfactory, optic and otic or auditory capsules).
3. Cartilages that evolved from and still partly develop from the cartilages of the branchial arches.
4. Bones that develop directly from mesenchymal connective tissue and which are not preceded by cartilage.

Bones derived from cartilage are described as being of endo-chondral (EC) origin. This includes some of the bones of the cranium and the jaws, although in mammals the EC bones of the jaws have all but disappeared. The bones derived from cartilage represent part of the primitive endoskeleton, whereas bones derived from mesenchyme connective tissue (dermal bones) represent the primitive exoskeleton. Dermal bones provide the roof of the mammalian skull, the bones of the hard palate in the roof of the mouth, the bones of the face and of the lower jaw.

Looking at the skull of an early amphibian Fig. 5.1, we can see that the skull comprises a braincase (yellow), derived from cartilage, and a cladding of dermal bones (pink). In A, we can see that the dermal bones:

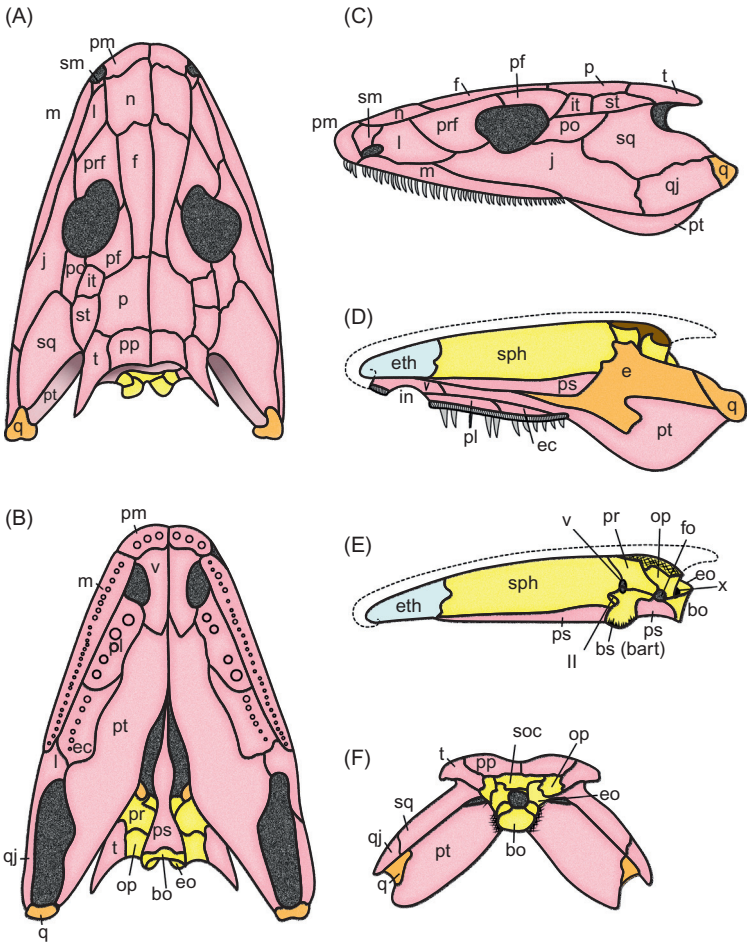


FIGURE 5.1 Skull of an ancestral land vertebrate, based primarily on the Carboniferous labyrinthodont, Palaeogyrinus. See list of abbreviations at the end of chapter for details of the labelling in Figures 5.1–5.4. *Reproduced with permission for Romer A.S. 1970. The Vertebrate Body, fourth ed. W B Saunders Company, Philadelphia, London, Toronto.*

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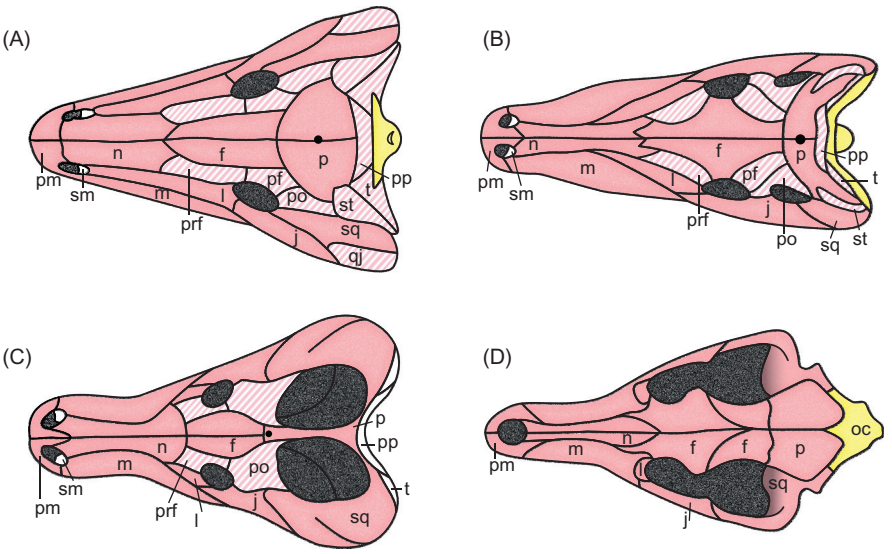


FIGURE 5.2 Diagrammatic dorsal views of the skull roof to show the evolution of the mammalian condition. *Reproduced with permission from Romer A.S. 1970. The Vertebrate Body, fourth ed. W B Saunders Company, Philadelphia, London, Toronto.*

premaxilla, nasal, frontal and parietal lie in anterior to posterior sequence on the top of the skull. These bones are still there in the mammalian skull. In E, we can see a good deal of the braincase: the sphenoid bone (named in human anatomy for its wedge shape) is large and obvious and there is a cluster of smaller bones ending with the occipital bone to the posterior of the braincase. If we look at B, we can see that there the roof of the mouth is provided predominantly by pairs of dermal bones: pterygoid, palatine, ectopterygoid, vomer, maxilla and premaxilla. Note that all of these, except the pterygoid and vomer, bear teeth. The cartilage bones of the upper jaw are shown in orange in D: there are just two, the epipterygoid and the quadrate at the back of the skull. The quadrate is important as the articular bone of the lower jaw hinges on it to form the primitive jaw joint. The articular bone, like the epipterygoid and quadrate, forms in cartilage, in this case the cartilage of the first visceral arch. Looking again at the palate, the gaps to each side of the paired vomers can be seen to form the internal opening of the nasal passages. These lie well forwards and not, as in mammals, to the back of the palate. The ethmoid bone, shown in blue, remains cartilaginous in this early amphibian and develops from cartilage along with the sphenoid and occipital bones.

Let us now take a long step forwards in time and pursue the evolution of the roof of skull (Fig. 5.2).

Here we are looking at the roof of the skull and how it has changed from its condition in a primitive, but relevant, reptile to its condition in a placental mammal, for example a dog. A: a stem reptile (cotylosaur), B: a primitive mammal- like reptile (pelycosaur), C: an advanced mammal-like reptile (therapsid), D: a placental mammal. The first thing that has obviously changed is that the orbit in the dog is confluent with a large space behind that is bounded laterally by the jugal or zygomatic bone, and by a forwards extension from the squamous temporal bone. This forms the zygomatic arch that flares so dramatically in the rat, and which encloses the temporal muscle and provides part of the origin of the powerful masseter muscle (see Chapter 6: The Musculature of the Rat). Note also that although the number of bones in the roof of the skull has decreased, the sequence from premaxilla to parietal is still present. The occipital bone at the rear end of the skull is the only bone of the base of the skull that we can see from this angle.

Let us now follow the sequence in the roof of the mouth (Fig. 5.3).

Here, many changes have taken place from the primitive reptile to the dog. The forms illustrated are as in Fig. 5.2. Of these changes, the most important is the development of the secondary palate comprising the much extended horizontal flanges of the maxilla and palatine bones. The secondary palate has displaced the internal openings of the nasal passages back and they are now described as the posterior nares (one naris or nostril and two nares). The quadrate bone has disappeared (it has become the incus) and the epipterygoid (original the large cartilage of the upper jaw) has been converted into a part of the sphenoid bone (the alisphenoid bone). At least this is, or was, generally believed to be the case: for a detailed discussion see Presley and Steele (1976). Here, we have an interesting point: some bones disappear and some appear as the skull evolves. The parasphenoid of reptiles (labelled ps in A of the Figure, a dermal bone, coloured pink) has disappeared and the presphenoid (labelled ps in

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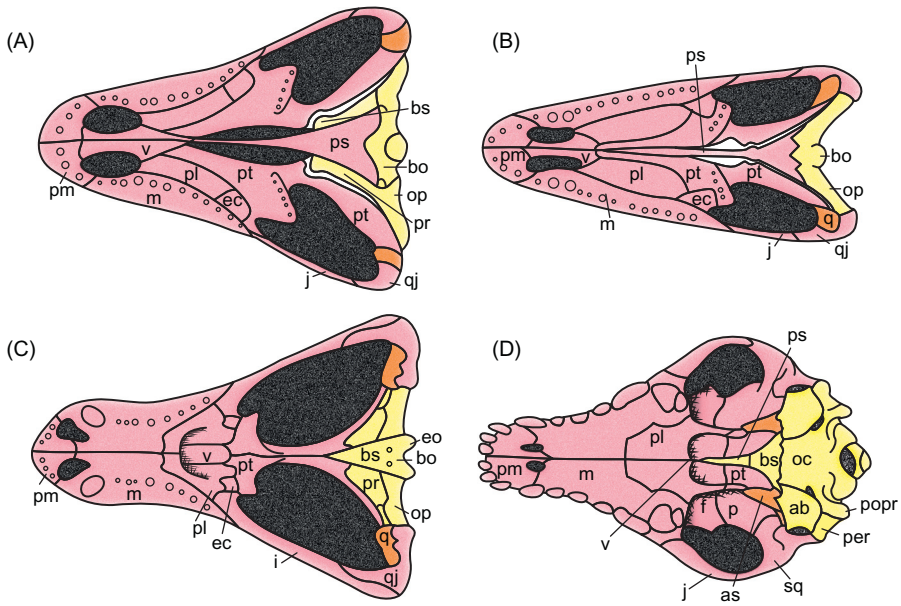


FIGURE 5.3 Diagrams to show the evolution of the ventral palate as seen in ventral view. *Reproduced with permission from Romer A.S. 1970. The Vertebrate Body, fourth ed. W B Saunders Company, Philadelphia, London, Toronto.*

D of the Figure – it should have been labelled prs – a cartilage bone, coloured yellow) has appeared. Note also that the vomers, originally placed anteriorly have fused in the mammalian skull and form a single bone that contributes to the nasal septum.

Our survey is completed by a lateral view (Fig. 5.4). Again, the forms illustrated are as in Fig. 5.2

No detailed description is needed, but note that the triangular area labelled pl (palatine), comprises the palatine bone anteriorly and the lateral pterygoid process, which arises from the sphenoid posteriorly.

Evolution of the Lower Jaw

We have noted that the jaw evolved from cartilages belonging to an anterior pair of branchial or visceral arches. That these were the most anterior arches of some early jawless form seems unlikely, indeed the cartilages of some more anterior pair of arches may reappear as the trabecular cartilages in the developing mammal, anterior to the parachordal cartilages. It has been suggested that these embryonic cartilages develop from neural crest material, as do all the visceral arch cartilages.

In a cartilaginous fish, the upper jaw is formed by the epipterygoid and quadrate cartilages; and the lower jaw by a single large mandibular cartilage (Meckel's cartilage), the posterior end of which sometimes ossifies to form a separate articular bone. Like the upper jaw, the lower acquires a cladding of dermal bones. The dentary is the largest and most important bone, in that it takes over carriage of the teeth forming the lower jaw and its joint with the cranium in mammals. There is a cluster of smaller less important bones, at least less important for us, including the splenial, the angular, surangular, prearticular and the coronoid at the posterior end of the jaw. The angular evolves to form the tympanic ring, a part of the external auditory meatus which in the rat is expanded and inflated to form the tympanic bulla. It will by now be clear that the original jaw joint between the articular and quadrate bones becomes the joint between the malleus and the incus.

SUMMARY

The mammalian skull has evolved from, and contains, many of the components of the skulls of earlier forms. Given this, it is tempting to look for homologies between the bones making up the mammalian skull and those of other forms. Although this preoccupation of comparative anatomists is important in tracing phylogenies, the task of finding of true homologies is strewn with difficulty. The following list sets out some features that might be considered when trying to decide whether a bone of the mammalian skull is a homologue of some bone in the skull of an earlier form.

The consistent location of the bone is important especially as regards its relations with other, adjacent, bones and with cranial nerves and arteries. The relationship to cranial nerves is very important.

Whether the bone consistently forms part of the neurocranium or viscerocranium is important.

Similarity of embryological origin might provide guidance, for example whether the bone originates from neural crest tissue or from paraxial mesoderm. We must be careful here, as it was thought that embryology could provide satisfactory criteria for homology but this is not the

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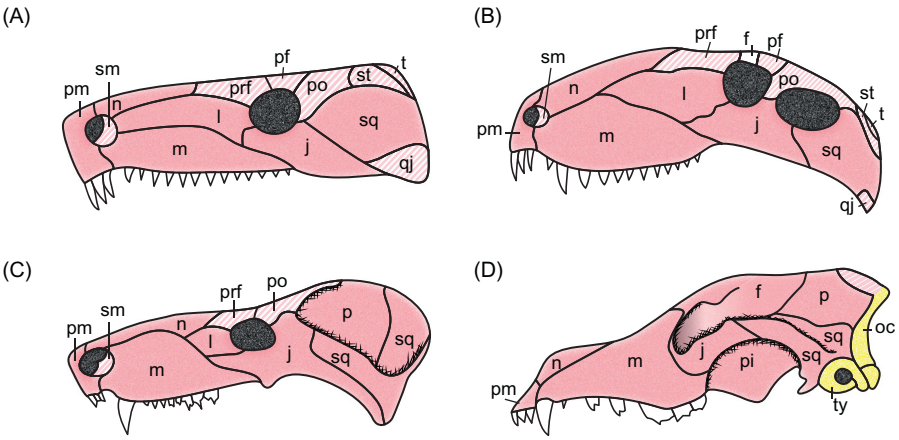


FIGURE 5.4 Diagrammatic views of the stages of the mammalian skull roof as shown in Fig. 5.2. *Reproduced with permission from Romer A.S. 1970. The Vertebrate Body, fourth ed. W B Saunders Company, Philadelphia, London, Toronto.*

case. Hall (2008) has provided a readable discussion of this problem in relation to the neural crest. The view that homologous structures have to develop from the same germ layer was upset by the appreciation of the importance of the neural crest as a source of many structures.

Similarity of mode of development, from cartilage or in membrane is important, but can be misleading. Bellairs and Gans (1983) discussed the origin of the orbitosphenoid in a group of reptiles and showed that it arose in membrane, rather than as had been expected from studies in other forms, in cartilage. This caused Presley (1983) to comment: 'The paper of Bellairs and Gans would have riveted the anatomical and zoological world of the 1920s. It should do so today'. Presley's point was that value of the developmental process as a basis for judging homology had been weakened.

Similarity as regards control of developmental processes is important: if the same genes are involved, and this can be demonstrated by work on mutants and in knock-out models, then that is helpful.

If an evolutionary series can be defined that allows the putative homology to be traced through a variety of forms, as we have followed some bones from early amphibians to mammals in the above account, then that is strong evidence.

What is unreliable is synonomy of name, meaning that two identically named bones from two different animals need not be homologues of one another. Presley (1983) makes this point with characteristic clarity. Function is an unreliable guide: the old example of the bat's wing and the bird's wing being functional but not structural homologues springs to mind.

EMBRYOLOGICAL DEVELOPMENT OF THE MAMMALIAN SKULL

The bones of the skull develop from primitive or early connective tissue. Some develop via a cartilaginous phase, others proceed direct to bone. The mesenchymatous tissue arises from two sources: the neural crest and the paraxial mesoderm. The mesenchyme from which the occipital bone develops arises from three or four conventional somites. That bone can arise from the neural crest tissue was a revelation in the mid-20th century, and there is still argument about the exact origin of some parts of some bones of the skull (Hall, 2008).

The skull can be divided into the neurocranium and the viscerocranium. The part of the neurocranium derived from cartilage is referred to as the chondrocranium, and develops from a group of cartilages that form in the floor of the developing skull around, and in front, of the notochord. The anterior end of the notochord is an important landmark. In front of it all cartilage seems to arise from the neural crest, but alongside the notochord paraxial mesoderm seems to be 'induced' by the notochord to form cartilage. The generally accepted grouping of cartilages is shown in Fig. 5.5.

The two parachordal cartilages that lie alongside the notochord expand, become confluent, and form a basal plate enclosing the notochord. At their posterior end they meet the cartilage developing from the occipital somites. This meeting becomes the articulation between the basisphenoid and basioccipital bones. As the occipital somites develop into cartilage and fuse with one another, they embrace the hypoglossal nerve, which leaves the skull via the hypoglossal foramen in the occipital bone. This inclusion of cranial nerves is an important feature of the development of the skull. To the sides of the parachordal cartilages, the developing otic (cartilaginous) capsules surround the developing inner ear.

Anterior to the notochord, there is a pair of cartilages called the trabecular cartilages. These develop from neural crest tissue and may represent a pair of visceral arch cartilages, which in the far distant past lay anterior to what we now recognise as the first or mandibular visceral arch. Each visceral arch is associated with a cranial nerve,

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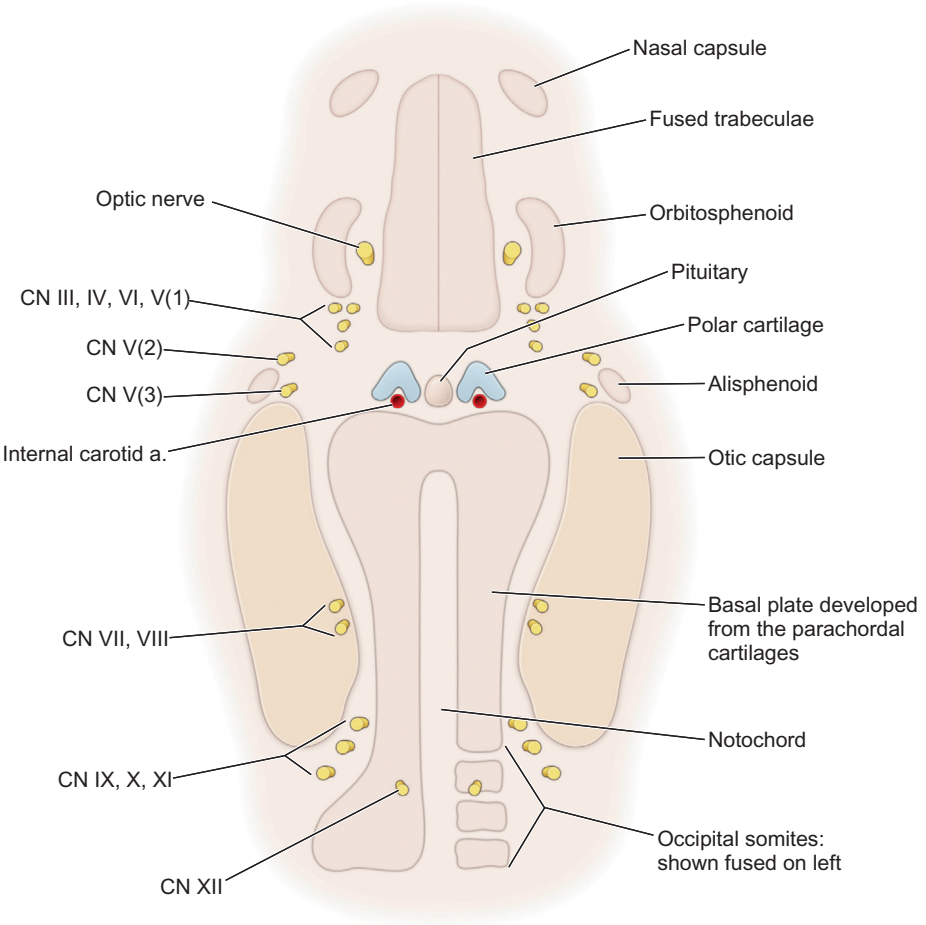


FIGURE 5.5 Diagram of the developing mammalian chondrocranium.

and this most anterior of the arches seems to be associated with a part of the ophthalmic division of the trigeminal nerve (see Chapter 20: The Brain and Spinal Cord). The trabecular cartilages give rise to the presphenoid bone: we shall meet this bone when we examine the rat skull.

To the sides of the space between the parachordal and trabecular cartilages, there are a number of small, apparently isolated, cartilages: the polar, alisphenoid and orbitosphenoid cartilages. The polar cartilages fuse with the trabeculi and parachordal cartilages to form the cartilage (and later bone) that surrounds the pituitary gland. The alisphenoid cartilages also fuse with the basisphenoid. This is a complex process with the gaps between the presphenoid, alisphenoid and basisphenoid cartilages, all enclosing cranial nerves. The alisphenoid cartilages contribute to the greater wing of the sphenoid bone (called the alisphenoid in rat anatomy), but part of that bone may be of dermal origin. In man, the orbitosphenoid cartilages also contribute to the sphenoid bone: as the lesser wings of the sphenoid. The fusion of presphenoid and orbitosphenoids is sometimes referred to as the anterior sphenoid bone. The posterior sphenoid comprises the basisphenoid and the alisphenoids. Rather similar to the otic capsule, the olfactory or nasal capsules form to the sides of the front ends of the trabeculi giving rise to the ethmoid bones. Here, we have the rudiments of the base of the skull, a series of bones running from the occipital to the basisphenoid, and on to the presphenoid and ethmoid.

We have mentioned a number of cranial nerves in relation to the developing bones of the base of the skull, Table 5.1 summarises the details.

We have mentioned the anterior and posterior lacerated foramina, but there is also a middle lacerated foramen (the foramen lacerum of human anatomy). This lies between the posterior corner of the greater wing of sphenoid, the basioccipital bone, and the petrous temporal bone that develops from the otic capsule. In man, the lower end of the foramen lacerum is closed by cartilage, but the carotid canal, which carries the internal carotid artery, runs into the foramen about half way up its posterior wall, turns upwards and enters the skull alongside the basisphenoid.

These are the bones of the base of the skull, to which we can add the dermal bones of the roof of the skull. The

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TABLE 5.1 Relationships Between Cranial Nerves and the Developing Skull

Cranial Nerve	Route in Embryo	Foramina
I: Olfactory	Through cartilage forming the ethmoid bone	The many foramina of the cribriform plate of the ethmoid bone
II: Optic	Between two processes of the orbitosphenoid cartilage which fuse with the remainder of the sphenoid	Optic foramen passing through the lesser wing of the sphenoid
III, IV, VI and V(1): Oculomotor, abducens, trochlear and the ophthalmic division of the trigeminal	Between the orbitosphenoid and alisphenoid cartilages	The superior orbital fissure also called the anterior lacerated foramen
V(2): Maxillary division of the trigeminal nerve	Originally as for V(1) but becomes separated from V(1) by a bar of bone	Foramen rotundum in the greater wing of sphenoid, combined with the anterior lacerated foramen in the rat to form the orbitorotundum foramen
V(3): Mandibular division of the trigeminal nerve	Between the alisphenoid and the otic capsule, comes to be surrounded by the alisphenoid	Foramen ovale in the greater wing of the sphenoid
VII and VIII: Facial and auditory nerves	VIII enters the otic capsule to innervate the organs of hearing and balance, the facial nerve passes through the otic capsule to reach the exterior	Facial nerve emerges via the stylomastoid foramen
IX, X, XI: Glossopharyngeal, vagus and spinal accessory nerves	Via a gap between the otic capsule and the developing occipital bone	Posterior lacerated foramen or jugular foramen in human anatomy
XII: Hypoglossal nerve	Surrounded by the developing cartilage of the occipital bone	Hypoglossal canal

nasal, frontal and parietal bones are still present in line, as they were in the early amphibians. To these, we should add the small lacrimal bones and the squamous temporal bone. The latter is a dermal bone that fuses with bone (either the periotic or petrous temporal bone) derived from the otic capsule to form the temporal bone. The squamous temporal bone is not one of the 'bones of the roof of the skull', but is derived from the dermal bones of the viscerocranium (see below).

The temporal bone is a little more complicated still. The ear canal (the external auditory meatus) is formed in part by the tympanic plate, which is derived in evolutionary terms from the angular bone of the lower jaw. This is the bone that inflates, so to speak, to form the tympanic bulla of the rat. The bones of the roof of the skull are joined one to another by fibrous joints (sutures). The occipital and basisphenoid bones are joined by secondary cartilaginous joints allowing growth to occur. In man the joint eventually ossifies to form a synostosis (see Chapter 3: Introduction to the Skeleton: Bone, Cartilage and Joints).

The viscerocranium of the adult is formed almost entirely from dermal bone. The bones formed in this way are the mandible, premaxilla, maxilla, the palatine, the vomer, the pterygoid bones (that fuse with the sphenoid to produce the pterygoid process with its internal and external medial and lateral plate) the zygomatic and the squamous temporal bones. The maxillary and mandibular cartilages of the viscerocranium appear in embryo, but are replaced by dermal bones, leaving only the incus and malleus, both formed in cartilage.

THE SKULL OF THE ADULT RAT

The skull of the rat is long in comparison with its breadth, and low from base to roof: a relationship that can be clearly seen in dorsal and lateral views of the skull. The small size of the rat means it does not lend itself to a general study of the anatomy of the mammalian skull, as many of the bones comprising the skull are very small and difficult to isolate and handle. We have found no detailed descriptions of, for example the palatine or sphenoid bones of the rat that compare with those available for the same bones in man or in the larger domestic animals. Such descriptions may exist in the older German literature, but we have been unable to pursue these sources. Although the bones of the rat skull are held together by sutures, distinguishing the suture lines is difficult. We

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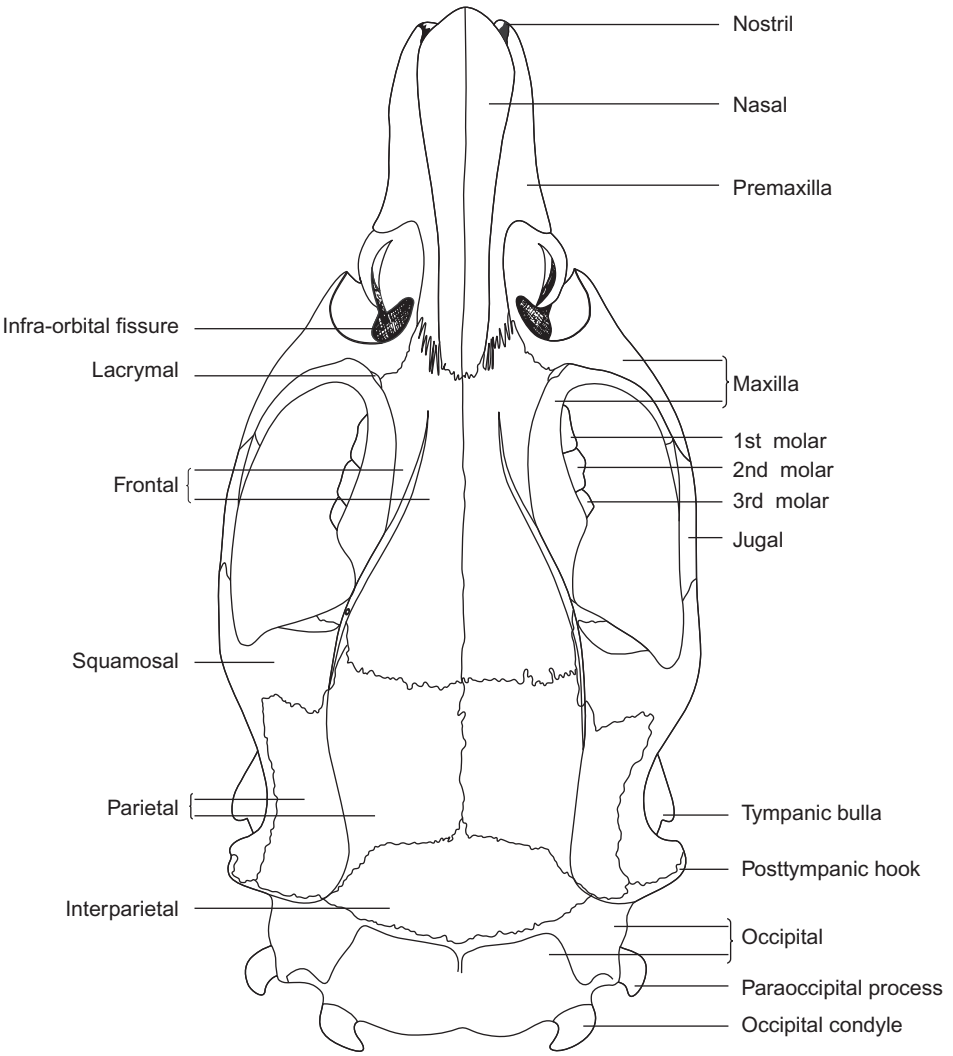


FIGURE 5.6 Skull (dorsal aspect).

shall say very little of the individual bones of the rat skull, but rather focus on the whole skull as viewed from a series of angles. When individual bones are mentioned for the first time a bracket (EC) and (IM) standing for endochondral or intra-membranous ossification, will be added.

Dorsal Surface of the Skull

The dorsal view of the skull (Fig. 5.6) presents few difficulties. Beginning at the anterior end and working back along the midline we may note on each side, the nasal (IM), the premaxilla (IM), to the side the maxilla (IM); then the frontal (IM) and parietal bones (IM) with the interparietal (IM) and occipital bone (EC) at the posterior end. The maxilla is the largest bone of the face, its upper surface is referred to as part of the rostrum of the skull. It can be seen that the maxilla is partially divided by the infra-orbital fissure that is wide in its upper part, but tapers towards the lower end. The anteorbital bar of the maxilla roofs the infra-orbital foramen, which is proportionately much larger in the rat than in man or the dog. The maxilla flares laterally forming the zygomatic plate or root of the zygomatic process. The zygomatic process of the maxilla sweeps back to form the anterior section of the zygomatic arch. The almost horizontal zygomatic plate is a notable feature of rodents such as the rat.

The frontal bone articulates with the nasal bone, the premaxilla and maxilla. The frontal bones roof the posterior part of the nasal cavity and the anterior part of the cranial cavity. Each fontal bone is divided by a prominent frontal crest, which divides the surface into curved superior part that roofs the skull, and an almost vertical lateral part that forms part of the side wall of the skull and thus of the medial wall of the orbit. The frontal crest also runs back to the next in the series of roof bones and the parietal bone.

Having mentioned the orbit, we should note that the orbit and the temporal fossa run into one another (see Fig. 5.7 lateral view). In man the orbit is only open at the

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front, but the arrangement in the rat allows the eyes to look laterally as well as forwards.

The cruciform arrangement of sutures between the frontal and parietal bones define a point called bregma, which is important because of its value as a landmark in stereotaxic work. The origin of the word 'bregma' is obscure: Wood-Jones (1946) noted that it was derived from the Greek meaning wet, and argued that this referred to the anterior fontanelle (the gap between the bones in the baby) which remained soft (or wet) longer than other parts of the skull (or perhaps it refers to the area made wet during a christening). Posterior to the parietal bones lies the single interparietal bone (IM). In man, this bone usually fuses with the occipital bone, but in the rat it remains separate. It articulates with the occipital bone at a sharp ridge, the superior nuchal line or crest, below which the muscles of the dorsal aspect of the neck are attached. The occipital bone is nearly vertical in the rat, and projecting from it are the occipital condyles that articulate with the first cervical vertebra, the atlas. At the sides the paraoccipital or exoccipital processes project outwards, and a little further forwards the hook at the posterior end of the squamous temporal or squamosal bone (IM) meets the periodic or petrous temporal bone.

Lateral Surface of the Skull

The premaxilla carries the incisors, the roots of which extend far back into the bone all the way to the maxilla. The lateral edges of the nasal bone can be seen above the premaxilla and the zygomatic plate and anteorbital bar of the maxilla are obvious.

The frontal bone provides much of the anterior part of the medial wall of the orbitotemporal fossa, and in man provides most of the roof of the orbit. At the upper, anterior corner of the fossa is the very small lacrimal bone. The squamous temporal bone lies posterior to it, with the parietal bone above and behind. The squamous temporal bone overlaps a good deal of the parietal bone. The area occupied by the squamous temporal bone is larger on the outside of the skull than on the inside.

The optic foramen is the most anterior of the larger foramina of the fossa and can be used as a landmark. In the rat the optic foramina from each side are linked together. In man, the optic foramen is surrounded by the orbitosphenoid, the roots of the lesser wing of sphenoid, (in Fig. 5.7, this bone is labelled presphenoid). The reason for this may be that the orbitosphenoid and the presphenoid are fused, and the nomenclature is perhaps arbitrary. In man, the optic foramen transmits the ophthalmic artery as well as the optic nerve. This artery arises inside the skull as a branch of the internal carotid artery, but in the rat the ophthalmic artery arises from the palatine branch of the pterygopalatine artery, finding its way into the fossa via the alisphenoid canal. The anterior end of the canal can be seen in the lower posterior corner of the fossa, but the entrance to the canal cannot easily be seen as it is hidden in the foramen ovale. Just anterior to and a

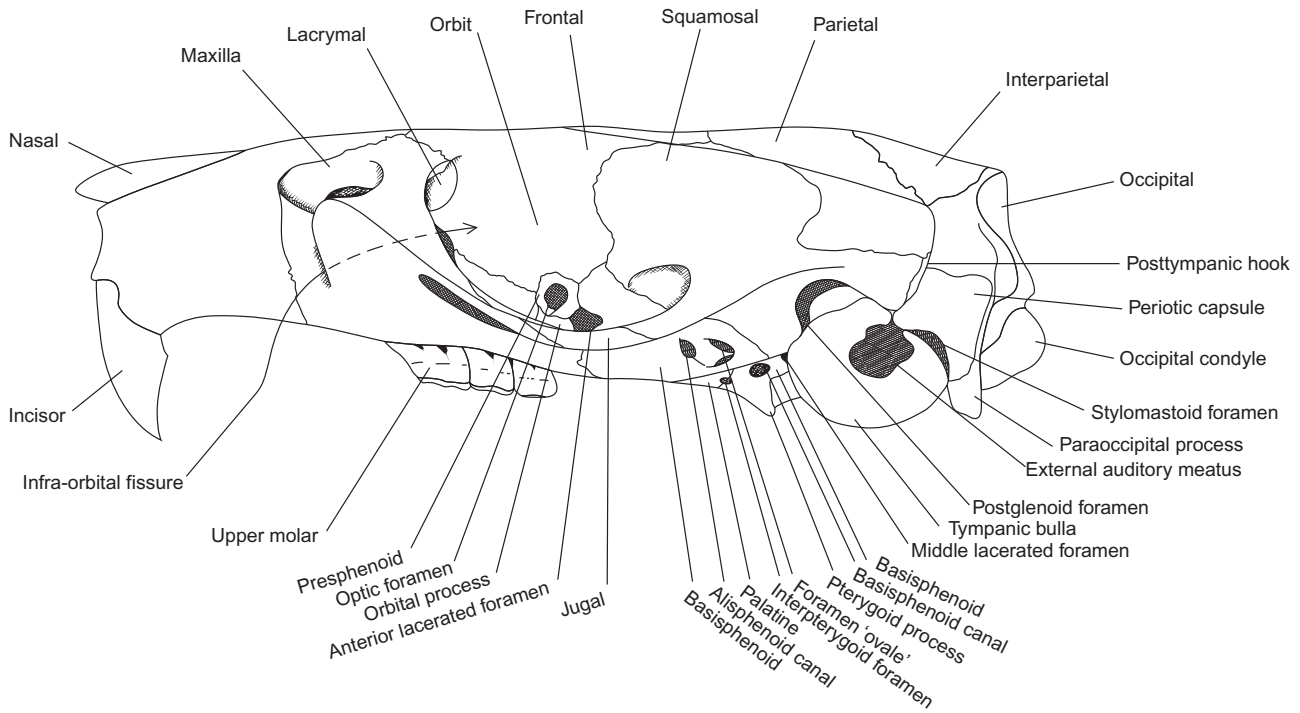


FIGURE 5.7 Skull (lateral aspect).

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little below the optic foramen lies the sphenopalatine foramen, connecting the fossa with the nasal cavity.

In the rat, the alisphenoid fails to fuse completely with the presphenoid, leaving a pair of gaps, one on each side. These gaps define the anterior lacerated foramen (superior orbital fissure in human anatomy) which lies just behind and below the optic foramen. The superior orbital fissure is the human equivalent of this foramen, and transmits the oculomotor, trochlear and abducens nerves (cranial III, IV and VI) that supply the extrinsic muscles of the eyeball. The foramen rotundum in the rat is confluent with the anterior lacerated foramen, a combination sometimes known as the foramen orbitorotundum. In man, the foramen rotundum presents a puzzle to medical students as it is fairly easy to find inside the skull, but its appearance on the outside is not because it emerges in the pterygopalatine fossa, a concealed region of the skull that is hidden below the floor of the orbit by the maxilla and the lateral pterygoid plate. In man, the pterygoid canal also emerges in the pterygopalatine fossa having tunnelled through the basisphenoid from a starting point in the wall of the foramen lacerum. This obscure little canal conveys the pterygoid nerve carrying preganglionic parasympathetic fibres from the facial nerve to the sphenopalatine ganglion. Enthusiasts who have got this far in this chapter may wish to note that the nerve of the pterygoid canal is actually a combination of the greater (superficial) petrosal nerve (carrying preganglionic parasympathetic fibres), and the deep petrosal nerve that carries sympathetic fibres from the plexus around the internal carotid artery. In the rat, the pterygoid canal emerges through a tiny orifice in the lower posterior corner of the orbitotemporal fossa. The pterygopalatine fossa of man is represented in the rat by a mere groove in the lower posterior corner of the orbitotemporal fossa, and it is here that we should expect to find the sphenopalatine ganglion. Before leaving the orbitotemporal fossa, note that the alisphenoid bone forms part of the medial wall of the fossa, and that the alisphenoid canal emerges from this bone.

The zygomatic arch sweeps laterally and backwards, and includes the jugal or zygomatic bone in its midsection. In man, the zygomatic bone provides much of the lateral wall of the orbit, and sends back a process that meets the forward running zygomatic process of the squamous temporal bone. Following the zygomatic arch back, it ends in a crest attached to the tympanic bulla. Above and behind the tympanic bulla, lies the periotic or petrous temporal bone, which fuses with the squamous temporal or squamosal bone. The posterior end of the squamous temporal bone forms the post-tympanic hook. The external auditory meatus is surrounded by the tympanic bulla, and just posterior to the meatus is the stylomastoid foramen, through which the facial nerve leaves the middle ear cavity. This foramen is named for the styloid process (a derivative of the second visceral arch) that is prominent in man but absent in the rat. The parietal bone and half of the interparietal bone can be seen above the posterior end of the squamous temporal bone. Finally, the occipital bone can be seen.

The diagram also shows the interpterygoid foramen and the basisphenoid canal, which will also be considered when describing the base of the skull. Although the foramen ovale is visible in lateral view, it really belongs to the base of the skull so will also be discussed there.

Inferior Surface of the Skull

This is perhaps the ‘least easy’ of the external surfaces of the skull: Fig. 5.8. In addition, it is fair to say in advance that the descriptions and diagrams provided in standard accounts (Rowett, 1960; Greene, 1935; Hebel and Stromberg, 1976) do not agree on all points.

Beginning at the incisors, the large premaxilla can be identified on each side, with the suture between the premaxilla and maxilla clearly visible. Adjacent to the midline the two large anterior palatine foramina are obvious. The front of the hard palate comprises the palatine processes, the premaxillae and maxillae, and behind the horizontal palatine processes of the palatine bones. The posterior nares are bounded by the palatine bones below, and the presphenoid above (EC). The palatine bone is a notoriously ‘difficult’ bone for students of human anatomy because so much of the bone is hidden by the maxilla. It forms part of the lateral wall of the nasal passages (nasopharyngeal duct in the rat), but this aspect of the bone cannot be seen from the exterior of the skull because of the overlap by the maxilla. In man it can be seen by looking into the anterior or posterior nares of the skull. In man, the posterior end of the palatine bone intervenes between the maxilla and the pterygoid process of the sphenoid, with the pterygoid processes appearing as an upside down flying buttresses to the maxilla. In mammals where the face lies in line with the base of the skull, the arrangement is different. Fig. 5.8 shows the palatine bones extending on each side back beyond the posterior nares. The outer aspect of the palatine bone is overlapped in front by the maxilla, and behind by the alisphenoid, and very little appears in the orbitotemporal fossa. In man a small part of the palatine bone appears at the very back of the floor of the orbit where it articulates with the maxilla (forming the floor of the orbit), the ethmoid bone (forming the medial wall of the orbit) and the sphenoid. In man, the palatine bone has a horizontal plate in the palate, a vertical plate that provides part of the lateral wall of the nasal cavity, and two processes arising from the top of the vertical plate. These are the orbital and sphenoid processes, both of which come into contact with the sphenoid bone and surround the sphenopalatine foramen. We have

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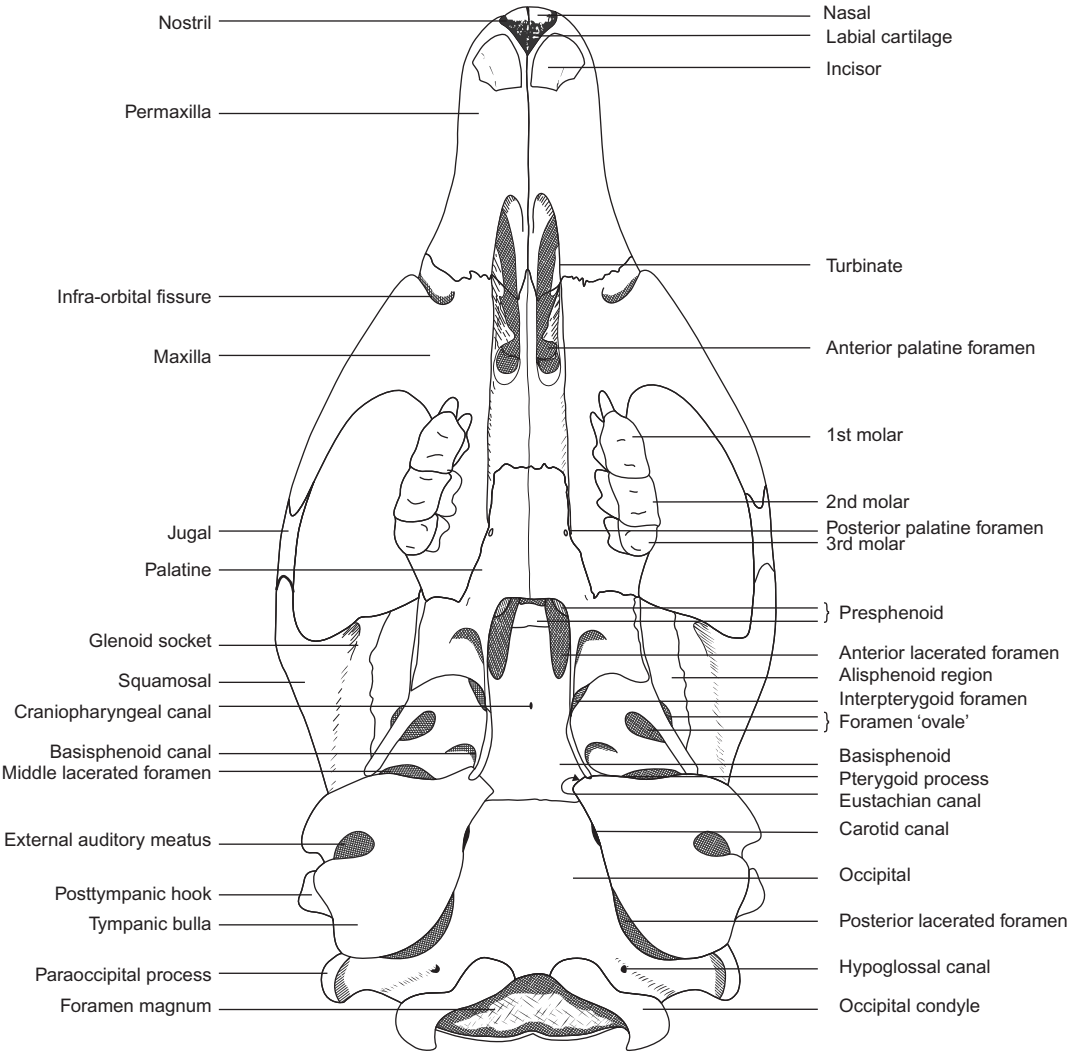


FIGURE 5.8 Skull (ventral aspect).

noted that the sphenopalatine foramen in the rat lies forwards in the orbitotemporal fossa: it is here that we should expect to find a bit of the palatine bone, but it is difficult to identify.

What of the pterygoid processes of the sphenoid? Hunt (1924) stated:

> *Two pairs of bony plates, the pterygoid processes, arise from the base of each alisphenoid bone. The outer plates are nearly horizontal in position. They extend from the posterior end of the maxillary bone caudad and laterad to the lateral surface of the tympanic bulla. The two inner pterygoid processes are vertical to the base of the cranium. Each expands posteriorly to the opening of the bony canal through which the Eustachian tube passes from the cavity of the middle ear (within the tympanic bone) to the pharynx.*

He continued:

> *Dorsal to the lateral pterygoid process is a depression into which the foramen ovale and two other foramina open. The alisphenoid expands, dorsal to the foramina, into a flat process which extends forward to the base of the zygomatic arch. This process runs posteriorly to the tympanic bulla but does not fuse with it. The process joins dorsally the squamosal and frontal bones. It forms a part of the posterior wall of the orbitotemporal fossa sending a process forward to the palatine bone and the alveolar process of the maxilla.*

Fig. 5.8 shows the internal (medial) pterygoid process clearly. The lateral pterygoid process is not labelled, but from Hunt's description we can see that it runs out from the lateral side of the medial process to the foramen ovale

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as a rather horizontal plate. Looking at Fig. 5.7, the alisphenoid can be seen to form an oblong bar of bone that reaches the squamosal, frontal and presphenoid bones. The diagram shows a small bone labelled 'orbital process', this is the orbital process of the palatine bone. Greene (1935) provided a diagram similar to Fig. 5.8. This shows the external pterygoid process sweeping back from the posterior corner of the maxilla to form a bar that passes below the foramen ovale (Rowett's diagram, Fig. 5.8, agrees with this).

The anatomy of the area just in front of the tympanic bulla is also complicated. Beginning at the anterior edge of the tympanic bulla, the petrotympanic fissure runs from close to the midline to the edge of the bulla. From its name, it would seem to lie between the petrous temporal (or perotic) bone and the tympanic bulla, but that is a little misleading. In man there is certainly an equivalent fissure between the tympanic plate and the squamous temporal bone, which is divided lengthwise into what we might call two subfissures in its medial part. The division of the fissure is caused by the intrusion of a tongue of belonging to the petrous temporal bone, which is an extension of the roof of the middle ear: the tegmen (roof) tympani. The anterior subfissure is the petro-squamous fissure and the posterior the petrotympanic fissure. In its lateral region the fissure remains undivided: the squamo-tympanic fissure.

Fig. 5.8 shows the middle lacerated foramen rather more laterally placed than it is usually shown, just lateral to the posterior tip of the internal pterygoid process or plate. All authorities agree that the foramen labelled basisphenoid canal in Fig. 5.8 exists. Hebel and Stromberg (1976) say that a branch of the pterygopalatine artery, which has emerged from the middle ear via the petrotympanic fissure, 'the pterygoid a. [artery] (a. Pterygoidea, Vidi's artery) turns medially to enter the basisphenoid canal, within which it runs to the roof of the nasopharynx'. In human, anatomy the pterygoid artery (Vidian artery) runs back through the pterygoid canal. It arises from the maxillary artery in the pterygopalatine fossa and runs in the opposite direction to the nerve of the pterygoid canal (the Vidian nerve), which runs forwards in the canal. This reverse of direction of the artery in man and the rat is plausible. The pterygopalatine artery of the rat, despite its complicated course via the middle ear, is the equivalent of the maxillary (internal maxillary) artery in man. In man, the pterygoid canal begins in the anterior wall of the foramen lacerum beneath the tubercle of the sphenoid bone, and ends by emerging from the anterior surface of the root of the pterygoid process in the posterior wall of the pterygopalatine fossa.

The anatomy of the pterygoid canal in the rat is more elusive. Hebel and Stromberg, in their drawing of the base of the skull, show what must surely be the posterior opening of the canal at the junction between the palatine and alisphenoid bones to the lateral side of the internal pterygoid process. The canal then goes forwards to emerge, as mentioned earlier, from the lower part of the medial wall of the orbitotemporal fossa. It is possible that adaptation of human anatomical terminology has led to confusion. The basisphenoid canal does not figure in descriptions of the human skull. The interpterygoid canal, which connects across the basisphenoid bone, is also labelled in Fig. 5.8. Hebel and Stromberg suggest that this is the same structure as the basisphenoid canal: 'At the middle of the body of its body the basisphenoid is perforated by a transverse canal (Greene, 1935 [a reprint of Greene, 1935]); basisphenoid canal (Rowett, 1960) which contains a branch of the internal carotid artery.' This seems to ignore the fact that Rowett labelled both the basisphenoid and interpterygoid canals, though in her table of structure passing through the foramina of the skull she omitted to include any structure for the interpterygoid canal. Rowett's Table of foramina is reproduced as Table 5.2.

Focussing on the midline, it can be seen that the occipital bone articulates with the basisphenoid, and that the basisphenoid articulates with the presphenoid. To the sides of the presphenoid, there is a gap separating the presphenoid from the alisphenoid, which represents the anterior lacerated foramen, but now seen from below. The craniopharyngeal canal is labelled as a point in the middle of the basisphenoid. Although this canal closes in man, it represents the embryological connection between the roof of the pharynx and the cavity on the upper surface of the basisphenoid, a vestige of the migratory route taken by the tissue that forms the anterior lobe of the pituitary gland, from Rathke's pit in the roof of the pharynx to its adult location.

The tympanic bulla is easily seen. On its medial aspect the large posterior lacerated foramen lies posterior to the much smaller opening of the carotid canal. Note the occipital bone, the elongated occipital condyles and the paraoccipital processes. The hypoglossal canals emerge lateral to the condyles, and lastly, note the foramen magnum encircled by the occipital bone, a foramen about which there is no doubt or argument.

Before leaving the inferior surface of the skull, probably with relief, note the elongated articular fossa, the glenoid fossa, on the lower surfaces of the squamous temporal bone. The articular surface of the condylar process of the mandible is elongated in an anterior–posterior direction and both rolls on and moves forwards and back on, the glenoid fossa. The reader may be wondering what has happened to the ethmoid bone and to the vomer: we shall return to these when we deal with the nasal cavity (see Chapter 10: Nasal Cavity).

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TABLE 5.2 Foramina Which Penetrate the Wall of the Cranium

Group I—Foramina Which Perforate the Wall of the Cranium

Foramen	Position	Structures Passing Through It
Foramen magnum	In occipital bone	Spinal cord
Olfactory foramina	Many in cribriform plate	Olfactory nerves
Optic foramen	In orbitosphenoid region of presphenoid	Optic nerve
Anterior lacerated foramen	Between the bodies of the basisphenoid and presphenoid and the palatine	Oculomotor, trochlear, abducens, nerves, and ophthalmic and maxillary branches of trigeminal nerve; palatine branch of internal carotid artery out of cranium
Foramen ovale	In alisphenoid region of basisphenoid	Mandibular branch of trigeminal nerve
Facial canal	In periotic	Facial nerve
Internal auditory meatus	In periotic	Auditory nerve
Posterior lacerated foramen	Between periotic and occipital	Glossopharyngeal, vagus and spinal accessory nerves; internal jugular vein
Hypoglossal canal	In occipital	Hypoglossal nerve
Postglenoid foramen	Between squamosal and periotic	Vein from transverse sinus
Cartoid canal	Between basioccipital and periotic with a deep groove in the tympanic	Main branch of internal carotid artery
Middle lacerated foramen	Between alisphenoid region of basisphenoid and tympanic	Pterygopalatine branch of internal carotid artery out of cranium
Alisphenoid canal	In alisphenoid region of basisphenoid	Palatine branch of internal carotid artery
Interpterygoid foramen	Between palatine and basisphenoid	

Group II—Foramina Which Do Not Perforate the Wall of the Cranium

Foramen	Position	Structures Passing Through It
Anterior palatine foramen	Between premaxilla and maxilla in the palate	Nasopalatine branch of trigeminal nerve
Posterior palatine foramen	In palatine	Palatine branch of trigeminal nerve
Infra-orbital fissure	In maxilla in front of orbit	Maxillary branch of trigeminal nerve and the anterior deep branch of the masseter muscle
Stylomastoid foramen	Between periotic and tympanic	Facial nerve from bulla
Posterior petrotympanic foramen	Between periotic and tympanic close to posterior lacerated foramen	Pterygopalatine branch of internal carotid artery into bulla
Petrotympanic fissure	Between periotic and tympanic	Pterygopalatine branch of internal carotid artery through bulla into cranium
Basisphenoid canal	In basisphenoid connecting with corresponding foramen of Other side within the bone	Pterygoid branch of internal carotid artery
Pterygopalatine foramen	In basisphenoid from basisphenoid canal to wall of anterior lacerated foramen	Pterygoid branch of internal carotid artery
Lacrimal groove	In lacrimal	Nasolacrimal duct
Eustachain canal	In tympanic	Eustachian tube
External auditory meatus	In tympanic	Outer ear passage
Mandibular foramen	In inner side of lower jaw	Mandibular branch of trigeminal nerve and mandibular blood-vessels
Mental foramen	In outer side of lower jaw	Mental nerve

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The Cranial Cavity

Removal of the roof of the skull and the brain reveals the upper surface of the floor of the cranial cavity. From anterior to posterior, the floor can be divided into the olfactory, cerebral and cerebellar fossae. The lesser wing of the sphenoid bone (the orbitosphenoid) and the presphenoid form the posterior edge of the olfactory fossa, and the orbitosphenoid and frontal bones form the floor of the olfactory fossa, which is closed at its anterior end by the sloping cribriform plate of the elusive ethmoid bone.

The rat is a macrosmatic animal that is heavily dependent on its sense of smell, which accounts for the large size of the olfactory bulbs that take up much of the olfactory fossa. The fibres of the olfactory nerve run through the holes in the cribriform plate (cribriform means sieve) to the olfactory bulbs, and from the bulbs the olfactory tracts (peduncles) run back to olfactory lobes of the brain (see Chapter 2: Introduction to Anatomical Terminology).

The cerebral fossa is floored by the basisphenoid running forwards to the presphenoid in the midline, and the alisphenoids that extend laterally from the basisphenoid to meet the squamous temporal (squamosal) and frontal bones, and posteriorly to meet the petrous temporal (periotic) bones. Between the alisphenoid and the presphenoid the orbital fissure (orbitorotundum foramen) runs more or less lengthwise in the medial wall of the orbitotemporal fossa in the rat. The orbital foramen lies close to the midline (the left and right orbital foramina are confluent in the rat). The foramen ovale lies well out from the midline in the alisphenoid, and behind it the petrotympanic fissure lies just at the anterior edge of the petrous temporal (periotic) bone. The pituitary gland (hypophysis) lies on the upper surface of the basosphenoid in a very shallow depression, unlike the deep sella turcica (Turkish saddle) of man. The pituitary gland sits further back in the rat than one might expect, beneath the pons and above the junction of the basisphenoid and occipital bones (see Chapter 21: The Autonomic Nervous System).

In man, the posterior cranial fossa is partly roofed by the tentorium cerebelli, a sheet of dura mater that is incomplete anteriorly, allowing the midbrain to pass through a notch at the front of the tentorium. In the rat the tentorium may be partly ossified and lies more or less vertically, as one would expect from the orientation of the long axis of the skull. The surface of the petrous temporal bone that forms part of the cerebellar fossa is a complex shape and forms a socket, the floccular fossa, which encloses the floccular lobe of the cerebellum. The walls of the socket must be nibbled away if the cerebellum is to be removed intact. The hypoglossal canals and the foramen magnum run from the cerebellar fossa to the exterior.

The Lower Jaw

The lower jaw or mandible of man is a single bone that arises from left and right halves that fuse at the mental symphysis see Figs. 5.9 and 5.10. In the rat the bones remain separate, so we can refer to the left and right mandibles. In man each half of the mandible comprises a roughly horizontal body that carries the teeth and a ramus (branch) set at an angle to the body. The ramus bears the coronoid process anteriorly, and the condyloid or articular process posteriorly. The angle of the mandible is at the lower posterior corner of the L shaped bone. Most of these terms are used when describing the rat mandible. The large incisor tooth extends far back into the mandible beyond the last molar tooth. Posterior to the incisor is a gap (the diastema) that is notably shorter in the lower jaw than in the upper. The diastema of the lower jaw is concave, and there is a sharp 'step up' to the plane of the molar teeth. Each mandible has one incisor that grows continuously, three molar teeth, but no premolar teeth or canines, giving a dental formula of I 1/1; C 0/0; PM 0/0; M 3/3.

The ramus is broad and runs back to the angle that lies in the same vertical plane as the incisor and its socket, the

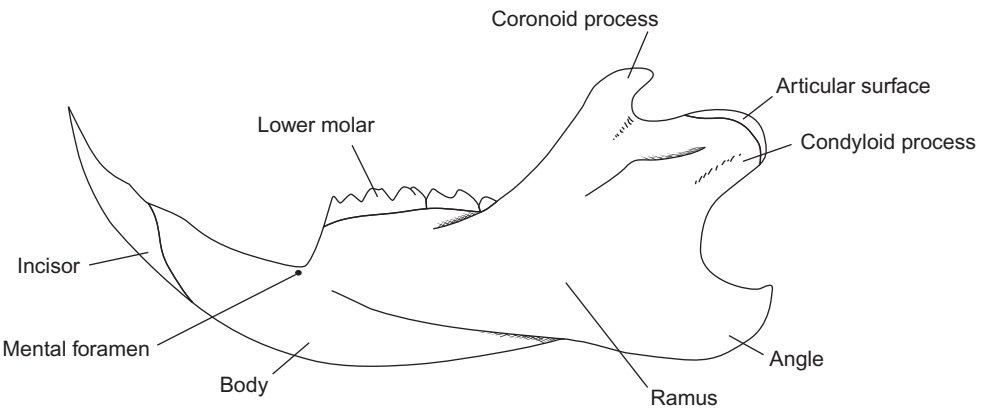


FIGURE 5.9 Lower jaw (lateral aspect).

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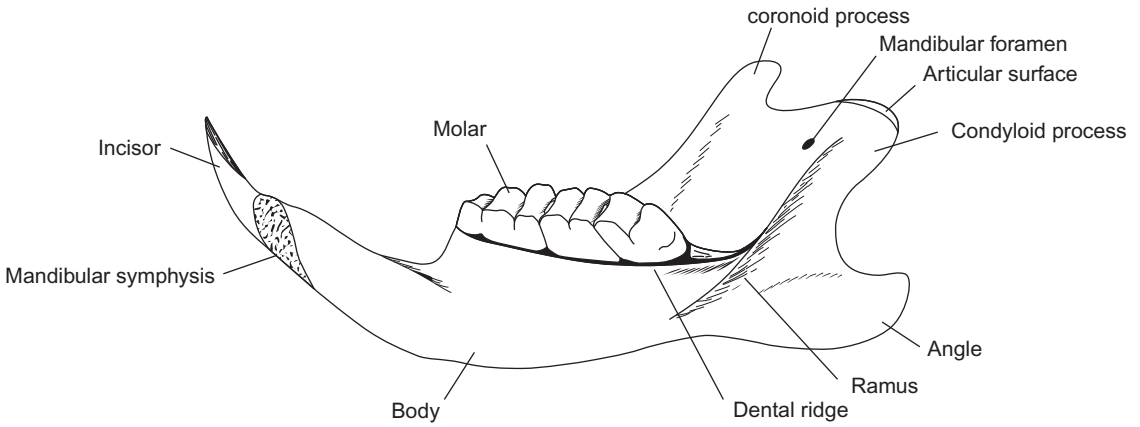


FIGURE 5.10 Lower jaw (medial aspect of one mandible).

defining characteristic of the sciurognathous rodents, which includes rats and mice. In the hystricognathous rodents, a group that includes guinea pigs (see Chapter 1: The Rat's Place in Nature and Chapter 6: The Musculature of the Rat) the angle flares out laterally. On the lateral surface of the mandible, the clearly defined horizontal line of the masseteric ridge can be seen running forwards from the angle. The mental foramen is found at the anterior end of the ridge beneath the posterior end of the diastema. A notch separates the angle from the condyloid process that bears the elongated articular surface. The coronoid process, to which is attached the temporal muscle, is much less well developed than in man, as the large masseter muscles of the rat have taken over much of the work of the temporal muscles. Examination of the medial surface of the mandible reveals a foramen at the base of the condyloid process, this is the alveolar or mandibular foramen through which passes the inferior alveolar nerve, a branch of the mandibular division of the trigeminal nerve (V3). The medial surface of the angle is concave and forms a marked depression.

ABBREVIATIONS USED IN FIGURES

bart basal articulation of braincase and palate
bo basioccipital
bs basisphenoid
con condyle
e epipterygoid
ec ectopterygoid
en external naris
eo exoccipital
eth ethmoid region
f frontal
fo fenestra ovalis
ic foramen for internal carotid artery
in internal naris
iptv inter-pterygoid vacuity
it intertemporal
j jugal
l lacrimal
m maxilla
n nasal
nc nasal capsule
on otic notch
op opisthotic
or orbit
p parietal
paf parietal foramen
pf postfrontal
pl palatine
pm premaxilla
po postorbital
pp postparietal
pr prootic
prf prefrontal
ps parasphenoid
prs presphenoid
pt pterygoid
ptf posttemporal fenestra
pv pituitary vein
q quadrate
qj quadrojugal
sm septo-maxilla
soc supraoccipital
sph sphenethmoid
sq squamosal
st supratemporal
stf subtemporal fossa
t tabular
v vomer

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Chapter 6

The Musculature of the Rat

INTRODUCTION

The arrangement of the skeletal muscles of the rat is not a subject of great interest to toxicologists. However, no account of the anatomy of the rat would be complete without a survey of this area. Toxicologists are of course interested in the effects of chemicals on muscle. A wide range of chemicals, including anaesthetics, narcotics, cholesterol-lowering drugs (statins), herbicides and insecticides, have been shown to have such effects. The clinical effects of such chemicals depend in part on the muscle groups affected, some knowledge of muscle groups, their function and their innervation, is therefore important. We have not attempted a muscle by muscle account as good accounts of this type are provided by Greene (1935) and Hebel and Stromberg (1976). Chiasson (1975) has also provided a well-illustrated account that lists all the major muscles giving details of their origins and insertions.

Muscular tissue can be divided into three histological types. Smooth or unstriated muscle is found in the walls of the gut, respiratory system and blood vessels; skeletal or striated muscle consists of the muscles attached to the skeleton (the flesh of the body) and cardiac muscle, which is found in the heart (as expected) and in some larger pulmonary vessels. In this chapter we deal only with skeletal muscle. The fundamental characteristic of all muscle cells is that they can contract or shorten, and thus do work. A short note on the histology of skeletal muscle is appended to this chapter.

EMBRYOLOGY OF SKELETAL MUSCLES

All skeletal muscle tissue is derived from mesoderm. The mesoderm closest to the sides of the axis of the embryo is defined as the paraxial mesoderm. Lateral to this is the intermediate mesoderm, and further lateral again, is the lateral plate mesoderm. Cavities develop in the lateral plate mesoderm and become confluent, leading to the formation of the intra-embryonic coelom. Formation of the intra-embryonic coelom splits the lateral plate mesoderm into an upper somatopleure (lies against the ectoderm) and a lower splanchnopleure (lies against the endoderm). The paraxial mesoderm now begins to form somitomeres, areas of condensation of the mesoderm that form in series along the axis of the embryo on both sides of the notochord, and recently formed neural tube. Once the somitomeres are well-defined they are recognised as somites, which define control the segmentation of the embryo.

Somites first appear towards the anterior end of the embryo, and then in sequence along the longitudinal axis. In man 42−44 pairs of somites appear. The somites cause the developing nervous system to become segmented creating a series of neuromeres (condensations of neural tissue) along the axis of the developing nervous system. The somites can be imagined as little blocks of solid mesoderm, but they are not solid for long, and soon develop small cavities (one per somite) called myocoeles, which also disappear quickly. Not all somitomeres form somites. At the anterior end of the embryo, the first seven somitomeres do not.

The somites are the key to the development of the skeletal and muscular systems. Each somite differentiates to form a sclerotome (‘sclero’ means hard, ‘tome’ means to cut: the sclerotome is hard to cut), myotome (myo means muscle) and a dermatome (derm = -skin). The sclerotome migrates around the notochord and the spinal cord (notochord, spinal cord) to form the vertebrae, ribs and sternum. Cells of the myotome migrate into the somatopleure and give rise to the muscles of the back, flanks, abdominal wall and limbs. The dermatome gives rise to the dermis underlying the epidermis of the skin, while the epidermis itself is formed from ectoderm.

Further Development of the Myotome

The next stage of development involves the division and migration of the cells of the myotome. One group, the epimere, spreads dorsally forming a series of muscle blocks alongside the vertebrae that will become the long extensor muscles of the back. The remainder of the myotome, the hypomere, migrates ventrally around the body forming a series of layers running round the body from just beneath the territory of the epimere almost as far as

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the ventral midline. Near the ventral midline blocks of muscle line up to form a series running forwards from the pelvis to throat. In the abdominal region, these form the rectus abdominis muscles (the 'six pack' seen in athletes). The lateral layers form the external and internal oblique muscles of the abdomen, and the external and internal intercostals muscles of the thorax. The exact origins of the intercostal muscles has long been debated. Romer (1970) argued that the deep layer of the external oblique forms the external intercostals, and that the internal oblique gave rise to the internal intercostals. Others have argued that the internal oblique layer gives rise to both sets of intercostals. The word oblique is important. The external oblique muscle layer runs obliquely towards the tail; the internal oblique layer runs obliquely forwards.

The segmental arrangement of the trunk muscles is reflected in their innervation. The spinal nerves leave the vertebral canal via the intervertebral foramina. They divide to form a posterior primary ramus (ramus means branch) supplying the longitudinal muscles of the back, and a ventral primary ramus that supplies the muscles of the remainder of the body wall.

The muscles of the limbs migrate into the limbs from the muscle of the lateral body wall. This is somewhat more complicated than it might appear at first sight as each limb muscle may receive contributions from a number of segments (ultimately a number of somites), as well as from the mesoderm within the developing limb. As a result most of these muscles have lost their segmentation, and so the innervation of the limb muscles is complex, with any one limb muscle likely to receive its innervation from a number of spinal segments.

This complication is the reason for the complex way in which spinal roots form brachial and lumbo-sacral plexuses to serve the limbs (see Chapter 21(2): Spinal Nerves).

THE ROTATION OF THE LIMBS

The arrangement of the muscles of the limbs is best understood by considering their evolution. In accordance with Haeckel's Recapitulation Theory (ontogeny reflects phylogeny – a theory much disparaged but still of some merit), we can see a pale reflection of the evolution of the limbs in their development.

If we go back to fish we can think of fins that are moved upwards and downwards by the dorsal and ventral muscles respectively. The dorsal muscles have evolved into the extensors of the limbs, the ventral muscles into the flexors. The primitive amphibian rested on its belly, the limbs extended out to the sides at about 90 degrees. Movement of the body was achieved by a sort of rowing action, with the limbs being moved forwards and backwards (protraction and retraction). No doubt, the limbs could be raised above the level of the trunk (adduction) or pressed to the ground (abduction). As these animals evolved the body became raised from the ground, and the limbs bent at what we can call the elbow and knee. However they still projected sideways from the body, and the weight of the body was slung between the legs. The sprawling stance of such creatures must have made rapid movement a fast waddle. More efficient movement was produced by pulling the limbs in under the body. The forelimb was turned back so that the first segment of the limb (the humerus) pointed back from its joint with the shoulder, and the hindlimb was turned forwards so that the first segment (the femur) pointed forwards. A moment's thought will show that the front limb now presented a difficulty, as the whole limb was now pointing in the wrong direction for forwards motion. However, if you bend the elbow and rotate the second segment (the forearm comprising the radius and ulna), the hand now lies palm down on the ground with the digits pointing forwards. The hindlimb presents no such difficult problem, because bending of the knee places the foot, toes forwards, sole to the ground and under the body.

The rotation of the forelimb just described is defined as lateral rotation, and the rotation of the hindlimb is as medial rotation. This can cause some confusion. If you were to lie face down on the floor with your arms stretched out at 90 degrees from your shoulders and your thumbs facing forwards, your arms would be in the position of the primitive amphibian forelimbs. From above the axis of your arm passes from the shoulder, down the arm, and through the middle finger with the thumb on what is reasonably described as the preaxial edge or surface of the arm. Now if you were to sweep your arms back towards your feet and bend your elbows you would find that your upper arm was tucked in beside your body, with your elbow a little in the air, and your little finger against your hip. Holding your upper arm firmly against your side, bend your elbow and flick your hand over so that it lies palm down and thumb in, and you have essentially modelled millennia of vertebrate evolution and your hand lies palm to the floor with the hand and forearm pronated.

Pronation of the hand, indeed of the bones of the forearm, is a characteristic of all four-footed mammals. Man and other primates have no difficulty in "unwinding" the bones of their forearms and their hands can be pronated or supinated at will. Rats and most habitual quadrupeds have only very limited powers of pronation and supination. Free rotation of the hand is invaluable in tree climbing and in manipulating objects with the hands. Rats certainly can manipulate objects with their forepaws but not as well as we can. Cats have even more limited powers of supination and pronation. Watch a cat with a

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mouse. The mouse can be flicked in the air with the paws, but the cat does not pick up the mouse and lift it to its mouth, on the contrary it lowers its mouth to bite the mouse.

TERMINOLOGY OF MOVEMENTS

Flexion and extension: typically described at hinge joints such as the elbow, flexion refers to the reduction of the angle at the joint and extension is the opening of the angle. Flexion is also defined as the approximation of volar surfaces. The volar surface of the arm includes the palmar surface of the fingers, the palm (volar means the hollow of the palm or foot), and the inner surface of the wrist, forearm and upper arm. Thus flexion at the elbow and knee approximates the volar surfaces of the arm and leg, respectively. At the hip flexion means the forwards movement of the leg (kicking a ball involves flexion at the hip) and extension means swinging the leg back as if getting ready to kick a ball.

Dorsi-Flexion and Plantar-Flexion: these are special terms used by human anatomists for the action that lifts the toes towards (dorsi) and moves them away (plantar) from the shin.

Abduction: movement away from the midline of the body, adduction is the converse. For instance, at the hip adduction means swinging the leg out sideways,

Protraction: swinging the limb forwards at the shoulder or hip, retraction is the converse, both of which are the essential movements when a four-footed animal runs.

Pronation: the turning of the hand so that the palm faces downwards, supination is the converse.

The shoulder and hip joints present special difficulties, because they are ball and socket joints, not hinge joints, so movement in a number of planes can occur. In human anatomy, flexion of the shoulder is defined as swinging the arm forwards and across the trunk, extension is the converse. Abduction means swinging the arm outwards from the body at 90 degrees to the plane of flexion and extension, adduction is the converse. Rotation can, of course, also occur at ball and socket joints.

TERMINOLOGY OF MUSCLES

Few aspects of anatomy are more likely to induce intellectual distress than the terminology applied to muscles. To make things worse, the terminology that has developed for human muscles tends to be applied to muscles in other vertebrate groups, despite the fact that exact evolutionary homologies between muscles may be difficult to define, or that the functions of the muscles may differ to some extent. Muscles are naming may be based on several different factors.

Shape:

Deltoid (shaped like a Greek Delta, Δ), trapezius (shaped like a trapezium), serratus anterior (shaped, in part, like the serrated edge of saw), piriformis (shaped like a pear), digastric (two bellies)

Origin and insertion:

Coracobrachialis (from the coracoid process of the scapula to the arm), sternocleidomastoid (from the clavicle and sternum to the mastoid process of the temporal bone)

Form:

Biceps (in two parts), triceps (in three parts), rectus femoris (a straight muscle on the front of the thigh), gracilis (a long thin, or gracile, muscle of the thigh)

Function as regards effects on a joint:

Flexor carpiulnaris (a muscle of the forearm that causes flexion of the wrist joint and which runs on the ulnar side of the forearm), extensor pollicis longus (the longer of two muscles that extend the thumb). Note the qualifications: if one function is served by both a long and a short muscle then the former is described as ". . .. . . . longus" and the short one as ". . .. . . brevis". Other qualifiers include external and internal.

Other functional descriptors:

Masseter (the chewing muscle), tensor tympani (a little muscle that tenses the ear drum or tympanic membrane).

Location:

Temporalis (the muscle of the temporal fossa of the skull)

By association:

Sartorius (the thigh muscle that helps us sit cross legged, like tailors who provide us with sartorial elegance).

HOW MUSCLES MOVE THE ELEMENTS OF THE SKELETON

All muscles act as contractile connections between two points drawing these points closer to one another. This is a rather mathematical or mechanical description, as muscles are often not attached to a point, but to a large area on the surface of a bone. Points of attachment are defined as the origin and insertion of the muscle, which can be rather arbitrary terms, although in general the origin is the more fixed of the two points.

By way of example consider the large muscles of the buttock, the gluteus muscle group. If the foot is raised from the ground, contraction of these extensors of the hip joint will cause the leg to swing backwards, but if the foot is firmly placed on the ground and the subject (human subject) is sitting down, then the gluteus muscles extend the hip joint, and the subject rises into a standing position. The hamstring muscles at the back of the thigh act in a similar

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way, providing the drive when a quadruped runs. Contraction, with the foot in contact with the ground, causes the pelvis to lift and the whole body to move forwards.

Muscles are often compared with levers. Archimedes' famous remark about being given 'a lever long enough and a fulcrum on which to place it, and I shall move the world' is well known. He might have added that he would need something firm to stand on. The point is that for a lever to work, the fulcrum must be fixed and so must the position of the operator. Imagine using a crow bar to lift a packing case full of bricks while standing on an ice-rink. Your feet would slip as you applied force to the lever. Fixing the origin of a muscle is very important to the function of skeletal muscles.

Muscles never work alone. The prime mover does the obvious work (e.g. the biceps in flexing the elbow), but its activity is accompanied by the activity of muscles acting as synergists, which often fix the position of the origin of the prime mover. When lifting a spoonful of soup to your mouth and drinking it from the spoon, the movement is of the forearm and hand. The shoulder joint is fixed by the short muscles around the shoulder providing a stable point for the origin of the biceps, and the elbow is held towards the side of the trunk. At the last moment, the hand is tipped by pronation of the forearm and the soup runs into the open mouth. Without the coordinated contraction and relaxation of a large number of the muscles of the arm, the upper arm would fly into the air when the biceps contracted. A complex business and one that takes some time to learn. Toddlers have to learn how to do this, and for a while eating jelly or cereal is a messy business.

The complexity of these movements suggests that movements of whole groups of muscles, rather than the activity of single muscles are represented in the brain, and that a good deal of feedback from the muscles and joints is needed to ensure a smooth movement.

Muscles are attached to bones in a variety of ways, often by a tendon that is firmly attached to the bone. The site of attachment may be raised producing a tubercle or tuberosity (again the terms are rather arbitrary) or may form a small pit. Alternatively the muscle may be attached to a large area of the bone without the aid of a tendon. In this case, the connective tissue sheath of the muscle (see Appendix on histology of muscle) blends with the superficial periosteum of the bone. Such attachments leave little in the way of marks on the bone.

A SPECIAL GROUP OF SKELETAL MUSCLES: THE SKELETAL MUSCLES DERIVED FROM THE PHARYNGEAL ARCHES

Vertebrates are characterised by the development of a series of pharyngeal arches and intervening slits that connect the pharynx with the exterior. In mammals the slits appear only as pouches of the pharynx during development, and never actually break through to the exterior. Each arch has an arterial supply, which in fish constitutes an arch running from the ventral aorta to the dorsal aorta, muscle tissue, skeletal components and innervation by a cranial nerve. Comparative anatomists used to revel in the details of the pharyngeal arches, tracing the components of the arches from fish to mammals is one of the triumphs of their subject. Though this is a complicated and difficult topic, it is quite impossible to understand the anatomy of the head and neck without it.

The series of pharyngeal arches suggests a link with the segmentation of the animal, although whether the arches actually have anything to do with segmentation, as defined by the series of somites, is debatable. But it is certainly true that in the developing head (including the neck in this context) the series of pharyngeal arches runs alongside a series of somitomeres, which run alongside a series of neuromeres within the developing nervous system. The correlation between the pharyngeal arches, the somitomeres and the neuromeres (or if we are considering the lower part of the brain, the rhombencephalon and the rhombomeres) has been worked out in detail and has been described by Larsen (1993).

Table 6.1 sets out the details of the derivatives of the pharyngeal arches. It should be noted that the muscles of the first arch provide the muscles of mastication (the chewing and gnawing muscles) and that those of the second arch provide the muscles of the face. The muscles of mastication require special consideration along with short account of the facial muscles controlling movement of the sensory vibrissae (see below).

A SECOND SPECIAL GROUP OF SKELETAL MUSCLES: THE MUSCLES THAT MOVE THE EYEBALL

The six extra-ocular muscles that move the eyeball are derived from the first three somitomeres mentioned earlier. All the extra-ocular muscles have the bones of the orbital wall as their origin, and are inserted into the sclera of the eyeball. These muscles are innervated by three cranial nerves: the oculomotor (CN III), the trochlear (CN IV) and the abducent (or abducens) (CN VI). There are four rectus muscles (superior, inferior, external and internal), which move the eye upwards, downwards, outwards and inwards respectively; and two oblique muscles, superior that moves the eye downwards and outwards, and the inferior that moves the eye upwards and inwards.

Remembering the innervation is easy if you remember that all the muscles are innervated by the oculomotor nerve (III) except ER6SO4. The bit of pseudo-chemistry

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TABLE 6.1 Derivatives of Pharyngeal Arches

Pharyngeal Arch	Artery of the Arch	Skeletal Derivatives	Muscles Derived From the Arch	Cranial Nerve Supply
1	Maxillary artery See chapter on the circulatory system for an account of the blood supply to the head in the rat	Incus, alisphenoid (from maxillary cartilage) malleus (from mandibular cartilage). These elements formed by endo-chondral ossification. All the cartilage is derived from the neural crest maxilla, zygomatic bone, squamous temporal bone, mandible (by direct ossification of mesenchyme): membrane bones	Masseter, temporalis, medial and lateral pterygoids, mylohyoid, anterior belly of digastric, tensor tympani, tensor veli palatini. All these are derived from somitomere 4	Maxillary and mandibular divisions of the trigeminal nerve: CN V
2	Stapedial artery in the embryo, corticotympanic artery (from the internal carotid) in the adult: a small artery	Stapes, styloid process (not found in the rat), Stylohyoid ligament, lesser horns and part of the body of the hyoid bone. All derived from cartilage: neural crest origin	Muscles of facial expression: derived from somitomere 6	Facial nerve. CN VII
3	Common carotid artery, root of internal carotid artery	Greater horns of hyoid and part of the body of the bone	Stylopharyngeus; derived from somitomere 7	Glossopharyngeal nerve. CN IX
4	Arch of aorta, right subclavian artery, original sprouts of pulmonary arteries	Laryngeal cartilages: derived from 4th arch cartilage which is derived from lateral plate mesoderm and not from the neural crest	Constrictors of the pharynx, levator palati: derived from occipital somite 2,3 & 4	Superior laryngeal branch of vagus nerve. CN X
6	Ductus arteriosus, roots of definitive pulmonary arteries	Laryngeal cartilages: derived from 6th arch cartilage: lat. plate mesoderm	Intrinsic muscles of larynx: from occipital somites 1 & 2	Recurrent laryngeal branch of vagus nerve. CN X

means: ER_6: external rectus, the muscle that abducts the eye is supplied by the abducent nerve (VI); SO_4: superior oblique, whose tendon runs through a pulley (Latin for pulley = trochlea), is innervated by the trochlear nerve (IV).

THE ARRANGEMENT OF THE MAJOR GROUPS OF SKELETAL MUSCLES

The Muscles of Mastication: Gnawing and Chewing

Rodents are characterised by their capacity to gnaw. The rat has a formidable set of muscles that control the movements of its jaw. The main muscles that move the lower jaw are: masseter, temporalis and the internal and external pterygoids, although the supra and infra-hyoid muscles also play a role. All the muscles of mastication have more than one function, and with the exception of the digastric and the external pterygoid, all can raise the mandible. The deep masseter for example raises the mandible and also everts (turns outwards) the lower edges of the mandible. This everting action is opposed by the internal pterygoid.

The 'power stroke' of chewing is one of protraction (forwards movement of the lower jaw) and medial movement, rather like a file being moved forwards and across a metal surface. The mandibular symphysis is also a point of mobility in the rat, as the junction between the left and right parts of the mandible is fibrous. In man, the left and right parts of the mandible are fused at the symphysis. Hiiemae (1967) studied the movements of the rat jaw by use of cine-photography and cine-radiography. The reader is referred to her work for a more detailed analysis that can be presented here. Hiiemae also contributed a series of more detailed papers on this subject (1971a,b,c). Further studies by Gans (1985), Weijs (1975), Satoh and Iwaku (2008) and Cox et al. (2012) provide detailed discussions of mastication. Perhaps, the most recent detailed and comparative account has been provided by Turnbull

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(1970): a 356 page monograph, 'Mammalian Masticatory Apparatus'.

Masseter

Masseter is a complex muscle that comprises three layers or parts. These layers have been given a bewildering number of names (Cox and Jeffery, 2011; Druzinsky et al., 2011). Here, we have adopted the terminology used by Cox and Jeffrey and take the three layers in turn, beginning with the deepest.

Zygomaticomandibularis

This muscle arises from the rostrum, runs back and down through the infraorbital foramen and fissure where it picks up fibres from the zygomatic arch before insertion onto the coronoid process and the lateral surface of the lateral crest of the mandible, just below the second and third molar teeth. The part arising from the rostrum, described as the antero-dorsal expansion of the muscle, is an expansion of modest size in the rat, but relatively large in the guinea pig. This distinguishes a myomorph (rat) from a hystricomorph (guinea pig) rodent. In the sciuromorphs, including the squirrels, there is no antero-dorsal expansion.

Deep Masseter

The deep masseter arises from the ventrolateral surface of the zygomatic arch and is inserted onto the masseteric ridge on the lateral surface of the mandible. In the rat, an anterior part takes its origin from the lateral side of the zygomatic plate and the adjacent rostrum (maxilla) as far forwards as the maxilla–premaxilla boundary, and a posterior part further back on the arch that inserts onto the angle of the mandible.

Superficial Masseter

The superficial masseter runs almost horizontally from anterior to posterior and protracts the mandible. The muscle originates from a small tubercle just below the infraorbital foramen and runs downwards and back over the lateral surface of the mandible before turning inwards around the lower edge of the bone and is inserting low down on its medial surface.

These parts of masseter are shown in Fig. 6.1.

Temporalis

The posterior part of the temporal muscle (also called the medial part of the temporal muscle) is a major retractor of the mandible. Temporalis is a fan-shaped superficial muscle that arises from an arc stretching from the fronto-parietal suture via the lambdoid crest to the temporal ridge and sweeps down to the zygomatic process of the temporal bone. The narrow handle of the fan is inserted via its tendon into a small area between the posterior molar tooth and the coronoid process. The muscle may be divided into medial and lateral components. The medial component arises from bone and the lateral arises from the fascia that covers the medial component. The temporalis muscle of the rat is not very different from that of man, though in man the muscle occupies all the temporal fossa as far forwards as the postero-lateral wall of the orbit. Although temporalis has been divided into two parts

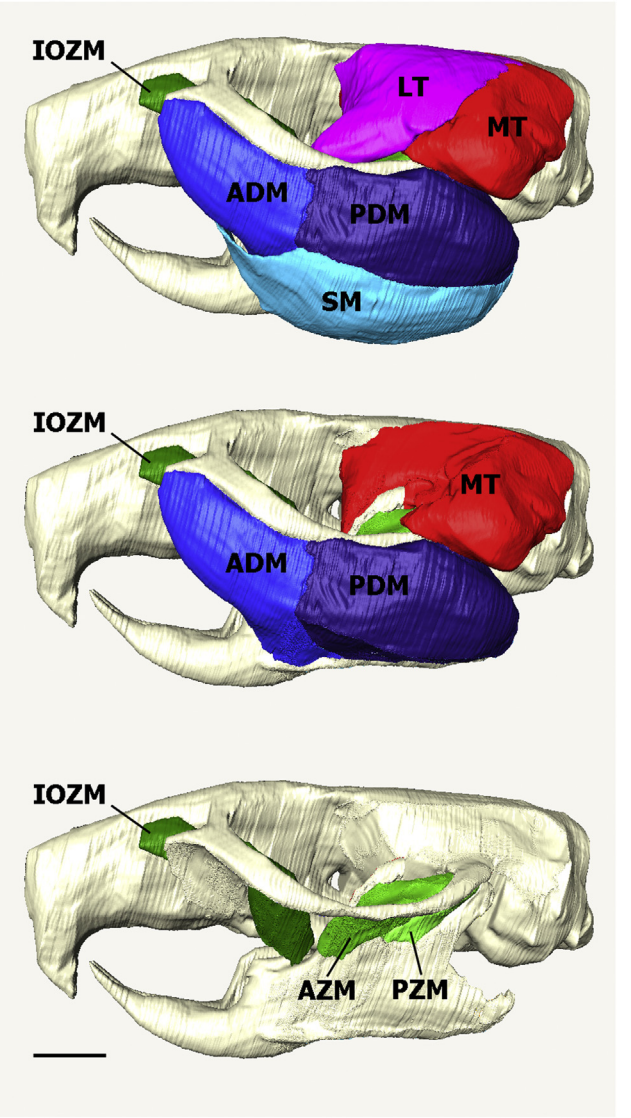


FIGURE 6.1 Left lateral view of 3D reconstruction of muscles of mastication of the rat. The three images, from the top, show progressively deep layers.
adm: anterior deep masseter, azm: anterior part of zygomaticomandibularis, iozm: infraorbital part of zygomaticomandibularis, lt: lateral temporalis, mt: medial temporalis, pdm posterior deep masseter, pzm: posterior part of Zygomaticomandibularis, sm: superficial masseter, t: temporalis. Scale bar: 10 mm. *Images kindly provided by Dr Philip Cox.*

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by many authors there is no clear evidence of these parts (Cox and Jeffrey, 2011). This confusion has probably arisen because the lateral part of temporalis, which overlaps the medial part is also called the anterior temporalis muscle, and the medial part is called the posterior temporalis muscle.

The Pterygoid Muscles

The internal and external pterygoid muscles arise largely from the pterygoid ridges of the sphenoid bone. The pterygoid ridges (internal and external) of the rat are the equivalent of the pterygoid plates (medial and lateral) of man. In man, these plates are well-developed, forming a conspicuous landmark on the inferior aspect of the skull where they spring from the pterygoid process of the sphenoid bone, their anterior edges providing a buttress for the posterior surface of the maxilla. In case any experts in human anatomy read this book we should say that, in man, the plates do not actually abut onto the maxilla as the tubercle of the palatine bone intervenes. In man, the medial (internal) pterygoid muscle arises from the medial surface of the lateral pterygoid plate and from the tuberosity of the maxilla, whereas in the rat the internal pterygoid arises from the pterygoid fossa (between the pterygoid ridges) posterior to the molar teeth, and runs ventrolaterally to a wide insertion on the medial surface of the angular process of the mandible just above the insertion of the superficial masseter (Cox and Jeffrey, 2011). Greene (1935) described the origin of the internal pterygoid as 'from the lateral surface of the feebly marked internal pterygoid ridge' and its insertion as being into 'the ridge which runs posterior from the molar teeth below the mandibular foramen'.

In the rat, the external pterygoid is a small muscle that arises from the external pterygoid ridge (or lateral pterygoid process) and the alisphenoid bone (part of the sphenoid bone in the orbitotemporal region), and is inserted onto the medial condylar process of the mandible just below the medial condyle. In man it arises from the lateral surface of the lateral pterygoid plate and the infratemporal surface and crest of the greater wing of the sphenoid bone, and is inserted onto the anterior surface of the neck of the mandible and the capsule of the mandibular joint. The similarity between the arrangement of the pterygoid muscles in man and the rat should be obvious.

All these muscles of mastication are muscles of the first pharyngeal arch and thus derive their nerve supply from the Vth cranial nerve: the trigeminal nerve.

The Supra and Infra-Hyoid Muscles

These small muscles are divided into suprahyoid and infra-hyoid muscles with reference to their position in relation to the hyoid bone (Greene, 1935).

Suprahyoid Muscles

The digastric is perhaps the most important of this subgroup. It takes its origin from the paroccipital process of the skull and is inserted onto the mandible just behind the symphysis. The muscle has two bellies separated by a tendon attached to the hyoid bone. The posterior belly is innervated by CN VII, and the anterior belly by CN V.

The Stylohyoid is named for its origin from the styloid process which is obvious in man but absent in the rat, and runs from just medial to the paroccipital process to the hyoid bone (CN VII).

The Mylohyoid is innervated by CN V and runs from the mylohyoid line on the internal surface of the mandible to the hyoid bone. Its attachment to the hyoid bone forms an arch under which the intermediate tendon of digastrics runs.

The Geniohyoid runs from the internal surface of the mandible, from close to the symphysis, to the hyoid bone.

Transversus mandibularis is a muscle that does not occur in man, but is present in all rodents with a movable joint at the mandibular symphysis (Greene, 1935). It runs across the angle between the two rami of the mandible, just behind the symphysis and is innervated by CN V.

Infra-hyoid Muscles

This is a group of strap muscles mostly supplied by branches of the upper cervical spinal nerves, (thyrohyoid is supplied by CN XII, Greene, 1935).

The sternohyoid runs from the manubrium of the sternum to the hyoid bone, and the sternothyroid runs from the manubrium of the sternum to the thyroid cartilage. The thyrohyoid runs from the thyroid cartilage to the hyoid bone. In an arrangement reminiscent of the digastric muscle, the omohyoid has two bellies in man, the first of which runs from the scapula via an intermediate tendon under a loop of fascia to an attachment on the clavicle, first costal cartilage and the posterior surface of the manubrium, and then via the second belly to the hyoid bone. In the rat, the muscle has only one belly that runs from the scapula direct to the hyoid bone.

Gnawing and Chewing

It may be a relief from the details of the anatomy of the muscles of mastication to consider what they do. A rat eats by nibbling or gnawing small pieces of food with its incisors and then chewing or masticating those morsels with the molars. Gnawing of nonfood material also occurs, under which circumstances the skin to the sides of the mouth is drawn inwards, into the gap between the incisors and the molars (the diastema) and detritus is expelled laterally from behind the incisors so that no

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detritus enters the mouth: the rat does not need to spit out material it cannot use as food.

In the rat, gnawing and chewing are two separate processes. For the incisors to function correctly the mandible has to be protruded and lowered. This can be mimicked by moving your own jaw so that the tips of your front teeth touch tip to tip. You will have had to push your lower jaw down and forwards from its resting position, with the result that the upper and lower premolars and molars are no longer in contact. In the rat the diastema of the mandible is shorter than that of the maxilla, which means that when the incisors are in action the lower molars lie in front of the upper molars and no chewing is possible. For chewing to be possible the mandible has to be retracted and raised to bring the molars into contact, but now the lower incisors are well behind the upper incisors.

The incisors of the rat are covered with enamel only on their anterior surfaces, so the softer dentine on the posterior surfaces is worn away by the gnawing process. Wearing of the posterior surface of the upper incisor is easily explained by the attrition of the lower incisor on the posterior surface of the upper incisor, but the wear on the posterior surface of the lower incisor is less easily explained. Although not fully researched it would appear that the gnawing allows further protraction of the lower jaw, so that the upper incisors impinge on and scrape away the posterior surfaces of the lower incisors.

Consider a rat that has detected a block of Parmesan cheese inside a wooden cupboard. Gnawing a hole in the door requires the removal of indigestible wood. The mandible is protruded and lowered, skin folds occlude the oral cavity, the wooden door is gnawed and bits of wood are expelled to each side from behind the chisel-like incisors. Once inside the cupboard the gnawing is transferred to the cheese. As soon as a few bits of cheese have been detached from the block these are transferred to further back in the mouth by the tongue, and the mandible is retracted and raised so the cheese can be chewed between the molars. This process continues until a number of boluses of cheese, mixed with saliva, have been formed, pushed towards the pharynx by the tongue, and swallowed. The cycle of gnawing cheese, chewing cheese and swallowing cheese is then repeated.

It will be obvious that a rather complicated series of movements are involved. The articular condyle of the mandible moves forwards on, and also rotates against, the surface of the articular fossa of the temporal bone whenever the mouth is opened. The articular condyle of the mandible and the articular fossa are described in Chapter 5, The Skull. The condyle is elongated in an anterior–posterior direction and can move forwards and backwards. The anterior part of the articular fossa lies lower than the posterior end, so as the condyle moves forwards to articulate with the anterior end of the fossa the mandible is depressed or lowered. In a reversal of this process, the mandible is raised and retracted. The process is similar in man when the condyle moves forwards (drawn by the lateral pterygoid muscle) in company with the articular disc that divides the temporo-mandibular joint (TMJ) into upper and lower compartments. This forwards movement transfers the axis of rotation of the lower jaw away from the joint. In man the lower jaw is said to rotate around an axis through the mandibular foramina where the right and left inferior alveolar nerves (inferior dental nerves) enter the mandible via its medial surfaces. Had the axis of rotation not been moved to this plane the nerves would be badly stretched whenever the mouth was opened.

The rat TMJ also has an intra-articular disc: see Miyako et al. (2011) for a discussion of the cell types within the disk and for references to earlier work. A well-illustrated comparative account of the rat and human TMJ has been contributed by Orset et al. (2014).

MUSCLES OF THE PECTORAL AND PELVIC GIRDLES

When discussing the origin and development of the skeletal muscular system, we divided the muscles controlling the fins of fish into dorsal and ventral groups and stated that these had evolved into the extensor and flexor muscles of the limbs of the mammal. Rotation of the limbs and the drawing in of the limbs under the trunk from a straddle position to form pillars supporting the body well off the ground complicates the picture, and it is not easy to say which of the muscles controlling the proximal part of the limb were derived from the original dorsal, and which from the original ventral groups. The picture is clearer as one moves distally along the limb as the extensor muscles are easy to distinguish from the flexors. Around the shoulder and hip a number of muscles have changed their functions, some have disappeared during evolution, and some have evolved into 'new' muscles. This makes tracing the history of individual muscles attached to the pectoral and pelvic girdles exceptionally difficult.

Let us begin with a proposition: the original function of the pectoral and pelvic girdles was to provide a firm, fixed base on which the fins (limbs) could move. This function was served by the development of the skeletal components of the girdles. The pectoral girdle developed from both dermal and endo-chondral bones (see Chapter 3: Introduction to the Skeleton: Bone, Cartilage, and Joints) and the pelvic girdle developed entirely from endo-chondral bone.

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Pectoral Girdle

In early fish, the pectoral girdle was attached to the skull, although this connection was lost in early amphibians. The pectoral girdle formed a horseshoe shaped system of bones that ran vertically on each side of the body, but was not connected ventrally, as there is no sternum in fish. The first segment of the forelimb (or pectoral limb) articulated with the endo-chondral part of the girdle, but as it was unattached to the vertebral column or skull, the girdle could move on the chest wall. The pectoral girdle has become less complicated during the process of evolution, but it has acquired a ventral linkage to the sternum (see Chapter 4: Vertebrae, Ribs, Sternum, Pectoral and Pelvic Girdles and Bones of the Limbs).

Pelvic Girdle

In contrast, the pelvic girdle began as a very simple structure, evolved into a more complicated one. The efforts of authors to establish identities between elements of the pectoral and pelvic girdles were described by Romer (1970) as 'purely fanciful'.

The key difference between the mammalian pectoral and pelvic girdles is that the former is not attached to the vertebral column but the latter is. As described in Chapter 4, due to fusion of sacral Vertebrae, Ribs, Sternum, Pectoral and Pelvic Girdles and Bones of the Limbs, what were sacral ribs are now fused with the transverse processes of the sacral vertebrae to produce a lateral mass that articulates with the ilium. This articulation is a synovial joint, at least in man, in which the uneven congruities of the bearing surfaces and the strong ligaments associated with the joint allow of very little movement.

The key difference between the two is that the pelvic girdle is fixed, but the pectoral girdle can move.

MUSCLES OF THE PECTORAL REGION

When the skin over the chest, shoulder and back of the rat is removed the superficial layer of muscle revealed is the panniculus carnosus, a muscle that is inserted into the dermis and which arises from the fascia covering deeper muscles. There are two major parts: the cutaneous maximus that surrounds the trunk, and the platysma that runs forwards from the upper, ventral chest region to the lower jaw and face (cervical part of platysma) and from the dorsal aspect of the shoulder to the sides of the face (facial part of platysma). In man, only the cervical part is found. In the rat, the cutaneous maximus arises partly from the lesser tubercle of the humerus.

Once the cutaneous muscles and underlying fascia are removed, a mass of muscles surrounding the shoulder joint is revealed. These are illustrated in Figs. 6.2 and 6.3.

Not all the muscles that have been described in the rat are described below; those requiring a muscle by muscle account are referred to Greene (1935), or for the real enthusiast the work of Parsons (1894, 1896 and 1898).

Muscles Connecting the Pectoral Girdle to the Axial Skeleton

It will be apparent that the pectoral girdle is attached to the axial skeleton by muscle rather than by bone. In mammals with a clavicle (including rat and man), there is an indirect bony connection, clavicle – sternum – ribs – vertebrae. In other quadrupeds, the trunk is suspended from the pectoral girdle by the serratus muscle.

Serratus, so named for the saw tooth interdigitations between its origins from the ribs and those of the eternal oblique muscle, runs from the medial surface of the scapula, close to the vertebral border, to the ribs. It lies between the scapula and the chest wall, but is separated from much of the medial surface of the scapula by the subscapularis muscle. In man, a habitual biped, the function of serratus is to draw the scapula forwards around the chest wall.

Three other muscles connect the pectoral girdle to the axial skeleton. The rhomboids run from the spines of the thoracic vertebrae to the scapula, the levator scapula runs from the spines of the cervical vertebrae to the scapula, and the superficially placed trapezius runs from the occipital bone and the spines of the thoracic and cervical vertebrae to the scapula and clavicle.

MUSCLES OF THE FORELIMB

Muscles Connecting the Humerus With the Pectoral Girdle

Recalling the original division of the muscles, controlling the limbs into dorsal and ventral groups allows these muscles to be divided, with a little effort, into dorsal and ventral groups.

Dorsal Group

Latissimus dorsi is a large superficial muscle that runs from the ilio-lumbar fascia and the spines of the lower thoracic vertebrae to the humerus. It is not attached to the pectoral girdle. It pulls the forelimb back, and is responsible for part of the forwards push on the body during locomotion in quadrupeds such as the rat. In man and monkeys, it is a climbing muscle and raises the body

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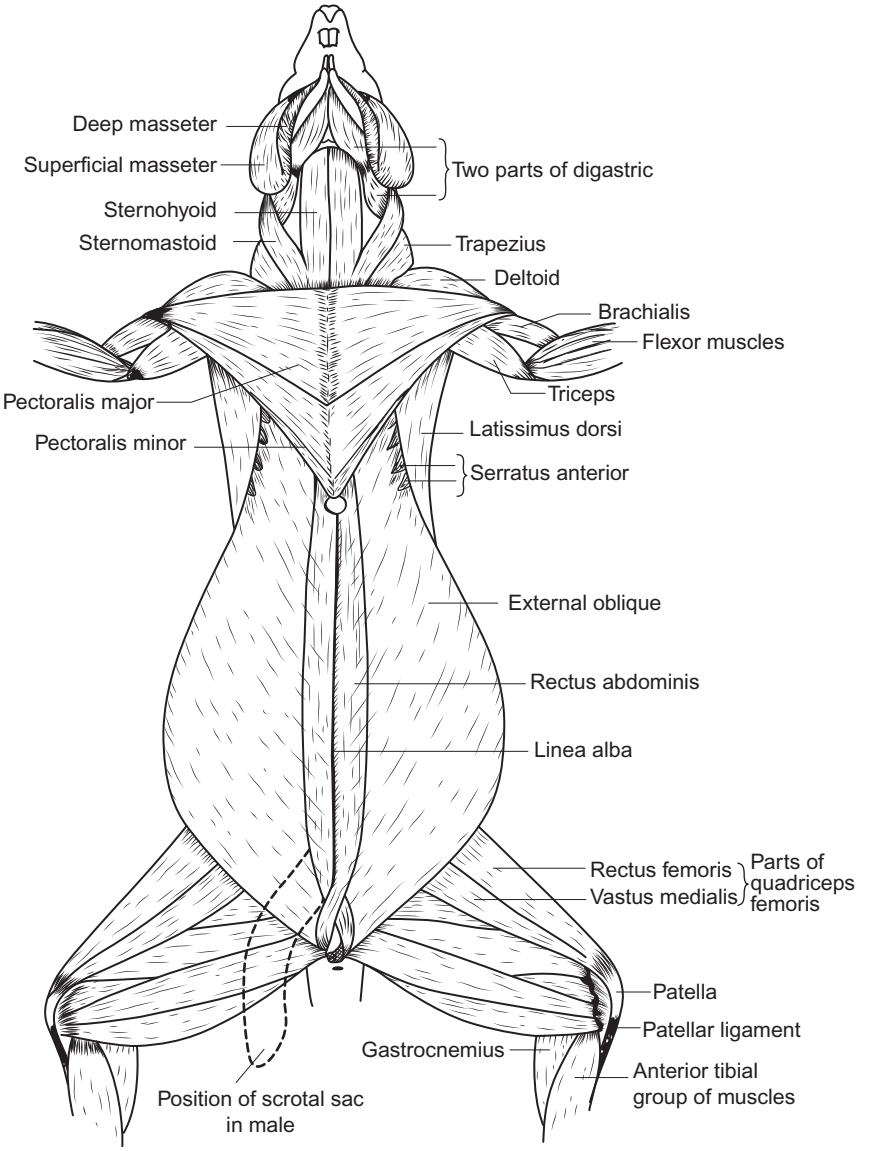


FIGURE 6.2 Muscles (ventral view with plastysma and cutaneous and muscles and glands of the neck removed).

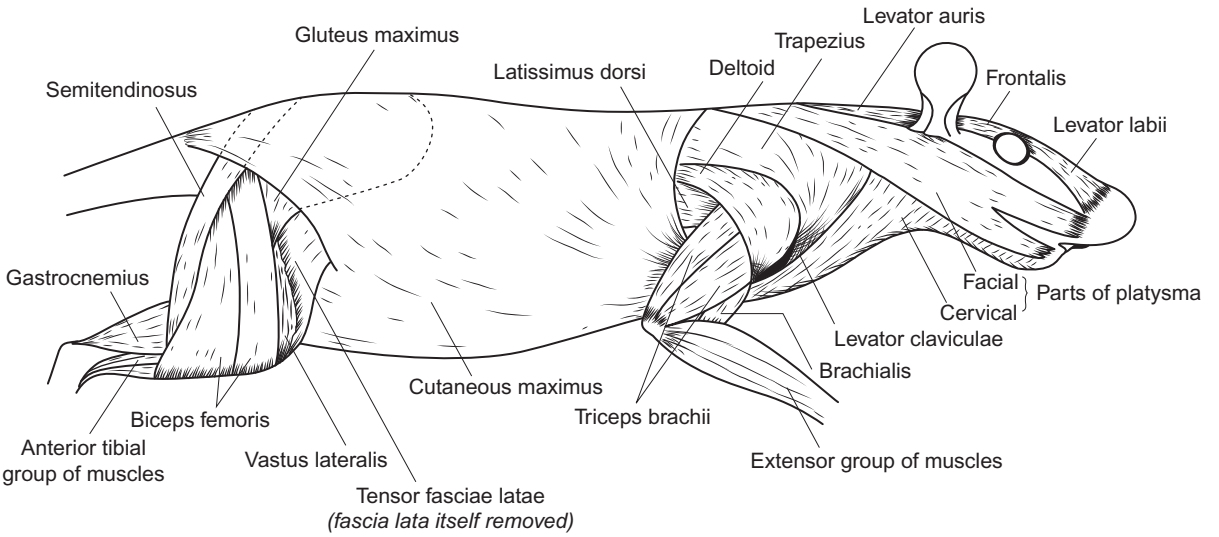


FIGURE 6.3 Chief superficial muscles (lateral view with fascial sheath removed from head and limbs).

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when the animal is hanging by its hands from a branch of a tree.

Teres major is a muscle that has become separated from latissimus dorsi during evolution and become attached to the scapula, from where it runs to the humerus, which it pulls back.

Deltoid is a large muscle originating from the clavicle, the spine of the scapula and the acromion and inserted into the large deltoid crest of the humerus. Its wide origin gives its parts different functions; the anterior part (acromio-deltoideus) pulls the humerus forwards, and the posterior part (spino-deltoideus) pulls the humerus back.

Subscapularis lies beneath the scapula, originating on the medial surface of the blade of the bone and inserting into the humerus.

Ventral Group

The largest muscle of the ventral group is pectoralis, and includes coracobrachialis, biceps and two oddities; supra and infra-spinatus.

Supra-spinatus and infra-spinatus arise from the lateral (dorsal) surface of the scapula and look as though they belong with the dorsal group. In fact, they have evolved from a muscle found in reptiles, but not in mammals, and have migrated to their current position. Supra-spinatus has an important role in man as it is the only muscle that can begin the process of abduction of the arm, although deltoid soon takes over. In the rat, it swings the forelimb forwards.

In human, anatomy the subscapularis, supra-spinatus, infra-spinatus and teres minor (posterior margin of scapula to humerus) are collectively known as the rotator cuff muscles. This group holds the head of the humerus firmly in the glenoid fossa and rotates the humerus medially or laterally depending on the details of their insertions.

Pectoralis is an important muscle of locomotion in the rat that can be divided into a number of parts. The major part runs from the sternum and ribs to the humerus at its upper end, and pulls the forelimb down and back.

Coracobrachialis and the short head of biceps (biceps has two heads, triceps has three) are attached to the coracoid process by a single tendon, and run together down the medial side of the humerus where they separate. The short head of biceps joins the long head of biceps and forms one muscle before inserting into the radius. The long head of biceps comes from the supra-glenoid tubercle of the scapula, its tendon running though the shoulder joint enveloped in a sheath of synovial membrane.

Coracobrachialis inserts onto the humerus and pulls the forelimb forwards. The biceps also does that, as well as flexing the elbow joint. In man, the attachment of biceps allows the muscle to act as a powerful supinator of the forearm, but in the rat, supination and pronation are very limited.

Muscles Connecting the First and Second Segments of the Forelimb

These muscles act on the elbow joint and can be divided into extensors and flexors.

Extensors

The triceps is the only important extensor muscle in this region. The muscle has three heads: a long head arising from the ventral third of the axillary border of the scapula, and medial and lateral heads that both arise from the posterior surface of the shaft of the humerus. The muscle is inserted into the olecranon process of the ulna, which is long in the rat (see Chapter 4: Vertebrae, Ribs, Sternum, Pectoral and Pelvic Girdles and Bones of the Limbs).

Flexors

We have already mentioned biceps and coracobrachialis, the third of this group is brachialis; a major flexor of the elbow joint that arises from the greater tubercle (tuberosity) of the humerus and inserts into the coronoid process of the ulna. In man, this muscle has a broad origin on the anterior surface of the humerus.

Muscles of the Second and Third Segments of the Forelimb: Muscles of the Forearm and Forepaw (Hand or Manus)

The problem for the nonspecialist dealing with the muscles of this region is that there are so many of them. A detailed account has been provided by Greene (1935) and by Hebel and Stromberg (1976) but this will not be attempted here.

Muscles Acting on the Articulations Between the Radius and the Ulna

The radius articulates with the ulna in three ways.

The head of the radius can rotate (to a greater or lesser extent depending on species) on the floor of the radial notch that lies just lateral to the coronoid process of the ulna (see Chapter 4: Vertebrae, Ribs, Sternum, Pectoral and Pelvic Girdles and Bones of the Limbs).

The shafts of the radius and ulna are held together by a long interosseus ligament with the distal end of the radius articulating with the head of the ulna. In man, the radius rotates around the ulna, and the distal end comes into direct articulation with carpal bones, but a fibrocartilage disc prevents direct articulation between ulna and carpal bones. In the rat, the ulna articulates with the triquetrum and pisiform bones.

The normal position of rat forearm of the rat is pronation, with the palm of the forepaw resting on the ground. This means that the radius is habitually crossed over the

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ulna, and the flexor muscles are on the posterior side of the forelimb rather than on the anterior side as described in human anatomy (see Chapter 2: Introduction to Anatomical Terminology for a discussion of the anatomical position and its implications for terminology in man and in the rat). The rat possesses a supinator muscle in the forearm that originates from the ulna, and winds around the lateral side of the forearm before inserting onto the radius. Given the habitual pronation of the forearm, it is difficult to believe that the supinators and pronators have much to do, but pronator teres runs on the flexor surface from the medial epicondyle of the humerus to the radius.

Flexor Muscles Acting on the Wrist Joint and the Joints of the Forepaw

The flexor muscles of the wrist joint arise from the medial epicondyle of the humerus, radius and ulna; and are inserted into the metacarpal bones. As a group, these muscles are called the long flexors (e.g. flexor digitorum superficialis) to distinguish them from shorter flexors found more distally. The long flexors run to metacarpals and phalanges. The exact sites of insertion are described by Greene (1935) and by Hebel and Stromberg (1976). Although the palm of the rat is small it contains a set of short muscles controlling the first digit (thumb or pollux) and the fifth. In addition there are palmar and dorsal interosseus muscles arising from metacarpals and running to phalanges. These adduct and abduct the digits, respectively. Lastly there are the very small lumbrical muscles (like worms: lumbricoides) that take their origins from the tendons of the deep long flexor (flexor digitorum profundus) and run round the radial sides of the digits 2–4 to reach their extensor surfaces.

This level of complexity is consistent with the fine control present in man (think of a concert pianist), but it is more difficult to see what they do in the rat. The higher primates are characterised by opposable thumbs: opposing the thumb to the tip of another digit is something humans do better than any other primate, and long thumbs are the key to the movement. Also required is a special saddle shaped joint between the first metacarpal and the proximal phalanx of the thumb to allow rotation of the phalanx on the metacarpal. The rat's thumb is very short, and though a rat can certainly manipulate small objects it cannot properly oppose its thumb and fingers, and in fact lacks two short muscles, the abductor pollicis brevis and the opponens pollicis that are present in man.

Extensor Muscles Acting on the Wrist Joint and on the Joints of the Forepaw

The extensors in this region arise in the forearm. Although none arise on the upper surface of the forepaw, there are a considerable number of extensors including those of the wrist joint (extensors carpi radialis and ulnaris: one on each side) and six controlling the digits. The lack of short extensors represents a change from the arrangement found in reptiles, which have a set of short distal extensors that have been lost in mammals (Romer, 1970). The reason for their loss is unknown, at least to us.

MUSCLES OF THE PELVIC GIRDLE

The muscles of the pelvic girdle present certain problems. In the distal hindlimb, the separation of muscle groups into ventral and dorsal (flexor and extensor) is obvious, but less so around the hip joint, although separation into ventral and dorsal is possible on the basis of the embryology of the muscles (Romer, 1970). It is tempting, but incorrect, to assume that are similarities between the muscles of the pelvic and pectoral girdles. A recent paper by Diogo and Molnar (2014), with the formidable title: 'Comparative anatomy, evolution and homologies of tetrapod hindlimb muscles, comparison with forelimb muscles, and deconstruction of the forelimb-hindlimb serial homology hypothesis' is available online, and is worth looking at, if only as an example of the level of scholarship still being applied to the study of comparative anatomy. Abandoning any attempt to homologise between the fore and hindlimbs musculature, we shall merely outline briefly the arrangement of muscles in the rat, and compare with man where possible.

The most obvious difference between the pelvic and pectoral girdles is that the pectoral girdle lies outside the body wall (outside the ribs), whereas the pelvic girdle forms a part of the body wall itself. No muscles run from within the chest to the forelimb, but a number of muscles run from the ventral surfaces of the lumbar vertebrae to the hindlimb. In man, psoas runs from the ventral surfaces of lumbar vertebrae where it is joined by iliacus, running from the inner surface of the ilium, to form a joint tendon that inserts into the lesser trochanter of the femur. Both act as flexors of the hip joint. Obturator internus also arises inside the pelvis and runs out to the femur.

Rats, unlike men, have tails for which seven muscles that move the tail have been described (Hebel and Stromberg, 1976). The ilioischiopubo-coccygeus (as Romer said: 'what names these muscles have') is the largest and runs from the inner surfaces of the three bones of the pelvic girdle to the vertebrae of the tail. This muscle, rather surprisingly as men have no tails, is of interest to human anatomists as it slips down the inner walls of the pelvis and forms the levator ani muscle, the important muscle of the pelvic floor.

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Muscles Arising From the Pelvis and Causing the Hindlimb to Move at the Hip Joint

Extensors of the Hip Joint

Extension of the hip joint, defined as occurring when the hindlimb is pulled back, is a key movement of locomotion: as the limb is pressed back against the ground, the body is propelled forwards. The gluteal muscles run from the outer surface of the ilium to the great trochanter of the femur, and extend the leg at the hip joint. The mechanical advantage of these muscles is small because the site of insertion is close to the hip joint, but these are powerful muscles in man, though rather less so in the rat. In the rat, gluteus maximus blends with another muscle, tensor fasciae latae, which is attached to the strong fascia running down the outer side of the thigh.

Muscles of the Thigh

These muscles can be divided into the hamstrings (behind), the extensors of the knee (in front) and the adductors of the hip joint (on the inner or medial aspect). Some cross both the hip and knee joints and therefore act on both.

The Hamstrings

These comprise three muscles, biceps femoris, semimembranosus and semitendinosus. All run from the ischial tuberosity to the lower, posterior, surface of the femur and the upper posterior surfaces of the tibia and/or fibula. In the rat, there are small medial and lateral fabellae (sesamoid bones) lying behind the knee into which the hamstrings insert en route to the bones of the second segment of the limb. The rat also has a caudo-femoralis muscle associated with biceps that inserts across the lower posterior surface of the femur from the medial fabella to the lateral fabella (Greene, 1935). All these muscles extend the hip and flex the knee.

Muscles of the Anterior Aspect of the Thigh

Four muscles; rectus femoris, vastus lateralis, vastus intermedius and vastus medialis, make up the muscle usually referred to as the quadriceps femoris. All arise from the upper end of the femur (rectus femoris also arises from the pubis) and are inserted into the patella. The patella is a sesamoid bone that develops in the tendon of the quadriceps. The part of the tendon below the patella, the patellar ligament, is attached to the upper anterior part of the tibia. Although all these muscles mainly extend the knee joint, rectus is also a weak flexor of the hip joint.

Muscles of the Medial Aspect of the Thigh

There are adductors, longus, magnus and brevis (long, large and short) and gracilis (thin) that run from the pubis and from the shaft of the femur to the tibia. The attachment to the shaft of the femur is long, and in man is marked by the linea aspera (roughened line) on the posterior surface of the bone.

In addition, there are the lateral rotators piriformis, obturator externus and internus, quadratus femoris or gemelli; short muscles that run from the pelvis to the upper end of the femur and rotate the femur and stabilise the hip joint.

Muscles of the Second Segment of the Hindlimb

Human anatomists make a great song and dance about reserving the term ‘leg’ for the second segment of the hindlimb, and ‘arm’ for the first segment of the forelimb. When thinking about rats we all know that they have four legs. The muscles of the second segment (hindlimb understood) are arranged as extensors and flexors of the ankle joint.

Veterinary anatomists refer to the knee joint as the stifle joint, and to the ankle joint as the tarsal joint or hock. This confusing terminology led Dr. Johnson astray when he defined the pastern of a horse as the knee (the pastern is the proximal phalanx). His contempt for such trifles is recorded in his reply to the lady who asked him why he had done this: ‘Ignorance, Madam, sheer ignorance’ But movement of the ankle joint also presents what Winston Churchill would no doubt have called further terminological inexactitudes. If you think of your own ankle, it seems logical to call a downward movement of the foot extension, and an upward movement as flexion. To the anatomist this is incorrect, because flexion means the approximation of the flexor or volar surfaces (the sole of the foot and the back of the leg), so an upward movement of the foot is a further extension of an already extended joint, and a downward movement is flexion. Clinicians, not being obsessed with anatomical correctitude (the Johnsonian style is catching), define the movements as plantar-flexion (foot moved down) and dorsi-flexion (foot moved up). By these definitions, gastrocnemius and soleus are plantar-flexors, although Hebel and Stromberg defined them as extensors of the ankle joint, and combined them as triceps surae.

Muscles of the Posterior Aspect of the Second Segment

These include gastrocnemius, soleus, tibialis posterior and a group of long flexors of the digits. Gastrocnemius arises as two heads from the medial and lateral femoral

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epicondyles and fabellae, and soleus by a slender tendon from the head of the fibula (Greene, 1935). Gastrocnemius lies superficial to soleus, and together they combine to produce a single tendon (of Achilles) that is inserted into the calcaneum, from where they plantar-flex (or flex) the ankle. The rat has a long hindfoot, so the calcaneus projects well behind the ankle joint, providing good leverage when the digits are pressed to the ground, as in running. Tibialis posterior (Geene, using human anatomy as a basis for describing rat muscles, uses this term, Hebel and Stromberg prefer tibialis cranius and add tibialis caudalis as a separate muscle), flexor halluicis longus and flexor digitorum longus arise from the upper posterior surfaces of the tibia and fibula, run down on the medial side of the ankle joint, and on into the sole of the foot to reach bones of the tarsus (in the case of tibialis posterior) or of the digits. This is another area of confusion. In man, there is a large superficial muscle called flexor digitorum brevis in the sole of the foot, but in the rat, the equivalent of this muscle occurs with the long flexors described as flexor digitorum superficialis (see above) or plantaris (see below), and is shown by Hebel and Stromberg as a large muscle of the posterior aspect of the second segment. Table 6.2 may help.

Although Hebel and Stromberg make no mention of plantaris, it is mentioned in works on human anatomy, and like palmaris in the forelimb, is said to be a remnant of a superficial flexor of the digits that fails to reach beyond the sole or the palm.

Muscles of the Lateral Aspect of the Second Segment

This group is formed by peroneus longus and brevis, and two muscles not described in man, peroneus digiti quarti and peroneus digiti quinti. Each arises from the fibula (p. longus also arises from the lateral condyle of the tibia) and run to metatarsals having passed around the lateral side of the ankle joint and entered the sole of the foot. They lift the lateral edge of the foot (eversion) and plantar flex the ankle joint.

Muscles of the Anterior Aspect of the Second Segment

Here, we find the extensors of the ankle joint and digits: tibialis anterior, extensor digitorum longus and extensor hallucis longus. The major origins of all three muscles are from the anterior surfaces of the tibia and fibula, but extensor digitorum longus also arises in part from the lateral epicondyle of the femur. Tibialis anterior runs to the first (medial) cuneiform bone and to the first metatarsal and the long extensors run to the distal phalanges.

Short Muscles of the Foot

Interest in these small muscles is likely to limited, not just among toxicologists. They are similar to those of the hand, but in the rat, unlike man, flexor digitorum brevis is a small muscle. In the rat the second digit has its own adductor (not the case in man), and there are no dorsal interossei in the rat hindfoot (Parsons, 1896). The plantar (ventral) interossei are well-developed muscles, and as in the forelimb, the rat has no opponens muscles for the hallux or fifth digit (Greene, 1935).

THE MUSCLES OF THE BACK

Towards the beginning of this rather long chapter, we noted that the muscles of the back were derived from the epimeres, each a derivative of a myomere, itself a derivative of a somite. In simple terms, the epimeres give rise to all the dorsal muscles running along the long axis of the body. These muscles lie in the groove between the posterior spines of the vertebrae, the transverse processes and angles of the ribs, thus forming a long column of muscle running from sacrum to skull. These extensors of the vertebral column lie on both sides of the column and can bend the vertebral column laterally by contracting unilaterally.

Although this seems straightforward, once the system is examined in detail, complications emerge because the column of muscle is broken into subcomponents, each having a large number of attachments to the vertebrae and ribs, and several reach the skull. A detailed muscle by

TABLE 6.2 Terminology Applied to Muscles of the Foot in Man and Rat

Man	Rat	Rat
	Greene (1935)	Hebel and Stromberg (1976)
Flexor digitorum longus	Flexor digitorum longus and flexor hallucis longus	Flexor digitorum profundus and flexor digiti primi
Flexor hallucis longus	–	–
Flexor digitorum brevis (in the sole)	Plantaris: fusing with flexor digitorum brevis in the sole of the foot	Flexor digistorum superficialis: fusing with flexor digitorum brevis in the sole of the foot

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muscle account is not likely to be of great interest to toxicologists, but if required Greene (1935) and Hebel and Stromberg (1976) provide such an account. We shall limit ourselves to a few general points and a short summary.

General Points

All the muscles run from bone to bone, with origins posterior to insertions. The components of the system are arranged in rather ill-defined layers, with the short muscles lying deeper than the long muscles. As the system runs forward from the sacrum, components are inserted into vertebrae and ribs until it reaches the skull. In the upper cervical region, a special group of muscles connects the first cervical vertebra (the atlas) to the skull, and the second cervical vertebra (the axis) to the first. These are important in that they move the skull on the vertebral column and allow the head to turn. All the muscles are supplied from the posterior (dorsal) primary rami of spinal nerves.

Several methods for summarising the components of this group of muscles have been produced. As good a method as any was put forwards by Leeson and Leeson (1972) in their admirably short text book of human anatomy. Of course, we might expect the rat to differ a little from man in terms of details but this summary remains helpful. We may think in terms of four groups making up the whole complex system.

Splenius Group

Not really a group as it consists of only the splenius muscle, which is divided into splenius capitus and splenius cervicis. These are large, superficial muscles that run from the ligamentum nuchae and the posterior spines of the upper thoracic and cervical vertebrae, to the posterior spines of more anterior vertebrae and the skull.

The Sacrospinalis Group (Sometimes Known, in Human Anatomy, as the Erector Spinae Group)

This is a superficial group of three muscles: ilio-costalis (subdivided into three parts: lumborum, thoracis and cervicis), longissimus (subdivided into three parts: thoracis, cervicis and capitus) and spinalis (also divided into three parts: thoracis, cervicis and capitus). Ilio-costalis is the most lateral of the three, spinalis lies medially, and longissimus lies in between. This group of muscles runs from the sacrum,iliac crest and the spines of the more posterior vertebrae to more anterior vertebrae and the skull.

The Transversospinalis Group

This is a group of long and short muscles that lies deeper than the sacrospinalis group. It is divided into three: semispinalis (subdivided into: thoracis, cervicis and capitus), multifidus and the rotators (rotatores). Semispinalis is a long muscle that runs from transverse processes to spinous processes and to the skull. There are a lot of mutifidus and rotator muscles running from vertebrae to adjacent vertebrae.

The Segmental Muscle Group

The segmental group comprises short, deeply placed muscles; the interspinales (running from spinous process to spinous process), intertransversarii (running from transverse process to transverse process) and the special suboccipital muscles that move the first cervical vertebra on the second (rectus capitus posterior major and minor) or the skull on the first (obliquus capitus superior and inferior).

A detailed knowledge of these muscle groups is not required by the toxicologist, but what should be remembered is that the muscles of the back form a complicated column of muscles, arranged in ill-defined layers with many attachments to the vertebral column and skull. For the readers, who need more detail (probably a small group) Hebel and Stromberg (1976) provide it and include details of the muscles described in the rat.

MUSCLES OF RESPIRATION

The most important muscle of respiration is the diaphragm, a thin, dome shaped sheet of muscle and connective tissue that separates the thoracic and abdominal cavities. Contraction of the diaphragm causes the central tendinous region to press down in man and back in the rat, onto the liver, compressing the abdominal contents. Further contraction causes the lower ribs to move outwards and forwards (out and up in man). De Troyer et al. (1981) studied the movements of the diaphragm in the dog and in man in great detail and argued that the diaphragm should be considered as two muscles, the peripheral sheet and the crura.

Structure of the Diaphragm

The diaphragm is attached to the xiphisternum and ribs, forming the margins of the posterior outlet of the thorax, and to the lumbar vertebrae by the crura (narrow extensions of the muscle). The diaphragm is shaped like a dome, the dorsal parts of which extend further posteriorly (caudad) than the anterior. The inferior vena cava passes through the central tendinous part of the diaphragm; the aorta lies at the very dorsal edge of the diaphragm. The

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right crus branches and surrounds the lower end of the oesophagus forming a sort of pinch-cock, which is supposedly particularly effective in rats, and in part explains why rats cannot vomit. The rat diaphragm contains a mixture of skeletal muscle fibre types, with the ratio varying according to location (Kilarski and Sjöström, 1990). The diaphragm is innervated by the phrenic nerve that arises from spinal segments C3, 4 &5 in man, but from C5 and 6 in the rat.

Other Muscles of Respiration

Although the diaphragm is the most important muscle of respiration, and probably the one of most interests to toxicologists, the external and internal intercostal muscles running between the ribs, move the ribs are forwards during inspiration. Less important muscles include the levator costae muscles, running from the transverse processes of the thoracic vertebrae to the subjacent ribs, and the dorsal serrator muscles (in two parts in the rat: cranialis and caudalis) and rectus thoracis (a continuation of the midline ventral muscles represented in man by rectus abdominis) that help move the sternum forwards.

FACIAL MUSCLES

Facial expression plays an important part in communication in man, primates and many other mammals such as the dog, although mammals such as cows and sheep are largely inscrutable to our eyes. A fascinating account of facial expressions in man and animals has been provided by Charles Darwin ('The expression of emotions in man and animals', 1872) and Gregory's book 'Our face from fish to man' (1929) is an extraordinarily interesting work from one of the greatest of all comparative anatomists.

Facial muscles are also needed for blinking, wrinkling of the nose when sniffing, and twitching of the whiskers and ears. All these movements are controlled by the facial muscles, which are derived from the second branchial arch, and thus innervated by the VII (facial) cranial nerve (see Chapter 21(1): The Cranial Nerves).

Although the facial muscles of the rat have not been studied in the same detail as those of man and the dog, recent work on the muscles controlling the mystacial pad (see below) has superseded earlier descriptions. Greene (1935) provided a short description of the facial muscles of the rat and deployed a terminology based on human anatomy; Hebel and Stromberg (1976) also provide a short account but used a different terminology. We shall consider the facial muscles in groups: those controlling the whiskers or vibrissae, the muscles controlling the nares (the external openings of the nasal cavity), the muscles around the mouth, the muscles around the eye and those of the external ear (auricle or pinna).

Muscles Controlling the Whiskers

In common with other rodents, rats have long whiskers that are part of the mystacial pad. This complex sensory organ is found on either side of the rostrum of the head, lateral to the nasal cavity, and posterior to the nares. The pad comprises about 35 large hair follicles that pass through a well-developed dermis (or corium) to reach, and in some cases penetrate, the mystacial plate, a deeply placed layer of connective tissue. Each follicle is characterised by a well-developed capsule and associated with a venous sinus. Movements of the whiskers are controlled by two sets of muscles, the intrinsic muscles of the pad, which run from one follicle to another, and a complex series of extrinsic muscles that are inserted into both the dermis and the mystacial plate.

The anatomy has been described by Haidarliu and colleagues in a series of papers (Haidarliu et al., 2010, 2011 and 2012). Haidarliu has also contributed a summary which can be found on Scholarpedia: Haidarliu S, Scholarpedia, 10(4): 32331. An account by Hill et al. (2008) describes the biomechanics of the system in detail, describing no fewer than four named superficial muscles and five named parts of the deeper muscle, nasolabialis profundus. Haidarliu et al have described the process of environmental searching by the whiskers, whereby contact resulting from large rostrocaudal sweeps (controlled by the intrinsic muscles) lead to convergence of the whiskers and more whiskers being brought into contact. This leads to better discrimination of the surface of the object, a process described as foveal scanning. The parallel with vision is obvious, as foveal vision provides high resolution. As stated above, movement of the whiskers is controlled by muscles innervated by the Vllth cranial nerves, and the sensory input provided by contact of the whiskers with objects is transmitted to the brain via the Vth (trigeminal) cranial nerve.

Dilation (or Dilatation) of the Nares

When compared with the mouth, the nose in man is a high resistance pathway for inspired air. Everyday observation shows that the nares widen with excitement and when sniffing, and that people switch from nasal to oral breathing during exercise to reduce airway resistance. The rat cannot do this as it is an obligate nasal breather. Nasal dilation in the rat might reasonably be expected to be controlled by the dilator naris (or dilator nasi) that runs from the lower edge of the orbit (maxilla) to an aponeurosis above the nasal cartilage, but this seems not to be so, as it appears to deflect the nose to one side rather than dilate the nares. There is however a division of nasolabialis profundus that dilates the naris. This runs from the nasal cartilage and premaxilla to the plate of the mystacial pad

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(see above). Contraction of this, and perhaps other muscles, causes retraction of the whiskers and dilation of the naris. It has been argued that dilator naris should be renamed as deflector nasi, a good example of the dangers of deducing the function of a muscle from its official name (Deschenes et al., 2015).

In man, there is no muscle called the dilator naris, but the splendidly named levator labii superioris alaeque nasi that has a similar origin and insertion, and perhaps plays the same role.

Muscles Around the Mouth

Greene (1935) recorded, '. . .orbicularis oculi (see below) and orbicularis oris are both poorly defined, present no peculiarities and need no description'.

This may be the case in the rat, where the poor development of is probably linked to the fact that the mouth of the rat is never completely closed over the large incisor teeth (Parsons, 1896). By contrast, in man orbicularis oris is a complex sphincter running beneath the skin (deep to the dermis) and mucous membrane of the lips, with which no fewer than 11 muscles are associated.

Muscles Around the Eye

Just as the orbicularis oris surrounds the mouth, the orbicularis oculi surrounds the eye. The muscle comprises a palpebral part that controls blinking, and a circumferential part that allows the eyes to be tightly closed. The circumferential part is only attached at the medial margin of the orbit, but the palpebral part is also attached to the lacrimal bone. This muscle appears to be poorly developed in the rat.

Muscles of the Auricle of the Ear (of the Pinna)

There seems to be little agreement with regard to the extrinsic muscles of the rat auricles (pinnae). Greene (1935) described two muscles: levator auris longus (no mention was made of a 'brevis') and interscutularis, running across the top of the head from auricle to auricle. She separated the levator auris into a cranial part, originating from the cervical spines of vertebrae C1−C4 and running to the anterior aspect of the base of the auricle, and a caudal part originating from the spines of C4 & 5 and running to the posterior aspect of the base of the auricle.

In contrast, Hebel and Stromberg (1976) described interscutularis as poorly developed, and described frontoscutularis as running forwards to the eyelids, with levator auris longus referred to as cervicoauricularis.

In man, a set of functionless intrinsic muscles arranged on the cartilage of the pinna are described by Parsons (1896), but there is no mention of these in his account of the muscles of the rat. Kiernan and Mitchell (1974) noted the presence of skeletal muscle fibres in the rat pinna, but commented that their arrangement did not correspond with the individual and identifiable intrinsic muscles of man.

MUSCLES OF THE ABDOMINAL WALL

The toxicologist's interest in the abdominal wall of the rat is likely to be limited to the initial midline incision of the postmortem examination. The abdominal wall of the rat is thin, and although the thick layer of fat found in obese people is not always encountered in the normal laboratory rat, animals in long-term studies may weigh up to 1.2 kg, and are equally obese. There are three thin layers of muscle, from outside to inside, the external and internal oblique muscles and the transversus abdominis. In addition, the rectus abdominis forms part of a linear arrangement of muscles that run from the pubis to the clavicle, first rib and manubrium sternum.

The external oblique arises from the outer surfaces of the ribs where it interdigitate with serratus anterior (serratus ventralis). It runs as a broad sheet to the pelvis where it is inserted into the iliac crest. The posterior edge of the muscle (remembering that the rat is described in a horizontal position) spans across from the ilium to the pubis where its posterior border forms an aponeurosis and is turned inwards. This thickened edge forms the inguinal ligament.

The internal oblique runs from the ilium and lateral part of the inguinal ligament and the thoraco-lumbar fascia, deep to and across the external oblique, forwards to the lower ribs and costal cartilages, and medially towards the midline. The posterior edge of the internal oblique 'steps over' the inguinal canal.

Transversus abdominis is difficult to differentiate from the internal oblique. It runs from the lateral part of the inguinal ligament, the ilium and thoraco-lumbar fascia, directly across the abdomen towards the midline.

Muscles of the Anterior Aspect of the Neck

While conducting a postmortem examination of the rat at least one muscle of the anterior aspect of the neck, the sternohyoid, will be noticeable as it has to be reflected or resected to allow access to the trachea and thyroid gland. This long strap of muscle runs posterior to anterior from the manubrium sternum and the medial end of the clavicle to the hyoid bone near the midline, and pulls the hyoid bone and the tongue, which is attached to the hyoid bone, downwards and towards the chest.

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Superficial Muscles

Sternomastoid is another large muscle, arising from the manubrium just ventral to sternohyoid, it runs to the mastoid region of the temporal bone. In man it is attached to the mastoid process. When the right and left sternomastoid muscles contract together the neck is flexed, and when only one contracts, the head is turned to the side. Lateral to sternomastoid lies trapezius clavicularis, the part of the trapezius muscle that runs round the side of the neck from the occipital bone to the clavicle. Behind and dorsal to trapezius clavicularis lies cleidomastoid, running from the clavicle to the mastoid region of the temporal bone.

Deeper Muscles

Omohyoid runs from the scapula to the hyoid and can be seen running in at an angle to the midline dorsal to sternomastoid and cleidomastoid. Deeper again, and lying dorsal to the subclavian artery and the roots of the brachial plexus, lie the scalene muscles (medius and dorsalis), running from the transverse processes of the cervical vertebrae to the ribs. Medius runs to the first rib and dorsalis runs to ribs 3 and 5. Deepest of all, longus colli lies on the ventral surface of the vertebral bodies and connects them with the transverse processes of the vertebrae. Again, this muscle flexes the neck.

There are a number of small muscles that we have not included in this account: rectus capitis, longus capitis, scalenus anterior (perhaps absent in the rat), sternothyroid and thryrohyoid. The reader who needs details of these muscles is referred to Greene (1935) and Parsons (1896).

APPENDIX

The Histology of Skeletal Muscle

Skeletal muscle is one of the three histological types of muscle found in mammals: the other types are smooth muscle (found in the walls of the gut and blood vessels) and cardiac muscle found only in the heart and some pulmonary vessels.

Skeletal muscle fibres are cross-banded: the origin of the alternative term ‘striated muscle’. Cardiac muscle is also striated, but smooth muscle lacks striations. Good accounts of the histology of skeletal muscle can be found in standard textbooks of histology. Fawcett (1994) provides an excellent well-illustrated account including a wealth of information on ultrastructure, and some splendid electron micrographs.

We should note that the term ‘muscle fibre’ actually means muscle cell. The cells are long and thin and look like fibres. Large muscles have long fibres, which may be up to 600 mm in length in man (Harris et al., 2005). In the rat they are of course much shorter than that, but may run the full length of a skeletal muscle. Alnaqeeb and Goldspink (1987) reported that in rat soleus muscle, fibres ranged from about 25 to about 75 μm in diameter and noted that the range of diameters increased in old age. These long thin cells contain many nuclei that lie beneath the cell membrane at the periphery of the cells. In a study of mouse muscle, Bruusgaard et al. (2003) have reported that muscle cells could contain 100 nuclei, and although the majority were spread throughout the cell clustering under the cell membrane occurred at neuromuscular junctions (where nerve fibres contacted the cells). Hoppeler and Flück (2002) reported that muscle fibre cross sectional area hardly varied across a series of mammals of widely varying body mass. Their paper, and a review by Weibel (1987) should be consulted for information on the scaling of factors including mitochondrial volume and capillary density with metabolic rate.

The most obvious and important feature of muscle cells is that they can contract. Contraction is mediated by a system of interdigitating myofilaments. These myofilaments are of two types: those made of the protein myosin and those of the protein actin. A group of six (thin) actin filaments surround each (thick) myosin filament. During contraction, the actin filaments are drawn along the myosin filaments (see textbooks of physiology for details of this process, e.g. Bray et al., 1999). As the overlap between the filaments increases the muscle fibre shortens, relaxation is a reverse of this process. Energy for the contraction process is provided in the form of ATP produced by the mitochondria, so it is hardly surprising that muscle mitochondria are large in size and many in number. As mitochondria require oxygen, it is again hardly surprising that the capillary density of muscle is high.

Histochemical techniques allow three types of muscle fibre to be identified. These are the slow twitch, fast twitch and intermediate fibres (also known as the red, white and intermediate fibres). Most muscles contain a mixture of fibre types. Muscles containing mainly slow twitch fibres are resistant to fatigue and rich in myoglobin. For details of the distribution of fibre types in the rat see: Kilarski and Sjöström (1990) and Armstrong et al. (1984).

Structure of a Whole Skeletal Muscle

A single muscle is wrapped in an epimysium, a connective tissue sheath containing collagen fibres, the usual cells of loose connective tissue including fat cells, capillaries and nerve fibres. Septae of connective tissue radiate from the epimysium into the muscle dividing it into bundles of muscle fibres known as the fascicles. Each fascicle is wrapped in connective tissue: the perimysium. The process is repeated within the fascicle, with each muscle fibre surrounded by an endomysium. Blood vessels and nerve

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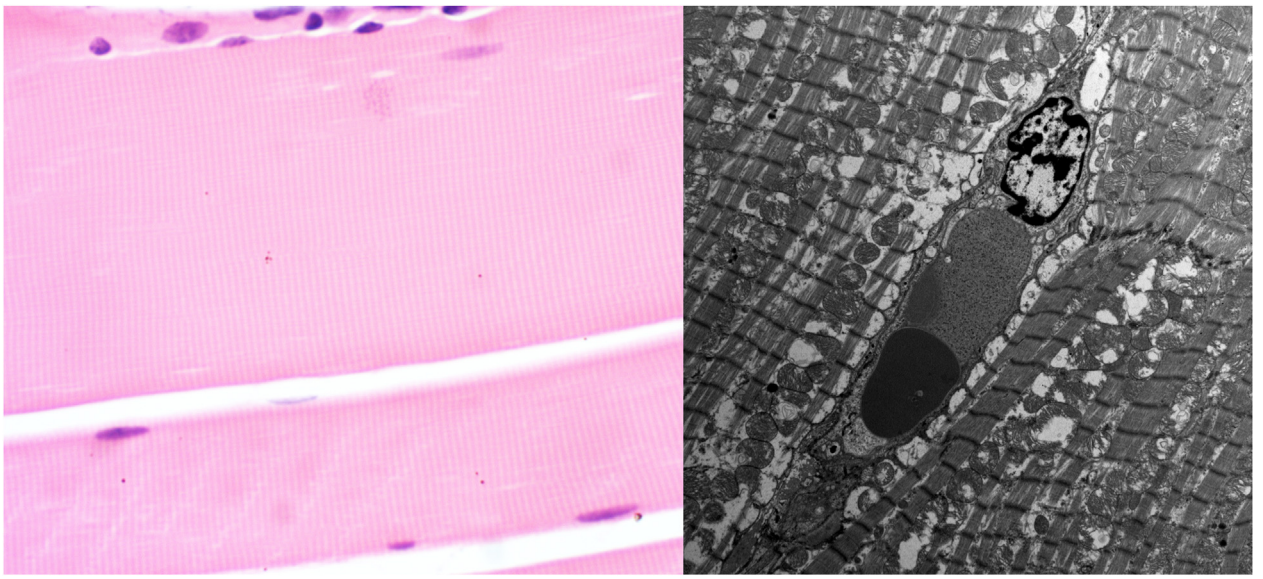


FIGURE 6.4 Microscopy of muscle. Although the bands are just about visible in the H and E section, electron microscopy is required for proper visualisation.
The sarcomere structure is common to skeletal and cardiac muscle. The band structure of the sarcomere is just discernible at high power in H and E of skeletal muscle, but can be clearly seen in the electron micrograph of cardiac muscle on the left.

fibres enter the muscle via this connective tissue network. The epimysium blends with the connective tissue of tendon if the muscle is inserted onto bone via tendon, but if inserted directly onto bone then the epimysium blends with the periosteum of the bone (Fig. 6.4).

Skeletal muscle is one of the more 'difficult' tissues for the light microscopist. A frame for holding the muscle at its normal length to avoid contraction when placed in fixative, rapid but thorough embedding in wax (vacuum embedding is recommended) and thin sections are needed if the subcellular structure of the cells is to be made out clearly. Muscle stains well with H&E, and the connective tissue stains (e.g. Masson's trichrome) show the connective tissue clearly, but iron haematoxylin stains (Heidenhain's or Weigert's) are best for examination of the cross banding of the cells, the nuclei and mitochondria. Really good sections allow the deeply staining A bands (include the full length of the thick filaments and part of the length of the thin filaments) and the paler staining I bands (containing only thin filaments) to be identified. In very good sections, the Z lines at the midpoints of the I bands can be clearly seen as thin dark lines. Transverse sections allow the fascicles to be identified and the nuclei to be recognised under the cell membrane of the fibres.

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Chapter 7

The Cardiovascular System

Few satisfactory accounts of the cardiovascular system (CVS) of the rat exist, although good accounts of the CVS in man, cat, dog and domestic animals are available (Williams, 1995; Reighard and Jennings, 1929; Evans and Christensen, 1979; Getty, 1975). Hebel and Stromberg (1976) provided an admirably short account that tabulates the major arteries, but said little about the venous system. Greene (1935) provided a longer, well-illustrated account that uses the terminology of human anatomy. Neither discusses the development or histology of the system. Early accounts in the German literature were not accessible to us, but references to these works may be found in Hebel and Stromberg (1976). In this chapter, we provide an introductory account of the CVS followed by a more detailed account of the heart, arteries and veins of the rat. The histology of the CVS is discussed in Chapter 8, Histology of the Vascular System.

INTRODUCTION TO THE ANATOMY OF THE CARDIOVASCULAR SYSTEM

There can be few readers who do not know that the CVS comprises the heart, arteries, capillaries and veins. The heart provides the energy to propel the blood around the system, the arteries carry blood from the heart to the capillaries, and the veins return the blood from the capillaries to the heart. It will also be widely known that all mammals have a double circulation.

The systemic circulation comprises the left ventricle of the heart, the aorta and its branches, the systemic arteries, the systemic capillaries and the veins that join to carry the blood back to the right atrium of the heart via the venae cavae (one vena cava, two or more venae cavae). In the rat, there are two superior (cranial) venae cavae and one inferior (caudal) vena cava; in man there is only one superior vena cava.

In the pulmonary circulation, blood from the right ventricle of the heart passes into the pulmonary arteries and is carried to the lungs. Once oxygenated, the blood flows back to the heart via the pulmonary veins and is delivered to the left atrium. Although the pulmonary arteries carry venous blood, they are described as arteries on the basis that they carry blood away from the heart, and the pulmonary veins, which carry oxygenated blood, are described as veins, on the basis that they carry blood towards the heart. The evolution of the lungs was an early event in the evolution of fish, and the swim bladder found in many fish evolved from the lungs, and not vice versa (Romer, 1970).

There is almost no limit to the details that could be provided regarding the arteries and veins of the body. In man nearly every vessel visible to the naked eye has been named. In the rat a dissecting microscope is necessary if the finer branches of arteries and tributaries of veins are to be traced. Greene (1935) has provided the most detailed, and certainly the best illustrated account of the rat vascular system currently available. Our less ambitious approach includes by no means all the arteries and veins that have been identified and named in the rat. The blood supply of the brain has been considered in more detail than that of other regions and organs, and here the discussion of the arterial supply and the venous drainage have been combined. It is hoped that this will be helpful to the reader (Fig. 7.1).

THE SYSTEMIC ARTERIAL SYSTEM: DEVELOPMENT AND GENERAL ARRANGEMENT

In fish, the blood flows forwards from the single ventricle in the ventral aorta. A series of branches arch around the pharynx to supply the capillaries of the gills. These arches run between the gill slits in the tissue of the pharyngeal arches, and are known as the aortic arches. The efferent branchial vessels running from the gills combine to form a pair of dorsal aortae that run cranially to the head and caudally before joining to produce a single dorsal aorta, from which the rest of the body is supplied.

Each arch should have its own artery branching from the ventral aorta, but as Romer (1970) explains, even in fish, things are not quite so simple. The relevance of this to the rat and to man is that aortic arches one to six (but not number five) make an appearance during embryonic development. At no time are all the arches present. The

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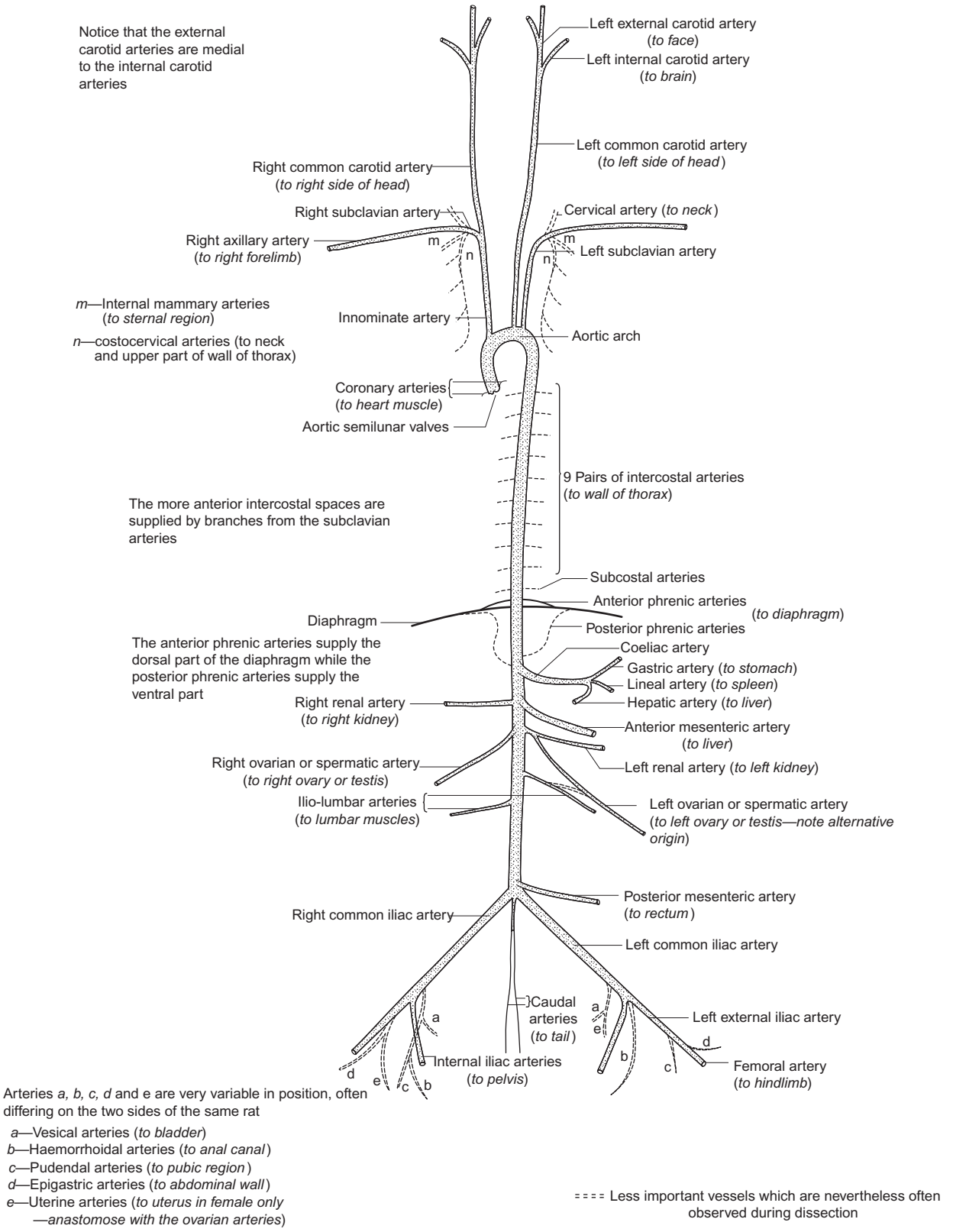


FIGURE 7.1 The major arteries of the rat.

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anterior members disappear as the posterior members appear. It is also important to remember that the adult arrangement includes bits and pieces from arches that have largely disappeared.

The two dorsal aortae in the cervical region largely disappear (completely so on the right), but on the left the fourth arch provides part of the adult aortic arch and descending aorta. The sixth arches provide the pulmonary arteries, but even this is more complicated that it might appear. In man, the pulmonary arteries sprout from the fourth arch, and become secondarily attached to the sixth arch arteries. Part of the connection between these arches persists until just after birth as the ductus arteriosus, which shunts blood from the pulmonary artery to the aorta. This is useful arrangement in foetal life as the pulmonary artery is carrying oxygenated blood that has been returned from the umbilical circulation, and there would be little merit in sending this oxygenated blood through the nonfunctioning lungs, so instead it is shunted into the aorta. The ductus usually closes soon after birth once the lungs are functioning, but does occasionally remain patent in man, requiring surgery to close the defect.

Each primitive dorsal aorta gives off a series of lateral branches that supply the body wall. These are the intersegmental arteries. Of this series the seventh forms, on each side, much of the subclavian artery that supplies the forelimb. Those that follow it provide the intercostal arteries that run between the ribs and around the thoracic cavity to anastomose with the intercostal branches of the internal thoracic arteries that arise from the subclavian arteries and run down the ventral surface of the chest wall near the sides of the sternum (Mizukami and Tanaka, 1991). The fate of the first six lateral branches is peculiar and interesting. Though they lose contact with the dorsal aortae, their remnants join to produce the left and right vertebral arteries that run via the foramina in the transverse processes of the first six cervical vertebrae to enter the cranial cavity via the foramen magnum to supply the brain. Each vertebral artery arises from a subclavian artery.

The fate of the aortic arches in man is set out in Table 7.1.

The stapedial artery, the artery that runs through the stirrup shaped stapes, is not of much importance in man, but in the rat, it is of great importance: see below.

TABLE 7.1 Derivatives of Aortic Arches and Associated Structures in Man

	Left	Right
First aortic arch	Part of L maxillary artery, a branch of the L external carotid artery	Part of the R maxillary artery, a branch of the R external carotid artery
Second aortic arch	L stapedial artery in the embryo. A branch of the external carotid artery. Stapedial artery disappears leaving a hole in the stapes	R stapedial artery in the embryo. A branch of the external carotid artery. Stapedial artery disappears leaving a hole in the stapes
Third aortic arch	Cervical part of L common carotid artery	Cervical part of R common carotid artery
Fourth aortic arch	That part of the arch of the aorta between the origin of the L common carotid artery and the L subclavian artery	Proximal part of R subclavian artery
Fifth aortic arch	Not developed	Not developed
Sixth aortic arch	L pulmonary artery and ductus arteriosus	R pulmonary artery
Dorsal aorta. Originally two: L and R, running forwards and linking the dorsal, efferent, ends of the aortic arches. The L and R dorsal aortae ran back and joined to form the descending aorta than runs dorsally through the thorax and abdomen	Left dorsal aorta contributes to the L internal carotid artery distal to third arch contribution	Right dorsal aorta contributes to the R internal carotid artery distal to third arch contribution
Aortic sac	A midline structure that contributes to the arch of the aorta and to the L common carotid artery and R brachiocephalic artery	

Source: Larsen W.J. Human Embryology. Churchill Livingstone. New York, Edinburgh, London. 1993.

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BRANCHES OF THE ABDOMINAL AORTA

The abdominal aorta provides three groups of branches:

1. Intersegmental arteries to the body wall (ilio-lumbar arteries)
2. Branches to the organs of the genito-urinary system (renal and ovarian/testicular arteries)
3. Branches to the gut

Three major unpaired arteries supply the gut: the coeliac artery, the anterior (cranial) mesenteric artery and the posterior (caudal) mesenteric artery. The abdominal aorta ends by dividing into the common iliac arteries (sometimes described simply as the iliac arteries) that supply the organs and tissues of the pelvis and hindlimb and tail. A terminal branch of the internal thoracic artery, the anterior (cranial) epigastric artery, runs caudally down the ventral abdominal wall to anastomose with a branch of the iliac artery, the posterior (caudal) epigastric artery. The femoral artery, another branch of the iliac artery, is the great artery of the hindlimb.

Having outlined the arterial system, we shall now turn to the heart.

THE HEART

The rat heart lies in the ventral part of the thorax, above the sternum, between the lungs and somewhat further from the diaphragm than in man. In man the heart sits on the diaphragm, and as a result has a flattened base, whereas in the rat the heart has a more ovoid shape (Carvalho and Thomazini, 2013). Mediastinal connective tissue connects the pericardium to the diaphragm and the thymus. The pericardium of the rat is a delicate structure compared with that of man. An outer fibrous layer blends with the walls of the great vessels entering and leaving the heart, and an inner layer parietal layer is reflected over the surface of the heart as the visceral pericardium, forming part of the wall of the heart: the epicardium. The opposing surfaces of the parietal and visceral pericardia are covered with a single layer of mesothelial cells, separated by a small amount of fluid. Like the lung, the heart has been invaginated into the pericardium.

The rat heart, like the hearts of all mammals, is four chambered and comprises left and right ventricles and atria. The atria are sometimes mistakenly referred to as auricles, but this term should be reserved for the ear shaped parts of the atria that curve ventrally around the aorta and pulmonary trunk. The left ventricle has a thick wall that continues as the interventricular septum. The right ventricle has a thinner wall, and in cross sections of the heart appears to be plastered onto the left ventricle and interventricular septum. Both atria are thin walled. The right atrium receives blood from the paired anterior or cranial venae cavae, from the single posterior vena cava and from the coronary sinus which collects blood from the intrinsic veins of the heart, the coronary veins (Kresakova et al., 2015). The left atrium receives blood from the pulmonary veins. In man, there are four pulmonary veins that enter the left atrium by four separate orifices, but in the rat there is just one opening into the atrium, as the pulmonary veins join outside the atrium. This is the condition in the developing human heart, but the confluence is absorbed into the wall of the atrium leaving the pulmonary veins to open directly and separately into the left atrium. The arrangement of the atria and ventricles is shown in Fig. 7.2.

Valves separate the ventricles from the atria and prevent back-flow of blood into the atria during ventricular contraction. Valves also guard the entrances to the aorta and pulmonary artery and prevent reflux of blood from these vessels into the ventricles during relaxation of the ventricles. These valves have semilunar shaped cusps, and are located at the root of each great artery. The tricuspid valve, on the right, has three cusps; the mitral valve, on the left has two cusps. A bishop's hat, his mitre, has two peaks: hence mitral valve. Each cusp is attached to wall of the ventricle by thin tendinous strings (chordae tendineae) that run from muscular elevations of the ventricular walls (Figs. 7.3 and 7.4).

Viewing the heart from the ventral aspect, the pulmonary artery can be clearly seen, but the root of the aorta is largely hidden by the pulmonary artery as it crosses the aorta and then passes under the arch of the aorta, runs for a short distance down the dorsal surface of the heart, before dividing into right and left pulmonary arteries. The arch of aorta gives off three large arteries, the brachiocephalic (or innominate) artery, the left common carotid artery and the left subclavian artery. All these run cranially towards the inlet of the thorax. Note from Fig. 7.4 the arrangement of the veins on the dorsal surface of the heart. The pulmonary veins overlap the coronary sinus.

The blood supply to the heart is provided by the left and right coronary arteries (so named because they from a crown or coronet around the heart). In man they lie on the surface of the heart, but in the rat they are buried more deeply in the wall of the heart (Carvalho and Thomazini, 2013, Ratajska et al., 2003).

The left coronary artery arises from the root of the aorta, just above the valve that separates the aorta from the left ventricle. It supplies the left ventricle via a ventral branch that runs in the groove between the left and right ventricles on the ventral surface of the heart, and the left circumflex artery that curls around to the dorsal surface of the ventricle. The right coronary artery runs in the groove between the right ventricle and atrium before curling around to the dorsal surface of the ventricle having given off a branch (the marginal artery in

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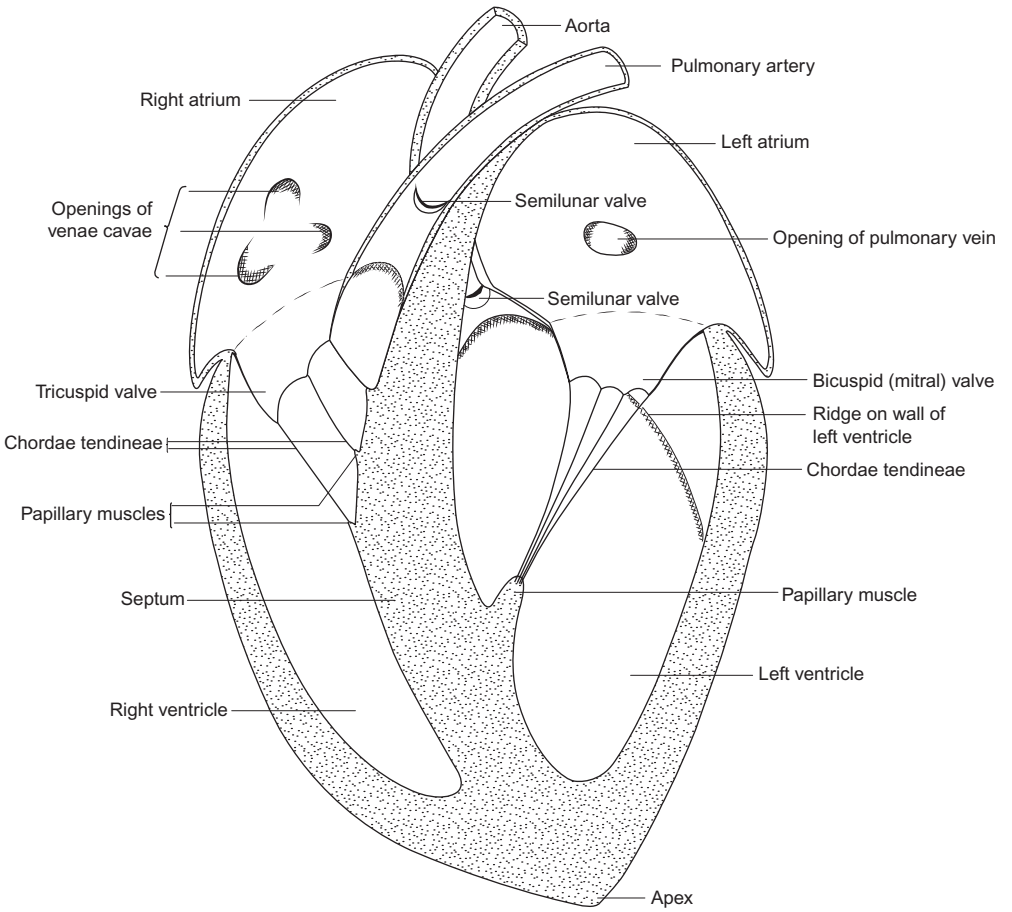


FIGURE 7.2 Heart (dissected).

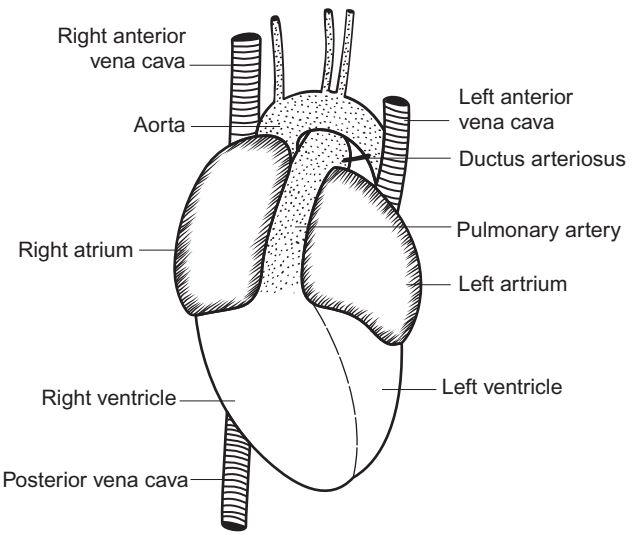


FIGURE 7.3 Heart (ventral view).

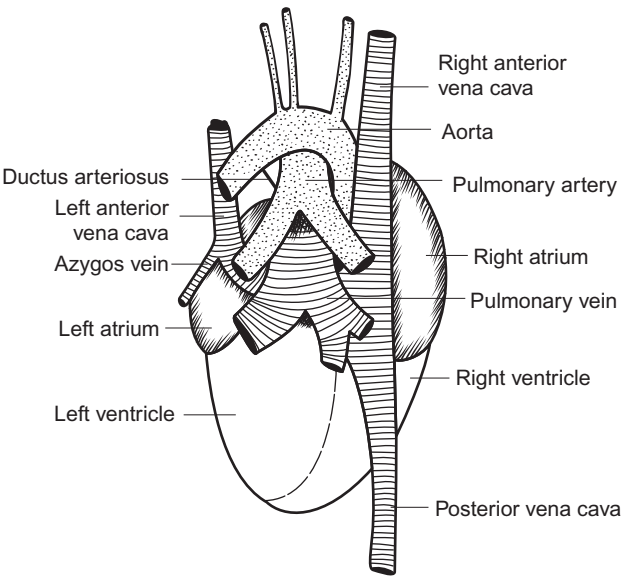


FIGURE 7.4 Heart (dorsal view).

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man) that runs along the right ventricle towards the apex of the heart.

In man the interventricular septum is supplied by both the right and left coronary arteries, but in the rat it is supplied by either one or the other. In about 50% of animals the supply is from the right, and in about 50% the supply is from the left (Ahmed et al., 1978). In man the atria are supplied by branches of the right and left coronary arteries, and an important branch of the right coronary artery supplies the pacemaker of the heart, the sino-atrial (SA) node (see below).

The atria and SA node in the rat have a double blood supply from the coronary vessels and the internal thoracic artery, and a matching double drainage (Mizukami et al., 1992). The extra-coronary cardiac veins run from the dorsal surface of the heart to the superior (anterior or cranial) venae cavae (Halpern, 1953a).

The true coronary veins accompany the coronary arteries. In man the vein accompanying the interventricular branch of the left coronary artery is the great cardiac vein; that accompanying the marginal branch of the right coronary artery is the small cardiac vein. The middle cardiac vein lies on the posterior surface of the ventricles in man. Enthusiasts will wish to know that in man the vein of the left atrium (the vein of Marshall) is a remnant of the left anterior cardinal vein; in the rat the left anterior cardinal vein persists as the left anterior vena cava.

It will be well known that the heart shows spontaneous contractile activity driven by impulse from the SA node (sinu-atrial is correct but sino-atrial is widely used). In the rat the SA node is found partly in the wall of the branch of the internal thoracic artery, where it enters the right atrium near the entry into the atrium of the right superior (anterior or cranial) vena cava (Anderson et al., 1979, Taylor, 1980). Impulses are conducted via the walls of the right atrium to the left atrium, and thence to the atrio-ventricular node, a small mass of specialised conducting tissue at the junction of the atria and the ventricles around the atrio-ventricular orifices (Anderson, 1972). Like all mammals the rat heart has a fibrous skeleton surrounding the atrio-ventricular orifices, the roots of the aorta, and pulmonary artery. Cartilage cells can be found in this tissue in the rat, and in some animals, such as the cow, bone (the os cordis) is present.

The histology of the heart is described in Chapter 8, Histology of the Vascular System.

The Systemic Venous System: Development and General Arrangement

The major veins of the rat are shown in Fig. 7.5.

In some ways, the development of the venous system is more complicated than that of the arterial system. In the development of the arterial system, the area of greatest complexity and interest is provided by the aortic arches and their derivatives; the complexity of the development of the venous system is due to there being three systems to consider. These are the vitelline, umbilical and systemic venous systems. The first two of these are discussed in Chapter 14, Liver, here we focus on the systemic veins.

Early development of the systemic veins produces paired anterior and posterior cardinal veins that run dorsally, from cranial to caudal, through the thoracic and abdominal cavities. On each side, the anterior and posterior cardinal veins join to create the common cardinal veins that run to the sinus venosus of the heart via the septum transversum. In man, the right common cardinal vein forms the superior vena cava, and the left common cardinal vein disappears, but in the rat the left anterior cardinal vein persists and forms the left anterior vena cava. A bridge between the anterior part of the left anterior cardinal vein and the right anterior cardinal vein forms in the region of the developing thymus.

The posterior cardinal veins are in Larsen's words 'augmented by' a two further pairs of veins, the sub and supra-cardinal veins that develop parallel with, and close, to the posterior cardinal veins (Larsen, 1993). Most of each posterior cardinal vein disappears. The inferior vena cava is put together from the remains of the right subcardinal vein, parts of the right supra-cardinal vein, and anterior to the liver, from a part of the right vitelline vein. It might be useful to remember the posterior vena cava is formed from parts of the right vitelline vein, right subcardinal vein, right supra-cardinal vein, that it lies on the right of the abdominal aorta, and delivers blood to the right atrium. The azygous and hemiazygous veins and their continuations into the abdomen, the ascending lumbar veins, are formed from the supra-cardinal veins. The iliac veins join a linkage between the caudal ends of the right and left posterior cardinal veins that contribute to the caudal end of the posterior vena cava.

The internal and external jugular veins, whose relative importance in the rat is the converse of that in man, drain to the anterior venae cavae. Details of the development of the veins of the head and cranial cavity and brain lie outside the scope of this account. The significant differences between the arrangements in the rat and man suggest that human embryology might be a poor guide to rat embryology in this area. See Szabo (1990 and 1995) for further details.

Arteries of the Lower Part of the Neck and Shoulder Region: The Territory of the Subclavian Artery

The right subclavian artery arises with the right common carotid artery from the brachiocephalic (innominate)

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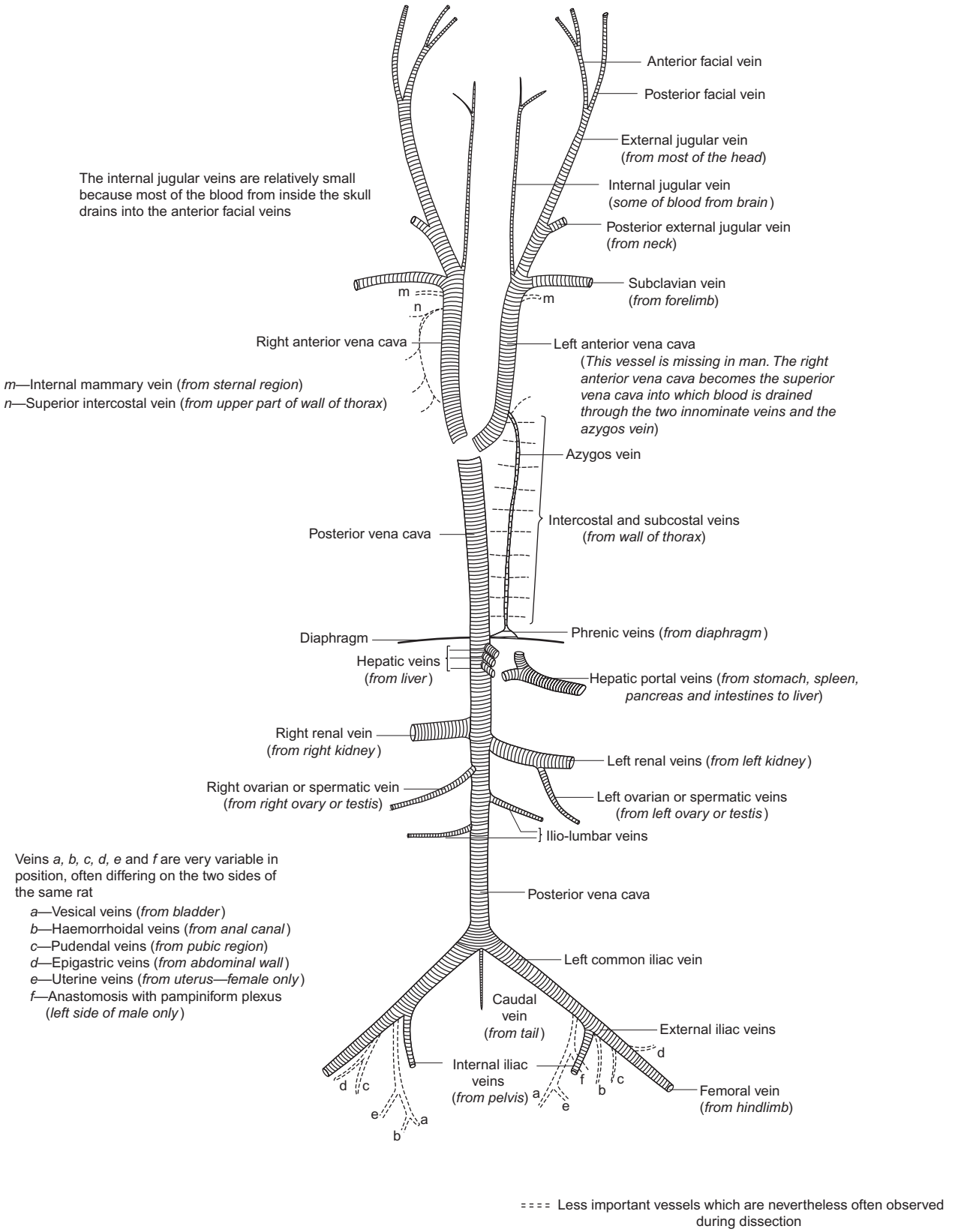


FIGURE 7.5 Principal veins of the rat.

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artery. The left subclavian artery and the left common carotid artery both arise from the arch of the aorta. Both subclavian arteries turn laterally to pass between the anterior end of the first rib, and the medial end of the clavicle. As the subclavian artery crosses the first rib, it becomes the axillary artery. Before and during its passage between the clavicle and the first rib, the subclavian artery gives off the following branches:

1. The costo-cervical trunk. This branch turns caudally and runs down the inner surface of the dorsal wall of the thoracic cavity. It supplies branches to muscles ventral to the vertebral bodies, to the thyroid gland (inferior or caudal thyroid artery) and to the anterior intercostal spaces.
2. The internal thoracic artery runs caudally down the inner surface of the ventral wall of the thorax, but outside the parietal pleura, just to the side of the sternum. It supplies branches to the anterior ends of intercostal spaces before dividing into the musculo-phrenic artery to the ventral part of the diaphragm and the anterior epigastric artery that runs caudally in the ventral abdominal wall.
3. The vertebral artery turns dorsally from its origin, crosses the roots of the brachial plexus (see Chapter 21 (2): The Spinal Nerves) and runs to the foramen in the transverse process of the sixth cervical vertebra. It then turns cranially and runs forwards through all the transverse foramina anterior to the sixth. Passing through the foramen magnum, it then moves towards the midline and joins with its colleague to form the basilar artery of the brain stem.
4. The cervical trunk (thyro-cervical trunk in man) provides the inferior thyroid artery. In the rat the inferior (caudal) thyroid artery springs from the costo-cervical trunk. The cervical trunk supplies muscles of the neck and shoulder.

The Common Carotid Arteries

The common carotid arteries run forwards in the neck accompanied by the internal jugular veins and vagus nerves. These three structures are enclosed by the carotid sheath, medial to which lie the recurrent laryngeal nerves and the cervical sympathetic trunks. Ventrally the common carotid arteries are covered by the sternothyroid muscles. Both common carotid arteries divide at the level of the thyroid gland to produce the internal and external carotid arteries.

The External Carotid Artery

This artery could be called the artery of many branches. Greene's account considers the artery in three parts.

The first part runs forwards to the angle of the jaw and provides five branches. These are the occipital, superior (cranial) thyroid, ascending pharyngeal, lingual and ascending palatine branches. The occipital artery supplies muscles and skin over the caudal/dorsal aspect of the skull; the destinations of the other branches are suggested by their names.

The second part makes a right angle turn and runs vertically to the ear and gives off three branches: the external maxillary or facial artery, the posterior auricular artery and the masseteric artery. The external maxillary artery supplies the facial area, the tonsil, the submandibular salivary gland and the tissue below the lower jaw (submental artery). It also sends a branch to the masseter muscle. The parotid gland is also supplied by branches from the external carotid artery. The rat has large masseter muscles and these receive a triple blood supply: two arteries from the second part of the external carotid, one from the internal maxillary artery.

The third part makes another right angle turn and runs forwards providing two terminal branches. The superficial temporal artery, which supplies the dorsal part of the face and the anterior dorsum of the scalp, and the complicated internal maxillary artery. The first branch of the internal maxillary artery is the inferior mandibular artery, which turns to the inner surface of the mandible, and along with the inferior mandibular nerve enters the mandibular foramen to supply the lower jaw and teeth. The second branch of the division is the pterygoid branch of the internal maxillary artery. In man the equivalent of this artery provides the very important middle meningeal artery before turning into the pterygo-palatine fissure. Once it has reached the pterygo-palatine fossa (see Chapter 5: The Skull), it gives off a further series of branches. In the rat the middle meningeal artery arises from the pterygo-palatine artery while it is in the tympanic bulla (Scremin, 2014): see below. This important observation fits well with the view that the pterygo-palatine artery of the rat is the equivalent of a good deal of the maxillary artery of man.

The Internal Carotid Artery

The internal carotid artery in man gives off no branches before entering the carotid canal just in front of the jugular foramen, but this is not the case in the rat. The internal carotid artery branches close to the tympanic bulla where one branch (still called the internal carotid artery) enters the carotid canal. The other branch, the pterygo-palatine artery, enters the jugular foramen and penetrates the tympanic bulla, then passes through the stapes bone (through the stirrup) and leaves the middle ear along with the chorda tympani branch of the facial nerve (VII cranial nerve) via the petro-tympanic fissure (see Chapter 5: The

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Skull). It then divides to produce a pterygoid branch and a palatine branch. The fate of the palatine branch is fascinating, passing through the foramen ovale and the alisphenoid foramen (canal) to enter the ventral, posterior part of the orbito-temporal fossa. In the rat this is equivalent to the pterygo-palatine fossa of man, so the palatine artery is well placed to take over the work of the last part of the maxillary artery of man. It provides descending palatine arteries, the infra-orbital artery (that leaves the orbit via the infra-orbital foramen) and the sphenopalatine artery (that passes through the sphenopalatine foramen into the nasal cavity). Much more remarkably the palatine branch of the pterygo-palatine artery provides the ophthalmic artery, which supplies the extrinsic muscles of the eye and the interior of the eyeball via the ciliary arteries. Further details of the development of the ophthalmic artery of the rat may be found in Moffat (1961). In man this artery arises from the internal carotid artery after passing through the dura and arachnoid on the medial side of the anterior clinoid process.

The less important branch of the pterygo-palatine artery, the pterygoid branch, described by several authorities as 'variable', supplies the roof of the pharynx. It is described by Hebel and Stromberg (1976) as Vidi's artery, suggesting perhaps that it runs in the Vidian or pterygoid canal. This rather fine point of rat anatomy is not likely to be of great interest to the majority of readers.

BLOOD SUPPLY OF THE BRAIN

The blood supply of the brain is both complicated and important. In this section we shall deal with both the arterial supply of the brain and its venous drainage. Scremin (2014) has provided an excellent, though demanding, description of the blood supply of the brain. *Craigie's Neuroanatomy of the Rat* (Zeman and Innes, 1963) provides a less detailed account.

The Arteries of the Brain

After passing through the carotid canal, the internal carotid artery appears inside the skull near the caudal border of the pituitary gland, and having given off the posterior communicating artery, runs forwards to the level of the optic chiasma. Here the internal carotid artery divides into the middle cerebral artery, which runs laterally across the inferior surface of the cerebral hemisphere to reach and ramify over its lateral surface; and the anterior cerebral artery, which runs forwards and joins with its partner. The joining of the anterior cerebral arteries produces the anterior cerebral trunk (the azygous cerebral trunk) that arches up and back over the anterior end of the corpus callosum to supply the medial aspects of both cerebral hemispheres. In man both the right and left anterior cerebral arteries persist and are joined together by the anterior communicating artery, which does not occur in the rat.

The posterior communicating artery runs back and reaches either the basilar artery or the superior cerebellar artery, a terminal branch of the basilar artery. The long posterior communicating artery is important and gives off the posterior cerebral artery that supplies part of the cerebral hemisphere and mesencephalon (see Chapter 20: The Brain and Spinal Cord).

The choroid plexus of the third and lateral ventricles has its own blood supply. The anterior choroidal arteries arise directly from the internal carotid arteries, and supply the posterior–inferior parts of the choroid plexuses (plexuses not plexi) of the lateral ventricles. The posterior choroidal arteries arise from the posterior cerebral artery, supplying the choroid plexus of the third ventricle and the remaining parts of the plexuses of the lateral ventricles. The inferior horns of the lateral ventricles lie just above the internal carotid arteries, hence their supply via the anterior choroidal arteries.

The basilar artery is formed by fusion of the right and left vertebral arteries. Prior to fusion these arteries give off the posterior–inferior cerebellar arteries, and arteries to the spinal cord. The basilar artery gives off a palisade of lateral branches to the brain stem, the internal auditory arteries, which run with the VIIth and VIIIth cranial nerves to the inner ear (see Chapter 23: The Ear), and the anterior inferior cerebellar arteries. The basilar artery also runs far enough forwards to provide small branches that surround the stalk of the pituitary gland before ending by dividing into the two superior cerebellar arteries.

It is important to remember that there is considerable intersubject variation in the arterial supply of the brain. This especially affects the relationship between the posterior communicating artery, the posterior cerebral artery and the superior cerebellar artery (Moffat, 1957, 1961).

The Venous Drainage of the Brain

The venous drainage of the rat brain is in principle similar to that of the human brain. The key principles are:

1. Blood from the capillaries of the brain is collected by venules that join to form veins that run on the surface of the brain.
2. In this context, the surface includes the obvious outer surfaces of the brain and what might be called an internal surface between the hemispheres and the central parts of the brain around which the hemispheres are folded.
3. Veins from these surfaces join the venous sinuses that run in the dura mater lining the skull. The dura has an

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outer layer made up of the periosteum of the inner surface of the skull, and an inner layer or true dura covering the brain. The outer layer is firmly adherent to bone, while the inner layer forms a number of partitions of the cranial cavity (falx cerebri and tentorium cerebelli), before continuing into the vertebral canal as the dura of the spinal cord. There it is separated from the periosteum of the vertebrae by the extradural space. The venous sinuses of the cranial cavity are lined by endothelium and lie between the layers of the cranial dura, but the venous plexuses of the spinal cord lie in the extradural space.

4. Blood collected by the venous sinuses leaves the cranial cavity via veins. In man the internal jugular vein is by far the most important of these, but in the rat the internal jugular veins are very small and were described by Scremin (2014) as 'almost vestigial'. Their place is taken by the retroglenoid veins, which leave the skull via the postglenoid foramina between the tympanic bullae and the edges of the retrotympanic processes of the squamous parts of the temporal bones (see Chapter 5: The Skull). Another difference between rat and man is illustrated by the comparatively large interpterygoid vein of the rat, which passes through the interpterygoid foramen (see Chapter 5: The Skull). Compare this with the small vein that passes through the foramen of Vesalius (emissary sphenoidal foramen) adjacent to the foramen ovale in man.
5. In general, it can be said that the arterial supply to the brain lies on its ventral aspect; the venous drainage runs on its dorsal surface, although as always, there are several exceptions to the rule.

The venous drainage of the brain can be divided into a superficial system, involving veins that run from the outer surface of the brain to adjacent sinuses, for example the superior sagittal sinus running in the upper margin of the falx cerebri; and a deep system, in which veins from the interior run to the vein of Galen (the great cerebral vein or the azygous internal cerebral vein). This large vein emerges below the posterior end of the corpus callosum (the splenium of the corpus callosum) and joins the inferior sagittal sinus, which runs in the lower margin of the falx cerebri to form the straight sinus. The straight sinus and superior sagittal sinus join to form the transverse sinuses, which run in the margin of the tentorium cerebelli and drain to the retroglenoid veins that leave the skull via the postglenoid foramina. It is important to remember that the tentorium cerebelli is almost vertical in the rat. There are many small sinuses in both rat and man, but one that is important is the cavernous sinus, which lies on either side of the basisphenoid bone and in the rat drains to the interpterygoid veins.

THE ARTERIES OF THE ABDOMEN AND PELVIS

The aorta enters the abdominal cavity by passing between the right and left crura of the diaphragm and the ventral surface of the first lumbar vertebra. The abdominal aorta runs back below the bodies of the vertebra, to the left of the inferior vena cava, and divides to form the common iliac arteries at the level of the last, sixth, lumbar vertebra. It is important to recall that the abdominal aorta runs above the parietal peritoneum lining the dorsal abdominal wall, and the branches within the abdominal cavity either remain in this retroperitoneal plane, for example the renal arteries, or if they supply the gut, run between the layers of the mesentery.

The diaphragm receives its blood supply from paired cranial phrenic arteries that leave the thoracic aorta before it crosses the diaphragm; from the caudal phrenic arteries that leave the aorta just as it appears in the abdominal cavity, and from the musculo-phrenic branches of the internal thoracic arteries. The nomenclature of these arteries is derived from phrenicus, the Latin word for diaphragm.

The abdominal aorta provides blood to the gut via the coeliac, the cranial (superior) mesenteric artery and the caudal (inferior) mesenteric artery. These unpaired arteries arise from the ventral aspect of the aorta.

The short coeliac artery leaves the aorta close to the diaphragm, and branches to create the splenic, hepatic and left gastric arteries. The splenic artery runs to the left to reach the spleen, which in man involves a route via a fold of peritoneum called the lieno-renal ligament (lien is Latin word for spleen) that connects the spleen to the left kidney. Before branching and entering the spleen, the splenic artery gives off the short gastric arteries that supply adjacent parts of the stomach and oesophagus. The left gastric artery supplies the lesser curvature of the stomach and runs forwards to the oesophagus which it also supplies. Running via the lesser omentum, which stretches from the lesser curvature of the stomach and the first part of the duodenum to the liver, the hepatic artery runs alongside the portal veins and common bile duct to the liver (see Chapter 14: Liver). The hepatic artery gives off the gastroduodenal artery, which itself divides to produce the right gastroepiploic artery, and the pancreaticoduodenal artery (Epiploön is Greek for the omentum). In man the left gastroepiploic artery arises from the splenic artery and anastomoses with its partner along the greater curvature of the stomach.

The cranial mesenteric artery is the largest branch of the abdominal aorta. Leaving the ventral aspect of the aorta 3–5 mm caudal to the coeliac artery, it turns to the right and crosses the inferior vena cava, whereas in man it crosses the left renal vein. It supplies the small intestine

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and the large intestine, including part of the descending colon (rather further along the colon than in man). There are two major branches of the cranial mesenteric artery. The right colic artery serves the ascending colon, and the ileocolic artery supplies the ileum, caecum and the colon, as well as a leash of branches to the jejunum and ileum.

The caudal mesenteric artery arises near the bifurcation of the abdominal aorta and supplies the rectum and part of the descending colon.

Arterial Supply of the Kidneys, Adrenal Glands and Gonads (Testis or Ovary)

The renal arteries leave the aorta between the coeliac and cranial mesenteric arteries. The right renal artery is longer than the left, because it has to pass behind the inferior vena cava to reach the right kidney (see Chapter 17: Urinary Tract).

The adrenal arteries arise either from the renal arteries or directly from the aorta. The origins of the ovarian and testicular (spermatic) arteries are also subject to some variation. On the right, they arise from the aorta at about the level of the left renal artery, but on the left they more often arise from the left renal artery itself. Some authors describe both left and right spermatic arteries as arising from the aorta (Hunt, 1924, Hebel and Stromberg, 1976), but Rowett shows the left spermatic artery arising from the left renal artery.

The testis, epididymis and vas deferens (ductus deferens) have a complicated blood supply. Greene states that the spermatic artery (internal spermatic artery in her terminology) passes back along the dorsal abdominal wall, crosses ventral to the ureter and enters the inguinal canal where it joins the vas deferens as part of the spermatic cord. In the scrotum it divides into the testicular arteries, which wind round the pampiniform plexus of veins before reaching the testis, and the epididymal arteries. The vas deferens has its own artery from a branch of the internal iliac artery. In the female, the ovarian arteries supply the ovaries, and the uterus draws its blood supply from the uterine arteries, branches of the internal iliac arteries.

Blood Supply of the Tail

The middle caudal artery (middle sacral artery of Hebel and Stromberg, 1976) leaves the aorta close to its bifurcation and runs to the tail. The blood vessels of the tail are of interest to physiologists and toxicologists because the dorsal veins can be cannulated for blood sampling and for intra-venous dosing. The blood supply to the tail is also of interest because 20% of heat loss in the rat occurs via the tail. The ventral artery is the main artery of the tail, running in a groove formed by the incomplete haemal arches of the caudal (coccygeal) vertebrae. This artery has a thick, muscular wall all the way to the tip of the tail. There are smaller arteries that lie dorsally and laterally (Staszyk et al., 2003) and the major veins lie to each side of the midline of the dorsal surface.

The Ilio-Lumbar Arteries

One aspect of terminology that is practically guaranteed to create confusion is with regard to the iliac arteries. In this instance, 'iliac' refers to the ilium, a part of the hip bone and has nothing to do with the ileum. The adjective associated with ileum is ileal, as in the ileal branch of the ileocolic artery.

Lateral branches of the aorta supply the tissues of the body wall and the spinal cord. There are about five of these arteries in the rat, each arising from the dorsal aspect of the aorta. These arteries may arise as paired arteries, or as single branches that divide into left and right. Branches to spinal cord pass through the intervertebral canals and pass the spinal nerves as they leave the vertebral canal.

The Iliac Arteries

The key word regarding the iliac arteries is 'variation'. The details of the branches of the iliac arteries vary from animal to animal and from account to account (Greene, 1935; Hebel and Stromberg, 1976). Each common iliac artery divides to form the external iliac artery and the internal iliac or hypogastric artery.

The external iliac artery provides the femoral artery, which runs beneath the inguinal ligament to the hindlimb, and the external spermatic artery that supplies the cremaster muscle. Sometimes it is also the source of the inferior or caudal epigastric artery. The internal iliac artery is a large artery that supplies the urinary bladder (superior and inferior vesical arteries), the uterus in the female, the ductus deferens in the male and usually the inferior or caudal epigastric artery mentioned above. Branches also run to the muscles associated with the hip joint.

ARTERIES OF THE LIMBS

The arterial supply to the adult limb (forelimb or hindlimb) differs from that which first appears in the embryo. In the developing embryo an axis artery runs down the centre of the developing limb. This artery is, to a greater or lesser extent, replaced by other arteries that develop later. Parts of the axis artery remain and are incorporated into the final system, other parts are lost. The joints receive an excellent collateral blood supply from a system of branches of the major arteries that surround and supply each joint. The brachial and femoral arteries give off deep or profunda branches, and the ulnar

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and popliteal arteries give off branches that pass to the extensor compartments of the second segments of the limbs. Generally the major arteries run of the flexor sides of the limbs and send branches to the extensor compartment.

There are many short arteries involved in this collateral supply, the names of these which need not concern us: see Greene (1935) for details.

The Forelimb Arteries

The axillary artery is the continuation of the subclavian artery that runs to the axilla (arm pit in man), on its way to supplying the forelimb. On its way, it gives off the thoraco-acromial and lateral thoracic arteries and the circumflex scapular trunk. The lateral thoracic artery, as the name implies, supplies the lateral chest wall. Other branches are concerned with the shoulder joint and with the muscles acting on that joint, which include the pectoral muscles of the ventral chest wall (see Chapter 6: The Musculature of the Rat). The axillary artery is closely associated with nerves of the brachial plexus (see Chapter 21 (2): The Spinal Nerves).

The axillary artery continues into the forelimb as the brachial artery, running on the medial side of the humerus and giving off the profunda brachii artery that penetrates to the extensor compartment of the limb and supplies the triceps muscle. Other more superficial branches also leave the brachial artery. At the elbow, having given off a group of recurrent and circumflex branches around the joint, it divides into the median and ulnar arteries. The median artery represents part of the original axis artery, and nearly always disappears in man, but persists in the rat. It runs straight down the arm in the company of the median nerve. The ulnar artery, running on the ulnar side of the flexor surface of the limb, provides the interosseus artery: a deep branch that passes between the radius and ulna to reach the extensor compartment. The median and ulnar arteries contribute to the plexus of small arteries of the palm of the paw, and it from this plexus that the arteries to the digits arise. Greene describes these arteries in detail: they are many.

The Hindlimb Arteries

The great artery of the hindlimb, the femoral artery, is a continuation of the external iliac artery that appears on the flexor aspect of the thigh from beneath the inguinal ligament. It is close to the surface, so can be exposed and cannulated. The old axis artery of the hindlimb was the sciatic artery, which left the pelvis via the greater sciatic foramen with the sciatic nerve. The femoral artery gives off minor circumflex branches near its origin in the thigh. In man the profunda femoris artery is an important branch that supplies muscles at the back of the thigh. Greene (1935) states that the profunda femoris artery is absent in the rat, but Hebel and Stromberg (1976) record its presence. Greene identifies a single muscular branch of the femoral artery, but unlike the profunda femoris of man, this does not appear to pass through the adductor magnus muscle. Greene gives the blood supply of the hamstring muscles (back of the thigh) as coming from smaller arteries: the medial femoral circumflex artery, the posterior scrotal artery and the inferior gluteal artery. A significant artery of the rat, not found in man, is the large saphenous artery. It leaves the femoral artery high up in the thigh, and runs with the saphenous vein all the way down the medial surface of the hindlimb, ending on the extensor surface of the foot (dorsal surface) and sending branches round behind the medial malleolus of the tibia to enter the sole of the foot.

The femoral artery passes between the muscles of the thigh to emerge behind the knee as the short popliteal artery. After supplying branches to the knee joint, the popliteal artery divides to produce the posterior and anterior tibial arteries. The posterior tibial artery is less important in the rat than it is in man, and runs down in the flexor compartment of the limb. In man the posterior tibial artery gives off the peroneal artery, from which the anterior peroneal artery penetrates the interosseus membrane between the tibia and fibula, and which may appear on the dorsum of the foot replacing the dorsalis pedis artery.

The anterior tibial artery passes between the tibia and fibula to reach the extensor compartment. It runs on to provide the dorsalis pedis artery of the dorsum of the foot. The peroneal artery arises from the anterior tibial artery to supply the muscles of the lateral side of the limb. The distal part may be homologous with the anterior peroneal artery of man, see Greene (1935) for a discussion of this point. The arteries of the sole of the foot (hindpaw) of the rat come from the saphenous artery and not, as in man, from the posterior tibial artery.

THE VENOUS SYSTEM

Only the larger veins will be described in this section.

Veins of the Head and Neck

The external jugular vein is the most important vein draining blood from the head and neck in the rat, in contrast to man where the internal jugular is the most important vein. In the rat, the external jugular vein collects blood from the posterior and anterior facial veins. The posterior facial vein drains the retroglenoid vein (internal maxillary vein in Greene's terminology: Greene, 1935) and the superficial temporal vein that drains the scalp.

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The anterior facial vein appears at the corner of the mouth and collects blood from the face and lower jaw. The external jugular and axillary veins join to produce the subclavian veins, which in turn join with the smaller internal jugular veins to form the anterior (cranial) venae cavae. The entry of the venae cavae into the right atrium is complicated. The right anterior vena cava opens into the anterior of the right atrium; the left anterior vena cava continues in a posterior direction, crossing ventral to the arch of the aorta before entering the right atrium in company with the posterior (caudal) vena cava. The left anterior vena cava receives blood from the azygous vein, which in turn receives blood from the hemiazygous vein (on the right in the rat) that has crossed the midline (Halpern, 1953b).

Veins of the Abdomen and Pelvis

Although there is no coeliac vein, many of the veins of the gut have names similar to those of the arteries of the gut, and all drain into the portal vein that runs to the liver. The splenic vein also drains to the portal vein, even though the spleen is no part of the gut (Braithwaite and Adams, 1957). From the liver, the seven hepatic veins of the rat join the posterior vena cava just before it passes through the diaphragm (Hebel and Stromberg, 1976).

The posterior vena cava is formed by the junction of the common iliac veins, which in turn are formed from the internal and external iliac veins. It lies to the right of the abdominal aorta and veers away from the aorta in the anterior part of the abdomen.

Both renal veins join the posterior vena cava. The left renal vein passes ventral to the aorta, and is longer than the right renal vein. The gonadal veins drain to the posterior vena cava on the right, and to the left renal vein on the left. Greene (1935) states that the left testicular (spermatic) vein anastomoses with the left vesical vein (from the urinary bladder), and thus with the internal iliac vein. The ilio-lumbar veins that drain the dorsal body wall combine to form the ascending lumbar veins, which are the abdominal equivalent of, and link with, the azygous and hemiazygous veins.

The many tributaries of the internal iliac vein correspond with the branches of the internal iliac artery. In both rat and man, the external iliac vein is a continuation of the femoral vein, which passes under the inguinal ligament in company with the femoral artery. In the rat a number of the short veins that feed into the femoral vein in man, run to the pudic-epigastric vein which passes through the 'subcutaneous inguinal ring', presumably the superficial inguinal ring of man. The pudic-epigastric vein is the largest tributary of the external iliac vein in the rat.

Veins of the Forelimb

Three veins are important. The median vein accompanies the median artery, the cephalic vein runs up the radial surface of the limb before draining into the external jugular vein, and the basilic vein runs up the ulnar side of the limb to become the axillary vein at the axilla. It is noteworthy that the venous drainage of the limb is superficial, and no large vein accompanies the brachial artery, though two small veins, the venae comitantes, may run with the artery. The cephalic and basilica veins anastomose on the flexor side of the elbow. It is here that blood samples are taken in human clinical work.

Veins of the Hindlimb

The femoral vein is the upward continuation of the popliteal vein that drains blood from the short (or small) saphenous vein and the anterior and posterior tibial veins. In addition, the long saphenous vein runs up the medial side of the limb to join the femoral vein just before the latter passes under the inguinal ligament. Those interested in the small veins of the fore and hindpaws of the rat are referred to Greene (1935) and to her uniquely detailed illustrations.

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Chapter 8

Histology of the Vascular System

Good accounts of the histology of the cardiovascular system can be found in standard textbooks of Histology (Fawcett, 1994; Simionescu and Simionescu, 1988). These accounts provide extensive information on both the light microscopic and electron microscopic appearances of blood vessels; here we focus on light microscopy, but make reference to EM findings in the section on capillaries. Textbooks of Histology tend to focus on human tissues, with the result that specific descriptions of the histology of the vasculature of the rat are lacking. In truth this is not much of a draw-back, as the structure of the blood vessels and overall structure of vascular system are very similar. However, one should always keep in mind that there will be functional differences related to the bipedal versus quadrupedal anatomies. We begin at the periphery, with the capillaries.

CAPILLARIES

Malpighi, in 1661, was the first person to report the existence of capillaries, completing William Harvey's discovery of the circulation of the blood by providing the essential link between the arterial and venous systems. Malpighi named very small vessels 'capilli', the Latin word for hairs. In fact hairs are much larger in diameter than capillaries: an average mammalian capillary is about 8 μm in diameter, about the same diameter as a red blood cell. An effective way of visualising how small capillaries actually are is to draw a square: 1 mm × 1 mm. Imagine each side being divided into 100 parts, each of length 10 μm. There will be 10,000 of the small 10 μm × 10 μm squares. In skeletal muscle about 2000 of these are occupied by capillaries; in the dermis the number is only 50 or so. Unsurprisingly, this means that capillaries cannot be seen with the naked eye, and even under the light microscope it is difficult to make out the details of the structure of capillaries. The nuclei of the endothelium can be seen, but not much of the cytoplasm of the endothelial cells can be defined. Careful examination of sections is needed to ensure that small spaces actually represent capillaries, and not artefacts of shrinkage: the nuclei of the endothelial cells are, of course, the clue.

All capillaries are surrounded by a basal lamina that also encloses cells found lying on the outer surface of the endothelial cells. These are the pericytes and probably represent modified fibroblasts that have some capacity for contraction. Pericytes are sometimes called the cells of Rouget, though the cells that Rouget described may have been associated with arterioles rather than with true capillaries.

Capillaries are arranged as networks. These networks are fed by arterioles, the terminal and smallest components of the arterial system, and drained by venules, the first and smallest tributaries of the venous system. These networks are quite variable, dependent on the tissue. In the interalveolar septum of the lung, the capillary network comprises short, interconnecting segments of capillaries. This results in an almost continuous lake of blood, but in other tissues, the capillaries are longer and a more conventional net-like appearance is seen. Often only a fraction of the capillaries of the network are open: in skeletal muscle, only a low percentage of the capillaries are functioning when the muscle is at rest, but when the muscle is in action more capillaries open up and blood flow through the muscle increases sharply.

Capillaries are the essential exchange zone of the cardiovascular system, where oxygen and carbon dioxide are exchanged with the tissues, nutrients flow from the blood to the tissues, and hormones reach their target tissues. Fluid exchange at the capillaries is a subject of endless fascination to the physiologist: from the ground breaking work of Starling to the recent studies of Michel, the field is full of interest. Levick (2010) has provided an admirable and accessible account of the development and current thinking in this area. The permeability of capillaries is not fixed; on the contrary it can vary considerably. Injury, inflammation and allergic reactions all increase the permeability of capillaries and increase the leakage of fluid, and in some cases, leucocytes.

All capillaries can be regarded as tubes of endothelium. Endothelium is a term used to denote the simple epithelium of mesenchymal origin (mesoderm), and to distinguish it from the epithelium derived either from

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ectoderm, as in the epidermis, or from endoderm, as in the gut. This is quite counter-intuitive as one might imagine that endothelium is derived from endoderm.

Each capillary develops from a cord of mesenchyme which acquires a lumen. Endothelium is not restricted to capillaries, on the contrary, it lines the entire cardiovascular system including the heart. Endothelial cells are about 20 μm in length and 8 μm or so in width and very thin, often in the order of a micron or so. Endothelial cells are held together by tight junctions, where the cell membranes are brought into close proximity and the membranes themselves are modified and thickened. One such junction is sometimes described as a zonula occludens (plural: zonulae occludentes), though this implies that the junction runs all the way round the cell as a sort of belt. This is the case in the columnar epithelium of the gut (see Chapter 13: Alimentary Canal or Gastrointestinal Tract), but there is some doubt as to whether this is the case in capillaries. The term fascia occludens would be more appropriate if the junction is limited in area.

Capillaries can be classified in terms of the permeability of their tight junctions.

Continuous Capillaries

These capillaries are found in skeletal muscle, the lung, the testes and the brain (and many other tissues). Their tight junctions are effectively impermeable to fluid. The capillaries of the brain and testes have particularly tight junctions that are of great importance to the toxicologist as they effectively shield tissues like the brain and testes from many new chemical entities, and in the clinic have created difficulties, when attempting to deliver novel therapeutic agents to the brain. The cytoplasm of the endothelial cells contains many microvesicles and these are believed to be the key to the transport or fluid across these vessels. Electron micrographs showing these vesicles can be found in Fawcett (1994).

Fenestrated Capillaries

Fenestrated capillaries are to be found in the gut, endocrine glands and the glomeruli of the kidney. The cytoplasm of the endothelial cells is penetrated by fenestrae of about 80 nm diameter, which are arranged in a clear geometrical pattern and often occupy discrete areas of the capillary walls. The endothelial cells contain fewer microvesicles than do those of continuous capillaries, and with the exception of those in the renal glomeruli, are closed by a delicate diaphragm. The functional significance of the diaphragm is unknown. See Fawcett (1994) and Simionescu and Simionescu (1988) for further details of the fine structure of these capillaries.

Discontinuous Capillaries or Sinusoids

Sinusoids lack the fine connective tissue that surrounds true capillaries. Vessels are larger, 20 μm or so in diameter, than conventional capillaries, and their endothelial cells are separated by gaps. Such vessels are found in the liver (Chapter 14: Liver), the spleen and the bone marrow (Chapter 9: The Lymphatic System). Sinusoids are leaky, in the liver the plasma within the sinusoids is in contact with the fluid of the space of Disse.

Sinusoids are not formed in the same way as capillaries, but by the subdivision of blood filled spaces. In some sinusoids, the endothelial cells are interspersed with macrophages of the reticuloendothelial system. Fig. 8.1 is stained with IBA-1, a stain specific for the macrophage family, which is staining the Kupffer cells, and has the effect of outlining the sinusoids at the same time.

ARTERIES

Arteries are divided into elastic arteries (the aorta and the large arteries of the trunk) and the muscular arteries that comprise the smaller arteries and are those most likely to be seen in histological sections of tissues and organs. Elastic arteries expand with each influx of blood from the left ventricle of the heart and then recoil thus adding to the force that propels the blood to the muscular arteries. In both types, the arterial wall comprises three coats or 'tunics':

Tunica Intima

This inner coat comprises the endothelium, the subendothelial connective tissue, which may contain occasional smooth muscle cells and the internal elastic lamina, which is a thin layer of elastic tissue defining the outer edge of the tunica intima. The internal elastic lamina is often

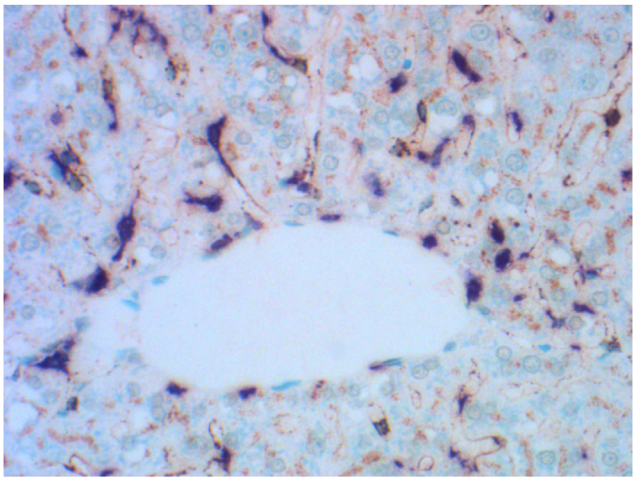


FIGURE 8.1 Liver sinusoids.

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corrugated in appearance in histological sections. This is an artefact caused by postmortem contraction of the smooth muscle of the tunica media and/or by fixation. The nuclei of the endothelial cells are small and stain darkly.

Tunica Media

In muscular arteries, this is a thick layer of smooth muscle interwoven with collagen, reticulin and elastic fibres. In elastic arteries, a series of fenestrated elastic laminae (sheets of elastic tissue with holes) predominate, but smooth muscle cells are also present. These are most easily seen in sections of the aorta.

Tunica Adventitia

This is an outer layer of connective tissue comprising mainly collagen, although there may be some occasional elastic fibres in some larger vessels.

The blood supply to the vessels themselves is provided by a network of small arteries and veins known as the vasa vasorum that penetrate the tunica adventitia and the outer part of the tunica media (McGeachie et al., 1982). The inner part of the wall exchanges oxygen and carbon dioxide and receives nutrients directly from the blood in the lumen of the vessel. Efferent and afferent fibres of the autonomic nervous system penetrate the walls of arteries. It is assumed that pain fibres run with the afferent autonomic fibres. Evidence of pain fibres is provided by every human arterial cannulation done with inadequate local anaesthesia (Fig. 8.2).

ARTERIOLES

The terminal branches of the arterial system are the arterioles. Larger arterioles have a relatively thick tunica media comprising mainly smooth muscle, and a well-developed internal elastic lamina. Both features disappear as the smallest arterioles approach capillary dimensions. The term meta-arteriole is sometimes used to describe the smallest arterioles: the muscle of the walls of these vessels is incomplete.

An interesting modification of the wall of small arteries of the rat has been described by Fourman and Moffat (1961). The red cells flowing through narrow arteries form an axial stream that leaves the periphery of the blood flowing through the vessels depleted of cells. Axial streaming is a result of the Magnus effect: see Burton (1965) for further details. Intra-arterial cushions that project into the lumen of the vessel at branch points allow the axial stream to flow into the side branches.

VEINS

Veins have the same basic structure as arteries, but with no internal elastic lamina. The tunic media is relatively thin, and the tunica adventitia relatively thick. The pulmonary vein of the rat is unusual in that the tunica media contains cardiac muscle cells. Peripheral veins contain semilunar valves arranged so as to prevent retrograde flow of blood. These are pocket shaped elaborations of the tunica intima comprising a connective tissue core covered by endothelium. Large veins are also supplied by a vasa vasorum. It is said that the arterial supply penetrates further into the wall of large veins than in the case of arteries.

Special Regions of the Vasculature

The rather brief account given above deals with blood vessels in general. A few special areas are worth noting.

Dural venous Sinus

The venous sinuses of the dura are thin-walled veins that are held open by the dura. The sinuses are lined by endothelium (Fig. 8.3).

Erectile Tissue

The erectile tissue of the penis is supplied by helicine arteries that spiral their way through the connective tissue of the organ and empty into large venous filled spaces. The arteries straighten during erection and the veins that drain the sinuses are thin-walled and easily compressed preventing blood flowing out of the organ.

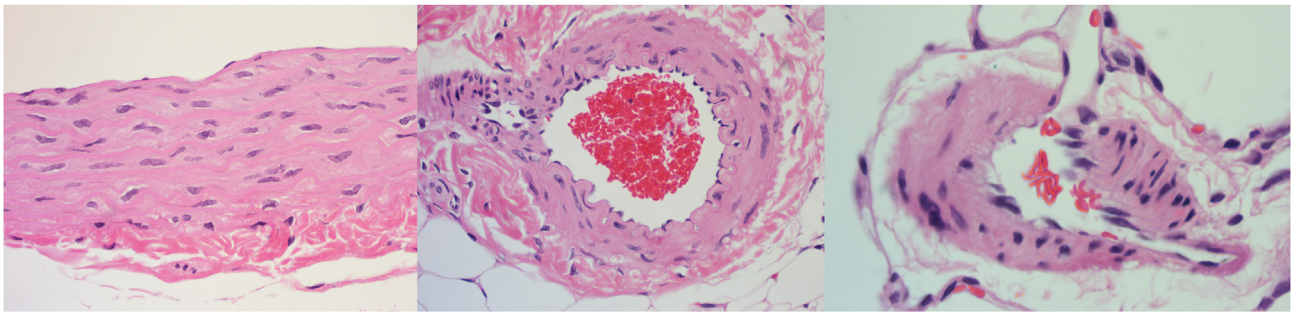


FIGURE 8.2 Examples of arteries. On the left is the wall of the aorta, an elastic artery, centre is a muscular artery and to the right a terminal arteriole in the lung leading to a capillary.

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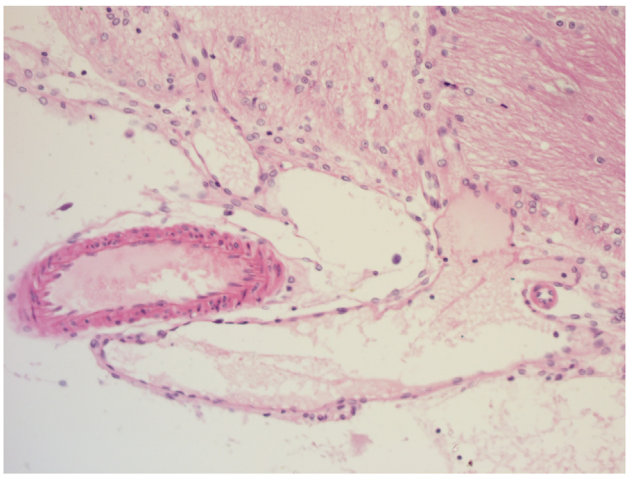


FIGURE 8.3 Dural sinus.

Nasal Muco-Periosteum

The vascular spaces of the nasal muco-periosteum. These are described in Chapter 10, Nasal Cavity.

Carotid Body and Sinus

The carotid bodies are small organs attached to the bifurcation of each common carotid artery. They are an expansion of the first part of the internal carotid artery. They have a thin tunica media and a tunica adventitia that is thick and well innervated. The carotid body is a chemoreceptor derived from the third branchial arch, supplied by afferent fibres of the glossopharyngeal nerve. It has a remarkably large blood supply and responds to a reduction in oxygen levels in the blood.

These organs are very small in the rat and cannot easily be identified without the aid of a dissecting microscope. At light microscopy, the carotid body is seen to contain a mass of pale-staining cells: the glomus cells, and many rather wide capillaries, which are in fact sinusoids. At the EM level, two types of cell have been identified: the Type I or glomus cells and the Type II or sheath cells that enclose the glomus cells.

The carotid sinus is a pressor-receptor that responds to changes in blood pressure.

Other chemo-receptor tissue is found in the aortic bodies that are innervated by the vagus nerve.

HISTOLOGICAL TECHNIQUES FOR BLOOD VESSELS

Conventional H&E staining shows the structure of the walls of blood vessels in fair detail. The internal elastic lamina often has a refractile appearance in well-stained sections. Elastic tissue can be stained with orcein (a light counter stain, safranin e.g. is often recommended though not actually required); Weigert's stain for elastic tissue combined with Van Gieson's stain for collagen and smooth muscle produces attractive sections. The internal elastic lamina of arteries stains jet black and contrasts well with the lemon yellow staining of the smooth muscle of the tunica media and the red staining of the tunica adventitia. Care should be taken to prevent red staining of the collagenous tunica adventitia 'bleeding' into the pale yellow tunica media. Masson's trichrome is also effective, but overstaining needs to be avoided as a heavy trichrome stain can obscure a light elastic stain such as orcein. Reticulin fibres wrap around the smooth muscle cells of the tunica media and may be stained by silver impregnation, but again we would warn that silver stains tend to be both expensive to perform and capricious in nature. For details of staining techniques, see Maynard et al. (2014), Drury and Wallington (1980) and Suvarna et al. (2012).

HISTOLOGY OF THE HEART

The walls of the atria and ventricles are made up of an endocardium, a myocardium and an epicardium. These are fundamentally similar to the three tunicae of the blood vessels, though the myocardium contains the specialised cardiac muscle fibres rather than smooth muscle. The endocardium comprises the endothelium that lines the chambers of the heart, a subendothelial layer of connective tissue, which may include some smooth muscle cells and a subendocardial layer, also of connective tissue, but containing some elastic fibres. In the human heart, the endocardium is thicker in the atria than in the ventricles.

The myocardium is made up of chains of cardiac muscle cells interspersed with connective tissue forming myocardial fibres, in which run the blood vessels and nerve fibres that supply the muscle cells.

Myocardial cells essentially contain the same contractile apparatus as the cells of skeletal muscle. They do not form an anatomical syncytium, but they do form a physiological syncytium, and contract in a coordinated manner.

In the heart, the individual cells abut on one another, the ends of the cells are irregular displaying elevations and depressions that fit with those of the adjacent cell. Between the ends of two cells is an intercalated disc that has an undulating appearance at electron microscopy. The intercalated discs always occur at what would otherwise be a Z line, in the middle of the I band of the bundles of myofibrils within the cells. Intercalated discs show a range of specialised cell to cell contacts including gap junctions, which allow the flow of ions from cell to cell, desmosomes and fasciae adherentes. The latter, which are said to be unique to the myocardium, are localised versions of the zonulae adherents found in the junctional complexes of for example epithelial cells of the gut.

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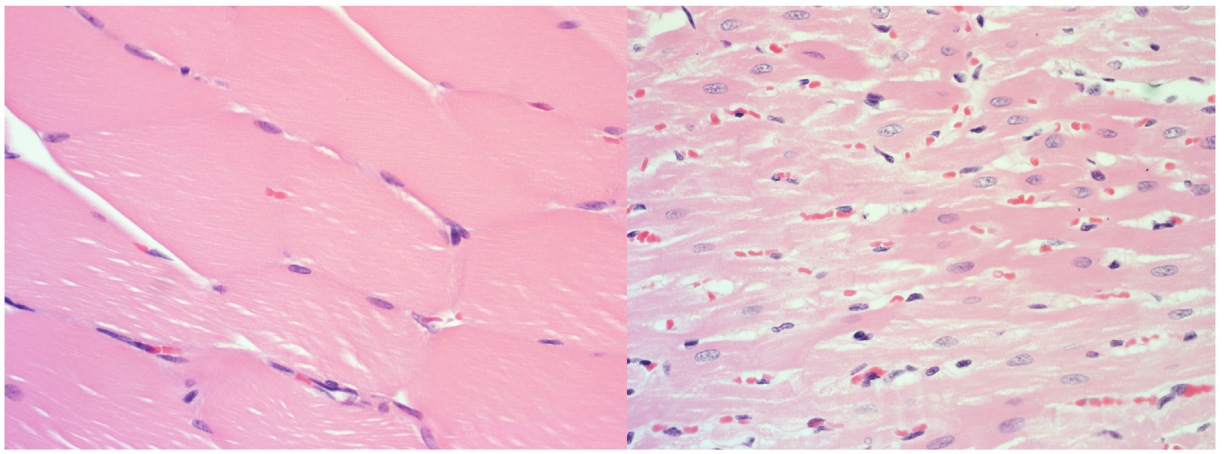


FIGURE 8.4 Comparison of skeletal muscle on the left with cardiac muscle to the right.

Myocardial cells are not arranged in perfect register, and thus the intercalated discs have a stepped appearance when seen at light microscopy. Cells are cylindrical and branch frequently, with one or more nuclei located among bundles of myofibrils, in contrast to skeletal muscle where many nuclei are located at the periphery of the cells, beneath the sarcoplasmic membrane.

Myocardial cells contain many mitochondria that make up 23% of the cell volume, as compared with 2% in skeletal muscle (Griepp, 1988). The transverse tubules of myocardial cells are large but the sarcoplasmic reticulum is not as well developed as that of skeletal muscle. In myocardial cells, the T tubules occur at the Z lines rather than at the junctions between the A and I bands (Fig. 8.4).

The epicardium comprises a single, superficial layer of mesothelial cells of the visceral pericardium and an underlying layer of connective tissue. The latter contains adipose tissue, blood vessels and bundles of nerve fibres. Parasympathetic ganglia also occur.

The specialised impulse generating tissue of the sinu-atrial node is made up of modified myocardial cells. These cells are smaller than the ordinary cells of the atrial myocardium and are said to lack intercalated discs. The cells of the specialised conducting tissue of the atrio-ventricular nodal system (see Chapter 7: The Cardiovascular System) are similar to those of the SA node. It is unlikely that these will be discernible in a standard preparation.

The fibrous skeleton of the heart comprises a dense, collagen-rich connective tissue in which cartilage cells may develop as the animal ages.

For further details of the ultra-structure of the heart, including details of the endocrine cells of the atria (producing atrial natiuretic peptide) and chemo-sensory cells (not identified in all mammalian species) see Griepp (1988). A valuable account of degenerative changes in the rat myocardium has been provided by Jokinen et al. (2011).

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Chapter 9

The Lymphatic System

INTRODUCTION

The lymphatic system has three essential functions:

1. Production of appropriately programmed lymphocytes that defend the body against infection and destroy abnormal cells including those that have undergone mutation leading to a malignant change.
2. Reabsorption of fluid that has passed from blood capillaries into tissue spaces (in man five litres of fluid are returned to the venous system per day).
3. Absorption of fat from the small intestine.

Beginning in the tissues, the lymphatic capillaries drain into larger lymphatic vessels with a structure rather similar to that of veins. These large vessels from all parts of the body join to produce two vessels, the thoracic duct and the right lymphatic duct, that pour lymph into the venous system at the root of the neck. The direction of flow is controlled by valves occurring at short intervals in lymphatic vessels. Lymph is propelled through the lymphatic vessels by pressure applied to the vessels by adjacent skeletal muscles, and contraction of smooth muscle in the walls of the larger vessels. Flow may also be aided by the subatmospheric pressure of the tissue around the large lymphatic ducts of the thorax. Lymph hearts are found in amphibians and reptiles, specialised regions of the system that contain much smooth muscle in their walls. No lymph hearts are found in mammals.

Lymphatic vessels are interrupted by lymph nodes that filter the lymph and in which lymphocytes multiply. The enlarged lymph nodes that can be felt in patients suffering from infection are an indication of that proliferative response. Nearly all lymph passes through at least one lymph node on its way from the periphery to the venous system, and usually there is more than one lymph node along the pathway.

The lymphatic system also includes the spleen, the thymus gland, mucosa associated lymphoid tissue (MALT) and the bone marrow.

The thymus is defined as a primary lymphatic organ, the lymph nodes, MALT and spleen are secondary lymphatic organs. This requires an explanation that takes us into the vast, complex and rapidly expanding field of immunology. This book is about anatomy, and only the briefest outline of the role of the lymphatic system will be provided here. Further details can be found in textbooks of immunology: *Roitt's Essentials of Immunology* (Delves et al., 2011) provides a detailed, readable up to date account. Staines et al. (1993) have provided a short and readable account that is still useful though becoming a little dated. The following points are generally agreed.

1. In early life, lymphocytes are derived from stem cells that migrate from the tissues of the yolk sac to the bone marrow early in the development of the embryo.
2. Lymphocytes are produced from stem cells in the bone marrow and some migrate to the thymus. Other lymphocytes remain in the bone marrow and undergo further maturation there. The bone marrow is thus also a primary lymphatic organ. In birds, lymphocytes migrate to the Bursa of Fabricius, a primary lymphatic organ associated with the hind gut, but this primary lymphatic organ is not found in mammals. The reason from mentioning the Bursa is that lymphocytes are divided into B lymphocytes, which in birds are programmed in the Bursa. In mammals, B lymphocytes are programmed in the bone marrow.
3. Lymphocytes move from the primary lymphatic organs to the secondary lymphatic organs, the lymph nodes, MALT, Bronchus associated lymphatic tissue (BALT), tonsils and spleen. It is here that final maturation takes place. It is also here that the multiplication of lymphocytes that represents the essential process of the immune response takes place. Secondary lymphatic organs contain lymphoid follicles where B lymphocytes divide and multiply, and interfollicular tissue where T lymphocytes divide and multiply. Lymphoid follicles are not found in the primary lymphatic organs.
4. B lymphocytes differentiate into plasma cells that produce antibodies, and into memory cells that 'remember' the antigenic stimulus that caused them to be produced and are thus prepared to mount a rapid immunological response if triggered by second and further exposures to the original antigenic stimulus.

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5. T lymphocytes differentiate to produce lymphocytes with a range of functions including (1) 'helping' B lymphocytes respond the antigen, (2) 'suppressing' the B lymphocyte response to antigen, (3) killing cells that have been infected by viruses.
6. Other lymphocytes develop into 'natural killer' lymphocytes that destroy malignant cells.
7. Antigen is presented to T lymphocytes by antigen presenting cells including dendritic cells and macrophages. T lymphocytes produce cytokines that stimulate production of antibody by B lymphocytes. Little antigen appears to be presented directly to B lymphocytes, at least in a primary immunological response.
8. Antigen presenting cells travel via both the lymphatic and vascular systems to secondary lymphatic organs. Dendritic cells are the 'professional' antigen presenting cells; macrophages are less specialised for this role (Milling et al., 2006).
9. Lymphocytes circulate to and from the secondary lymphatic organs. They enter these organs from the blood stream via specialised venules (see below) and leave via efferent lymphatic and blood vessels.

See Margaris and Black (2012) and Haley (2003) for further information.

THE GENERAL ANATOMY OF THE LYMPHATIC SYSTEM

Lymphatic capillaries form a network beneath the epithelial surfaces of the epidermis and the mucosa of the gut, respiratory and genitourinary systems. They also occur in all connective tissue planes for example between bundles of skeletal muscle, and around the secretory tissue of glands. In the liver, the fluid that escapes from the sinusoids to the space of Disse (see Chapter 14: Liver) drains to the periphery of the lobules and enters lymphatic capillaries of the portal tracts (Ohtani and Ohtano, 2008).

Lymphatic vessels are also found in the meninges of the central nervous system (Louveau et al., 2015), the periosteum of bone and the perichondrium of cartilage. Lymphatic capillaries are NOT found in the neural tissue of the central nervous system, in cartilage or in the cortex of the thymus gland.

Lymphatic capillaries are more variable in diameter than blood capillaries, and the endothelial cells of their walls are less firmly attached to one another. Like blood capillaries, lymphatic capillaries do not, in general, penetrate between the cells of an epithelium. Trzewick et al. (2001) reported a system of microvalves in lymphatic capillaries formed by the overlapping of the thin endothelial cells. Fine collagen fibres (reticular fibres) attached to the walls of lymphatic capillaries are thought to play a role in holding the vessels open. Plexuses of lymphatic capillaries normally lie in a deeper plane than the associated plexus of blood capillaries, this is certainly the case in the dermis and the gut.

Larger lymphatic vessels have a structure similar to that of veins; the tunicae intima, media and adventitia can all be defined. Sacchi et al. (1997) described precollector vessels between lymphatic capillaries and larger lymphatic vessels. The smooth muscle layer of the walls of these vessels is incomplete, exposing sections where the endothelium is essentially naked and takes part in fluid exchange. Large lymphatic vessels often run with blood vessels; a plexus of small lymphatic vessels often forming around adjacent blood vessels.

TOPOGRAPHICAL ANATOMY OF THE LYMPHATIC SYSTEM OF THE RAT

Tilney (1971) has provided an admirable description of the anatomy of the lymphatic system of the rat. The following tables are based on his work. These tables describe both the major collections of lymph nodes found in the rat and the major lymphatic vessels. The tables are arranged by region. L and R stand for left and right.

Head and Neck

Type	Lymph Node Group	Location	Area Drained	Efferent Drainage
Peripheral nodes	Superficial cervical: 2 on L, 2 on R	Cranial pole of submandibular gland	Tongue and naso-labial plexus. Lymphatic vessels run between masseter and digastric muscles. Also connect with facial nodes and/or their efferent vessels	Central cervical nodes
	Facial: 2 on L, 2 on R	Caudal pole of submandibular gland, close to junction of anterior and posterior facial veins with external jugular vein	Head and neck	Central cervical nodes via large lymphatic vessel looping lateral to sternomastoid muscle

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	Internal jugular: 1 on L, 1 on R	Ventral to brachial plexus, lateral to carotid sheath	Deep cervical viscera: pharynx, larynx, anterior part of oesophagus	Central cervical nodes
Central nodes	Posterior cervical: 1 on L, 1 on R	Dorsal to brachial plexus	Peripheral nodes, deep cervical viscera: pharynx, larynx and anterior part of oesophagus	Cervical duct running dorsal to carotid sheath: to subclavian vein

Forelimb and Trunk

Type	Lymph Node Group	Location	Area Drained	Efferent Drainage
Peripheral nodes	Brachial: 3 on L, 3 on R	Superficial to triceps muscle, within fascial sheath of muscle	Forelimb via cephalic lymphatic vessel accompanying cephalic vein, shoulder and chest	Axillary nodes
Central nodes	Axillary: 4 on L, 4 on R	Axilla, on chest wall	Forelimb via brachial lymphatic vessel accompanying brachial vein, trunk. Brachial nodes. Caudal axillary node receives lymph from inguinal nodes via lymphatic vessel running along the milk line: drains nipples and ventral chest and abdominal wall	Subclavian duct: to subclavian vein

Hindlimb

Type	Lymph Node Group	Location	Area Drained	Efferent Drainage
Peripheral nodes	Popliteal: 1 on L, 1 on R	Popliteal space, flexor surface of knee joint	Foot, hindlimb via vessels accompanying greater and lesser saphenous veins	Efferent vessels follow femoral vein to plexus dorsal to iliac vessels and on to iliac nodes. Minor supply to inguinal nodes
Inguinal nodes	Inguinal: 2 on L, 2 on R	Flank (lateral lower abdominal wall), anterior to inguinal ligament	Thigh, haunches, scrotum, lateral parts of tail (via the caudal nodes) and popliteal nodes	Axillary nodes via superficial channel following the nipple line

Tail

Type	Lymph Node Group	Location	Area Drained	Efferent Drainage
Peripheral nodes	Gluteal: 1 on L, 1 on R	Sciatic foramen	Tail	Paired vessels run on lateral aspects of tail and follow superior gluteal veins to gluteal nodes. Gluteal nodes drain to caudal nodes
				Single ventral lymphatic vessel runs direct to caudal nodes. Other lymphatic vessels cross inguinal area and wall of scrotum to reach inguinal nodes. Minor pathway with inferior gluteal vein to popliteal nodes

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Visceral Lymph Nodes

Region	Lymph Node Group	Location	Area Drained	Efferent Drainage
Thorax	Parathymic: 2 or 3 on L, 2 or 3 on R	Embedded in fat on lateral aspects of thymic capsule	Peritoneal cavity, superior surface of liver and liver capsule via lymphatic plexus on pleural surface of diaphragm, pericardium and thymus (see Section on thymus)	Mediastinal duct or ducts (R and L) to subclavian vein or veins
	Posterior mediastinal: 1 on L, 1 on R	Paravertebral gutter adjacent to oesophagus and aorta	Thoracic viscera, pleural space, pericardium and thymus	Mediastinal duct or ducts (R and L) to subclavian vein or veins
	Paravertebral: several on L and R	Dorsal to pulmonary vessels	Diaphragm, thoracic viscera	Posterior mediastinal nodes
Pelvis and retroperitoneal tissues	Caudal (several)	Adjacent to median sacral vein	Ventral part of tail, anus and gluteal nodes	Iliac nodes
	Iliac (single or paired on each side)	Adjacent to aortic bifurcation	Pelvic viscera, Popliteal, gluteal and caudal nodes	Renal nodes via para-aortic trunks or via para-aortic nodes
	Para-aortic nodes (inconsistent)	Along the aorta: para-aortic area	Pelvic viscera, Popliteal, gluteal and caudal nodes	Renal nodes via para-aortic trunks
	Renal: 1 on L, 1 on R	Dorsal to renal veins	Kidney, adrenal glands, lumbar lymphatic vessels, iliac and para-aortic nodes	Renal duct(s) to cisterna chyli. Efferent vessel from L renal node often runs to R renal node
	External lumbar (several on L and R)	Retroperitoneal fat close to lumbar veins	Retroperitoneal fat, psoas muscles and pelvic viscera	Lumbar lymphatic vessels
Abdomen	Splenic (several)	Adjacent to splenic vein	Splenic capsule and trabeculae	Posterior gastric nodes
	Posterior gastric (several)	Along the gastro-duodenal vein	Distal part of oesophagus, stomach, pancreas and splenic nodes	Portal nodes
	Portal (several)	Adjacent to portal vein	Liver, splenic and posterior gastric nodes	Portal duct to cisterna chyli
	Superior mesenteric (many, arranged as a chain of nodes)	Root of mesentery	Duodenum, small intestine, caecum, ascending and transverse colon	Superior mesenteric duct to cisterna chyli
	Inferior mesenteric: 1 only	Mesentery of descending colon	Descending and sigmoid colon	Inferior mesenteric duct to cisterna chyli

The numbers of lymph nodes in each group are given for guidance only and individual variation should be expected.

The thoracic duct begins at the cisterna chyli, a small thin walled sac found medial to the left adrenal gland (Hebel and Stromberg, 1976). The thoracic duct passes through the diaphragm with the aorta and runs on the dorsal surface of the thoracic cavity, dorsal to the oesophagus, to the junction of the left subclavian and internal jugular veins. The termination of the thoracic duct is subject to much variation: see Hebel and Stromberg (1976) for details.

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HISTOLOGY OF LYMPHATIC TISSUES

Thymus

The thymus is a pale pink, soft and fleshy organ located in the anterior part of the mediastinum, ventral to the trachea and great vessels. In young animals, it is quite large and easy to identify on removal of the ventral chest wall. It reaches a maximum size around sexual maturity, but involutes with age, and may be difficult to identify in animals older than 18 months. The thymus is often referred to as the thymus gland; terminology dating from the days when lymph nodes were called lymph glands. However, unlike lymph nodes, the thymus really is a gland, an endocrine gland that produces a number of peptide hormones that act both within the gland, and on more distant tissues. Pearse (2006) has provided a valuable review of the structure of the thymus.

In the young rat, the thymus (Fig. 9.1) comprises two large lobes that are connected ventrally. It develops from the endoderm of the third and fourth pharyngeal pouches which provides the epithelial scaffolding of the gland. It is an unusual epithelium in that the cells are not generally arranged as sheets, but as a reticulum or network that is infiltrated by lymphocytes. A thin sheet of epithelial cells is formed at the periphery of the cortex of the thymic lobules and around the postcapillary venules of the corticomedullary boundary (see below). Whether ectodermal cells play a part in the development of the thymus is uncertain, but it is known that interaction between endodermal cells and mesenchymal cells derived from the neural crest is necessary for normal development (Bockman, 1997). The thymus receives its blood supply from the internal thoracic arteries; its venous drainage is to the anterior vena cava on each side. Lymphatic vessels in the capsule and connective tissue of the thymus penetrate into the medulla, but not the cortex (Kato, 1997). No afferent lymphatic vessels run to the thymus, but efferent lymphatic vessels from the thymus drain to the parathymic lymph nodes.

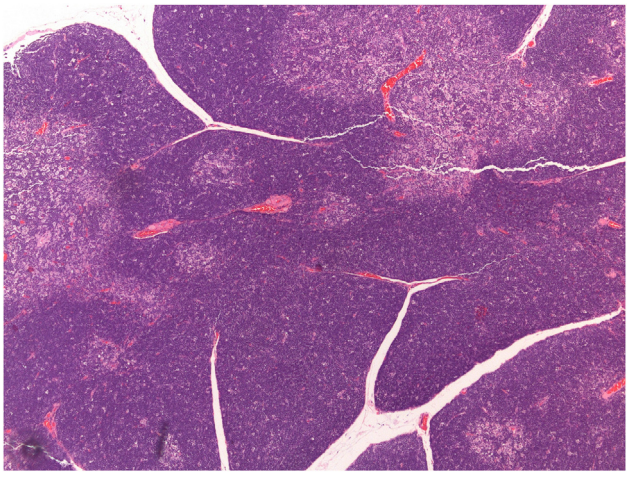


FIGURE 9.1 Thymus. As noted below this is from a relatively young rat, in the older animal only scraps of tissue may be evident.

The rat thymus is divided into lobules by thin septa of connective tissue. Each lobule comprises a dark staining cortex and a paler medulla. At first sight the tissue appears to be a mass of lobules, but the medulla is in fact continuous from lobule to lobule, and the gland might be better considered as an intricately folded structure with a continuous medulla and cortex. Although the cortex is packed with lymphocytes, there are no follicles, a feature that allows the thymus to be easily distinguished from lymph nodes and spleen. The lymphocytes of the thymus (thymocytes) migrate to the thymus from the bone marrow and are transformed into T (thymic) lymphocytes.

The passage of lymphocytes through the thymus has been studied in detail. It appears that lymphocytes leave capillaries at the corticomedullary border, and migrate into the cortex, where they interact with epithelial cells. This interaction is decidedly complex, whereby a host of recognition factors and intercellular messengers (chemokines) allow the epithelial cells to 'instruct' the lymphocytes, and the lymphocytes to 'instruct' the epithelial cells.

Most of the lymphocytes that make their way into the cortex die there through apoptosis, but those that escape this fate locate to its periphery where they divide. The progeny of these cells migrate towards the corticomedullary boundary, but most fail to reach the boundary, and again undergo apoptosis (Takahama, 2006). This process is thought to weed out the lymphocytes that have become 'confused' about 'self or nonself' (Weiss, 1988a). Those reaching the boundary make their way into the medulla where further interaction between lymphocytes and epithelial cells occurs. This highly selected population of lymphocytes make their way back to the boundary and enter postcapillary venules. In the rat these venules have a flat endothelium, but in the mouse and in man, venules with a cuboidal epithelium similar to that seen in lymph nodes have been described (Kato, 1997).

The postcapillary venules are surrounded by a perivascular space between the basal lamina of the endothelial cells and that of the surrounding epithelial cells. The capillaries of the cortex are closely wrapped by epithelial cells, and arrangement thought to be responsible for the blood–thymus barrier that prevents antigen from reaching the developing lymphocytes of the cortex. Whether this barrier is quite as efficient as was once thought is debatable.

A number of different types of thymic epithelial cell have been described (see De Waal and Rademakers (1997) for a detailed account of the epithelial cells of the rat thymus). There seems to be four different types of epithelial cell in the cortex and two more in the medulla.

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One well-defined type is the subcapsular/perivascular cell that limits access to the cortical lymphocytes. The subtypes cannot be identified at light microscopy, indeed it is not easy to identify any epithelial cells among the densely packed lymphocytes of the cortex. The identifying feature of these cells is their pale nuclei, not much cytoplasm can be seen. Macrophages and dendritic cells are also found in the cortex [see Schuurman et al. (1997) for further details].

The medulla contains small lymphocytes and epithelial cells, with macrophages said to be rare. In man, Hassall's corpuscles, epithelial bodies comprising whorls of epithelial cells and cellular debris are found in the thymic medulla. The cells of these corpuscles contain keratohyalin granules, perhaps suggesting an ectodermal origin. Their function is obscure, and few are found in the rat thymus. In the human thymus, occasional skeletal muscle cells (Hammar's myoid cells) are also found in the medulla; such cells are occasionally found in the rat thymus.

The epithelial cells of the thymus secrete a number of peptide hormones: thymulin, thymic humoral factor, thymopoietin and thymosin-1. These are thought to be involved in the control of T lymphocyte development. Thymopoietin binds to acetylcholine receptors and might play a role in the disease myasthenia gravis, although autoantibodies to acetylcholine receptors are regarded as a more likely explanation for the muscle weakness found in patients with this disease. Recent studies have shown that interactions between the thymus and the nervous system are more complicated that was thought. Moll (1997) has reviewed the neuroendocrine activities of the thymus in some detail.

Lymph Node

Lymph nodes (Fig. 9.2) are small, solid and rounded bodies that lie along lymphatic pathways, occurring singly or as well-defined groups. Lymph nodes have a complicated structure, much of which has been discovered during the past 20 or so years. Willard-Mack (2006) has contributed an authoritative review from the perspective of a toxicological pathologist. Original work by Kowala and Schoefl (1986) and Roozendaal et al. (2008) should be consulted for further information. Much of the recent thinking about the structure of lymph nodes has been based on work in rats.

Rat lymph nodes are surrounded by a thin capsule of connective tissue, which is thicker and contains smooth muscle fibres in larger species. In some species, the capsule has been shown to contract and is thought to play a part in propelling lymph along the lymphatic vessels. Trabeculae of connective tissue run from the capsule into the centre of the node, though this is not a conspicuous

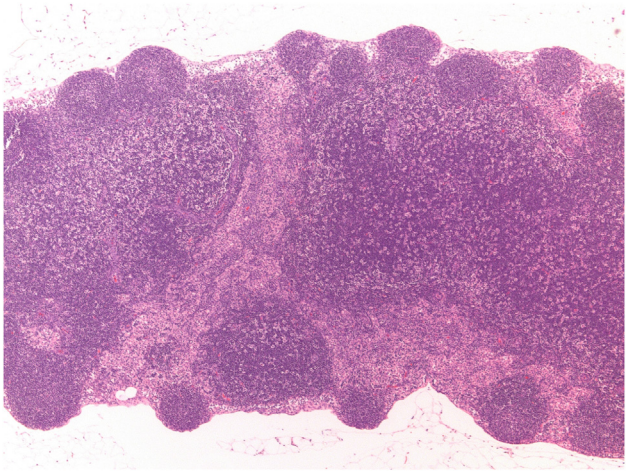


FIGURE 9.2 Mesenteric lymph node.

feature of rat lymph nodes. Multiple afferent lymphatic vessels run into the node at its periphery and a single efferent lymphatic vessel leaves at its hilus (or hilum), where the blood vessels of the node enter and leave. The afferent lymphatic vessels form a subcapsular sinus that runs round the node under its capsule. The predominant cell in the lymph node is the lymphocyte, a small cell readily identified by its dark staining nucleus and sparse cytoplasm (Fig. 9.3).

The cortex can be divided into two zones: the superficial cortex (follicles and interfollicular cortex) and the deep cortex (or paracortex), which contain no follicles, but which has a distinct function of its own. The follicles are the home of the B lymphocytes; the deep cortex contains the T lymphocytes, which also, rather confusingly, also occur in the interfollicular cortex. There are generally more lymphocytes per unit volume of tissue in the outer part of the node (the cortex) than the interior or medulla. The cortex contains collections of lymphocytes called lymphatic or lymphoid follicles. Active follicles have pale staining centres (the germinal centres) where mitotic division of lymphocytes takes place: the dividing cells have pale nuclei. Between the follicles lies an interfollicular cortex containing many lymphocytes. The lymphatic tissue of the medulla of the lymph node is arranged as a network of cords of cells. These cords are separated from one another by a labyrinth of lymphatic sinuses. Lymphocytes and, importantly, plasma cells are found in the medullary cords.

The Lymphatic Lobule

Modern thinking defines the lymphatic lobule as the functional unit of the lymph node; some nodes contain only one lobule, other nodes contain several or in the case of large mammals, many. The lobule comprises cortex and medulla and is surrounded by a confluent lymphatic sinus

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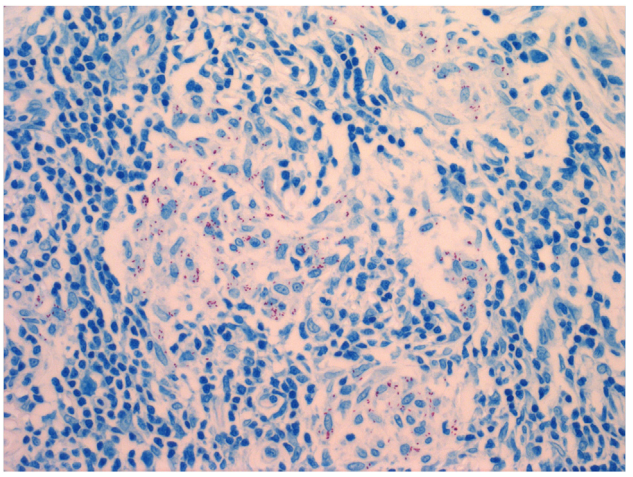


FIGURE 9.3 Lymphoid follicles. In contrast to the above this node has some active germinal centers. The nodes are stained with Ziehl Neelson stain, a stain for acid fast bacteria, which can be seen as small red dots within the germinal centers.

that arises from the subcapsular sinus. Perilobular sinus would be an appropriate term for the sinus that surrounds the lobule, but it is usually known as the transverse sinus. Small lymphatic sinuses begin as blind-ended vessels within the interfollicular cortex, and the deep cortex and run from the interior of the lobule to the sinuses of the medulla. The deep cortex itself is subdivided into the central deep cortical unit, and a peripheral deep cortical unit that surrounds the central deep cortical unit. This distinction is important because no lymphatic sinuses are found within either the follicles of the superficial cortex, or the deep cortical unit. The lymphatic sinuses of the interfollicular and the peripheral deep cortical unit are efferent sinuses, carrying fluid and lymphocytes from the follicles and central deep cortical units to the lymphatic sinuses of the medulla. We can now see that the lymphatic vessels of the node are complex. First, there are the vessels that carry lymph to the node, feed into the subcapsular sinus and drain to the medullary sinuses, and second, there are the sinuses that arise in the interfollicular cortex and the peripheral deep cortical unit, which also empty into the medullary labyrinth of sinuses. Lymph leaving the node comes from both sources.

The Reticular Meshwork of the Node

The entire scaffolding of the lymph node is made up of reticular tissue. Fibroblastic reticular cells (FRCs) line the lymphatic sinuses and provide the scaffolding for the remainder of the node (note that the lymphatic sinuses are not lined by endothelium). The FRCs are remarkable cells, although they appear indistinguishable from endothelial cells, they can also produce fine collagen fibres traditionally called reticulin fibres. These fibres are associated with glycoproteins and can be stained by silver impregnation techniques. The lymphocytes of the node are always, and everywhere, in contact with the FRC. There is little intercellular fluid in the follicles and the lymphocytes can be thought of as sliding along the reticulum as they migrate.

The network of reticulin fibres forms a spider's web of fibres cross the lumina of the lymphatic sinuses and holds the macrophages (histiocytes) that take up particles and particulate antigen from the lymph. It used to the thought that the sinuses had leaky walls, and that lymph percolated from these vessels into the spaces between the lymphocytes of the cortex and medulla. This is not so, only two things leave the lymphatic sinuses. Fluid with a low protein content leaves via a system of conduits formed by the collagen fibres enwrapped by FRC, and dendritic cells (e.g. Langerhans cells from the skin) that have travelled to the node in the lymph stream migrate from the lymphatic sinuses into the lymphocyte-rich parenchyma of the node. The conduits are intriguing structures, comprising a bundle of fine collagen fibres wrapped in FRC, with the basement membrane of the FRC lying against the collagen fibres. The conduits run from the lymphatic sinuses into the follicles and central deep cortical zone. Fluid creeps along the collagen fibres, but is not allowed to come into contact with the lymphocytes of the tissue around the conduit. In some parts of the conduit, the FRC and the underlying basement membrane are replaced by dendritic cells that are in contact with the fluid in the conduit. These dendritic cells, the 'professional' antigen presenting cells of the immune system, are perfectly placed so as to be able to sample soluble antigens within the fluid creeping along the conduits, which is eventually delivered to a perivenular space adjacent to the high endothelium venules that we have yet to describe. Thus fluid leaves the lymphatic sinuses via the conduits, and returns to the blood by entering venules. This accounts for the higher concentration of protein in the efferent than the afferent lymph vessels of lymph nodes. Fluid, but not protein, has been lost from the lymphatic vessels, and has entered the venous drainage system of the node.

The Blood Vessels of the Lymph Node

Arteries enter the node at the hilus and divide into the smaller arteries that run through the medullary cords to the boundary between the medulla and cortex. Further branches penetrate the peripheral deep cortical unit and send branches to the superficial cortex, penetrating the follicles. Capillary branches of the vessels of the peripheral deep cortex penetrate the central deep cortical unit. Blood from the capillaries of these tissues is collected by venules, some of which have become specialised as high endothelial

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venules (HEV). These have a cuboidal endothelium that expresses receptors that attract the lymphocytes that migrate between the endothelial cells to the parenchyma of the node. This is the route taken by lymphocytes passing from the blood into the node. The advantages conferred by the cuboidal endothelial cells are unknown. At first glance they would seem to provide a more formidable barrier than the usual squamous endothelial cells. The conduits drain into the perivenular space that surrounds these special venules.

Haemolymph Nodes of the Rat

Haemolymph nodes are enigmatic structures that in both histological and functional terms appear to be intermediate between ordinary lymph nodes and the spleen. Whether they occur in man is debatable, but they certainly occur in the pig, sheep, horse and rodents, including the rat (Sakita et al., 1997; Kazeem et al., 1982; Kazeem and Scothorne, 1982; Hogg et al., 1982). Turner (1969) reported a detailed study of the vascular tree of the haemal nodes of the rat. Haemolymph nodes are characteristically dark red or brown in colour, and have been reported at the edge of the thymus, between the spleen and the pancreas, and bilaterally close to the aorta near the origin of the renal arteries. Haemolymph nodes differ from ordinary lymph nodes in that their lymphatic sinuses contain many red blood cells, which are thought to pass through the walls of rather permeable venous blood vessels. Sakita et al. (1997) suggested an open circulation (see discussion of the spleen) with blood cells entering the lymphatic sinuses by crossing their walls. The function of these unusual nodes is obscure.

MUCOSA ASSOCIATED LYMPHOID TISSUE

Collections of lymphocytes occur beneath many mucosal surfaces. These collections are referred to as MALT. A number of location-specific terms have been coined. These can be summarised as 'X'ALT, where 'X' defines the tissue, and ALT means Associated Lymphoid Tissue. Thus GALT is Gut Associated Lymphoid Tissue, TALT refers to the tear duct (also known as LDALT where LD stands for lacrimal duct), BALT refers to the bronchial tree, CALT refers to the conjunctiva, DALT refers to the salivary ducts, NALT, refers to the nasopharynx. There is also lymphoid tissue in fat, which is termed FALC (Fat Associated Lymphatic Clusters). The so called milky spots of the peritoneum, the Kampmeier foci of the pleura and the isolated lymphatic follicles that occur in the submucosa of the gut and in the connective tissue around blood vessels can be regarded as members of the same family. They have two characteristics that differentiate them from lymph nodes: lack of a capsule and afferent lymphatic vessels (FALC may be an exception to the latter point, see below).

The MALT of the gut includes Peyer's patches in the small intestine, isolated lymphoid follicles found throughout the gut, the cryptopatches of the colon and the gastric associated lymphoid tissue. Detailed discussions of these tissues have been provided by Cesta (2006a) and by Randall and Mebius (2014). The literature dealing with these tissues is extensive. If this were a book on human anatomy we should have to add the tonsil and the appendix, but these structures are not found in the rat. Different types of MALT differ in terms of their detailed histology, and in terms of the ratio of volumes occupied by B and T lymphocytes. Readers requiring details of these variations should consult the references given above.

The General Histology of Mucosa Associated Lymphoid Tissue

MALT is found as isolated collections of lymphoid tissue in the submucosa beneath epithelial surfaces. The epithelial surface over the collection of lymphocytes is often domed and contains, in addition to the epithelial cells characteristic of the host tissue, modified epithelial cells called M cells, so called for the microfolds of the surface cell membrane. The cell membrane of the basal surface of the M cells is also modified, with deep indentations occupied by dendritic cells and lymphocytes. This close relationship allows the M cells to transport antigen from the surface to these other cells. A sequence: M cell (antigen transport), dendritic cell (antigen presentation) and lymphocyte (the response cell) is thus established. The lymphoid mass contains lymphoid follicles, often with germinal centres and interfollicular lymphoid tissue. In common with lymph nodes, the germinal centres are the source of B cells, with T lymphocytes are found in the tissue around the follicles. The postcapillary venules of the tissue around the follicles are of the HEV type, from which lymphocytes escape into the surrounding tissue. Efferent lymphatic vessels drain the tissue, carrying away lymphocytes produced in the tissue. Perhaps the one key fact that should be retained about MALT is that it is here that induction of an immune response occurs. Lymphocytes produced in MALT are carried away from the MALT, by lymph and blood to the location where execution of the response is required: often in the mucosal surface that was challenged. Some types of MALT, for example Peyer's patches, develop before birth, others, including BALT, TALT and NALT develop only after birth.

Fat Associated Lymphoid Clusters

These resemble MALT in that they lack a capsule, contain lymphoid follicles, HEV and interfollicular tissue,

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but differ in that they do not lie adjacent to an epithelial surface. Examples of FALC are found in the mesenteric fat where they are said to acquire antigens via intestinal lymphatic vessels. This suggests that they, unlike MALT, do enjoy an afferent lymphatic supply

Milky Spots

These collections of lymphoid tissue have access to antigen via the stomata of the overlying mesothelium and so monitor peritoneal and pleural fluid.

THE SPLEEN

The spleen (Fig. 9.4) is an elongated dark red organ, covered by peritoneum and lying in the abdominal cavity to the left of the stomach. Blood is supplied by the splenic artery (a branch of the coeliac artery) that runs to the left across the dorsal abdominal wall under cover of the parietal peritoneum before entering the spleen at the hilus, where it divides into many branches. Venous blood drains through the hilus via the splenic vein to the portal vein. No afferent lymphatic vessels run to the spleen, but efferent lymphatic vessels drain to the splenic lymph nodes at the hilus of the organ.

The spleen has two major functions. As a secondary lymphatic organ it contains lymphatic follicles, in which B lymphocytes mature and multiply, interspersed with tissue in which T lymphocytes mature and multiply. Although this architecture is similar to a lymph node, it lacks afferent lymphatic vessels and is functionally different from the lymph nodes in that the spleen is not a lymphatic filter. Unlike lymph nodes and thymus, the spleen is not divided into a cortex and medulla.

The second function of the spleen is to act as a filter of the blood. The spleen contains tissue spaces through which blood percolates. Macrophages lie in these tissue spaces and remove particulate material and aged red blood cells from the circulating blood. The blood that has passed through this filtration system is collected by venous sinuses and drains to veins located in the connective tissue trabeculae of the organ.

In addition to these major functions, the spleen may store red blood cells and platelets, and in some animals provides an important reserve of both. Haemopoiesis, the formation of blood cells, is quite marked in the spleen of the younger rat, but decreases with age. However, this function is rapidly restored when red cell volume is decreased through blood loss or haemolysis. Given all these obviously important functions, it is curious that the spleen can be removed from animals and man without apparent ill-effects. This is probably because none of the functions of the spleen is unique to the spleen, and other tissues can take over its functions. Cesta (2006b) has provided an admirable review of the spleen from the perspective of a toxicological pathologist, and reviews by Mebius and Kraal (2005) and by Steiniger (2015), provide further information, especially regarding the immunological functions of the spleen.

Studies of the histology of the spleen are confounded by species differences. The spleen is not as important as a blood-storage organ in man and rat as it is in the cat and horse. Venous sinuses are a prominent feature in the spleens of men, dogs and rats, but are not found in cats or mice. This difference allows spleens to be classified as sinusal, with venous sinuses and nonsinusal, without. The arrangement of lymphatic tissue within the spleen is also subject to interspecies variation. Weiss, whose work on the vasculature of the spleen is of classical importance, has provided an excellent and comparative account (Weiss, 1988b). In passing, it is worth noting that Weiss's chapters in 1988 edition of *Cell and Tissue Biology* are among the best textbook accounts of the lymphatic system currently available.

The tissue, or parenchyma, of the spleen is divided by anatomists into white pulp and red pulp. The white pulp comprises the accumulations of lymphocytes, and is not white, but rather pinkish grey in fresh tissue. The red pulp

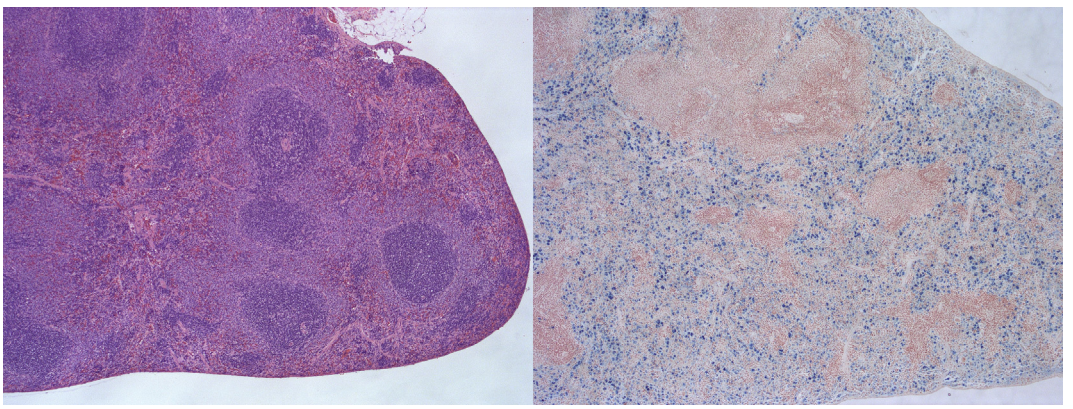


FIGURE 9.4 As the rat ages haemosiderin pigment accumulates, as is demonstrated by the Perl's stain on the left. The haemosiderin stains bright blue.

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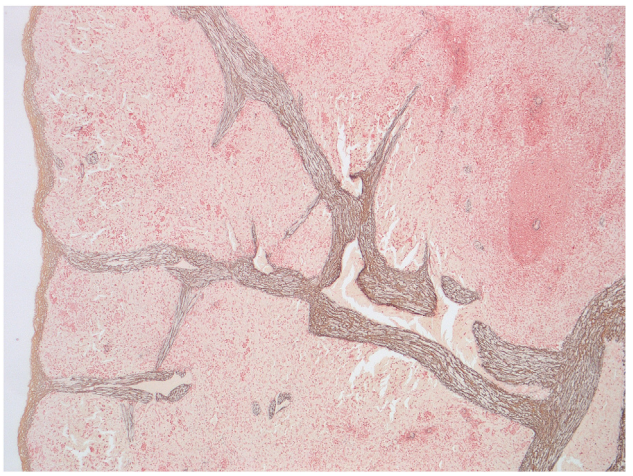


FIGURE 9.5 Spleen—reticulin stain.

is red in colour, and comprises the blood filled tissue spaces and the venous sinuses of the organ. The parenchyma is supported by a scaffolding of reticular tissue that becomes obvious when sections of spleen are impregnated with silver (Fig. 9.5).

The capsule of the spleen is thick in man, thicker in animals in which the spleen is an important blood-storage organ, but thin in the rat. It contains collagen, elastic fibres and a few smooth muscle cells. Trabeculae of connective tissue, extending from the capsule into the interior of the organ, contain the large blood vessels of the spleen and the efferent lymphatic vessels.

The histology of the spleen can perhaps be best understood by following the arteries. As the trabecular arteries run into the parenchyma they acquire a sheath of lymphocytes known as the periarterial lymphatic sheath (PALS). Lymphoid follicles occur within the lymphatic sheath, which is scaffolded by a delicate framework of reticular fibres. Each follicle is surrounded by a marginal (venous) sinus, which is well developed in the rat, but hardly detectable in man. A further layer of lymphatic tissue comprising the marginal zone of the follicle is wrapped around the marginal sinus [see Steiniger (2015) for a series of diagrams showing these relationships]. Active follicles contain a germinal centre similar to those of lymph nodes, with a pale centre where lymphoblasts divide to produce B lymphocytes. B lymphocytes locate to the follicles, T lymphocytes to the interfollicular lymphoid tissue and PALS. Lymphocytes leave the follicles and sheath by entering the lymphatic vessels that run with the central arteries i.e. they run back to the trabeculae. Lymphatic vessels join with others in the trabeculae and form the efferent lymphatic vessels of the spleen. Some lymphocytes also enter the venous sinuses of the red pulp. The flow of lymph is in the opposite direction to the flow of blood in the central arteries.

The artery that runs through the periarterial sheath is named the central artery. In man this runs through a lymphoid follicle (usually a little off centre), but in the rat, the follicles lie to the side of the artery. The capillaries from the central artery supply the tissue of the lymphoid sheath before running to the marginal sinus, where lymphocytes escape and make their way into the sheath. As the central artery leaves the white pulp, it loses its lymphoid sheath and enters the red pulp where the arteriolar capillaries supply the venous sinuses and spaces of the red pulp. The venous sinuses are lined by endothelium, and surrounded by bands of basement membrane that run at right angles to the axis of the sinus. The spaces of the red pulp are not lined by endothelium, here the blood percolates between the networks of fibroblasts and macrophage surrounding reticular fibres. These macrophages remove aged red blood cells and particulate material from the blood stream. Coal dust particles are found in the macrophages of the red pulp in coal miners, and in the aging rat quite heavy deposits of haemosiderin may be seen, particularly in females. Blood that has entered the red pulp finds its way back into the venous sinuses or the veins, the pulp veins, which drain both the venous sinuses and the red pulp. These veins run to the trabeculae and there join to form the tributaries of the splenic vein. The walls of the venous sinuses are regarded as permeable to plasma and cells of the blood. Some authors speak of a rapid transit of blood via the venous sinuses and a slower transit via the red pulp (see Weiss, 1988a for further discussion). Whether the spleen has an open or closed circulation has produced much argument. In a closed circulation, the blood is confined to vessels lined with endothelium and in an open circulation, the blood flows into tissue spaces. Weiss (1988b) concluded that the spleen has an open circulation: his authoritative account should be consulted for further details.

Recent work has shown there exists an extraordinary complexity of interactions between lymphocytes and other cells, including dendritic cells, in the lymphoid tissue of the spleen. Textbooks of immunology should be consulted for further details (see reference list).

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Two classical accounts of the lymphatic system which have not been mentioned in this chapter provide a wealth of information. These are the monographs by Yoffey and Courtice and by Rusznyák, Földi and Szabó. One of the earliest modern works on the lymphatic system, by Drinker and Field, is also worth consulting. Rusznak and colleagues provide more than 2000 references including many from East Europe and from Russia. Not all of these are easily accessible to English speakers but they are summarised in the monograph.

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Chapter 10

Nasal Cavity

The nasal cavity of the rat is important to toxicologists interested in the effects of inhaled toxicants and differs, as regards structure, rather significantly from that of man. The anatomy of the rat nasal cavity is very much more complicated than that of man. Published descriptions vary, both with regard to descriptions of anatomical features, and terminology. Difficulties are most marked when the ethmoturbinates (or ethmoturbinals) are considered, although attempts to simplify the nomenclature have been made. It may be that the detailed topography of these structures will be of less interest to the toxicologist, than the histological structure of the mucosa with which they are covered. However, even the generalist may find themselves confronted by a nasal instillation study at some point in their career, at which time this chapter may become a useful reference.

The nasal cavity begins at the anterior nares (nostrils) and ends where the nasal cavity passes into the nasopharynx or epipharynx. Essentially, the cavity is a space surrounded, divided and subdivided, by bone and cartilage. The inner surface of the cavity is lined by a mucoperiosteum that is more complex than ordinary periosteum, and comprises a surface epithelium, a supporting lamina propria, and the periosteum itself. The lamina propria contains glandular tissue, and is well supplied with blood vessels, and well innervated by the autonomic nervous system. Five types of epithelium are described in detail a little later, but for the moment, we shall simply list them: olfactory epithelium, respiratory epithelium, squamous epithelium, transitional epithelium and lymphoepithelium. Detailed reviews aimed at the toxicologist have been provided by Harkema (1990, 1991, 2006) and by Chamanza and Wright (2015).

THE ANTERIOR REGION OR VESTIBULE OF THE NASAL CAVITY

This part of the rat nasal cavity does not appear to have been described in detail until Haidarliu et al. (2013) published an admirable description with good illustrations. The rostral part of the rat nose is a hairless rhinarium that is attached to, and moves with, the nasal cartilages. The nares are divided by a cartilaginous septum that spreads dorsally to roof the nostrils. This roofing cartilage is partly covered by dorsal cartilages that extend rostrally from the long nasal bones. Haidarliu et al. (2013) described the roofing cartilages (the nasal tectum) as being fused with lateral cartilages, and these in turn fused with the cartilage forming the inferior or ventral margin of the anterior nares (the anterior transverse lamina). This lamina is itself fused to the nasal septum, so the nasal cartilages form a complete ring around each naris. Two further cartilages arise from the inner, lower parts of the side walls. These accessory nasal cartilages incompletely divide each nasal passage into two. Hebel and Stromberg (1976) refer to these as the medial accessory cartilages, and point out that they form ridges that run to the ventral nasal conchae (see below). They define this ridge as the plica (fold) alaris. Hebel and Stromberg also describe a bilateral plica recta (a straight fold) that runs from the dorsal nasal cartilage to the dorsal nasal concha. These plicae divide the airflow into one that follows a lateral route to the lateral meatuses (dorsal, middle and ventral: see below), and one that follows a more medial path via the vertically disposed medial meatus alongside the nasal septum. Hebel and Stromberg describe this medial meatus as the paramedian or common meatus.

The anterior part of the nasal cavity is described as the vestibule. Chamanza and Wright (2015) have provided a satisfying description of this region, and define the medial accessory cartilages as the atrio-turbinates. These are seldom seen by toxicological pathologists who tend to begin their examination of the nasal cavity with a coronal section taken through, or just behind, the upper incisor teeth. This is a pity because two important structures, the nasolacrimal duct and the duct of the lateral nasal gland (Steno's gland), open into the vestibule. In conventional sections these structures can be see, but not their openings into the nasal cavity. Fortunately, this is an area in which few toxicological effects are seen, and only highly irritant materials will affect the epithelium in this area as it is protected by a stratified squamous epithelium.

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THE NASAL CAVITY PROPER

This is the space into which the vestibule opens, is the part of the nasal cavity that most interests toxicologists. The space is divided into two by the nasal septum, and in rodents the ventral edge part of the septum is incomplete and a gap, the septal window, allows continuity between the right and left nasal passages. This gap can often be seen in coronal sections. No such gap occurs in man. Each nasal passage (left or right) has a floor, a roof, a lateral wall and a medial wall. The floor and medial wall are easy to understand; the roof and lateral wall present a few difficulties.

The floor is formed by the upper surface of the hard palate and comprises the palatal or horizontal processes of the premaxilla, maxilla and palatine bones (see Chapter 5: The Skull). The floor forms a gutter with a smooth surface. The septum articulates with the floor via a longitudinal ridge that rises from the bones forming the floor. Fig. 10.1 shows the septum in diagrammatic form. It is largely cartilaginous in its rostral part. The vomer runs forwards and downwards from its superior articulation with the ethmoid, and perhaps sphenoid bone, to articulate at its lower edge with the hard palate. In man, the vomer certainly does articulate with the sphenoid. An articulation that is rather complicated as the upper edges of the vomer flare laterally to produce two wings (alae, singular: ala) that articulate with the vaginal processes of the sphenoid and enclose the pterygopharyngeal (palatinovaginal) canal that runs in the roof of the nasopharynx.

The medial wall is formed by the septum. As stated above this is largely cartilaginous, but includes the vomer and the vertical plate of the ethmoid bone (ethmoid plate in Fig. 10.1). The septum in man is smooth, but in the rat, the vomeronasal organ produces a bulge at its lower, rostral end (see below).

The lateral wall and roof are complicated and cannot be understood without considering the ethmoid bone. In Chapter 5, The Skull, we described the ethmoid bone as elusive: it is not only elusive, it is invisible from the outside of the skull.

THE ETHMOID BONE

The ethmoid bone formed from the paired cartilaginous nasal capsules that fuse to form one bone at the rostral end of the cartilages that form the base of the skull (see Chapter 5: The Skull). Valkenburgh et al. (2014) point out that it forms from three centres of ossification: anterior, intermediate and posterior.

THE HUMAN ETHMOID BONE

The human ethmoid bone is far easier to understand than the ethmoid bone of the rat. At least a part of the human ethmoid bone can be seen from the outside of the skull. Both Grant (1944) and Moffat (1987) have provided useful descriptions of the human ethmoid bone.

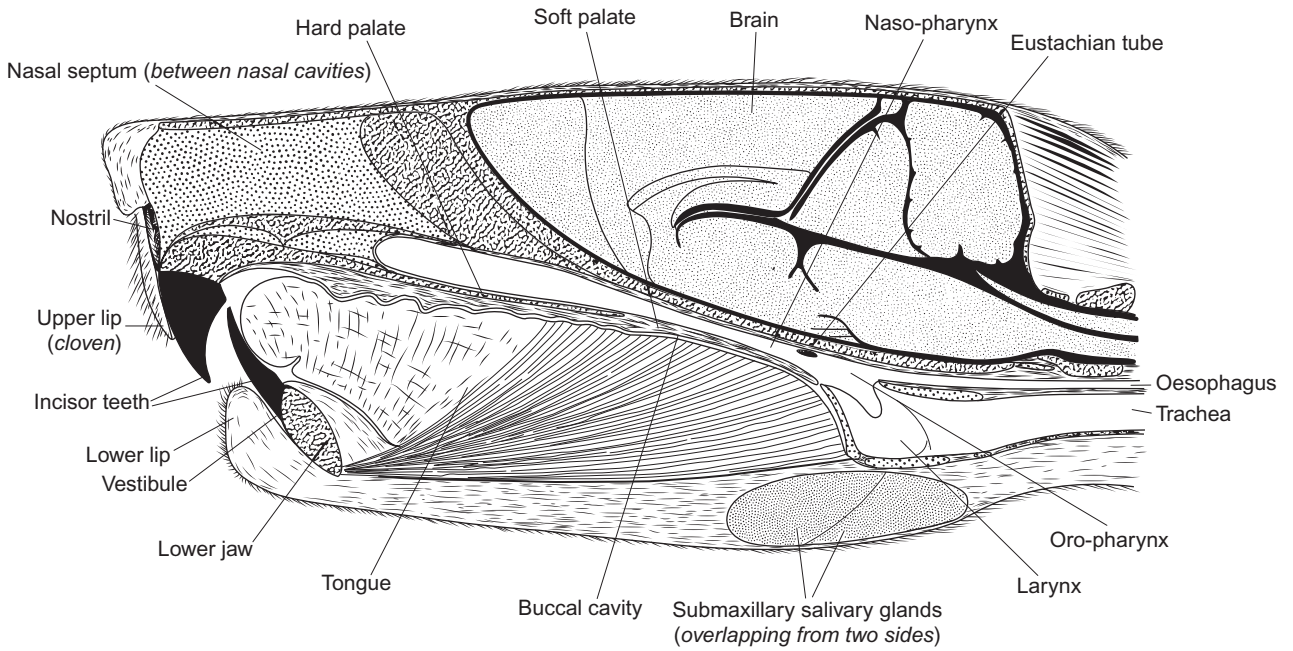


FIGURE 10.1 Median vertical section through the head showing the relationship of the buccal cavity, pharynx and oesophagus to the respiratory passages.

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The human ethmoid bone comprises two very delicate, hollow, blocks of bone; the lateral surfaces of the blocks contribute to the medial walls of the orbits. The interior of each block is filled with air contained by the ethmoid sinuses. The blocks are joined, superiorly, by a perforated plate of bone: the cribriform plate (cribriform meaning a sieve). The olfactory nerves run through the perforations from the nasal cavity to the olfactory bulbs which, in man, lie above the cribriform plate. The space between the blocks forms the upper part of the nasal cavity and is divided, in the sagittal plane, by a plate of bone which contributes, below, to the nasal septum and rises through the cribriform plate to form the crista galli (Latin for cock's comb) which projects into the anterior cranial fossa. Two projections extend for some way into the nasal cavity from the medial surfaces of the blocks, these are the superior and middle nasal conchae. Understanding the human ethmoid bone is a useful starting point to understanding the bone in the rat. It is important to remember that in man the cribriform plate lies above the nasal cavity, but in the rat it lies behind it.

THE ETHMOID BONE OF THE RAT

The ethmoid bone of the rat makes no contribution to the wall of the orbito-temporal fossa, which is made up of parts of the maxilla, lacrimal and frontal bones; with additions from the palatine, alisphenoid and pterygoid bones. The posterior part of the fossa is formed by the parietal and squamous temporal bones (see Chapter 5: The Skull). The ethmoid bone is completely hidden by the maxilla and nasal bones (and part of the frontal bones), indeed the ethmoid and maxilla form a double wall, which we shall return to when we describe the maxillary sinus of the rat. This hiding of the ethmoid is also found other mammals that lack a wide gap between the orbits, for example the dog (Evans and Christensen, 1979). Detailed descriptions of the articulation of the rat ethmoid with other bones of the skull do not appear to have been published, at least not in English.

Another important difference between the rat and human ethmoid bones is related to the fact that the rat's face lies in a line with the base of the skull, whereas in man in hangs from the front end of the base. The rat's nasal cavity is proportionally much longer than that of man, and contains a large posterior recess that is cut off from the main passageway carrying air from the nostrils to the nasopharynx. This space is largely filled by the ethmoturbinates, complex bony processes that arise from the ethmoid. These are not separate bones, but part of the ethmoid bone. This is also the case with regard to the upper two conchae or turbinates of man. Only the inferior turbinate, the maxilloturbinate, is a separate bone, one that articulates with the maxilla, lacrimal and ethmoid bones. In the rat the vomer, in the midline, and a pair of shelf-like plates from the maxilla to the front, and from the ethmoid to the back, form the floor of the posterior part of the nasal cavity. This part of the floor of the nasal cavity forms the roof of the nasopharyngeal duct as it runs back to the posterior, bony, openings of the nasal cavity into the nasopharynx. This duct has a floor, the hard palate to the front and the soft palate to the rear. The side walls of the nasopharyngeal duct are provided by the perpendicular plates of the palatine bones to the front and the medial surfaces of the medial plates of the pterygoid processes to the rear. The vomer articulates with the long presphenoid bone (not seen in man), which continues in the midline of the roof of the duct. All this is rather difficult to make out in the rat skull but is easy to see in the skull of a breed of dog with a long skull, a greyhound for example. Evans and Christensen (1979) provide a well-illustrated account of the anatomy in the dog skull.

The vomer forms the posterior edge of the septum in man. In the rat, this edge is made of cartilage, and rat the posterior edge sweeps down and forwards (see Fig. 10.1). In its rostral part the vomer is shaped, in vertical section, like a Y or perhaps a T. Towards the back the long stem of the T disappears and is replaced by cartilage.

LATERAL WALL OF THE NASAL CAVITY

The lateral wall of the nasal cavity comprises the premaxilla and maxilla in front, and the ethmoid behind. The maxilla runs back to the orbit and provides the zygomatic processes that flare laterally and back as the anterior parts of the zygomatic arches. Fig. 10.2 has been modified from Hebel and Stromberg (after Liebich) and shows the complexity of the lateral walls of the nasal cavity. Fig. 10.2 should be studied with the following description.

Turbinates and Meatuses

'Meatus' means a passage, although the correct Latin plural of meatus is, rather confusingly, meatus, for the sake of clarity we shall use meatuses as the plural. If we think back to the desk model, we can define a meatus as the space between the shelves fitted to the medial walls of the pedestals and below the lower shelf. The first thing to notice is that there are two single turbinates towards the front of the lateral wall (the nasal turbinate or nasoturbinate and maxilloturbinate) and a complex group of ethmoturbinates to the rear. In man, there are three all told: the superior and middle turbinates arise from the ethmoid bone, and the inferior turbinate, a separate bone that articulates with the maxilla, the lacrimal bone and the uncinate process of the ethmoid. The uncinate process is a hook shaped process that curves down

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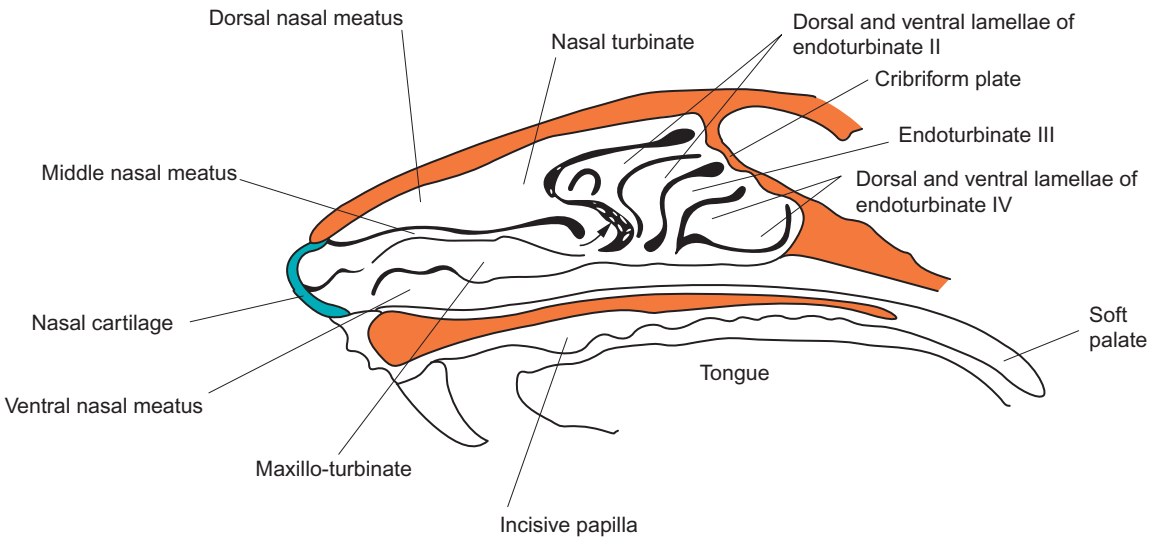


FIGURE 10.2 Lateral wall of the rat nasal cavity.
Section of head of rat showing lateral wall of nasal cavity.

and back from the under surface of the ethmoid and forms part of the lateral wall of the nasal cavity of man.

In man, the three conchae define the meatuses:

Inferior meatus: between inferior concha/turbinate and floor of nasal cavity

Middle meatus: between middle concha/turbinate and inferior concha

Superior meatus: between superior concha/turbinate and middle concha

The spheno-ethmoidal recess is found above the superior concha towards the back of the cavity. This rather remote corner is important because it is here that the sphenopalatine foramen connects the nasal cavity with the pterygo-palatine fossa. Chapter 5, The Skull explains that the rat does not have the sort of pterygo-palatine fossa found in man, but it does have a sphenopalatine foramen located towards the lower anterior corner of the orbito-temporal fossa and leading, of course, into the nasal cavity.

The rat has a more complicated set of turbinate bones than man. From anterior to posterior, there are:

The atrio-turbinates (see above)

The nasal turbinate – located high up on upper end of the lateral wall of the nasal cavity.

The maxilloturbinate – located on the lateral wall below the nasal turbinate.

A complicated set of ethmoturbinates located posterior to the others.

Man lacks a nasal turbinate, a ridge known as the agger nasi (agger meaning an eminence) is perhaps its equivalent. This ridge is covered by mucosa in the living subject, and lies high on the lateral wall towards the rostral end of the cavity. More complex turbinates mean more complex meatuses. These are divided into middle or median meatuses (between the nasal septum and nasal turbinate/maxilla-turbinate) and the lateral meatuses (between the edges of the nasal turbinate/maxilla-turbinate and the lateral wall)

Ventral medial meatus: between maxilloturbinate and the floor of the nasal cavity, vental lateral meatus: between maxilloturbinate and the lateral wall

Middle median meatus: between the free ends of the nasal/maxilloturbinates, middle lateral meatus: between the free ends of the nasal/maxilloturbinates and the lateral wall

Dorsal medial meatus: between the nasal turbinate and dorsal wall, dorsal lateral meatus: between the nasal turbinate and lateral wall

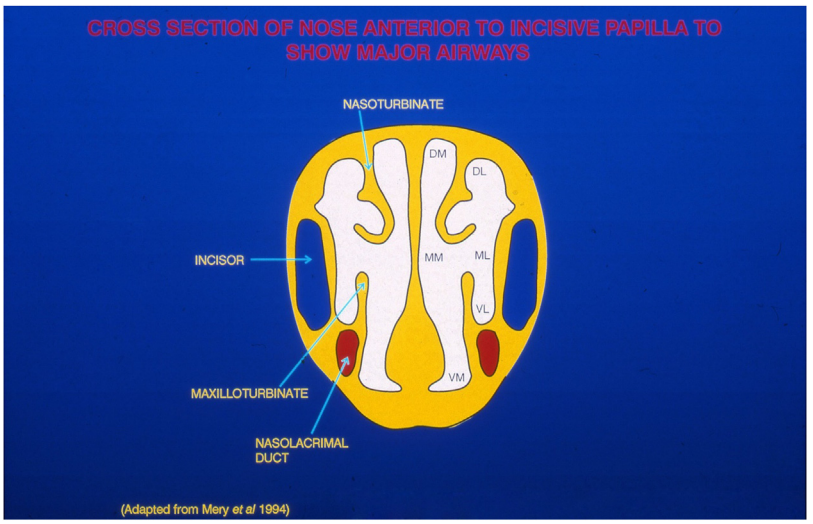
The meatuses are shown in simplified form.

Although this may appear to be an unnecessary complication of an already complex anatomical area, this is important from the aspect of the toxicity of inhaled toxicants. Lesions may be confined to the medial meatuses as this is the area through which airflow is most rapid, and as such receives the largest volume of air and consequently is most highly exposed to an inhaled toxicant.

DM dorsal meatus
MM median meatus
VM medial meatus
DL dorsolateral meatus
ML median lateral meatus
VL ventrolateral meatus

The middle meatus expands posteriorly and is overhung by the nasal turbinate. A coronal section taken at position 1 on Fig. 10.3 would show only the nasal turbinate and the maxilloturbinate. This section, taken just behind the upper incisor teeth is usually the first of the series taken by the toxicological pathologist.

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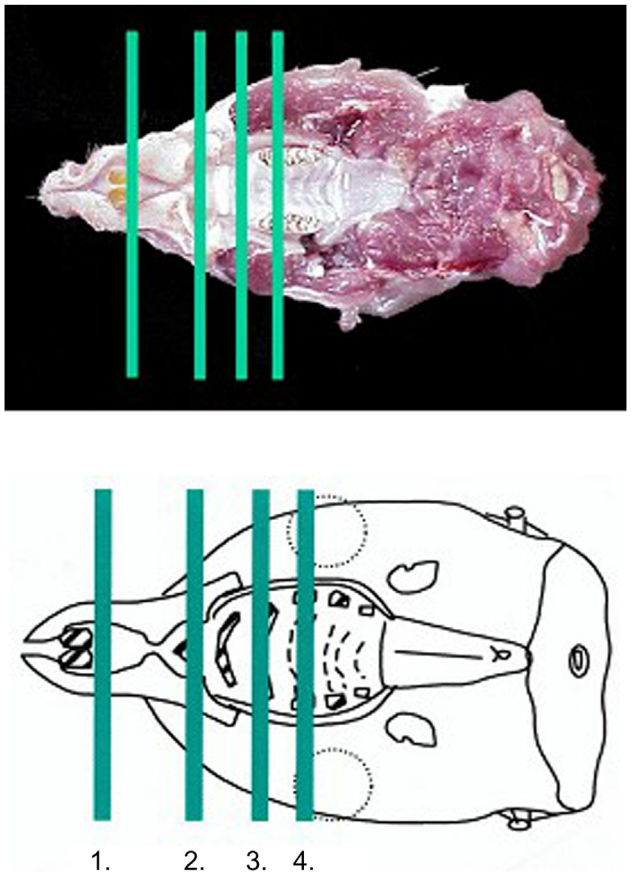


FIGURE 10.3 Ventral view of hard palate showing positions of conventional sections.

The Ethmoid Turbinates (or Turbinals)

In man, the middle and superior conchae arise from the ethmoid bone. In the rat, a whole set of angled turbinates spring from the ethmoid, and lie within the cavity above the nasopharyngeal duct (see Fig. 10.2). Description of the ethmoturbinates begins with Allen (1882) and Paulli (1900a,b): see Moore (1981) for references to Pauli's publication, which are in German, and an admirable summary of the work of Allen and of Paulli. Liebich's account (1975) is valuable and provides the basis of the account provided by Hebel and Stromberg (1976). A complete description has not been attempted here but the following points should be noted.

All the ethmoturbinates spring from the ethmoid bone.

Some authors include the nasoturbinate with the ethmoturbinates. This may lead to confusion when the ethmoturbinates are numbered.

Each ethmoturbinate projects into the nasal cavity from the lateral wall of the cavity and may branch to form lamellae for example dorsal and ventral lamellae that may curl to form scrolls. The nasal turbinate hangs down into the cavity from above.

The ethmoturbinates that project well into the cavity and approach the nasal septum were defined by Paulli as the endoturbinals, while those projecting for only a short distance were defined as ectoturbinals. The endoturbinals were given numbers, printed as Roman numerals (I, II, III, etc.) starting from above. Calling the nasoturbinate (or nasoturbinal) 'ethmoturbinal I' has caused endless confusion. The ectoturbinals were also numbered from above, but given Arabic numerals (1,2,3, etc.) Hebel and Stromberg (1976) use Roman numerals for the ectoturbinals. The series of ethmoturbinates is angled with the lower members lying, of course, below but also posterior to the upper members.

The comparative anatomy of the ethmoturbinates is fascinating. In the dog, some ethmoturbinates extend not into the nasal cavity, but into the sinuses of the bones around

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the cavity. In the pig, there are as many as 20 ectoturbinals, and in the seal the complex of ethmoturbinates fills the cavity almost completely. In the rat, and probably in other mammals, there is subject to subject variation in the exact form of the ethmoturbinates. Identifying the ethmoturbinates as a group in a coronal section of the rat skull is not difficult, but naming them is a challenge. Recall that the series forms an angled set, so not all will appear in any one coronal section, and any one may present a varying appearance from section to section.

Mery et al. (1994) have provided a series of blank diagrams showing the appearance of sections taken through the rat skull. These diagrams are intended to be used by pathologists wishing to record abnormalities of the epithelium covering the turbinates. A total of 17 diagrams are provided, taken from a series of 30 sections. Note that the authors describe the sections as NOT being taken vertically. Usually, the decalcified skull (minus the lower jaw) is placed on a board and cut in a series of vertical planes from anterior to posterior (see Fig. 10.3). In Mery et al. (1994), the plane of the sections slope back from its superior to its inferior border. This choice of sectioning plane is not explained by the authors, though from their illustrations it may be conjectured that it was chosen to allow the sections of the ethmoturbinates to be cut at right angles. In the widely used 'Revised guides for organ sampling and trimming in rats and mice Part 2' [A joint publication of the RITA (Register of Industrial Toxicology Animal-data)] and NACAD (North American Control Animal Database groups) Kittel et al. (2004) recommend vertical sectioning at the following levels:

1. Immediately posterior to the upper incisors
2. Incisive papilla
3. Second palatine crest
4. First molar tooth

For most toxicity studies, the rather more pragmatic approach advocated in the RITA proposals is used. It requires a great deal of skill to cut the samples free-hand in a true vertical plane, or in a sloping plane, so the pathologist must be able to recognise all turbinates present whatever planes of sectioning have been used.

We note in passing that the RITA Guides refer to the ethmoidal bullae when defining a plane of sectioning to show Zymbal's gland. This seems to be misprint: the authors probably meant the tympanic bullae. The ethmoid bulla is not described in standard accounts of rat anatomy, but is well defined in human anatomy as a bulge of the medial wall of the ethmoid labyrinth that projects into the middle nasal meatus.

Mery et al. (1994) discussed the ethmoturbinates in some detail and proposed a new terminology. This terminology, which is now adopted by most toxicologic pathologists, has involved abandoning Paulli's distinction between endo- and ectoturbinals and simply calling them all ethmoturbinates. Six ethmoturbinates have been defined:

Ethmoturbinate 1: medial scroll, lateral scroll
Ethmoturbinate 2: dorsal scroll, ventral scroll
Ethmoturbinate 3: dorsal scroll, ventral scroll
Ethmoturbinate 4: dorsal scroll, ventral scroll
Ethmoturbinate 5
Ethmoturbinate 6: dorsal scroll, ventral scroll

The authors did not include the nasal turbinate with the ethmoturbinates. They pointed out, having discussed their proposed terminology and having compared it with that of Allen (1882, the authors give 1894, in error) and Young (1981), that the nasoturbinate 'of the F-344 rat appears to have only a single scroll, in addition to a ridge lateral to this scroll [the lateral ridge reported by Morgan et al. (35)]. In certain strains of rats, however, this ridge may be extended to form a clear scroll and the terms dorsal and ventral scroll may be more appropriate for these portions of the nasoturbinate'. Ref 35 of Mery et al. is to Morgan et al., 1986. The authors discussed Young's paper (1981) and disagreed with his description of what they regarded as an ectoturbinate. Table 10.1 compares the terminologies.

The reader may be wondering how important all this osteological detail actually is. Of course it is of interest to anatomists, especially to comparative anatomists interested in defining homologies, but to the toxicologist it may seem a little arcane. What is certainly important to the toxicologist is the distribution of the types of epithelium found in the nasal cavity. Because different types of epithelium respond differently to toxic materials, the location of pathological changes within the nasal cavity is

TABLE 10.1 Comparison of Terminologies Applied to the Turbinate Bones

Mery et al. (1994)	Allen (1882)	Young (1981)
Nasal turbinate		
Ethmoturbinate 1	Ectoturbinate 1	Ectoturbinate 1
Medial scroll		
Lateral scroll		
Ethmoturbinate 2	Ectoturbinate 2	Ectoturbinate 2
Dorsal scroll		
Ventral scroll		
Ethmoturbinate 3	Endoturbinate 1	Endoturbinate 1
Dorsal scroll	Dorsal scroll	Dorsal scroll
Ventral scroll	Ventral scroll	Ventral scroll
Ethmoturbinate 4	Ectoturbinate 3	Endoturbinate 2
Dorsal scroll		
Ventral scroll		
Ethmoturbinate 5	Endoturbinate 2	Endoturbinate 3
Ethmoturbinate 6	Endoturbinate 3	Endoturbinate 4
Dorsal scroll		
Ventral scroll		

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important. We shall return to the epithelia shortly. It is also important to recognise the different turbinates and understand how the effects of airflow and particulate deposition may influence the distribution of lesions.

Openings Into the Nasal Cavity in Addition to the Anterior Nares and the Nasopharyngeal Duct

The incisive foramen (shown as two foramina by Hebel and Stromberg (1976) lies in the midline of the hard palate, between the left and right premaxillae and just behind the upper incisor teeth. The foramen leads to a short canal that connects the nasal and oral cavities. The terminal branches of the greater palatine arteries pass through the canal into the nose, and branches of the sphenopalatine nerves pass down through the canals to the anterior surface of the hard palate. The canal also connects the oral cavity with the vomeronasal organs, one of which can be found either side of the lower, anterior part of the nasal septum. The vomeronasal organ has attracted much attention and we shall describe it with the olfactory epithelium.

The nasolacrimal duct passes down from the inner corner of the eye to open into the nasal cavity towards the anterior end of the ventral meatus. The canal can be recognised in histological sections by its nonciliated pseudostratified columnar epithelium. The opening of the duct cannot be seen unless sections are taken through the anterior part of the nasal cavity.

The maxillary sinus is large in man and opens via a gap in the part of the maxilla that provides part of the lateral wall of the nasal cavity. The opening is reduced in size by a group of bones: the inferior concha, the uncinate process of the ethmoid and the palatine bone, all of which partially overlap the opening. In the rat, the maxillary sinus lies between the ethmoid bone and the overlying maxilla. Hebel and Stromberg (1976) state that the ethmoid provides the medial wall of the sinus, that the maxilla provides the lateral wall, which may be, in part, composed of connective tissue. The opening of the sinus lies just under the anterior end of the ethmoturbinates. In man, there are other sinuses: the frontal sinus opens via the infundibulum into the middle meatus, and the sphenoid sinus open into the spheno-ethmoidal recess, but these are not described in the rat.

The sphenopalatine foramen has been mentioned earlier. The sphenopalatine nerve (a branch of the maxillary nerve V2) carries post ganglionic parasympathetic fibres from the sphenopalatine ganglion via the foramen into the nasal cavity. It passes down the lateral wall of the septum to reach the upper opening of the incisive foramen.

Glands Associated With the Nasal Cavity

The olfactory mucosa contains Bowman's glands (see discussion of the olfactory epithelium). Except for the substantial portion covered by the olfactory epithelium, and the small parts covered by squamous and transitional epithelia, the mucosal covering of the interior surface of the nasal cavity could be regarded as a gland. Glandular tissue is widely scattered throughout the lamina propria of the mucoperiosteum and closely resembles that of the salivary glands: both serous and mucous secretory cells are present. In the rat, two well-defined, localised glands are described. Steno's gland and the anterior medial glands of the septum. Steno (whose name, Niels Stensen, is better known to human anatomists from the duct of the parotid gland: Stensen's duct) described a large gland that lies beneath the mucosa of the maxillary sinus. The gland occupies the rostroventral part of the sinus and can be distinguished from glandular tissue in the caudal-dorsal part of the sinus. The latter is described by Hebel and Stromberg (1976) as the maxillary gland. Steno's gland is not found in man, but is probably homologous with the salt glands found in marine birds, and connects to the nasal cavity by a duct that opens into the posterior part of the middle meatus.

The anterior medial glands of the nasal septum (see Tandler and Bojsen-Moller, 1978) comprise a long duct with acini connected to it by short intercalated ducts. The gland lies beneath the mucosa of the anterior part of the septum. See Moe and Bojsen-Moller (1971) and Tandler et al. (2000) for further discussion.

An admirable account of the submucosal glands of the respiratory system has been provided by May and Tucker (2015).

Blood Vessels of the Nasal Cavity

The mucosa of the nasal cavity is well supplied with blood vessels. Arteries enter the cavity through the sphenopalatine and incisive foramina. Large, thin walled veins can be seen in the lamina propria of the mucosa. The tissue has been described as erectile, and marked swelling of the mucosa can be produced by engorgement of these veins, allowing airflow to be redirected, directing inspired air towards the olfactory mucosa. The organisation of the tissue differs from that of classical erectile tissue in that it lacks septa containing smooth muscle fibres (Bloom and Fawcett, 1968). The vasculature is under autonomic control, and interestingly displays cyclical activity, with airflow switching between the right and left nasal passages at regular intervals. This nasal cycle was described in rats by Bojsen-Moller and Fahrenkrug (1971), and in pigs by Eccles and Maynard (1975). In man, the cyclical

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obstruction of the right or left nasal passages is exaggerated during upper respiratory tract infections. In the rat, the large venous spaces on the sides of the nasal septum are described as the swell bodies.

HISTOLOGY OF THE NASAL CAVITY

Olfactory Epithelium

Macrosmatic animals such as the rat rely on olfaction to a large extent; man is a microsmatic mammal and the sense of smell is relatively less important. In the rat, most of the surface of the ethmoturbinates, and in total about 50% of the nasal cavity, is covered by olfactory epithelium (Gross et al., 1982); in man the figure is about 3% (Sorokin, 1988). Structurally, the olfactory epithelium comprises receptor cells that are stimulated by contact with molecules of odorous substances, and communicate with the olfactory bulbs of the brain via bundles of unmyelinated axons that pass to the olfactory bulbs via the cribriform plate of the ethmoid bone. The receptor cells may be regarded as neurones, but they are remarkable in that they are short lived and replaced about once every 28 days. A peripherally placed neurone that receives stimuli, in this case odour, and transmits information to the brain via its own axon represents a primitive arrangement. For all other sensory modalities, the receptor cell transmits information to a peripheral branch of a more centrally located neurone, for example the neurones of the dorsal root ganglia of the spinal nerves. The olfactory neurones are supported by sustentacular cells that appear to hold the neurones upright in the epithelium. Sustentacular cells may do a little more than that, as they are also thought to be involved in the detoxification of substances coming into contact with the olfactory epithelium.

Olfactory neurones send a peripheral process to the surface of the epithelium. This process ends in a knob from which spring 6–8 long immotile cilia. These cilia lie horizontally on the surface of the epithelium surrounded by surface fluid secreted by Bowman's glands. Near their bases these cilia have the usual 9 + 2 arrangement of microtubules, but more peripherally this arrangement is lost (Fawcett, 1994). Menco and Jackson (1997) have provided a detailed account of the ultrastructure of the olfactory epithelium. The cilia bear olfactory receptors. Much is now known of the physiology of these receptors and their control by the genome. It is not possible to review this large field here, but interested readers should consult the works of Gaillard et al. (2004), Fleischer et al. (2009) and DeMaria and Ngai (2010) for details. Richard Axel and Linda Buck were awarded the Nobel prize for Physiology and Medicine for work on the olfactory receptors in 2004. Their work is reviewed in their Nobel Lectures (Axel, 2004; Buck, 2004), which can be accessed via the Nobel web site (Nobelprize.org). Readers with a special interest in the olfactory system will wish to look back to the development of the field and are referred to Proetz (1941), Beidler (1971) and Stoddart (1980). Graziadei's 1971 account of the olfactory mucosa of vertebrates (in Volume IV of the *Handbook of Sensory Physiology*, Beidler, 1971) is especially valuable and provides a review of the classical literature from Massa'a discovery of the olfactory nerves in 1536 to the electron microscopic studies of the 1960s.

Light microscopy of the olfactory epithelium shows that the epithelium contains many nuclei. The nuclei forming a horizontal band nearest to the surface are of the sustentacular cells, those against the bases of the epithelium are of the basal cells, which provide the renewing population of olfactory neurones, and perhaps sustentacular cells as well. The other nuclei belong to the olfactory neurones themselves. The nuclei of the basal cells stain darkly in H&E preparations. See Fig. 10.4.

The olfactory epithelium lacks goblet cells and serous cells. The surface fluid is produced by the cells of Bowman's glands located in the lamina propria beneath the epithelium. These are branched tubulo-alveolar glands with rather pale staining cells. They produce a serous fluid that is delivered to the surface by short ducts lined by a low cuboidal epithelium.

In addition to the olfactory epithelium, there are other chemosensory structures, namely the vomeronasal organ (Jacobson's Organ), the septal olfactory organ (Organ of Masera) and the organ of Grüneberg. The function of each organ is subtlety different and is primarily dependent on the receptors they express, and whence in the brain they send information.

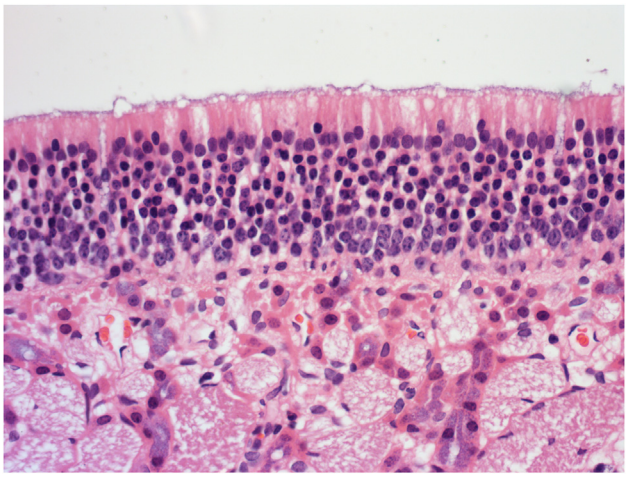


FIGURE 10.4 High power LM of olfactory epithelium.

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The Vomeronasal Organ

The vomeronasal organ has been reviewed by Doving and Trotier (1998), Meredith (2001) and by Salazar and Quinteiro (2009). Evans (2003) has contributed a valuable monograph: 'Vomeronasal Chemoreceptors in Vertebrates'. These reviews show that the vomeronasal organ is found in many tetrapods, but not in fish and birds, being particularly well developed in rodents, including the rat. There is a detailed account of the vomeronasal organ of the rat by Vaccarezza et al. (1981), the autonomic innervation of the organ has been reported by Meredith and O'Connell (1979) and by Eccles (1982). Details of the post-natal development of the vomeronasal organ of the rat may be found in Weiler (2005). Whether the vomeronasal organ plays a part in receiving olfactory signals in man has been much debated. In Meredith's account (2001) the evidence for and against is assessed. It was concluded that though the vomeronasal organ is found in 'the vast majority of human adults' it was impossible to be certain that it plays a part in chemoreception.

In the rat, the vomeronasal organ is a tubular structure about 7 mm long and 1.5 mm in diameter that lies in the infero-rostral part of the nasal septum, largely enclosed by cartilage. Bone may be present in the wall, probably derived from the vomer and/or the ridge on the upper surface of the hard palate with which the vomer articulates. There are two vomeronasal organs, one on each side of the septum, which open into the nasal cavity by a short duct at their anterior ends. The lumen of the organ begins at the duct as a slit but expands and rotates to assume a crescentic shape. The concave margin of the crescent is medial to the convex lateral margin (see Fig. 10.5).

The concave surface is covered by an epithelium similar to the main olfactory epithelium described earlier. This epithelium contains bipolar neurones (the olfactory receptor cells), supporting cells and basal cells. The bipolar neurones bear the olfactory receptors, but the long atypical cilia seen in the main olfactory epithelium appear to be lacking. Vaccarezza et al. (1981) describe capillaries running into the epithelium: this is unusual in that it is generally taught that of all epithelia, only the stria vascularis of the inner ear and, in some mammals, the maternal epithelium of the epiethlio-chorial placenta are vascularised. Sangari et al. (2002) have provided a detailed account of the intra-epithelial capillaries of the guinea pig vomeronasal organ, and noted that the main olfactory epithelium of man is similarly vascularised in the foetus, but not in the adult.

The bipolar neurones of the vomeronasal organ send their unmyelinated fibres via the surface of the nasal septum and the cribriform plate of the ethmoid to the accessory olfactory bulbs. The bundles of nerve fibres just described are sometimes referred to as Jacobson's nerve (Crosby et al. 1962), but this is potentially confusing as Jacobson's nerve is a name that is also applied to the branch (the tympanic nerve) of the IXth (glossopharyngeal) cranial nerve that leaves the IXth cranial nerve in the jugular fossa, passes through a canaliculus to enter the middle ear and there forms the lesser superficial petrosal nerve (Macalister, 1889). The bundles of nerve fibres from the vomeronasal organ should also not be confused with the nearby fibres of the nervus terminalis (see Crosby et al., 1962).

The convex surface of the lining of the vomeronasal organ is covered by a pseudostratified columnar epithelium, lacking cilia but bearing microvilli. The lamina

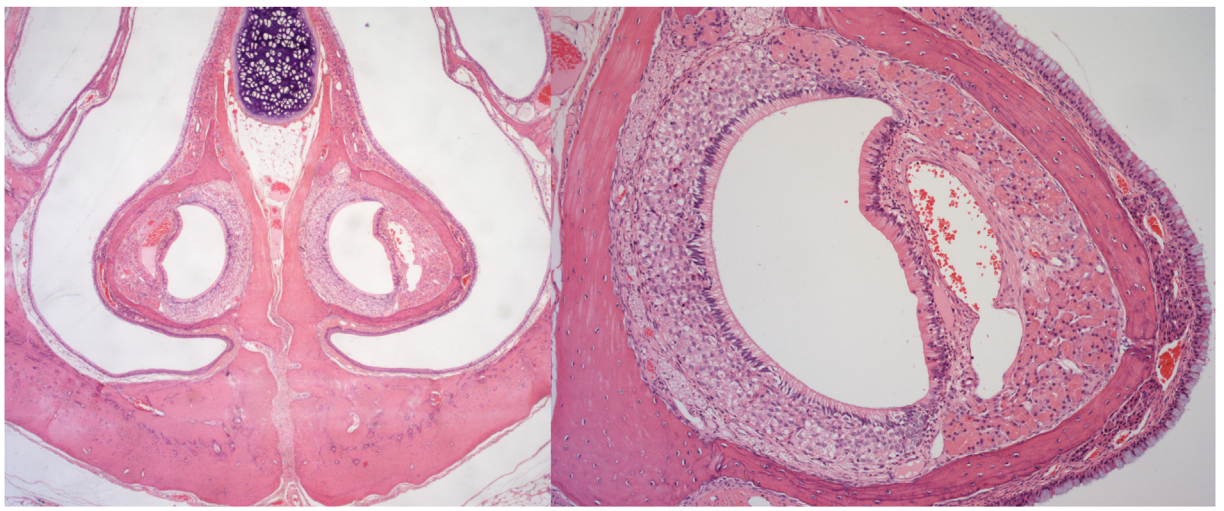


FIGURE 10.5 Photomicrograph showing VN organ. Although described as being largely enclosed by cartilage by many authors, here it seems to be enclosed by bone.

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propria beneath this convex surface contains large venous channels characterised by thin walls. This tissue also erectile. It has been suggested that alternating expansion and contraction of this tissue pumps fluid into and out of the lumen of the vomeronasal organ (Meredith and O'Connell, 1979; Eccles, 1982). The posterior end of the lumen of the vomeronasal organ is lined by a glandular epithelium.

The Septal Organ of Masera and the Organ of Grüneberg

This organ is found on the surface of the nasal septum posterior to the vomeronasal organ and adjacent to the septal window, a gap in the lower edge of the septal cartilage. Less is known of this structure than of the vomeronasal organ, although Ma et al. (2003) published an account of signal transduction in the mouse septal organ of Masera and Taniguchi et al. (1993) have provided a detailed account of the septal organ of the hamster. These authors describe the majority of the bipolar receptor cells of the epithelium as more similar to those of the main olfactory epithelium than to those of the vomeronasal organ, though some cells are likened to those of the vomeronasal organ.

Grueneberg (1973) (Grueneberg is sometimes given as Grüneberg) argued that the structure he discovered on the antero-dorsal surface of the interior of the nasal cavity close to the anterior nares was a ganglion of the nervus terminalis. It is now accepted as a small patch of 300–500 olfactory receptors (Fuss et al., 2005; Brechbühl et al., 2008).

Neither the septal organ of Masera nor the organ of Grüneberg has been as well studied in the rat as in the mouse.

EPITHELIUM OF THE NASAL CAVITY

Respiratory Epithelium of the Nasal Cavity

Pseudostratified ciliated columnar epithelium lines about 45% of the rat nasal cavity (Gross et al., 1982). This type of epithelium is described as 'respiratory', on the grounds that it lines much of the upper respiratory tract. The exact structure of the epithelium varies from region to region, and from species to species. The majority of the cells present are tall ciliated cells, goblet cells producing mucus, and basal cells that provide a reserve population for the other cells, and anchor them to the basal membrane. In addition to these are the rare brush cells, nonciliated columnar cells, serous cells, dendritic cells, neuro-endocrine cells and the related isolated chemosensitive cells. In the rat the greatest density of goblet cells is found on the surface of the nasal septum. Serous cells, found in the airways of pathogen-free rats, do not appear in the nasal respiratory epithelium (Harkema et al., 2006), but are found in the glands of the lamina propria of the nasal mucosa. Finger et al. (2003) and Tizzano and Finger (2013) have reviewed the role of chemosensitive cells and point that stimulation of these cells is linked with neurogenic inflammation.

Squamous Epithelium

The squamous epithelium of the vestibule is a thin stratified squamous epithelium. It extends as far as the rostral ends of the naso and maxilla-turbinates and is continuous with the epithelium of the hard palate via the incisive foramina.

Transitional Epithelium

This term is applied by older histologists to the epithelium that lines the urinary tract from the pelvis of the kidney to the first part of the urethra (see Chapter 17: Urinary Tract). This epithelium is stratified, but has the unusual property of being able to change its conformation on expansion: the number of layers is reduced to two and the cells become flattened. This 'transition' from one form to another is, presumably, the origin of the name 'transitional epithelium'. More modern histologists refer to the epithelium of the urinary tract as the urothelium, and use the term transitional epithelium to describe an epithelium that appears at the border between two contrasting epithelia. This is the sense in which the term is used to describe transitional epithelium in the nasal cavity. A narrow band of transitional epithelium separates the squamous epithelium of the vestibule from the respiratory epithelium of the nasal cavity. The change from squamous to transitional is well marked. Transitional epithelium comprises basal cells and nonciliated columnar cells with either dense or sparse microvilli (Chamanza and Wright, 2015). The change from transitional to respiratory is less obvious, the epithelia grade from one to the other. Harkema et al. (2006) pointed out that transitional epithelium lacked a source of mucus and argued that this increased its susceptibility to damage by inhaled toxicants, and also pointed out the 'abundant' smooth endoplasmic reticulum of the cells of the transitional epithelium that suggested the tissue played a role in detoxification of inhaled substances.

Lymphoepithelium: Nasal Associated Lymphatic Tissue

Mucosa Associated Lymphatic Tissue (MALT) is found in the bronchi (BALT) and gut (GALT) as well as in the nasal cavity (NALT) and other locations: see Chapter 9, Lymphatic System. In each location, the epithelium is

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infiltrated by lymphocytes so that the tissue looks rather like a portion of lymph node that has been mislocated at an epithelial surface. Lymphatic nodules (see Chapter 9: The Lymphatic System) are found in NALT. The surface epithelium contains ciliated cells, some goblet cells and a population of cuboidal cells that lack cilia, but which have plentiful microvilli. Although dendritic cells are found among the lymphocytes they cannot easily be seen in H&E sections. In man the palatine tonsils, which are the tonsils of everyday speech, lie in the side walls of the pharynx. The pharyngeal tonsil is an aggregation of lymphatic tissue that lies high up on the posterior wall of the nasopharynx and is described as 'adenoids' when enlarged. These, plus the lingual tonsil of the upper posterior surface of the tongue, and the collection of lymphatic tissue around the opening of the auditory (Eustachian) tube, form an incomplete ring of lymphatic tissue that older anatomists call Waldeyer's ring. Whether this is very effective at guarding the gastrointestinal and respiratory tracts from invasion by bacteria might be doubted, but it seems likely that NALT is involved in sampling the inspired air for antigenic material. No tonsils are found in the rat.

TOXICOLOGICAL PATHOLOGY OF THE NASAL CAVITY

The majority of toxicological effects seen in the nasal cavity are detected as effects on the epithelium. Although the nasal glands, blood vessels and underlying bone may be damaged, these are comparatively rare occurrences, and generally occur after epithelial damage. As such we will concentrate on the epithelial changes that may occur. Inflammatory responses in the nasal cavity are very much the same as in any other tissue, but oedema is perhaps a little more prominent than in other tissues.

The stratified squamous epithelium that lines the most anterior portion of the rat nasal cavity, presents the most straightforward diagnostic challenge. Squamous epithelium is the most robust of the various epithelia that line the nasal cavity, and is fairly resistant to the effects of inhaled toxicants. The commonly seen acute lesions of the squamous epithelium are erosion or ulceration, which are normally accompanied by inflammation, usually neutrophilic. Longer term chronic lesions in the squamous epithelium, such as hyperplasia and hyperkeratosis may develop. These are much the same type of defensive response that one sees in squamous epithelium in other areas.

In addition to the inflammatory responses that are the feature of squamous epithelium, a range of noninflammatory changes may be seen in the respiratory epithelium. One of the earliest changes to be seen is the loss of cilia, usually followed by swelling and eventually exfoliation. As noted above, these changes are often seen first in the lateral walls where the airflow is greatest. Secretory cell and respiratory cell hyperplasia are common, more chronic, responses seen after exposure to gaseous irritants such as formaldehyde and cigarette smoke. Where life becomes more difficult is when squamous metaplasia of the respiratory epithelium occurs. This is typically associated with cytotoxic irritants, and highlights the importance of knowing the anatomy of the nasal cavity. Although metaplastic epithelium may become keratinised, and can even go towards dysplasia when the insult is of sufficient length and severity, it may also be indistinguishable from the epithelium lining the anterior nasal cavity, and it is only the site that distinguishes abnormal from normal. Where epithelial hyperplasia and keratosis become severe keratin plugs can form, occluding the nasal cavity to a greater or lesser degree. To an obligate nose breather such as the rat this can represent a significant hazard and has been known to result in the deaths of animals.

The olfactory epithelium also presents challenges that require clear knowledge of the normal structure, as low grade toxicity may result in minimal changes to the epithelial height and underlying structures. More straightforwardly toxicants such as chlorine, sulphur dioxide and tobacco smoke result in ulceration and necrosis of the olfactory epithelium, but difficulties occur when repair begins. Although repair may result in a full regeneration of the epithelium, a respiratory or squamous metaplasia may also occur. Although this should be sufficiently distant from the anterior nasal cavity so as not to be confusing to the pathologist one needs to be very clear as to where the respiratory epithelium should normally be situated.

As examination of the nasal cavity is routinely performed in inhalation and nasal installation studies a detailed knowledge of the anatomy and histology of this region is essential when it comes to interpretation of changes, particularly those that may occur after chronic administration of toxicants. The anatomy and physiology of the rat are very different from that of man, and the distribution of inhaled or instilled toxicants may also be very different. Even though these species differences may be considerable, the rat is nevertheless a useful model for hazard identification as long as it is remembered that risk assessment may be complicated by the species differences.

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Chapter 11

Larynx

INTRODUCTION

The hyoid bone and the laryngeal cartilages have evolved from the cartilaginous supports of the branchial (pharyngeal) arches. In their early form these arches comprised right and left ventral elements that were bound together across the midline, and vertical elements that curved up and round the pharynx (Romer, 1970). The vertical elements were often divided into a number of small cartilages.

The hyoid bone of the rat is attached by ligament and muscle to the base of the skull, and to the thyroid cartilage, forming a sling that supports the posterior part of the tongue. The thyroid cartilage is the largest of the laryngeal cartilages, forming a sling that supports the anterior part of the larynx. Behind it, the cricoid cartilage forms a ring around the posterior end of the larynx. The hyoid bone is derived from second and third arch cartilages, the thyroid cartilage from the fourth arch cartilage, and the cricoid cartilage from the sixth. The smaller arytenoid cartilages articulate with the anterior surface of the cricoid and provide a dorsal origin for the vocal folds, which stretch ventrally to the thyroid cartilage as it sweeps around beneath the larynx.

At their anterior edges the vocal folds are braced with dense connective tissue. Calling the vocal folds the vocal cords, leads to the common misconception that the cords resemble the strings of a piano. Although a transverse section through the larynx in the region of the vocal folds does make the folds look like piano strings, this is an illusion produced by representing a three dimensional structure in two dimensions. The glottis is the opening between the vocal cords.

In essence, the larynx of the rat is similar to that of man. In man the arytenoids cartilages are perched on top of the cricoid cartilage, but in the rat they are attached to its anterior surface. If we turn a diagram of the human larynx through 90 degrees and close the gap between the hyoid bone and the thyroid cartilage (see Fig. 11.1), we will produce a rough outline of the rat larynx. Note that Fig. 11.1 is of a human larynx with a modified hyoid bone!

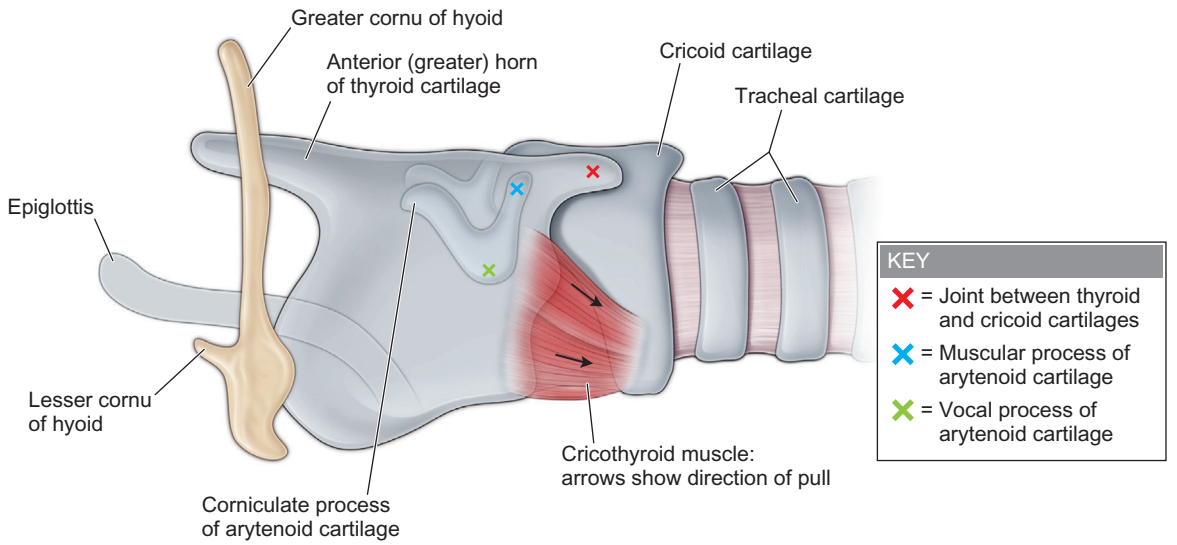


FIGURE 11.1 Lateral view of human larynx modified to resemble that of the rat.

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In the rat, the epiglottis (another laryngeal cartilage) is attached to the inner surface of the inverted arch of the thyroid cartilage, and lies in line with the ventral aspect of the larynx. It is often said that the human epiglottis folds back over the opening of the larynx during swallowing acting as a lid to the larynx. This incorrect idea appears in many accounts. During swallowing the epiglottis does not fold back, but is squeezed between the base of the tongue and the upper end of the larynx. Nutrients pass over the back of the tongue and posterior surface of the epiglottis (not over the anterior surface which would be the case had the epiglottis been folded back), over the tightly closed inlet of the larynx and into the pharynx (Negus, 1927). Negus showed that the human epiglottis is unnecessary for safe swallowing, and argued that it may be a degenerate structure of little functional importance. In macrosmatic animals such as the rat, the epiglottis is inserted into the posterior opening of the naso-pharyngeal duct, and thus allows breathing and olfaction during eating and swallowing. Nutrients flow around the epiglottis, via the lateral food channels, and then around (and over) the tightly closed inlet of the larynx (Negus, 1927). Whatever the reason for the placement of the tip of the epiglottis, the toxicologist should remember that exposure of the rat, an obligate nose breather, to excessive concentrations of particulate matter during inhalation studies can block the nasal airways and lead to suffocation.

THE LARYNGEAL CARTILAGES

Thyroid Cartilage

The thyroid cartilage is named for its shape (thyreos (Gr): shield shaped) rather than for its proximity to the thyroid gland (also named for its shape). Two lateral laminae join ventrally to form a U-shaped cartilaginous sling of hyaline cartilage. In man the lateral laminae meet anteriorly at a sharp angle, forming Adam's apple. The dorsal edges of the laminae lie well apart, and are extended anteriorly and posteriorly by the greater and lesser horns of the cartilage. The posterior horn articulates with the upper, lateral and surface of the cricoid cartilage via a synovial joint. The long anterior horns pass medial to, and are attached to, the posterior horns (usually called cornua; cornu = horn) of the hyoid bone. In man the greater horns of the thyroid cartilage are attached to the tips of the equivalent greater cornua of the hyoid by the thyrohyoid ligaments, which form the posterior edges of the large thyrohyoid membrane. In the rat, the gap between the hyoid and the thyroid cartilage is narrow, and the thyrohyoid membrane is short. In man the larynx appears to have descended away from the hyoid bone. Each lamina of the rat thyroid cartilage is perforated by a foramen that is not found in man.

Cricoid Cartilage

Overlapping the first tracheal ring dorsally and composed of hyaline cartilage (cricos = ring), this is the most caudal of the laryngeal cartilages, and forms a complete ring around the airway. Ventrally, it is narrow but expands laterally to form a dorsal plate of roughly hexagonal shape giving it the appearance of a signet ring. The cricoid cartilage articulates with the posterior horns of the thyroid cartilage, and the arytenoid cartilages. The thyroid cartilage can tilt on the cricoid, a movement that adjusts the length of the vocal folds through the action of the cricothyroid muscles, the only intrinsic muscles of the larynx that lie on the outside of the larynx: see Fig. 11.1.

Arytenoid Cartilages

Despite their name, this pair of hyaline cartilages are not particularly cup-shaped (arytenoid = cup or ladle). In the rat, they are V shaped (Smith, 1977) and in man they are like three sided pyramids with extended processes. The three processes provide attachment for the aryepiglottic folds (quadrangular membrane in man) that sweep up around the lateral margins of the inlet of the larynx to the epiglottis. In man the tiny cuneiform and corniculate cartilages are attached to the processes of the arytenoid cartilage and lie within the root of the aryepiglottic fold. The arytenoid and cricoid cartilage are connected by synovial joints, which allow each arytenoid to pivot on the cricoid and, by rotating about a horizontal axis (vertical axis in man), control the gap between the vocal folds.

Epiglottis

The epiglottis of the rat has been described by Hebel and Stromberg (1976) thus, 'The elastic epiglottic cartilage is shaped like a grooved triangular leaf with a straight caudal base and a long apex. Laterally near the base arises a posited and curved cuneiform process'. At its base, it is attached to the thyroid cartilage by thyroepiglottic ligament and there is a fat pad between the base of the epiglottis and the thyroid.

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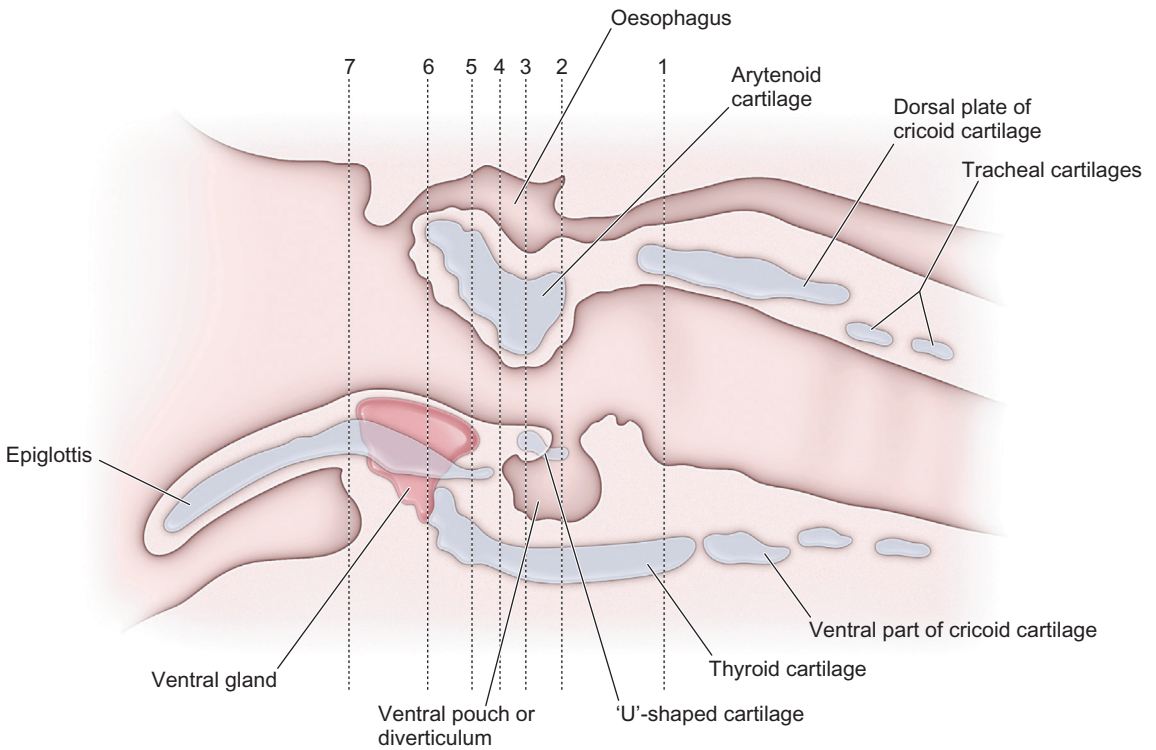


FIGURE 11.2 Mid-sagittal section of larynx. *Modified from Sagartz, J.W., Madarasz, A., Forsell, M.A., Burger, G.T., Ayres, P.H., Coggins, C.R.E., 1992. Histological sectioning of the rodent larynx for inhalation toxicity testing. Toxicol. Pathol. 20 (1), 118–121.*



FIGURE 11.3 Arytenoid cartilage.

The U-Shaped Cartilage

A small blind pouch extends ventrally from the posterior part of the supraglottic compartment of the rat larynx. The opening of the pouch is maintained by a U-shaped cartilage that does not seem to have attracted a specific name (Smith, 1977; Lewis, 1991).

THE HYOID BONE

The body of the hyoid bone is a horizontal rod of triangular cross section that runs across the midline beneath the base of the tongue. This rod, the basihyoid, expands at its lateral extremities and is attached on each side to a pair of cornua. The greater (posterior) cornua run upwards on

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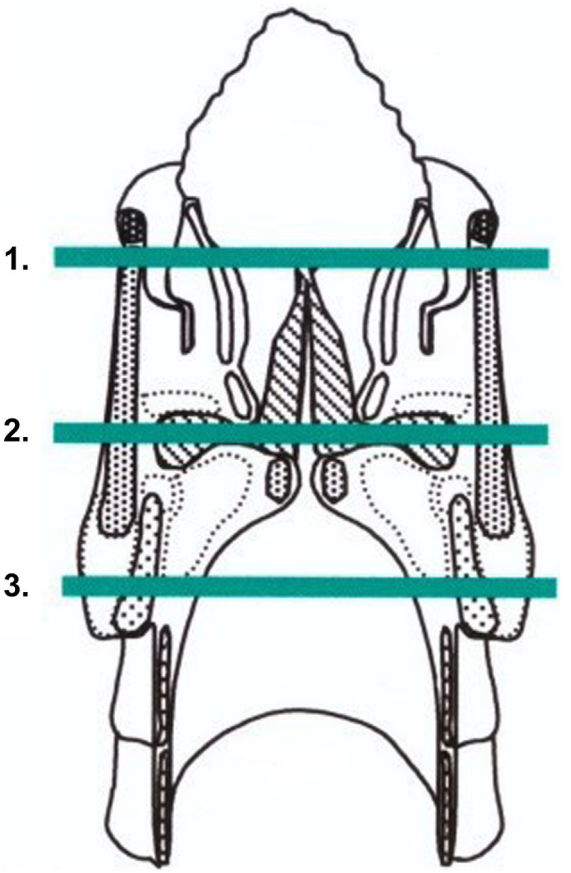


FIGURE 11.4 Trimming pattern for the larynx, as recommended for regulatory studies.

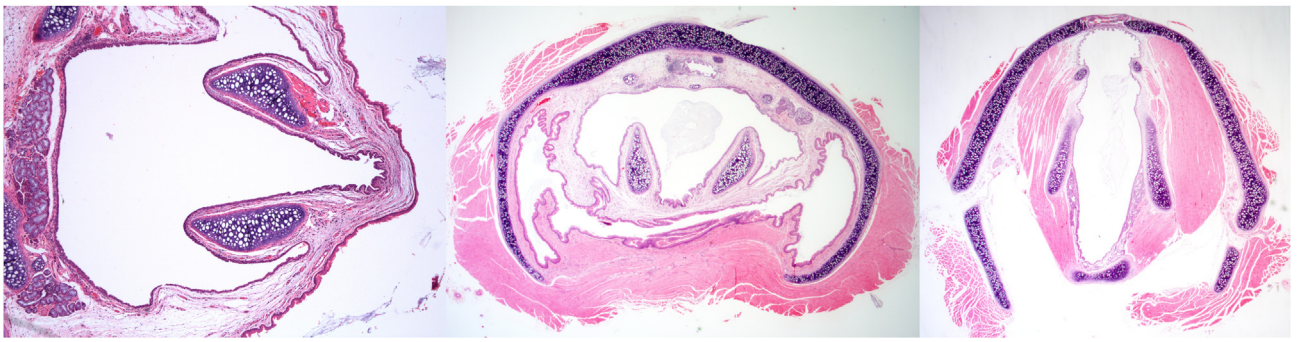


FIGURE 11.5 From left to right levels 1, 2 and 3 of the larynx that are produced from the trimming protocol outlined above.

each side and are attached to the anterior horns of the thyroid cartilage. The lesser cornua are attached by a ligament to the base of the skull near the jugular process. In man this is called the stylohyoid ligament, and in the rat the ligament is equivalent to the jugulo-hyoideus muscle of other species (Hebel and Stromberg, 1976). The cornua are jointed or fused to the basihyoid.

As might be expected from their position, the development of the hyoid bone and laryngeal cartilages relate to the cartilages of the pharyngeal arches as follows.

Second arch: contributes the anterior part of the basihyoid and the lesser cornua of the hyoid. The lesser cornua are short in man and in the rat, but long in carnivores and some other species, for example in the dog, is made up of a series of little bones.

Third arch: contributes the posterior part of the basihyoid and the greater cornua.

Fourth and sixth arches: contribute the thyroid cartilage, the cricoid cartilage and the arytenoid cartilages.

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Cartilage of the second and third arches is thought to be derived from neural crest mesenchyme, whereas that of the fourth and sixth arches are derived from lateral plate mesoderm. The epiglottis arises at the location of the fourth arch, but may not be formed from pharyngeal arch cartilage; its origin in man remains unsettled (Larsen, 1993, Katori et al., 2011).

MUSCLES OF THE LARYNX AND THEIR INNERVATION

The topographical details of the muscles of the rat larynx are less well described than those of man, and probably of limited interest. The most important function of the laryngeal muscles of the rat is the protection of the airway from inundation by food and water. This protection is provided by:

1. Approximation of the aryepiglottic folds and movement of the arytenoid cartilages towards the base of the epiglottis (tubercle of the epiglottis). The aryepiglottic folds are drawn together by the aryepiglottic muscles that run laterally in the folds from the arytenoids to the epiglottis. The two arytenoids are moved ventrally towards the thyroid cartilage by the thyroarytenoid muscles that lie beneath the mucosa to each side of the vocal folds.
2. Adduction and approximation of the edges of the vocal folds leading to closure of the glottis. Adduction of the vocal folds is caused by contraction of the lateral, transverse and oblique cricoarytenoid muscles.

These muscles comprise the intrinsic muscles of the larynx, running from one part of the larynx to another, are all innervated by branches of the vagus nerve (Xth CN). With the exception of the cricothyroid, all are supplied by the recurrent laryngeal nerves. The cricothyroid muscle on the outer surface of the larynx is innervated by the external laryngeal nerve, a branch of the superior laryngeal nerve, itself a branch of the vagus.

The Cavity or Lumen of the Larynx

The cavity of the larynx is lined by mucous membrane (mucosa) that sweeps around the inner surface and is raised into a number of folds. In man there are three, the aryepiglottic folds, the vocal folds and the vestibular folds. The vocal folds divide the cavity of the larynx into supraglottic and infraglottic compartments separated by the glottis, the narrow gap between the vocal folds.

The vestibular folds are not found in the rat but lie just above the vocal folds forming the 'false vocal cords' in man. Again in man, there is small space between the vocal and vestibular folds known as the laryngeal sinus, from which a narrow and blind-ended duct, the saccule of the larynx arises. This is huge in howler monkeys and invades the basihyoid bone to the extent that it takes on an egg-like shape and acts as an amplifying resonator for their very loud voices. Many primates, and some other mammals, also have air sacs connected with the cavity of the larynx, although the size of the air sacs appears to have little to do with the size of their voices (Negus, 1949). There is no saccule in the rat.

Innervation of the mucosa above the vocal folds is from the internal laryngeal nerves, which penetrate the thyrohyoid membrane in man, and below the glottis from the recurrent laryngeal nerves.

The Laryngeal Mucosa

Submucosal Glands

Seromucinous submucosal glands are found throughout the larynx, with those on the superior surface of the epiglottis being largely mucous in nature (Lewis, 1991). A large collection of glands occurs in the submucosa of the ventral wall between the epiglottis and the ventral diverticulum or pouch.

Epithelium

Four types of epithelium are found in the larynx.

1. Stratified squamous epithelium covers most of the epiglottis, the arytenoid projections and the edges of the vocal folds. A narrow strip of squamous epithelium also lies between the opening of the ventral pouch and the base of the epiglottis. The epithelium over the vocal folds is a two layered epithelium, with dark staining basal cells and lighter staining surface cells, described as 'squamoid epithelium', to distinguish this from the multilayered stratified squamous epithelium found elsewhere in the larynx (Lewis, 1991).
2. Respiratory epithelium (pseudostratified ciliated columnar epithelium) is found throughout the infraglottic compartment and as isolated patches at the sides of the base of the epiglottis. The usual cell types occur: ciliated, intermediate, serous and basal; with brush cells and enterochromaffin (Kulchitsky) cells being recognisable at the electron microscopic (EM) level. Goblet cells appear to be rather rare.
3. Transitional epithelium (stratified nonciliated cuboidal or columnar epithelium) occurs between areas of squamous and respiratory epithelium, for example between the base of the epiglottis and the ventral pouch. This epithelium is of special interest to toxicologists, for it is here that the earliest changes induced by exposure to toxic materials tend to occur.
4. A two layered stratified cuboidal epithelium with basal cells that stain more deeply than the superficial cells, lines the ventral pouch.

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A detailed account of the ultrastructure of the epithelium of the rat larynx has been provided by Lewis and Prentice (1980)

Sectioning of the Larynx

Although a single section may be sufficient for some regulatory purposes, at least three sections are recommended for inhalation studies. Kittel et al. (2004) proposed a scheme with sections to be taken at:

1. the base of the epiglottis,
2. through the ventral pouch, and
3. through the cricoid cartilage.

Note that in Kittel et al. (2004), the thyroid cartilage is mislabelled as the U-shaped cartilage. Although it is U shaped, the actual 'U-shaped cartilage', which is very small, can be seen guarding the mouth of the ventral pouch.

In an alternative scheme, Sagartz et al. (1992) cut serial sections and chose seven levels. Fig. 11.2, modified from Sagartz et al. shows a midline longitudinal section through the larynx and the location of the sections recommended (Figs. 11.3–11.5).

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Chapter 12

The Lung

The literature dealing with the structure of the lung is vast. The bibliography at the end of this chapter lists the standard works on the anatomy of the human lung in addition to works dealing with the rodent lung, and specifically with the rat lung. Relevant texts on inhalation toxicology are also listed. The reader is advised to be cautious about accepting any statement on the anatomy of the lung which is not qualified by identification of the species concerned, as even the lungs of the mouse and rat differ significantly as regards their histological structure.

DEVELOPMENT OF THE LUNG

It is not possible to understand the gross anatomy of the lung and its relations to other intrathoracic organs without some grasp of the embryology of the lung. An understanding of the embryology allows one to avoid incorrect statements such as, the lungs lie in the coelomic cavity, or the lungs lie in the pleural cavities.

To understand this we need to think of the early embryo as a trilaminate disc. The primitive coelom forms as a result of splitting of the lateral plate mesoderm, and runs forward from where the intra and extraembryonic coeloms are confluent at the right and left edges of the disc as a pair of canals that are linked across the midline anterior to the buccopharyngeal membrane. The two canals, plus their anterior linkage, have the shape of a horseshoe. The mesoderm that lies anterior to the bend of the horseshoe is the transverse septum. Folding of the embryo causes the anterior end of the disc to be tucked in under the ventral surface so that the two canals of the intraembryonic coelom now run, on each side, forward from what will become the peritoneal cavity, turn down (ventrally), run back and link across the midline just in front of what will become the diaphragm. The transverse septum contributes to the formation of the diaphragm. The developing heart is invaginated into the linking canal that crosses the midline to become the pericardial cavity. The original coelomic canals that ran forward to the horseshoe bend become the pleural cavities. These canals are called the pericardio-peritoneal canals: a very reasonable name as they connect the peritoneal cavity with the future pericardial cavity.

Separation of the Pericardial Cavity From the Pericardioperitoneal Canals

Two pleuropericardial folds grow in from the body wall in the coronal plane and run from anterior to posterior. These folds separate the future pericardial cavity (lying ventrally) from the future pleural cavities (lying dorsally). Once the folds have met and joined, the future pericardial cavity is cut off from the pericardioperitoneal canals. The canals should now be called the pleuroperitoneal canals, but the old name is retained by some authors, a source of much confusion. Another pair of folds now grows in laterally from the postero-lateral aspect of the embryo body wall towards the transverse septum. Once they meet the septum the separation of the thoracic cavity from the abdominal cavity will be complete. These folds are called the pleuroperitoneal membranes. As they grow in they nip the pericardioperitoneal canals and close them off, separating the peritoneal cavity is from the developing pleural cavities. Helpful diagrams explaining this rather complex process may be found in Larsen (1993).

The pericardioperitoneal canals expand within the developing thoracic cavity and form two sacs that envelop the developing lungs. The original lumen of each canal is preserved and becomes the pleural cavity. This process of invagination of a sac (of the expanding canal in this case) by an organ is a common theme in embryological development. In this circumstance the sac retains its lumen and the inner wall clothes the organ and the outer layer of the sac becomes the parietal layer. The parietes are the body walls. The parietal pleura lines the chest the parietal peritoneum lines the abdominal cavity, but the parietal pericardium does not line the body wall, but rather the outer, fibrous pericardium.

An easy way to think of this process of invagination is to image thrusting your fist into a soft, small, rubber balloon. Your fist will be covered by two layers of rubber,

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and the original cavity within the balloon will be confined to the now rather attenuated space lying between these layers. All the blood vessels and nerves that run to your hand will, of course, continue to run through your wrist. So it is that the blood vessels and nerves that supply an organ, such as the lung or gut, all enter via the point at which the organ was invaginated into the coelomic sac. With respect to the lung this is the hilum, and it is here that the visceral layer of the pleura is continuous with the parietal layer, just as the wall of the rubber balloon is continuous at this point around your wrist. The essential thing to remember is that no blood vessels or nerves reach the invaginated organ across the coelomic space, all enter and leave at the site of the invagination.

Pleura and Pleural Cavity

The visceral and parietal pleura are bound by connective tissue to the surface of the lung and inner surface of the chest wall respectively, leaving a narrow space between the visceral and parietal layers of the pleura that is the pleural cavity. The lung does not lie in the pleural cavity; rather it is enveloped by the pleural cavity. All that the pleural cavity contains is a little fluid and some free cells that have become detached from the opposed surfaces of the visceral and parietal pleurae.

The rat visceral pleura is notably thin, comprising a layer of squamous mesothelial cells at its surface characterised by microvilli at their free surface, and a supporting layer of connective tissue containing elastic fibres in which lymphatic vessels run. The visceral pleura of the rat is supplied by the pulmonary circulation; in man the supply is via the bronchial circulation.

The parietal pleura is similar to the visceral pleura in structure, though the surface cells are of two types: cuboidal and flattened. Stomata between the cuboidal cells open into the lymphatic system of the parietal pleura (Wang et al., 1997; Abu-Hijleh, 1994). The blood supply to the parietal pleura is from the intercostal vessels and branches of the oesophageal arteries arising from the aorta, that also supply the mediastinal pleura. Kampmeier foci ('milky spots') found on the mediastinal pleura represent collections of macrophages and lymphocytes.

DEVELOPMENT OF THE LUNG

Much more is known of the development of the human lung than of the rat lung. The development of knowledge of the embryology of the human lung can be traced through reviews and monographs: De Reuck and Porter (1967), Emery (1969), Gaultier et al. (1999). Burri's chapter in the last mentioned monograph sums up much of the work undertaken by Burri and colleagues over an extended period. This work has included some of the few definitive morphological studies on the development of the rat lung. Burri's earlier review (1984) is particularly important, although it deals mainly with the human lung it refers to the author's work on the rat lung. Burri and Moschopulos (1992) discuss the development of the rat lung in detail. In recent years the techniques of molecular biology have been applied to the development of the lung and the proliferation of concepts and terminology has been overwhelming. Warburton et al. (2000, 2010) have contributed lengthy reviews (the second being of 85 pages) to which the reader is referred, Morrisey and Hogan (2010) have also contributed a valuable review.

General Points

The mammalian lung develops from an outgrowth of the foregut. The outgrowth, which from the start contains a lumen, branches repeatedly and invades the local mesenchyme. Branching is dependent on interaction between mesodermal tissue and endothelium. In the early stages of lung development the tissue resembles a gland (e.g., a salivary gland), later it is invaded by capillaries and the smaller airways appear, lastly the epithelium involved in gas exchange appears. In man alveoli appear just before birth and continue to develop during childhood.

Development of the Rat Lung

Gestation in the rat lasts for 20–21 days, and all the development of the lung that occurs before birth is compressed into the period from about E11 to birth (E stands for embryonic). In man the process is more leisurely. Lung development begins at about 3.5 weeks and proceeds to birth and beyond. In neither rat nor man is the lung is fully developed at birth, in both the main period of alveoli development in terms of size and number, occurs after birth. Yamada et al. (2002) have reported a detailed study of the development of the rat lung, the following summary is based on their findings.

Days E11.5–E13. The embryonic stage. The lung develops from the ventral surface of the upper oesophagus, first signs of development are seen at about the 11th day of gestation. The bud does not grow out from the oesophagus as a simple tube, but forms from a groove in the ventral wall of the oesophagus. The future trachea and oesophagus separate as the result of the ingrowth of ridges of tissue running lengthwise along the sides of oesophagus. The upper end of the developing airway forms the larynx. The lung bud grows out into the mesenchymal tissue and is surrounded by developing blood vessels. The bud begins to branch at about day 13.

Days E13–E18.5. The pseudoglandular stage. Branching of the developing airway occurs and the lobar bronchi appear. At E 16.5 cartilage appears in the wall

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of the future trachea and extrapulmonary bronchi. The future intrapulmonary bronchi contain a complete ring of smooth muscle in their walls, and the local capillary networks are now particularly dense around the smaller bronchi.

Days E18.5–birth. The canalicular and saccular stages. Repeated branching occurs, and by day 19.5 all the branches of the conducting airways are in place. Saccular spaces representing prospective alveolar acini appear distally.

After birth: the alveolar stage. Alveoli appear and bronchioles are surrounded by smooth muscle cells that continue to increase in number.

Pinkerton and Joad (2000) reported slightly different timings and noted that alveolar proliferation continued in the rat for 2 weeks after birth and that alveolar expansion continued until 4 weeks after birth.

Development of the Blood and Nerve Supply of the Lung

The pulmonary arteries originate from the arteries of the sixth branchial arch, which grow into the mesenchyme around the developing lung. The developing pulmonary arteries follow the developing bronchi and lie close to them within the lung. Initially venous drainage is via a plexus of small venous capillaries that drain largely to the azygous vein. Later the developing pulmonary veins take over, and the drainage shifts from the systemic veins to the left atrium. Burri (1999) described the development of the alveolar capillary bed in detail and pointed out that the initially thick walls of the terminal air spaces contain two layers of capillaries, later reduced to one, that follows a serpentine course through each interalveolar septum and comes into close contact with the alveolar epithelia of contiguous alveoli. Burri also points out that the term 'canalicular phase' was based on the canalisation of the connective tissue of the developing lung by capillaries, but that the fine branches of the airways seen during this phase of development are also referred to as canaliculi.

The walls of the airways, and the vasa vasorum supplying the walls of the pulmonary vessels, receive their blood supply from bronchial arteries, which arise from the aorta, and perhaps from some intercostal arteries. Bronchial veins that drain the tissues supplied by the bronchial arteries in the central parts of the lung drain into the pulmonary veins and the azygous vein, creating a small anatomical shunt that mixes venous blood with arterial blood returning from the lungs via the pulmonary veins. A detailed account of the bronchial arteries of the rat has been provided by Ferreira et al. (2001).

TOPOGRAPHICAL ANATOMY OF THE LUNG: THE LUNG IN THE THORACIC CAVITY

The anterior inlet of the thoracic cavity is defined by the vertebral column, the first rib and the anterior end of the sternum. In man the heart 'sits' on the diaphragm and the pericardium is attached to the central tendon of the diaphragm. In the rat the heart lies further forward. The thoracic part of the inferior, or posterior, vena cava is very short in man, but proportionally much longer in the rat. At first sight the left lung is not obviously subdivided, but the right lung is fairly clearly divided into four lobes: the anterior or cranial, the middle, the posterior or caudal, and the accessory or postcaval. The postcaval lobe is named for its location behind the inferior vena cava. This small lobe is surrounded by a pocket of the pleural cavity (see above). The inferior vena cava, itself surrounded by a fold of the parietal pleura, lies between the posterior lobe and the accessory lobe (Fig. 12.1).

Upon removal from the thoracic cavity the lungs, and the lobes of the right lung, fall apart from one another. The lung and the chest walls are mechanically linked by the fluid in the pleural cavity, so when air enters the cavity the fluid linkage is broken and the lung collapses, producing what is effectively a pneumothorax. It is the balance between the inward pull of the lung, and the outward pull of the chest wall that sets the Functional Residual Capacity (FRC) of the lung. This balance point is reached at the end of every normal expiration. To move away from FRC, to expand or further compress the lung, work must be done: muscles must be employed. The inward pull of the lung is a combination of the elastic recoil of the lung tissue and the surface tension of the fluid lining the lung. The surface tension is much reduced by the presence of surfactant. It will be obvious that if there is a lack of surfactant then expanding the lung will require a great deal of force. This is the basis of the pathology of Neonatal Respiratory Distress Syndrome: when the lung is immature, insufficient surfactant is produced and the lung is difficult to expand.

If the collapsed lung is inflated with air, or expanded with formalin, the lobes expand and fit together as they do in the living animal. To allow for meaningful examination of the lung under the microscope, fixation for the lung in an expanded state is essential (see below).

The Mediastinum

The visceral pleura that covers the surface of the lung is reflected from the surface of the lung at the hilum, where it spreads over the surface of the thin septum of tissue that divides the thoracic cavity into right and left halves. This septum is in fact an anatomical space in which the

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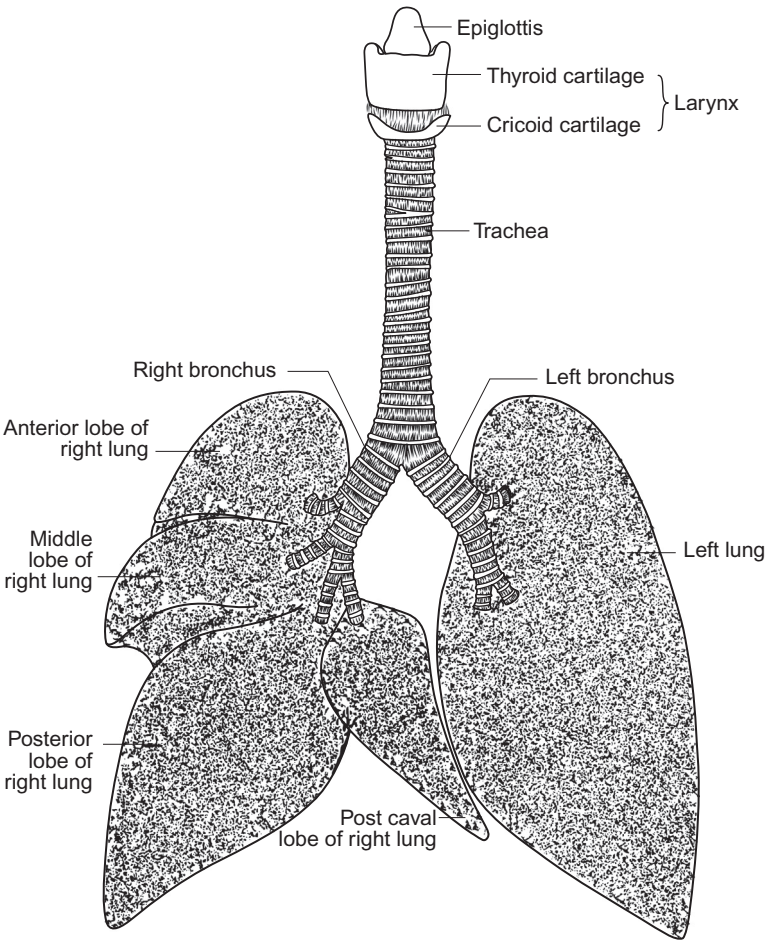


FIGURE 12.1 Respiratory system.

great vessels, oesophagus, trachea, thymus and heart are found. Very loose connective tissue fills the gaps between the contents of the space. The septum is described as the mediastinum, and the covering pleura is referred to as the mediastinal pleura. Having spread over the right and left surfaces of the mediastinum the pleura reaches the chest wall, where it becomes recognisable as the parietal pleura.

The anterior end of the mediastinum is open, and the structures in the mediastinum run into the neck along with the connective tissue of the mediastinum. The posterior end, in contrast, is closed by the diaphragm.

The Bronchial Tree

The bronchial tree comprises the trachea and all the branches of the airway. Much attention has been paid to the pattern of branching of the airways (Boyden, 1955), which is important for developing physical models of the pattern of deposition of inhaled materials in the airways. In man the pattern of branching is described as symmetrically dichotomous, each division leading to two daughter branches of about equal diameter. In the rat the pattern is one of asymmetric monopodial branching, with each branch point giving rise to one small daughter and one large daughter airway, leading to the appearance of the parent airway continuing with the small offshoots at the branch points. Monopodial branching has important effects on the distribution of path lengths from the trachea to the terminal airways, as a path along a series of minor daughter branches is likely to be shorter than that along a series of major daughter branches.

Classification of the Airways

Anatomy of the Airways in Man

In man the airways are classified in terms of generations, the trachea being generation 0, the main bronchi generation 1 and so on. The trachea divides to produce the left and right main bronchi, with further divisions producing the lobar bronchi supplying each of the lobes of the right lung. Thereafter, segmental bronchi supply an essentially isolated volume of lung tissue, and then divide into a series of bronchi of decreasing size until the bronchioles are reached. Bronchi in man are characterised histologically by the presence of cartilage and submucosal glands

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in their walls, but in the rat neither is found beyond the main bronchi, indeed submucosal glands are common only in the upper trachea. This gives rise to difficulties of terminology: how is a bronchus in a rat to be defined? We shall return to this point when we consider the histology of the airways.

The bronchioles comprise a series of airways with a layer of smooth muscle (but no cartilage) surrounding the mucosa, gradually decreasing in size until they terminate as the terminal bronchioles. The airways proximal to, and the terminal bronchiole itself, are called the conducting airways, and take no part in gas exchange. In man the terminal bronchiole is followed by a few generations of respiratory bronchioles, with alveoli appearing as pouches radiating from their walls. As one generation of respiratory bronchioles gives rise to another, the percentage of the wall occupied by the mouths of the alveoli increases. The respiratory bronchioles give way to the alveolar ducts, where the airway represents little more than a central path through a network of alveoli. After the alveolar ducts come the alveolar saccules and then the alveoli. Some authors define a space called the atrium from which the alveolar saccules radiate; others speak only of alveolar saccules and alveoli (Table 12.1).

Each alveolar sac (or alveolar saccule) gives rise to about 17 alveoli. Thus about 136×10^6 alveoli arise in this way. About as many again arise from the alveolar ducts and respiratory bronchioles, giving about 300×10^6 as the total number of alveoli in man. Other authors have given a range, from 274 to 790×10^6 as the total number (Ochs et al., 2004).

Anatomy of the Rat Airways

Despite the small size of lungs of the rat, there are about 16 generations of conducting airways, but rather fewer alveoli than the human lung. Hyde et al. (2004) estimated that the rat lung (right and left lungs combined) contained, on average, about 20.1×10^6 alveoli. The rat bronchial tree differs significantly from the human lung in that cartilage and submucosal glands only occur in the trachea and main bronchi, and respiratory bronchioles are absent, or occur only as a short, single, generation.

Yeh et al. (1979) have published a model of the rat lung based on laborious counting and measuring of airways. Data extracted from Yeh et al. (1979) is shown in Table 12.2.

Details of branching patterns and methods of analysis of these patterns see Horsfield (1986) and Kitaoka et al. (1999).

Structural Units of the Lung

The largest structural unit of the lung is the lobe, which is supplied by the lobar bronchi, and can be easily seen at dissection. Each lobe can be subdivided into more or less independent segments that are supplied by a segmental bronchus. These are easy to define in large animals with a thick pleura and well-developed septa of connective tissue that extend into the substance of the lung. The naming of segments is a source of confusion, and not perhaps a point of great interest to toxicologists. For reference see: Nomina Anatomica Veterinaria (2010). The segments of the right lung are named there in Latin. The following is a summary in English.

Anterior lobe: 4 segments: dorsal, ventral, medial and lateral

Middle lobe: the lobe comprises 1 segment

Posterior lobe: 4 segments: dorsal, ventral, medial and lateral

Accessory lobe (postcaval lobe in the rat): the lobe comprises a single segment.

TABLE 12.1 The Details of the Human Bronchial Tree

	Generation	Number	Diameter (mm)
Trachea	0	1	18
Main bronchi	1	4	13
Lobar bronchi	2–3	4–8	7–5
Segmental bronchi	4	16	4
Small bronchi	5–11	32–2000	3–1
Bronchioles to terminal bronchioles	12–16	4000–65,000	1–0.5
Respiratory bronchioles	17–19	130,000–500,000	0.4
Alveolar ducts	20–22	1×10^6–4×10^6	0.3
Alveolar sacs	23	8×10^6	0.3

Source: Data from Weibel, E.R., 1963. Morphometry of the Human Lung. Springer, Berlin.

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TABLE 12.2 The Rat Bronchial Tree

Generation, Trachea = 1	Number of Airways	Diameter (mm)	Length (mm)
1	1	3.40	26.80
2	2	2.90	7.15
3	3	2.63	4.00
4	5	2.03	1.76
5	8	1.63	2.08
6	14	1.34	1.17
7	23	1.23	1.14
8	38	1.12	1.30
9	65	0.95	0.99
10	109	0.87	0.91
11	184	0.78	0.96
12	309	0.70	0.73
13	521	0.58	0.75
14	877	0.49	0.60
15	1477	0.36	0.55
16	2487	0.20	0.35

It will be obvious that the above summary applies to the right lung of the rat. The Nomina (see above) divides the left lung into a pars cranialis and a pars caudalis. If we argue that these parts represent cranial and caudal lobes of the right lung in combination, then the segments defined for the cranial and caudal (anterior and posterior) lobes of the right lung should be present in the left lung.

Each segment can be divided into lobules. Here there is much scope for confusion and debate. In mammals with a thick pleura and well-developed septa of connective tissue within the lung, secondary lobules can easily be defined. Each represents a volume of lung tissue supplied by a small bronchus. The boundaries of these lobules are obvious at the surface of the human lung, but secondary lobules cannot easily be defined in the rat.

What can be agreed is that after the generations of bronchioles the terminal bronchiole occurs. A terminus can always be defined! This is the last of the purely conducting airways, and is not involved in gas exchange. It supplies a volume of lung tissue defined as an acinus. An acinus therefore comprises all the lung tissue distal to a terminal bronchiole. Miller (1947), in his great work on the anatomy of the human lung, defined a further 'lobule': the primary lobule. Miller's definition was characteristically precise:

"The ensemble of ductulus alveolaris, atria, sacculi alveolares and alveoli, together with their accompanying blood vessels, lymph vessels, and nerves, form a primary lobule."

Note that Miller did not include the respiratory bronchiole within the primary lobule. Tyler and Julian (1992) misquote Miller on this point. It will be obvious that, because the rat has essentially no respiratory bronchioles, Miller's primary lobule in the rat is supplied by a terminal bronchiole. This means that the acinus, as defined in human anatomy, is remarkably similar to the primary lobule (*sensu* Miller) of the rat. Miller's use of the term 'atria' is of some interest. He defined the atrium as a space that occurred at the end of an alveolar duct, and from which opened a group of alveolar saccules. Other workers have not found the atrium to be a particularly useful concept.

The reader may be wondering whether all these anatomical arcana are of much importance. The answer is that they are when choosing an animal model to study the possible effects of inhaled material on the human lung. The respiratory bronchiole has been identified as a site of particular sensitivity to inhaled toxicants. As the rat lacks respiratory bronchioles, it is likely to be a poor model for such effects, and the dog with several generations of respiratory bronchioles will likely provide a better model.

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The Histological Structure of the Airways

Enthusiastic lung histologists speak of 40 or more cell types within the lung, although some cells such as, chondrocytes and smooth muscle cells, are not of course unique to the lung. The epithelial lining of the airways is of the greatest interest to toxicologists, as it is here that the effects of inhaled toxicants are first seen.

Preparation of Lung Tissue for Histological Study

Because the lung collapses on removal from the thoracic cavity, histological sections cut from tissue that has not been expanded fail to reveal the normal structure of the lung. Lung tissue should always be fixed in an expanded state. Expansion with fixative fluid is the normal method, and is recommended unless there is a suspicion of pulmonary oedema, as the oedema fluid is diluted by fixative and becomes difficult to detect. Lungs can also be inflated with ethanol vapour, formalin vapour or a combination of formalin and steam (Wilson et al., 2001; Weibel and Vidone, 1961; Silverton, 1965). Vascular perfusion of the inflated lung is perhaps the ideal method, but is too complicated for use in routine toxicological pathology. However the lung is fixed, it should be vacuum-embedded in paraffin wax: careful control of the vacuum is needed to ensure that rupture of delicate interalveolar septa does not occur. Paraffin sections can be cut at 5 μm for study of cells, but thicker sections (15−20 μm) are useful for study of the three dimensional structure of the air spaces. Plastic sections cut at 1 μm and stained with toluidine blue are excellent for the study of cellular detail, and form a bridge between the light microscopic (LM) and electron microscopic (EM) levels of examination. EM sections can, of course, be prepared, and the scanning electron microscope has been used to examine the structure of the airway surface. Two frequently found background pathological changes should be mentioned: lymphocyte cuffing of small airways is often seen in section of lung from rats with viral infections. Goblet cells, see below, are rare in the airways of SPF (Specific Pathogen Free) rats but occur more frequently in others.

The Trachea and Main Bronchi

The rat trachea is about 2.5 cm in length (26.8 mm according the model produced by Yeh) (see above) and about 3.5 mm in diameter. Hofstetter et al. (2006) give a smaller value for the diameter: 1.6−1.7 mm. The rat trachea is relatively long and narrow having a length/diameter ratio of about 9, compared to one of about 6 in man. In the ferret the ratio is 20: a notable long and narrow trachea.

The wall of the trachea is thin and delicate, supported by 18−24 C shaped hoops of hyaline cartilage. The dorsal aspects of the hoops are incomplete, the gap being closed, so to speak, by smooth muscle (muscle of Reisseisen) that are attached to the perichondrium at the tips of the cartilages. The relationship between the muscle and the cartilage varies from species to species: see Towers (1953) for a discussion of this point. The rat trachea is readily extensible: Gatto and Houck (1989) reported that an increase in length of 50% could occur without significant narrowing of the lumen. As noted earlier, the cartilage of the trachea extends into the main or primary bronchi but not beyond that. At the bifurcation of the trachea, the edge between the lumina of the bronchi forms the carina (keel) which, in the rat, is long and shaped like a flap.

The terminology of airway histology is complicated: Krstic's excellent encyclopaedia should be consulted for details (Krstic, 1984). The following tables set out the official terminology and other widely used terms (Tables 12.3 and 12.4).

TABLE 12.3 Trachea and Primary (Main) Bronchi

Official Name	Unofficial Name	Notes
Tunica mucosa, divided into Epithelium Lamina propria	Mucous membrane	See below
Tela submucosa	Submucosa	Loose connective tissue containing many elastic fibres
Tunica fibromusculocartilaginea Divided into Paries cartilaginous Paries membranaceus	Cartilage and smooth muscle layers	Cartilage disposed as C shaped, incomplete hoops in the trachea. The paries membranaceus comprises the smooth muscle of the trachea
Tunica adventitia	Adventitia	Connective tissue

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TABLE 12.4 Secondary Bronchi: All Bronchi Between the Primary Bronchi and the First Bronchioles

Official Name	Unofficial Name	Notes
Tunica mucosa, divided into: Epithelium Lamina propria	Mucous membrane	See below
Tunica muscularis	Muscularis mucosa (used by some but not all histologists)	Spiral layer of smooth muscle, with connective tissue interspersed between the fascicles of muscle
Tela submucosa	Submucosa	
Tunica conjunctiva-cartilaginea	Cartilage plates	
Tunica adventitia	Adventitia	Connective tissue

There are only three layers in the bronchioles: Tunica mucosa (epithelium and lamina propria), Tunica muscularis (smooth muscle) and Tunica adventitia. Unlike the gut where there is a multilayered muscularis, there is no sign of an inner circular, outer longitudinal arrangement in any of the smooth muscle of the airways.

Epithelium

The epithelium begins as a pseudostratified, ciliated, columnar epithelium and decreases in height to become a simple ciliated columnar epithelium in the bronchioles. Once again terminology is debatable. Whether the epithelium of the bronchioles is simple or pseudostratified is a nice point of lung histology. If the presence of basal cells that do not reach the epithelial surface is taken as the criterion for the pseudostratified state, then the epithelium is pseudostratified throughout. Jeffery and Reid (1975) divided the cells of the epithelium into epithelial (endodermal origin) and mesenchymal, the latter comprising the migratory cells: globular leucocytes and lymphocytes. These authors classified the epithelial cells, excluding the mesenchymal cells as shown in the Table 12.5 (Fig. 12.2).

Cells of the Airways

The entire epithelium of the rat airway (trachea to bronchiole) has been studied at the LM, transmission electron microscopic (TEM) and scanning electron microscopic (SEM) levels: Jeffery and Reid (1975), Souma (1987), these accounts should be consulted for details. Souma's account includes some superb scanning electron micrographs. The account by Mercer et al. (1994) is also of exceptional value.

Ciliated Cells

The ciliated cells are tall, with a pale cytoplasm and a smooth nuclear outline. Cilia and microvilli are present on the apical surface. The cilia are about 5 μm in length, 0.5 μm in diameter and there are around 200–300 on each cell. They have the typical '9 + 2' arrangement of microtubules and possess small terminal projections. These claws engage with the overlying layer of airway mucus and were reported for the first time by Jeffery and Reid (1975).

Serous Cells

The serous cells are similarly tall cells, with a cytoplasm rich in rough endoplasmic reticulum located around 600 nm diameter, electron dense granules located mainly at the apices. The presence of these cells in SPF rats in large numbers was also first reported by Jeffery and Reid (1975).

Clara Cells (Bronchiolar Exocrine Cells)

Clara cells are similar in appearance to serous cells, with a granular appearance that is related to the plentiful smooth endoplasmic reticulum present at the apices of cells, which project above other epithelial cells. This is particularly evident in mouse airways, where it produces a saw-tooth appearance. See Plopper (1983) for an interesting discussion of marked species variations in Clara cell structure. The presence of large amounts of cytochrome p450 gives these cells an important role in the detoxification of inhaled toxicants (Fig. 12.3).

Many Clara cells have apical club-shaped cytoplasmic processes have been interpreted as indicating a secretory function. Initially the secretion was thought likely to be mucus, but more recently it has been suggested that the Clara cell the source of pulmonary surfactant.

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TABLE 12.5 The Epithelial Cells of the Airways

Epithelial Cells					Cell Types
	Superficial				
		Ciliated			Ciliated cells
		Nonciliated			
			Secretory granules present		
				Dense granules	
					Serous cells (rough ER present)
					Clara cells (agranular ER present)
				Lucent granules often with dense centres	
					Goblet cells
			No secretory granules		
					Intermediate cells
					Brush cells
	Basal				
					Kulchitsky cells (with secretory granules)
					Basal cells

ER, endoplasmic reticulum.

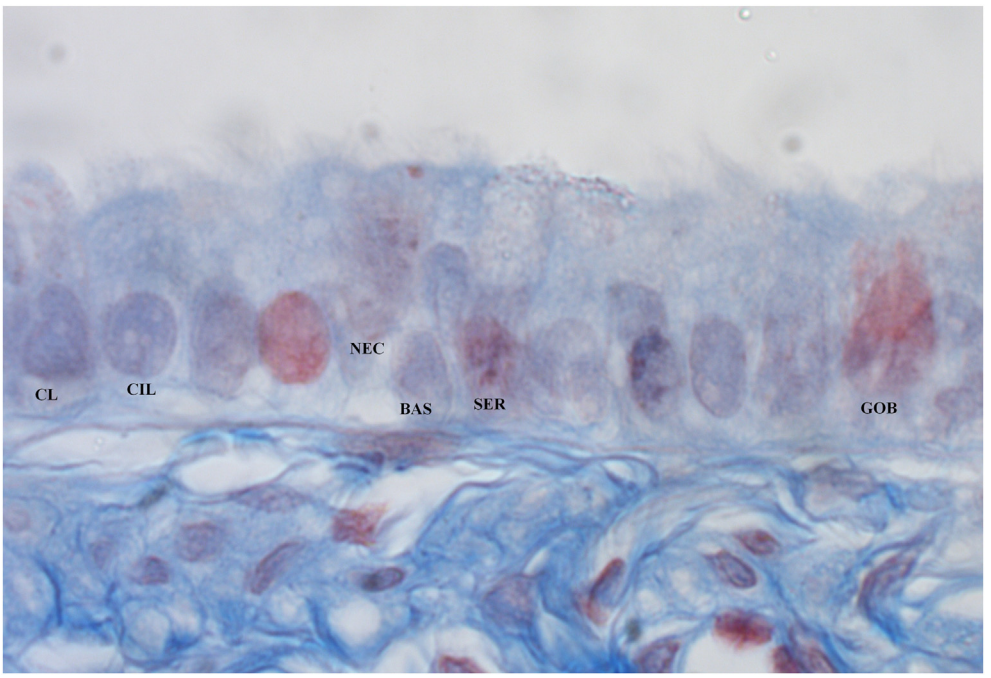


FIGURE 12.2 Cells of the airways (*CIL*, ciliated cell; *SER*, serous cell; *CL*, Clara cell; *BAS*: basal cell; *GOB*: goblet cell; and *NEC*, neuro-endocrine cluster).

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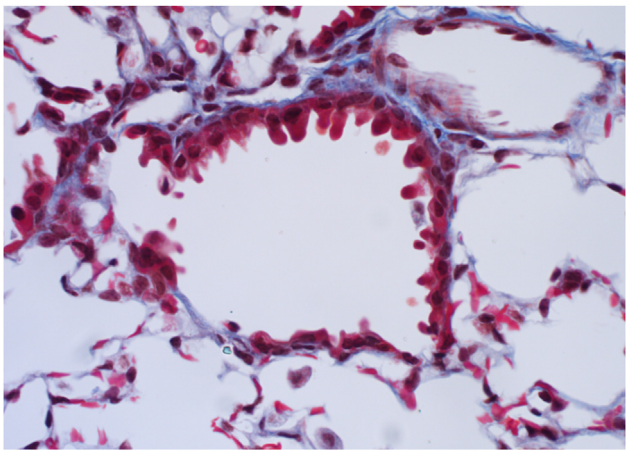


FIGURE 12.3 Clara cells in the mouse (masson's trichrome stain).

Goblet Cells

Goblet cells are rare in the airways of SPF rats. They are modified, simple, columnar and epithelial cells and very similar wherever they are found. In an H and E section, the mucins in the granules stain palely. PAS or Alcian blue staining reveals the mucus very clearly.

Neuro-Endocrine Cells

NECs exhibit a diverse array of functions within the lungs, which include airway oxygen sensing, regulation of pulmonary blood flow, control of bronchial tone and modulating immune responses.

Single NEC's are found throughout the respiratory epithelium, but also gather together in clusters called neuro-epithelial bodies, which are found at specific locations, often at airway bifurcations or bronchioalveolar duct junctions bodies. They are a rich source of a range of potent amines and peptides. These clusters are innervated at their bases and have apical microvilli.

Basal Cells

Basal cells derive their name from their proximity to the underlying basal lamina. They are a common feature of pseudostratified epithelium such as that seen in conducting airways of the lung. In man they comprise between 6%−30% of the epithelial cells, depending on location along the airway. There is sound evidence indicating that airway basal cells are a population of multipotent stem cells that drive both homoeostasis of the normal epithelium, and its orderly regeneration after injury. Basal cells probably also contribute to disease susceptibility, initiation and progression. It would seem plausible that any disposition towards preferential expression of a particular lineage could easily result in the mucous cell hyperplasia, metaplasia or squamous metaplasia seen in many respiratory disorders. As a stem cell population, alterations in their genome through mutations or epigenetic modifications induced by environmental agents might affect the long-term susceptibility of individuals to respiratory disease. Equally this makes them a prime target for gene cell therapies, and as such may well be of great interest to the toxicologist in the future.

Brush Cells

Brush cells are a rare cell type. These are columnar in shape with apical vesicles, and although they are seen throughout the airway, they are most numerous in the region of the terminal bronchiole and form part of the alveolar lining. They have squat microvilli around 0.5−1μm in length and 150−180 nm in width; around 120−140 are found on each cell. The microvilli extend into the alveolar space either perpendicularly or parallel to the alveolar wall and contain filaments that stretch into the cytoplasm forming a long root-like structure.

At present, the precise function of pulmonary brush cells is unknown. The increased surface area suggests an absorptive function for these cells, but the small number of brush cells in the lung would suggest that fluid transport is more likely to be a function of ciliated cells or type II pneumocytes. A chemoreceptor function seems plausible, but a detoxification role, immune surveillance, or as a sensor for alveolar fluid or alveolar air tension have all been suggested as functions. It has been reported that they increase after bleomycin-induced interstitial pneumonitis, which suggests there may also be a role in cell regeneration. See Reid et al. (2005) for a full discussion of these cells.

Intermediate Cells

The intermediate cells are nonciliated cells, with filiform projections from the apical surface that are evidence of incipient ciliogenesis. Little has been written but they are possibly an intermediate form between basal cells and ciliated cells.

Quantification of Epithelial Cells Along the Airway

Jeffery and Reid (1975) counted the different cells types: Table 12.6 is constructed from their data.

The Submucosal Glands of the Trachea

These are seromucous glands with typical mixed acini (serous and mucous cells present) and myoepithelial cells wrapped around the circumference of each acinus. The acini are connected to the airway surface by short ducts in which the epithelium is initially simple cuboidal, but which becomes ciliated near the orifice of the duct. These glands, their development and duct structure, have been

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TABLE 12.6 Percentages of Cells Types at Locations in the Airways of the SPF Rat

	Ciliated	Nonciliated			Basal Cells	Migratory Cells: Globular, Leucocytes and Lymphocytes
		Granules Present		Granules Absent	K Cells and Basal Cells	
		Dense Granules: Serous and Clara Cells	Lucent Granules: Goblet Cells	Intermediate Cells and Brush Cells	K Cells Rare	
				Brush Cells Rare		
Upper trachea	17	20	<1	27	36	14
Lower trachea	33	27	<1	13	27	8
Extrapulmonary bronchi	35	21	<1	16	27	4
Intrapulmonary bronchi	53	20	<1	12	14	4
Bronchioles	65	20	<1	15	<1	1

Notes:
1. The epithelium of the bronchioles is essentially a simple epithelium with few basal cells: thus the percentage of ciliated cells is high in these small airways.
2. The percentage of serous cells is remarkably stable throughout the airways.
3. Migratory cells are most frequently found in the large airways.

studied in detail (May and Tucker, 2015). The SPF rat, like all rats, has few submucosal airway glands and few goblet cells, raising the question of the origin of airway mucus in these rats. Sims and Horne (1997) have contributed an interesting discussion of tracheal mucus in the rat and point out that special techniques are required for its histological study (Fig. 12.4).

Features of the Airway Wall Excluding the Epithelium

Cartilage is only found in the walls upper airways: none is found beyond the main (primary) bronchi. It is replaced by smooth muscle, which forms a complete layer of spiralling fascicles of muscle interspersed with connective tissue.

The Vexed Question of What Is a Bronchus and What Is a Bronchiole in the Rat

While it is clearly sensible to call the larger airways, those supplying lobes and segments, bronchi, and the terminal bronchiole can also be clearly defined, the point at which one should make the distinction between bronchi and bronchioles seems to be arbitrary. Perhaps use of the terms large and small conducting airways is advisable.

Bronchus Associated Lymphatic Tissue (BALT) and Migratory Cells of the Airway Epithelium

Collections of lymphocytes are found beneath the airway epithelium of the adult rat, mainly at bifurcations of the airways. Murray and Driscoll (1992) have provided an instructive and short review of the aggregates that form part of the Mucosa Associated Lymphatic Tissue (MALT) system. The epithelium over the aggregates of lymphocytes comprises flattened, nonciliated cells and M cells (M for membranous) that transport antigen to the underlying lymphocytes. The epithelium itself is infiltrated by many lymphocytes. Lymphocytic follicles may appear in the aggregates, particularly in infection, or when the animal has been exposed to toxicologically active particulates, for example silica. Plesch (1982) reported that about 50 BALT aggregates were found in the adult rat lung.

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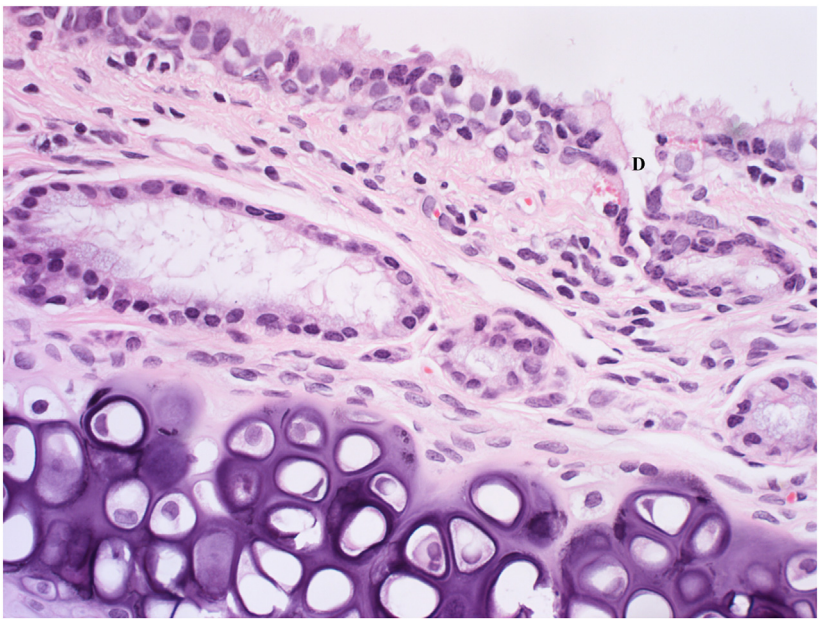


FIGURE 12.4 Tracheal submucosal glands. Duct (D) opening into lumen shows the transition from cuboidal to ciliated epithelium.

Acting in concert with the M cells of BALT a network of antigen presenting dendritic cells act as immunological guardians of the lung. These dendritic cells are the same cell type as those first described in the skin by Langerhans in 1868 (see Vermaelen and Pauwels, 2005 for a review of this cell type) and are characterised by 'dendritic' processes that extend between the other cells of the airway epithelium. Dendritic cells cannot be identified in ordinary H & E sections, but can be identified by immunohistochemical techniques. At the EM level dendritic cells can be recognised by the presence of characteristic Birbeck granules (shaped like tennis rackets) in their cytoplasm.

THE GAS EXCHANGE ZONE OF THE RAT

The alveoli are the essential structures for gas exchange. In the rat an alveolus is of about 150 μm diameter, about half the diameter of the human alveolus. A great deal of effort has been put into developing relationships between alveolar dimensions and the metabolic requirements of various mammalian species. For some years it was thought that alveolar surface area scaled with metabolic rate. Although this made intuitive sense (Tenney and Remmers, 1963) it turned out to be untrue. Diffusion capacity and alveolar surface area scale with body weight (Gehr et al., 1981), and it is now known that the key parameters of lung dimensions and function scale as follows: Lung volume = Body Mass$^{1.06}$, Capillary Volume = Body Mass$^{1.00}$, Alveolar Surface Area = Body Mass$^{0.95}$ and Diffusion Capacity = Body Mass$^{0.99}$ (Gehr et al., 1981). Log−log plots of these parameters and Body Mass produce straight lines, the slope being the index term in the relationships given previously. Weibel has contributed an interesting discussion of the evolutionary basis of these relationships (Weibel, 1987).

The Alveolar Wall

The alveolar wall is thin. Septa between adjacent alveoli consist of a sandwich of two layers of alveolar epithelium providing the bread, and a single layer of capillaries with supporting connective tissue providing the filling. The barrier to diffusion is yet thinner: the alveolar epithelium is separated from the capillary endothelium only by the fused basement membranes of the epithelium and the endothelium. In the rat, this barrier is about 1.5 μm in thickness, but in some areas as thin as 0.1 μm. This remarkably attenuated barrier is provided by the cytoplasm of the Type I alveolar cells and the endothelial cells. The existence of an epithelial lining of the alveoli was doubted for many years, and a controversy, fuelled by the lack of resolving power of the light microscope, developed. Low, using the recently developed electron microscope, settled the issue (Low, 1952, 1953). All textbooks of histology now include elegant transmission and scanning electron micrographs of the alveolar epithelium.

The Septal Interstitium

The capillaries of the alveolar septum wind back and forth from one side of the septum to the other. One can think

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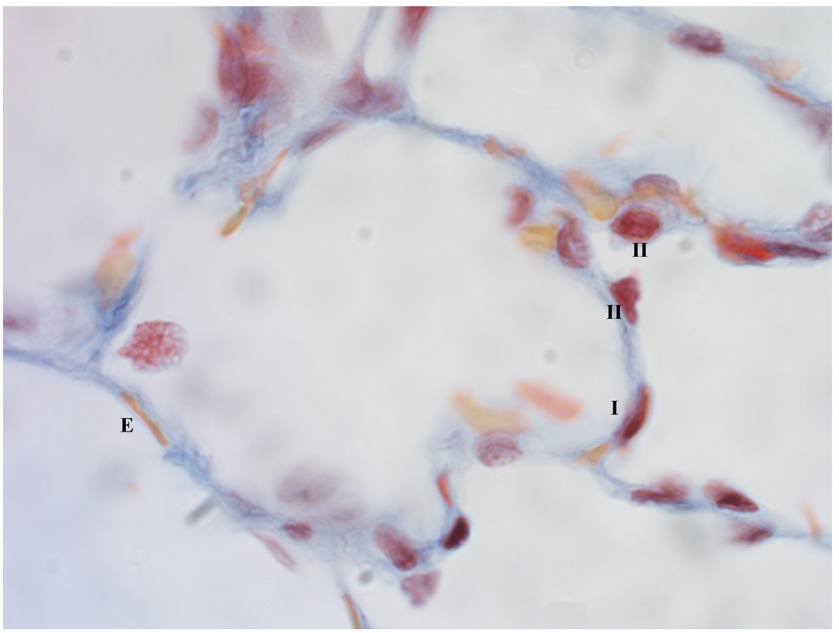


FIGURE 12.5 Type I and II alveolar cells (MSB Stain). Note how yellow stained erythrocytes (E) have flattened themselves to squeeze through the tiny capillaries.

of a pulmonary capillary as having two surfaces. One fused by basement membrane to the Type I alveolar cells, and the other that is exposed to the connective tissue of the interstitium. The interstitial connective tissue contains the fibrous and cellular components of loose connective tissue – collagen, elastic and reticulin fibres, fibroblasts, macrophages, plasma cells and leucocytes. Mast cells in this region are rare in the rat, but more common in the guinea pig.

The capillary wall is no more than the endothelium with occasional pericytes surrounding it. Endothelial cells are connected by tight junctions, resulting in a relatively tight endothelium with no fenestrations. The endothelial cells contain many micropinocytotic vesicles, which can also be seen occasionally in alveolar Type I epithelial cells. The presence of these vesicles suggests the movement of fluid, and perhaps other substances, across the air–blood barrier. Even though the capillary endothelium is relatively tight, it does leak, and fluid that has entered the septa moves to junction points between groups of alveoli, and from there to the terminal lymphatic capillaries at the level of the alveolar duct. As far as is known no lymphatic capillaries occur in the alveolar septa.

Interconnections Between Air Spaces

It has long been known that in some species collateral ventilation from part of the lung to another can occur. Three routes are described in various species – the pores of Kohn between alveoli, the canals of Lambert between bronchioles and alveoli, and the interbronchiolar pathways of Martin (Menkes et al., 1979). Pores of Kohn occur in the interalveolar septa of the rat, varying in size from a few microns to 10–15 μm in diameter. The larger pores are surrounded by reticular and elastic fibres (Fig. 12.5).

Cells of the Alveolar Region

Type I Alveolar Cells

Type I alveolar cells cover the great majority of the alveolar surface. With their central nucleus and a large expanse of remarkably thin cytoplasm, they look a little like fried eggs. The cytoplasm of Type I cells contains few mitochondria or other organelles, and those that are present tend to be grouped close to the nucleus.

Type I alveolar cells are not capable of division and are slowly replaced as Type II cells differentiate into Type I cells (Shami and Evans, 1992). If Type I cells are damaged by inhaled toxicants, a brisk expansion of the Type II cell population occurs and the alveoli become lined with a cuboidal epithelium that subsequently differentiates into the normal epithelium of Type I cells.

Rather interestingly, one alveolar Type I cell can extend into more than one alveolus. This caused great confusion in the early days of lung histology when what appeared to be anucleate (nonnucleated) plates of cytoplasm were observed (Weibel, 1971).

Type II Alveolar Cells

These cells, also sometimes called the Great Alveolar Cells, are cuboidal in appearance and are found, often in groups of two or three, in the corners of the alveoli. They

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can be recognised by light microscopy and stained for alkaline phosphatase. At the EM level they are seen to contain characteristic lamellar bodies that stain darkly, indeed they appear black with conventional EM techniques. These bodies contain a complex of lipoproteins that form tubular myelin that spreads over the alveolar surface when released. This dispersion of material forms the surfactant layer that lines the interior of the alveolus. Surfactant contains dipalmitoyl phosphatidylcholine as its key lipoprotein, and a range of surfactant proteins (SP, A−D). The surfactant layer has the property of lowering the surface tension of the liquid lining the alveoli (Pattle, 1955, 1966). In addition, it has the fascinating property of varying the surface tension with the area of the surface. This is the key to alveolar stability. The pressure within what is essentially a bubble (if we think of the alveolus as a bubble connected to the airway) is given according to the Law of LaPlace by the formula $P = 2T/R$ where, T is the surface tension and R the radius. A moment's thought, as the algebra textbooks used to say, suggests that if T is constant then small alveoli will have a higher internal pressure than large alveoli. Given that the alveoli are interconnected and of various sizes, it follows that the small alveoli will empty into the larger. Indeed, this can be demonstrated by connecting two soap bubbles of differing sizes: the larger expands, the smaller contracts. Such instability, which would be fatal for the lung (and individual), is avoided by the surface tension varying with the surface area of the film. As surface area increases, so does the surface tension. Thus, our expression 2T/R remains constant, and the pressure in alveoli of differing sizes is equalised. Surfactant reduces the surface tension of the lining film to extraordinarily low levels, reducing the 'pull' on fluid in the interstitium and preventing alveolar flooding, keeping the alveoli relatively dry. Surfactant is continuously produced, requiring some mechanism for its removal. It is thought that both alveolar macrophages and Type II alveolar cells play a role in this.

Type III Alveolar Cells

These are the brush cells that we have already mentioned as rare cells of the airways.

Alveolar Macrophages

The alveolar surface is patrolled by macrophages that migrate to the alveolar surface from the septal interstitium. These large cells remove bacteria and particulate matter from the alveolar space (Fig. 12.6). Having completed their house-keeping duties, alveolar macrophages leave the alveoli for the alveolar ducts and join the mucus escalator at the terminal bronchiole. Oberdörster et al. (1992) have argued that migration of alveolar macrophages from the alveolar surface to the septal interstitium also occurs; especially when ultrafine particulate matter is deposited in the lung and is moved into the interstitium.

Some mammals have significant resident population of macrophages within the pulmonary circulation. Large farm animals (cows, sheep, goats, horses) have many such cells, but the rat appears to have only a few (Staub,

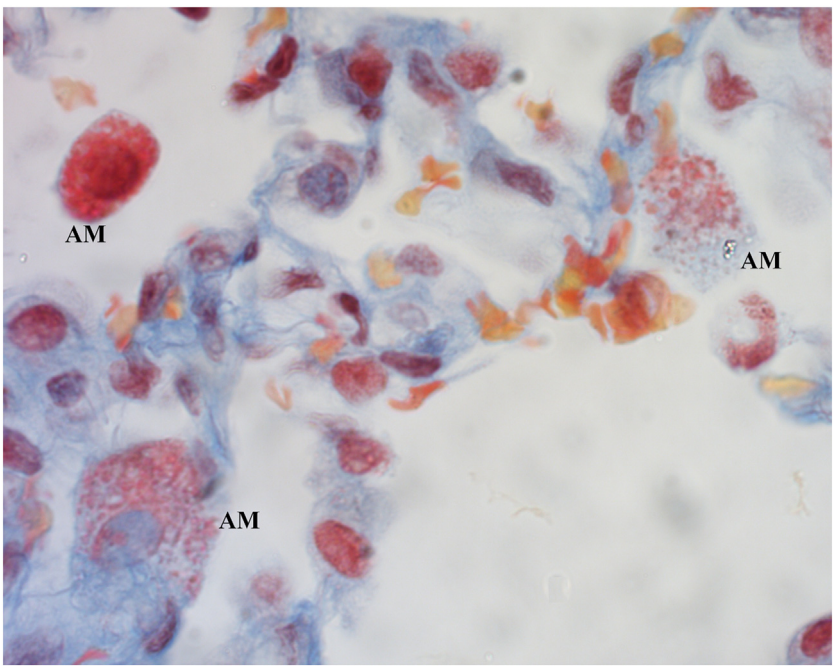


FIGURE 12.6 Alveolar macrophages (AM) stained with MSB. MSB is typically a stain for fibrin, but stains mucins bright red. These activated macrophages appear to have been cleaning up some excessive mucin production.

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TABLE 12.7 Numbers in Brackets: SE of Mean

	Fischer 344 Rat (SE)	Sprague-Dawley Rat (SE)
Body weight (kg)	0.29 (0.01)	0.36 (0.01)
Total cells/lung $\times 10^9$	0.67 (0.02)	0.89 (0.04)
Total cells (%)		
Type I alveolar cells	8.1 (0.3)	8.9 (0.9)
Type II alveolar cells	12.1 (0.7)	14.2 (0.7)
Endothelial cells	51.1 (1.7)	42.2 (1.1)
Interstitial cells	24.4 (0.7)	27.7 (1.78)
Macrophages	4.3 (1.0)	3.0 (0.3)
Alveolar surface area covered (%)		
Type I alveolar cells	96.4 (0.5)	96.2 (0.5)
Type II alveolar cells	3.6 (0.3)	3.8 (0.5)
Average cell surface area (μm^2)		
Type I alveolar cells	7287 (755)	5320 (694)
Type II alveolar cells	185 (56)	123 (20)
Endothelial cells	1.121 (95)	1105 (72)

Source: Data from Crapo, J.D., Young, S.L., Fram, E.K., Pinkerton, K.E., Barry, B.E., Crapo, R.O., 1983. Morphometric characteristics of cells in the alveolar region of mammalian lungs. Am. Rev. Respir. Dis. 128, S42–S46.

1994). It is generally believed that the rat alveolar macrophages migrate to the lung as monocytes and differentiate to macrophages in the septal interstitium. It seems unlikely that macrophages divide having reached the alveolar surface. Any division that may occur takes place in the interstitium.

Table 12.7 shows the distribution of cells in the rat alveolus.

THE CIRCULATORY SYSTEM OF THE LUNG

Pulmonary Artery

The pulmonary artery leaves the right ventricle, divides and sends one branch to each lung. In the foetus the pulmonary circulation is a high pressure system, and in man the foetal pulmonary artery is not very different in structure from the foetal aorta. Soon after birth, the circulatory changes that occur result in the pulmonary circulation becoming a low pressure circuit, and the pulmonary artery changes in its histological appearance.

In man and rat the pulmonary artery and its major branches are defined as elastic arteries. In man these soon give way to typical muscular arteries, but in the rat most of the branches of the pulmonary artery, down to very small arteries, remain thin walled with a good deal of elastic tissue in their walls. Chemoreceptor tissue, the pulmonary glomus (see Chapter 8: Histology of the Vascular System) has been described in the adventitia of the pulmonary artery near its origin.

The branching pattern of the pulmonary arteries has been studied in less detail than that of the airways, see Horsfield (1976) for discussion and references to the literature.

Pulmonary Capillaries

These have been discussed earlier.

Pulmonary Veins

The pulmonary capillaries of the gas exchange zone drain to pulmonary venules, and thence to pulmonary veins. In man and other mammals with well-developed interlobular septa, the pulmonary veins run in the septa and do not join the branches of the pulmonary artery and bronchi for some time. In the rat, the pulmonary veins run in association with the smaller bronchi and branches of the pulmonary artery. The larger pulmonary veins of the rat contain mainly cardiac muscle in their walls, smaller pulmonary veins contain a mixture of smooth and cardiac muscle. Sorokin (1988) has pointed out that the intercalated discs of this form of cardiac muscle are a little different from those of the cardiac muscle of the heart, having less

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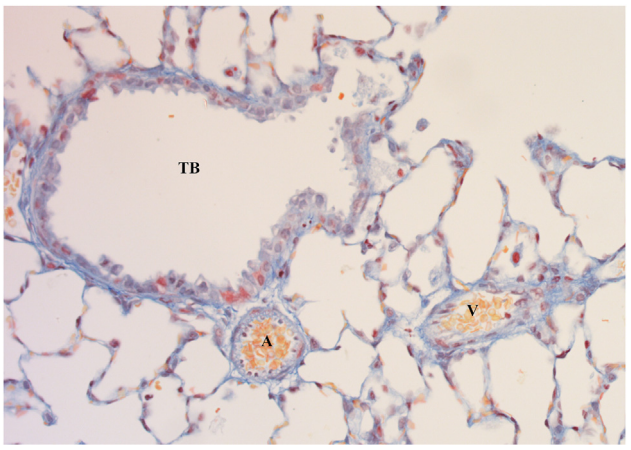


FIGURE 12.7 Terminal bronchiole with associated artery and vein. Note absence of any red staining muscle fibres, only blue stained collagen.

interdigitation. The smallest pulmonary veins have thin walls and have an endothelium that is supported by no more than a thin mesh of collagen fibres (Fig. 12.7).

Lymphatic Vessels

The lung has two systems of lymphatic vessels. One lies in the visceral pleura, draining the superficial alveoli and running to the hilum of the lung, and the other runs with the airways and pulmonary blood vessels. The hilar lymphatic vessels of both lungs drain largely to the right lymphatic duct.

Bronchial Arteries and Veins

These have been discussed above, but it is worth adding that the capillaries of the bronchial circulation, unlike those of the pulmonary circulation, may be fenestrated, especially where they supply glands of the airways. This appears to be the case in man (Sorokin, 1988), whether it is the case in the rat is unknown.

NERVE SUPPLY OF THE LUNG

The lung is supplied by the sympathetic and parasympathetic systems. The efferent component of the former is a postganglionic supply based on the thoracic sympathetic ganglia 2–5; the latter is via the vagus (Xth CN). The vagus nerves and sympathetic fibres contribute to a dorsal and ventral pulmonary plexus near the root of the lung. The right and left vagus nerves contribute to the nerve supply of both lungs. Chapter 21 (3), The Autonomic Nervous System has a good deal to say about the vagus nerve. Here we shall merely note that the parasympathetic efferent supply to the lung is from the Dorsal Motor Nucleus (General Visceral Efferent, GVE), and that the afferent input to the brain stem runs to the tractus solitarius and onward to the nucleus solitarius (General Visceral Afferent, GVA). Sorokin (1988) stated that the cell bodies of the afferent supply lie in both the superior and inferior vagal ganglia (presumably in man). If true this is unusual, in that the small superior ganglion is generally thought to be associated with the minimal General Somatic Afferent (GSA) activity of the nerve, and the much larger inferior ganglion with GVA activity. Afferent fibres also run in the sympathetic nerves: the cells bodies lie in the dorsal root ganglia of the spinal cord.

Unmyelinated postganglionic sympathetic fibres and myelinated preganglionic parasympathetic fibres enter the lung at the hilum in association with the bronchi. In the rat these nerve fibres follow the bronchial tree forming parasympathetic ganglia in the adventitia, most frequently near bifurcations of the airways. From here nerve fibres run into the smooth muscle of the airway walls, and to the submucosa. The smooth muscle of the rat airway wall and glands of the submucosa are supplied by cholinergic (parasympathetic) and adrenergic (sympathetic) fibres. The latter accompany the bronchial arteries and supply these and the pulmonary vasculature. The muscle of pulmonary veins (cardiac and smooth muscle) is also supplied by adrenergic fibres in the rat; in other mammals the supply to the cardiac muscle component is said to be cholinergic.

In addition to the above, a Non Adrenergic Non Cholinergic (NANC) efferent nerve system is found in the airways. The transmitter molecules of this system include peptides and purines. It is not possible to provide an account of this system here: Burnstock's review (2012) should be consulted for further information and references.

The afferent supply to the lung that innervates the airway receptors respond to irritants [toxicologists will be familiar with the Alarie response to inhaled irritants (Alarie, 1966)] by stretching of the airway walls. Widdicombe (2006) has provided an admirable survey of these receptors. Note that the term receptors is used in a physiological sense, as it has proved difficult or impossible to link function with structure. It is accepted that fine nerve fibres run between the cells of the lining epithelium of the airways: these sometimes lie in grooves in the epithelial cells. The neuro-endocrine cells of the airways (occurring singly and also grouped as neuro-epithelial bodies) receive a nerve supply. Such goblet cells as occur in the rat airway mucosa are likely to respond to local irritation rather than to cholinergic stimulation. The nerve supply to the gas exchange zone is sparse and its function obscure. Juxtapulmonary capillary receptors (the J

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receptors described by Paintal, 1969) respond to stretch produced, perhaps, by expansion of the interalveolar septa by fluid. These receptors have proved difficult to define in histological terms.

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Chapter 13

Alimentary Canal or Gastrointestinal Tract

This chapter describes the mouth, pharynx, oesophagus, stomach and intestine. The salivary glands and exocrine pancreas are described in Chapter 15, Exocrine Glands and the liver in Chapter 14, Liver. As a general reference the review by Kararli (1995) (dealing with stomach and intestine) contains a wealth of data on the anatomy, physiology and biochemistry of the gut.

THE MOUTH AND PHARYNX

The long, narrow oral cavity is bounded, anteriorly and antero-laterally, by the lips. The hard palate and soft palate make up the roof of the mouth and the tongue its floor, while posteriorly the oral cavity opens into the pharynx.

The Lips

The lips of the rat are, in comparison with those of man, immobile: rats do not smile. The upper and lower lips meet at the angles of the mouth, from whence the cheeks run back towards the bulge produced by the large masseter muscles.

The outer surfaces of the lips are covered with hairy skin; the inner surfaces are smooth and covered by a keratinised squamous epithelium. Human histologists divide stratified squamous epithelia into two types: SSEMMV or stratified squamous epithelium mucous membrane (unkeratinised) variety, and SSESV or stratified squamous epithelium skin (keratinised) variety. In man the mouth and oesophagus are lined with SSEMMV, but in the rat the surface layers of the epithelium of the mouth and oesophagus are keratinised. A thick keratinised layer is characteristic of the epithelia of the hard palate and dorsum of the tongue; elsewhere in the mouth the keratinised layer is less well developed.

Unlike the upper lip, the lower lip is not divided in the midline, and covers the lower incisor teeth almost completely. In the midline, the internal surface of the lower lip forms the fraenulum (frenum is Latin for bridle), a fold that joins the lip with the tissue of the floor of the mouth. The fraenulum is joined by two fleshy ridges running along the inner surfaces of the lower lips. These ridges occlude the diastema between the incisors and the molar teeth when the mouth is closed, and when the rat is gnawing. A fold covered with hairy skin, the inflexum pellitum, turns inwards at the angle of the mouth.

There is no fraenulum of the upper lip, which is divided medially exposing the upper incisors. The edge of the upper lip immediately behind the incisor teeth is turned inward, forming a lobe with a hairy lower surface that touches the dorsal surface of the tongue (Hunt, 1924). The upper surface of this lobe is hairless and lies against the hard palate (Fig. 13.1).

The Hard Palate

The hard palate separates the oral cavity from the nasal cavity and is characterised by a series of transverse ridges. The most anterior of these ridges lies posterior to the incisive papilla, a small fleshy projection found in the midline. The papilla provides an opening into the incisive canals that link the mouth with the nasal cavity. The papilla is covered with keratinised epithelium and strengthened by a small plate of cartilage.

A terminal branch of the maxillary nerve (CN V3), the nasopalatine nerve, runs from the nasal cavity, through the incisive canal and supplies the tissues of the anterior part of the hard palate. Small arteries run in the opposite direction. The first and second palatal ridges lie towards the posterior end of the diastema, and the remaining five ridges are arranged in a W formation and lie at the level of the molar teeth: see Fig. 13.1. These ridges are important landmarks for determining the planes of standard transverse sections through the head (Kittel et al., 2004). The epithelium of the ridges is well keratinised and hair follicles containing unerupted hairs can be seen in histological sections of the ridges (Jayaraj, 1972; Hebel and Stromberg, 1976). Between the epithelium and the bone of the hard palate (premaxilla, maxilla and palatine bones) there is a layer of dense connective tissue containing many mucous salivary glands. As in the nasal cavity, the tissue lying deep to the epithelium may be regarded as modified periosteum. There are unnamed strands of striated muscle running laterally from the midline of the hard palate to the upper lip and mystacial pad (Pavlikova et al., 2004).

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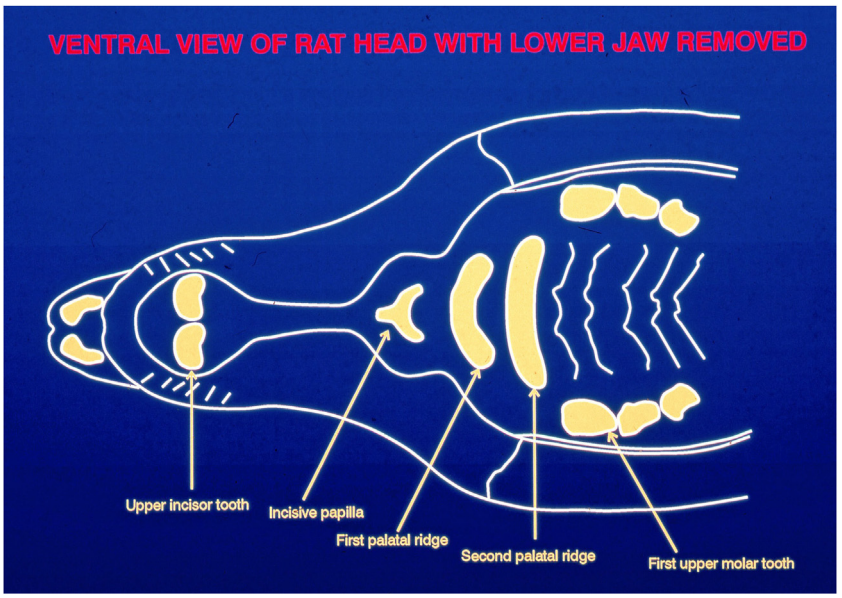


FIGURE 13.1 An enlarged macrophotograph of the hard palate.

The Soft Palate

The soft palate is the posterior continuation of the connective tissue and epithelial layers of the hard palate; its upper surface forms the floor of the nasopharynx. There is no uvula in the rat. Muscles are inserted into the soft palate of man and can both tense and raise it. These muscles are the tensor veli palatini and the levator veli palatini (veli from velum, Latin for curtain or covering: the palatine velum = the soft palate); whether the levator actually exists in the rat seems doubtful. The former is supplied by the mandibular division of the trigeminal nerve (CN V3). Both muscles arise from the sphenoid bone. The palatopharyngeus muscle takes origin from the soft palate and runs to the wall of the pharynx; the reader may be relieved to know that palatoglossus is poorly developed in the rat. Cleaton-Jones (1971) found no evidence of any striated muscle in the soft palate of the albino rat, with the exception of some belonging to the nasopharyngeal sphincter. Plentiful mucous salivary glands surrounded by elastic fibres are found in the ventral submucosa of the soft palate. The keratinised epithelium on the inferior surface of the soft palate can be divided into three subtypes: see Cleaton-Jones (1971) for further details.

The Tongue

The thick, narrow and pointed tongue of the rat is attached posteriorly to the hyoid bone, and posterolaterally to the floor of the mouth. The tip is free and not tethered to the floor of the mouth by a fraenulum. The anterior part of the tongue has a deep median groove on its upper surface; the dorsal prominence divides the middle from the posterior third of the tongue. The tongue is a bag of skeletal muscle (the intrinsic muscles), which alter the shape of the tongue, running in all directions to create an interwoven mass of muscle that is easy to recognise in histological sections. The extrinsic muscles of the tongue, which move it forwards, backwards, and from side to side; run from the hyoid bone (hyoglossus), from the jugular process of the temporal bone (styloglossus) and from the internal surface of the front of the right and left mandibles (genioglossus).

The tongue is covered by a keratinised stratified squamous epithelium, which is thick on the upper surface, but smoother and thinner over the inferior surface. The epithelium of the upper and lateral sides of the tongue carry a large number of prominent papillae that have a covering of epithelium and a core of connective tissue. On the inferior surface, the papillae are much less well developed or absent. There are four types of papillae.

Filiform Papillae

Filiform papillae are conical with a thread of keratin, sometime bifurcated, extending from their tips (filum, Latin for thread). This type of papilla is very well developed in the rat and cat, but less so in man. Filiform papillae allow soft substances to the licked into the mouth; you could not eat an ice cream without them! Carnivores have well-developed filiform papillae which function as a rasp. See Fig. 13.2, right hand panel.

Fungiform Papillae

The name is derived from fungus, Latin for a mushroom, and these papillae look like mushrooms in histological

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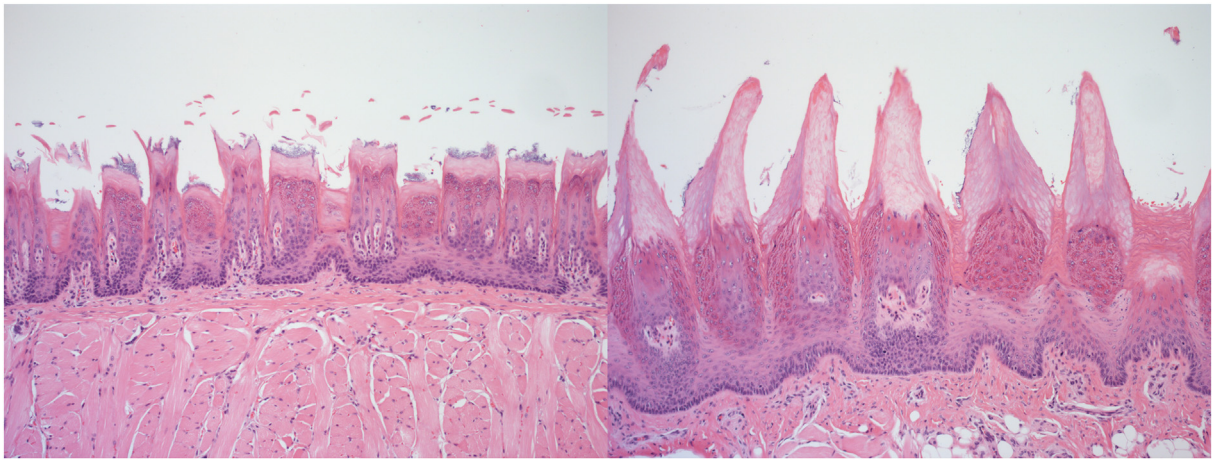


FIGURE 13.2 Tongue TS fungiform papillae to the left and filiform papillae to the right.

sections. Fungiform papillae are large enough to be seen with the naked eye. In man they are a brighter red than the remainder of the surface of the tongue, but in the rat they are pale. Most are found on the anterior part of the dorsum of the tongue with around half located near the tip and none in the midline. Two rows of fungiform papillae run from anterior to posterior, parallel to the midline, on each side of the dorsum of the tongue (Miller and Preslar, 1975). Taste buds, see below, are found among the epithelial cells of fungiform papillae. The epithelium over this type of papilla is not thickened as it is over the filiform type. See Fig. 13.2, left hand panel.

Foliate Papillae

Foliate papillae (foliate, Latin for lead shaped) are less numerous than the types mentioned above and are found at the dorsolateral corners of the tongue, where they define the edges of a series of grooves (Picut and Coleman, 2016). Many taste buds are found on these papillae.

Circumvallate Papilla

The rat has one circumvallate papilla at the posterior end of the dorsum of the tongue, whereas in man there are 10 to 12 arranged in a forward pointing V formation with a solitary representative between the arms of the V. The name is derived from the Latin word vallum, a rampart that defines the ditch that runs round the circumference of the papilla. The ducts of small salivary glands open into this ditch: see Chapter 15, Exocrine Glands. Taste buds are found on the sides of the papilla.

Taste Buds

Taste buds are small barrel shaped structures that lie within the epithelium and are made up of a group of elongated epithelial cells that lie at 90 degrees to a basement membrane. A small pore is present at the apex of the structure. Classical descriptions of the histology of taste buds define two types of cell. The type I cells stain darkly and have been regarded as supporting cells. Type II cells, which stain palely, are regarded as neuroepithelial cells. Bundles of long microvilli arise from the apices of the Type II cells and stick together to form the 'hairs' described by earlier microscopists.

It was always thought that receptors that respond to substances dissolved in saliva were present on the microvilli, but recently Type III cells have been described that may be the key receptor cells (Fawcett, 1994). These have dense-cored vesicles in their basal cytoplasm and a long apical cytoplasmic process.

It seems likely that the binding of materials to taste receptors causes depolarisation of the cell membrane and release of transmitters that interact with nerve endings at synaptic junctions (Herness and Gilbertson, 1999; Chaudhari and Roper, 2010) The latter authors consider that Type I cells should be regarded as glial-like cells and have identified umami as the fifth sensation of taste (the original four were: sweet, sour, salt and bitter; 'umami' is the Japanese word for 'deliciousness'), which is triggered by L-amino acids and nucleotides. Herness and Gilbertson (1998) do not describe Type III cells as a feature of rat taste buds though they pointed out that they had been described in mice and men, and that in the rabbit they are the major receptor cell.

Taste buds are not restricted to the lingual papillae: they occur in the epithelium of the palate, in the larger ducts of the major salivary glands and in the pharynx and larynx (Travers and Nicklas, 1990).

Innervation of the Tongue

The anterior two thirds of the tongue develops from the first branchial or pharyngeal arch. General sensation is

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served by the lingual nerve, a branch of the mandibular nerve (CN V3), with fibres running in the chorda tympani joining the lingual nerve to supply the taste buds and salivary glands. Afferent fibres, subserving touch and pain and temperature run in the lingual nerve to the trigeminal ganglion.

The posterior third of the tongue develops from the third arch, which grows over tissue from the second. Innervation with regards to taste and general sensation and parasympathetic supply to local salivary glands is provided by the glossopharyngeal nerve (CN IX). The vagus nerve plays a minor part in supplying the most posterior parts of the tongue.

The intrinsic and extrinsic muscles of the tongue are supplied by the hypoglossal nerve (CN XII).

Although not fully relevant to the rat, Mu and Saunders (2010) have provided a recent and comprehensive account of the motor innervation of the human tongue.

Teeth

The rat has 16 teeth: 4 incisors and 12 molars. The dental formula is:

I 1/1, C0/0, P0/0, M3/3 (incisors, canines, premolars and molars)

There is no deciduous dentition. The incisors (Fig. 13.3) erupt about 10 days after birth, with molars following on days 19, 23 and 35 in sequence, from anterior to posterior. Incisors continue to grow throughout life, continuously abrading each other during gnawing to maintain a chiselled edge. Incisors grow at a rate of 2.1 (upper) and 2.8 (lower) mm per week, and any damage to an incisor results in overgrowth of the opposing tooth. The yellow–orange colouration of the incisors that appears from about the 5th week after birth is due to the presence of iron containing pigment (Wen and Paine, 2013; Kuijpers et al., 1996). The enamel organ of the incisor teeth persists throughout life, but the molar enamel organ degenerates soon after contact with the opposing teeth is established. Formation of primary dentine of the molar teeth stops at about 125 days after birth, but production of cementum continues throughout life (Lefkowitz et al., 1953; Rowett, 1965). Each molar tooth has several roots (Fig. 13.4).

Upper molars, M1: 5 roots, M2: 4 roots, M3: 3 roots

Lower molars, M1: 4 roots, M2: 3 roots, M3: 3 roots. (Prasanth and Saraswathi, 2012)

In man, the upper molars have three roots, the lower molars, two. Comparisons between rat and human molars have been provided by Dammaschke (2010). The rat is subject to a range of dental disorders including proliferative lesions of the periodontal tissues (Kuijpers et al., 1996).

Nerve Supply of the Mouth

The inner surface of the oral cavity is innervated by branches of the trigeminal nerve (CN V). The palate and the teeth of the upper jaw are innervated by the major palatine nerve, which descends through the palatine foramen from the floor of the pterygo-palatine fossa. Branches of the infraorbital nerve supply the upper jaw and teeth. The

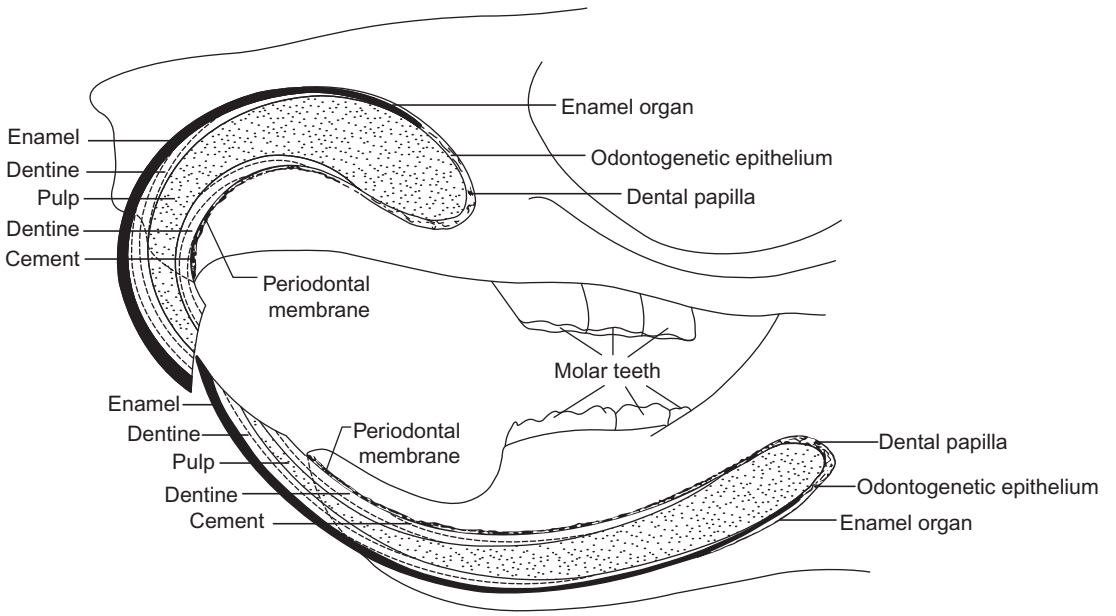


FIGURE 13.3 Parts of jaws with V.S. incisor teeth.

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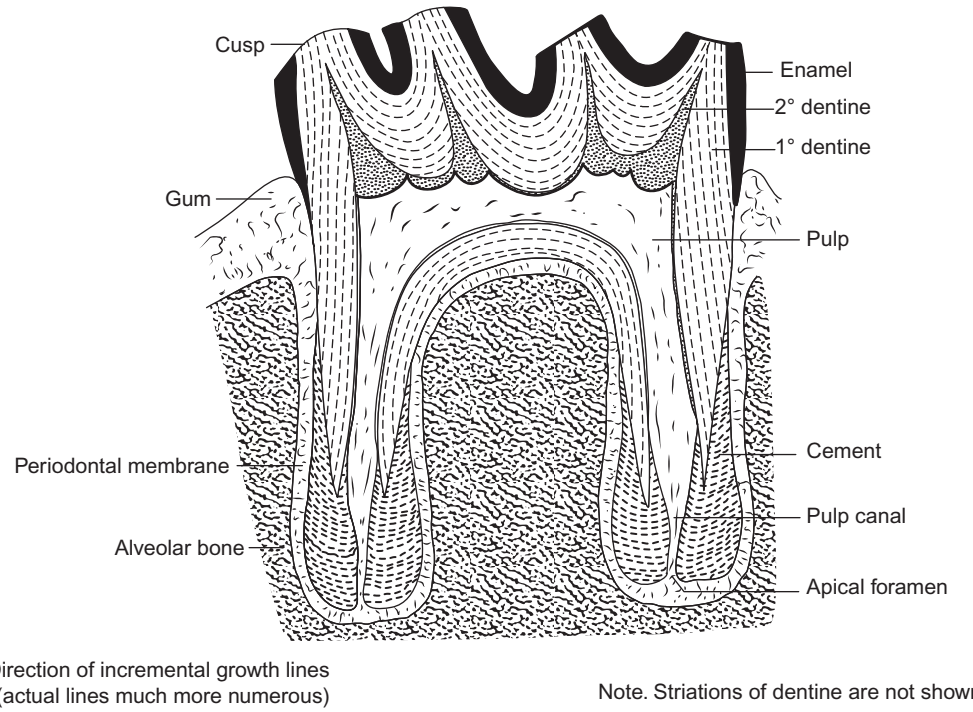


FIGURE 13.4 V.S. first molar of rat.

lower jaw and teeth are innervated by the inferior alveolar nerve, a branch of the mandibular nerve (CN V3) which runs inside the mandible, entering through the mandibular foramen and exiting at the mental foramen as the mental nerve (see Chapter 5: The Skull). These nerves are described in more detail in Chapter 21(1) Cranial Nerves.

PHARYNX

The pharynx is a space which opens anteriorly into the mouth and nasal duct, and posteriorly into the oesophagus and larynx. The passage above the soft palate is the nasopharynx; the space into which the cavity of the mouth opens is the oropharynx. The nasopharynx is bounded above by the basisphenoid laterally, by the palatine bone in front, and the medial (internal) pterygoid processes of the sphenoid bone behind. The floor is provided by the soft palate.

The pharynx is a wide, short tube with muscular dorsal and lateral walls. The muscles of these walls are inserted latero-ventrally into the hyoid bone and the cartilages of the larynx, and in the rat can only be separated with great difficulty (Hebel and Stromberg, 1976).

The muscles can be divided into constrictors that are attached to the base of the skull (to the midline, ventral crest of the basioccipital bone) and which sweep round the sides of the pharynx; and the muscles that draw the pharynx forwards, and which run to the wall of the pharynx from the palate (palatopharyngeus), the cartilage of the Eustachian tube (salpinopharyngeus) and the pterygoid process of the sphenoid bone (pterygopharyngeus). Stylopharyngeus (so named though there is no styloid process) runs from the jugular process of the temporal bone to the side wall of the pharynx. Motor innervation is provided by the glossopharyngeal and vagus nerves (CN IX and X). Sensory innervation of the anterior pharynx is by the palatine nerves; the posterior pharynx is innervated by the glossopharyngeal nerve. The ascending pharyngeal and inferior thyroid arteries supply blood to the pharynx.

The pharynx is lined with keratinised squamous epithelium like that of the mouth. The Eustachian tube opens into the lateral wall of the pharynx about 0.5 cm behind the edge of the soft palate (Hunt, 1924). The rat has no tonsils on the lateral walls of the pharynx, indeed the pillars of the fauces (folds contributed in man by palatoglossus and palatopharyngeus and between which the human tonsil lies) are poorly developed.

OESOPHAGUS

The oesophagus runs from the pharynx to the stomach and can be divided into cervical, thoracic and abdominal parts. The oesophagus runs through the neck just below the ventral surfaces of the bodies of the vertebrae, veers a little to the left as it enters the thorax, and so lies above and to the left of the trachea as it passes through the anterior thoracic inlet. It runs in the mediastinum, dorsal to the arch of the

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aorta and the bifurcation of the trachea, to the diaphragm (see Chapter 6: The Musculature of the Rat) before entering the stomach. The oesophagus is between 70 and 80 mm in length the adult rat, about 15 mm of which lies in the abdominal cavity (Hebel and Stromberg, 1976). Within the abdominal cavity the oesophagus is wrapped by a lobe of the liver: see Chapter 14, Liver.

The structure of the oesophagus (Fig. 13.5) is similar to that of the rest of the alimentary canal. Its wall comprises the four layers of mucosa, submucosa, muscular layer or muscularis and the adventitia; in the abdomen the outer surface of the oesophagus is covered with visceral peritoneum.

The mucosa comprises an epithelium, a lamina propria and muscularis mucosae. The epithelium is a keratinised stratified squamous epithelium, with dark staining prismatic basal cells resting on a well-defined basement membrane. There are several layers of spinous cells (tonofibrils are visible in their cytoplasm) and a thin layer of cells containing granules of developing keratin (granular layer) between these and the cornified cells of the surface layers (Meyer et al., 2014).

The lamina propria is a layer of dense connective tissue of interwoven with collagen and elastic fibres. Endophytic ingrowths of epithelial cells expand into the lamina propria, but never penetrate through this layer (Pozharisski, 1990a,b). These should not be confused with preneoplastic lesions. The muscularis mucosae is not well developed in the rat: some authors describe an inner circular and an outer longitudinal layer but others describe only patches of smooth muscle tissue and no continuous lamina (Pozharisski, 1990a,b; Hunt, 1924).

The submucosa is a layer of loose connective tissue: aglandular in the rat but not in man. In most parts of the gut a collection of autonomic fibres and ganglion cells known as Meisner's plexus is found in the submucosa, but this is not obvious in the rat oesophagus.

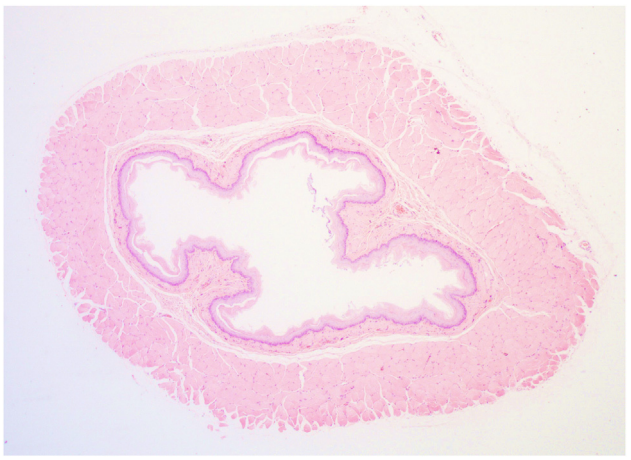


FIGURE 13.5 Oesophagus in transverse section, note the multiple layers of muscle.

The muscularis comprises an inner circular and outer longitudinal layer of mostly striated muscle, becoming smooth in the lower oesophagus. This muscle tissue is derived from the last few branchial arches and is supplied by the glossopharyngeal and vagus nerves (CN IX and X). In the lower oesophagus Auerbach's plexus of nerve fibres and ganglion cells can be found between the layers of muscle.

The adventitia is made up of loose connective tissue containing blood and lymphatic vessels and nerve fibres. Arterial blood reaches the oesophagus via the oesophageal branches of the inferior thyroid and internal mammary arteries and venous blood drains to the bronchial veins and thence to the anterior vena cava (Greene, 1935).

THE STOMACH

The stomach lies in the left, anterior part of the abdominal cavity, its anterior surface is overlapped by the liver and the left costal margin. To the left the stomach is in contact with the spleen. Visceral peritoneum covers the anterior and posterior surfaces of the stomach except for two narrow strips along the greater and lesser curvatures where an enveloping double layer of peritoneum separates to cover the anterior and posterior surfaces of the organ. The rat stomach is shaped like half a doughnut, with the greater curvature representing the outer edge of the half doughnut, and the lesser curvature the edge of the hole in the doughnut (Hunt, 1924). Innervation is from the vagus nerve (CN X): see Chapter 21(1) Cranial Nerves. The blood supply of the stomach is described in Chapter 7, The Cardiovascular System and its lymphatic drainage in Chapter 9, The Lymphatic System.

The oesophagus enters the fore-stomach at the midpoint of the lesser curvature. The fore-stomach is lined with keratinised stratified squamous epithelium, and is separated from the glandular stomach by a well-defined ridge that runs round the inner surface of the organ. To the right of the ridge is a strip of glandular epithelium characterised by the cardiac glands: see Fig. 13.6.

The terminology of the parts of the stomach and its glands is inconsistent. The terms fore-stomach, limiting ridge (between the fore-stomach and the glandular stomach), corpus (that part containing glands that produce enzymes and acid), the antrum (the part proximal to the pylorus that contains mucous glands that do not produce acid and enzymes) and the pylorus (characterised by the pyloric sphincter) are widely used but some authors refer to the glandular part of the stomach as being divided into the fundus and the pylorus (Robert, 1971; Takahashi and Hasegawa, 1990). The use of the term 'fundus' is confusing; in man the fundus is glandular but the anatomically equivalent part of the rat stomach is not.

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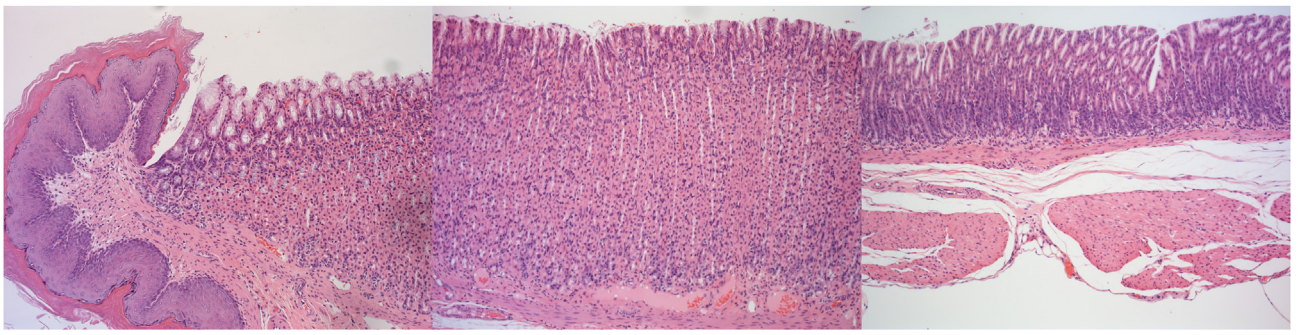


FIGURE 13.6 Stomach – From left to right the marginal ridge, corpus and antrum.

The Fore-Stomach

The thin walled fore-stomach is a storage sac for food that has been ingested and is awaiting digestion but it is not the anatomical equivalent to the rumen of ungulates. The inner surface is ridged and keratinised and has been referred to as the cutaneous zone of the stomach. The lamina propria is poorly developed, the submucosa comprises rather loose connective tissue, and the muscularis is well developed (Hebel and Stromberg, 1976). Typically the keratinised stratified squamous epithelium has a stratum germinativum, stratum spinosum, stratum granulosum and stratum corneum, although cornification is slight (Takahashi and Haegawa, 1990). Three layers of smooth muscle are found in the muscularis of the fore-stomach (Picut and Coleman, 2016).

Glandular Stomach

The epithelium of the glandular part of the stomach covers the surface and lines the tubular, often branching, and sometimes coiled, glands that produce hydrochloric acid and digestive enzymes.

The surface epithelium is described as foveolar, as it contains foveolae or small holes that represent the mouths of the glands. This is a simple columnar epithelium extending over the whole surface of the glandular stomach. Darkly staining nuclei are located towards the bases of the cells that rest on a basement membrane. Cells contain droplets of mucus can be seen, but goblet cells are absent.

The mucus neck cells found in the proximal or neck regions of the glands also produce mucus, but stain only with Alcian blue; by comparison the mucous cells of the surface epithelium stain with both Periodic acid Schiff (PAS) and Alcian Blue. Histologists describe gastric pits lined with surface epithelium leading to the glands that open from the distal ends of the pits.

The glands of the cardiac zone are straight, or branch only once, are seldom coiled, and produce only mucus. The cells of the glands are similar to those of the surface epithelium. The glands of the antrum (pyloric glands) are similar to the cardiac glands and produce only mucus.

The glands of what Robert (1971) defined as the corpus of the stomach [the fundic glands of Takahashi and Hasegawa (1990)] are more complicated: they are markedly branched and coiled. Four types of epithelial cell are found in the epithelium of the glands. These are listed below.

Chief Cells

The glands of the corpus contain more of these cells that of any other type. Chief cells produce enzymes and intrinsic factor essential for vitamin B12 absorption. They are pyramidal cells with basal nuclei and basal cytoplasm that stains blue–purple with H&E because of the high content of ribosomes and rough endoplasmic reticulum. The zymogen (enzyme containing) granules of these cells are better preserved by a chromate-formalin mix than neutral formalin fixation (Lillie and Fullmer, 1976). The authors also recommended staining with 0.05%–0.1% thionin buffered to pH 4–5 with 0.01 M acetate buffer, which results in the zymogen granules staining deep blue or green–blue, distinguishing them from the cytoplasm of the basal parts of the cells. The apical cytoplasm may appear vacuolated if zymogen granules have been lost during fixation.

The Oxyntic or Parietal Cells

These hydrochloric acid producing cells stain bright pink in H&E sections and are scattered throughout the epithelium of the glands. Less numerous than chief cells, they contain intracellular canaliculi (visible only at electron microscopy (EM)) that are lined by microvilli. Parietal cells often appear surrounded by chief cells pressed tightly against the basement membrane: apparently out of contact with the lumen of the gland. In fact these cells remain in contact with the lumen via narrow channels between the chief cells. EM examination shows that the

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parietal cells are packed with mitochondria, giving a granular appearance to the cytoplasm. In man the parietal cells are more frequently found in the neck regions than in the depths of the glands.

Enterochromaffin Cells

Historically, these have been known as argentaffin because of their capacity to reduce ammoniacal silver solutions, and argyrophil cells, which also reduce silver solutions, but only in the presence of a reducing agent such as hydroquinone. Argentaffin cells are not seen in the rat stomach but argyrophil cells are. For details of staining techniques for these interesting cells see Lillie and Fullmer (1976).

Today, immunocytochemical techniques can precisely identify the products of these cells which include serotonin (5HT), histamine, gastrin, bombesin, endorphin and somatostatin among others. These six products have all been identified in human gastric enterochromaffin cells, and even more have been identified in the enterochromaffin cells of the intestine (Fawcett, 1994). For detailed histology of the glands of the stomach the paper by Yoschida, published more than 100 years ago, in 1915, and available on the Internet, provides a superb description of the light microscopy of the glands of the rat and rabbit. More recent descriptions of rat stomach histology and the changes in the fundic mucosa of the albino rat that occur with aging (including apoptotic changes in secretory cells, fibrosis and loss of mucus from mucous neck cells) are available (Ghosal and Bal, 1989; El-Shall, 2010).

Lamina Propria and Muscularis Mucosae

The lamina propria is largely occupied by the glands of the stomach. Strands of smooth muscle from the muscularis mucosae extend between the glands.

Muscularis

The stomach has a well-developed muscular layer; in fact there are two layers, an inner circular layer and an outer longitudinal layer. To these an innermost oblique layer is added in the human stomach. At the pylorus circular layer is much thickened forming the pyloric sphincter.

THE SMALL INTESTINE

The small intestine runs from the pyloric sphincter to the caecum and can be divided into the duodenum, jejunum and ileum. The jejunum and ileum cannot be as easily identified in the rat as they can in man so sampling is usually based the measurements, shown in Table 13.1 (Hebel and Stromberg, 1976).

TABLE 13.1 The Small Intestine of the Rat

	Length (mm)	Diameter (mm)
Duodenum	95–100	2.5–3
Jejunum	900–1350	4–5
Ileum	25–30	3–5

Duodenum

Duodenum is a Latin corruption of the Greek dodekadaktulus meaning 12 finger breadths, and was first coined by Herophilus in 300BC to describe the length of the duodenum of animals then being dissected, presumably large domestic animals. The duodenum of the rat runs to the right when it leaves the stomach. It then runs dorsally and forms a wide loop with descending, transverse and ascending sections before continuing as the jejunum. In man much of the duodenum is plastered to the posterior abdominal wall, but in the rat this is less obvious. The loop of the duodenum is filled with mesentery containing the numerous ducts of the pancreas and the bile duct (Rowett, 1952; Hunt, 1924). The duodenum is covered in part by the mesothelium of the visceral peritoneum.

The mucosa (Fig. 13.7) is arranged as a series of shallow folds about 1 mm in height and 5 mm in width that run transversely to the long axis of the gut (Hebel and Stromberg, 1976). Some of these folds have a zigzag shape, while others have lobe-shaped ends. The rat has no folds of Kerckring (plicae circulares): folds that involve the submucosa and which are found in man. The mucosa projects into the lumen of the duodenum as leaf shaped villi, which greatly increase the surface are of the gut. Villi are found throughout the small intestine; indeed they are diagnostic of the small intestine. Between the villi the mucosa is continuous with that of the crypts of Lieberkühn, that is with the mucosal glands. The first part of the duodenum is characterised by the presence of Brunner's glands in the submucosa (Krause and Leeson, 1967). These branching, coiled, tubular glands are the only submucosal glands of the rat intestine, and are easily recognisable in the submucosa of the first part of the duodenum. The secretory tubules are lined by a columnar epithelium and secrete an alkaline fluid. Lymphatic follicles (Peyer's Patches) appear in the submucosa from around the midpoint of the small intestine, in contrast to man where they are restricted to the ileum (see Chapter 9: The Lymphatic System).

Jejunum and Ileum

It has been stated that the jejunum and ileum are not distinguishable in the rat, and that the small intestine, with

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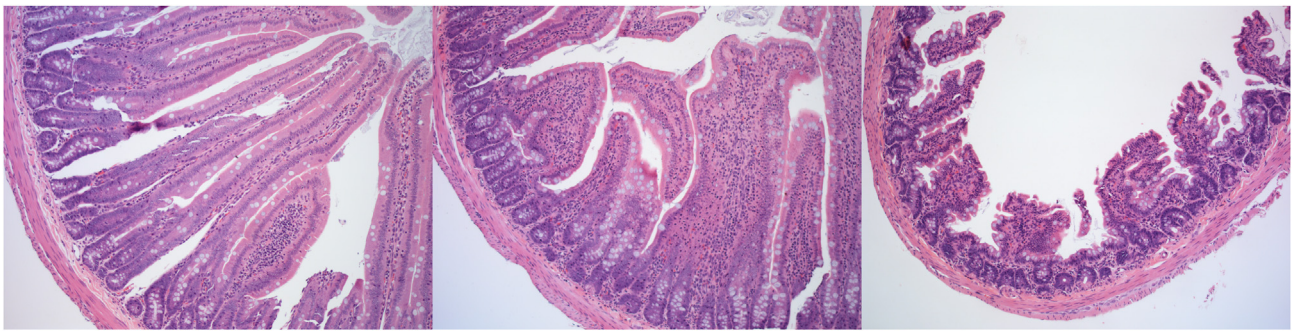


FIGURE 13.7 The small intestine. From left to right duodenum, jejenum and ileum.

the exception of the duodenum should be described as ileum (Rowett, 1965). Other authors have distinguished the jejunum (named for its empty appearance) from the ileum, noting that the ileum may appear darker than the jejunum because of faecal contents (Hunt, 1924). The small intestine is longer than its mesenteric attachment to the dorsal abdominal wall and falls into a series of garland-like loops (Hebel and Stromberg, 1976). Villi are present in the small intestine: about 0.6 mm in both height and width (Hebel and Stromberg, 1976). Crypts of Lieberkühn open between the villi: see Fig. 13.7. The submucosa contains the autonomic plexus of Meissner and a plexus of blood and lymphatic vessels. The muscularis comprises two layers (inner circular and outer longitudinal) separated by the myenteric plexus of Auerbach, which undergoes a dramatic reduction in the number of ganglion cells present in the first 14 days of life (Schäffer et al., 1999)

The adventitia is covered with the mesothelium of the visceral peritoneum; the blood and lymphatic vessels of the small intestine run in the mesentery as do the nerve fibres running to and from the gut.

The Epithelium of the Villi and the Crypts of Lieberkühn

The epithelium of the villi and crypts of Lieberkühn comprises mostly enterocytes and goblet cells, although there are also enteroendocrine (enterochromaffin) cells, Paneth cells and the occasional lymphocytes that wander through the epithelium prior to release into the lumen of the gut.

Enterocytes

Primarily functioning as absorptive cells, they also produce hydrolases that are associated with the brush border of these cells. Enterocytes are columnar cells with elongated nuclei located in the basal half of the cells. The apical surface of each cell is characterised by up to 3000 microvilli about 1 μm in length and 80 nm in diameter. Although individual microvilli cannot be seen at LM, collectively they constitute a brush border and amplify the surface area of the small intestine by a factor of about 30. Each microvillus contains a core of microfilaments attached to a terminal web of microfilaments located below the apical surface of the cells. The microvilli interact with the overlying glycocalyx, which contains glycoproteins and hydrolase enzymes. Junctional complexes hold the cells firmly together and provide a barrier that prevents intestinal contents infiltrating between the cells (Fawcett, 1994).

Goblet Cells

These are easy to recognise in histological sections with their basal nuclei and goblet-like appearance, particularly when stained for their mucus content. Their appearance is probably an artefact of fixation which leads to swelling of the apical part of the cells and distorts them from their true ovoid or columnar shape into the classic goblet shape (Fawcett, 1994).

Enteroendocrine Cells

These small cells that were identified by their affinity for chromium salts (hence enterochromaffin cells), and by their capacity to reduce ammoniacal silver solutions (hence argentaffin and argyrophil cells), are found among the enterocytes and goblet cells. They cannot be recognised in standard H&E sections. It is now known that there are a large number of subtypes of these cells: each producing specific hormones: see above. These cells are members of the APUD (Amine Precursor Uptake and Decarboxylation) series.

Paneth Cells

The Paneth cells found in the depths of the crypts of Lierberkühn are instantly recognisable by the brightly stained granules present in their cytoplasm. The granules stain bright pink in H&E sections, and even more clearly in sections stained with Orange G. What is remarkable is that the functions of these cells, described in the late

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19th century, were until recently completely unknown. It was known that they occurred in many mammals (not in the dog, cat, pig and raccoon; present in very large numbers in South American anteaters, and present in more modest numbers in man and laboratory rodents), showed no, or few, mitoses and had the appearance of secretory cells. It was suggested that they might secret lysozyme, or produce enzymes that digested the chitin of ant exoskeletons (the anteaters had great influence!) or that they might play some sort of defensive role. Much more is now known. Clevers and Bevins (2013), in a masterly review, described these cells as 'maestros of the small intestinal crypts'. Elphick and Mahida (2005) have also provided a valuable review. It seems that the functions of the Paneth cells include: production of antimicrobial peptides including defensins (the term cryptdins, from crypt defensins, has been introduced but is sometimes given, wrongly as criptidins) and creating the necessary environment for the stem cells of the epithelium (see below). Paneth cells appear to be susceptible to free radical induced oxidative stress, and respond by apoptosis. They also appear to express susceptibility genes for Crohn's disease (Clevers and Bevins, 2013).

Replacement of the Epithelium of the Small Intestine

The enterocyte cell cycle in laboratory rodents is only 10–17 hours and the entire epithelium is replaced every 2–3 days. Goblet cells hardly have time to refill after releasing their contents (Fawcett, 1994). Replacement is from the stem cells found in the depths of the crypts. The progeny of these cells differentiate into enterocytes and goblet cells as they migrate upwards. Effectively this means that the epithelium slides upwards as cells are lost from the tips of the villi. The process appears to be driven by the fibroblasts beneath the basement membrane, which seem to pull the surface along with them as they move, but more remains to be discovered about this process.

The Lamina Propria of the Small Intestine

The subepithelial tissue contains strands of smooth muscle extending from the muscularis mucosae into the villi, and a network of specialised interconnected fibroblasts that have the capacity to contract. Together these cells 'milk' the lacteals, the lymphatic vessels of the villi, so named for their absorption of fat from milk. The lacteals are linked with the submucosal lymphatic plexus. In addition, mast cells and plasma cells are present in the lamina propria. Arterioles run into the villi and also provide the capillary plexus deep to the epithelium. Venules draining the capillary plexus and the vessels of the villi run to larger vessels in the submucosa.

THE LARGE INTESTINE

Hebel and Stromberg (1976) gave the following dimensions for the rat large intestine (Table 13.2).

The colon and rectum are, of course, widened by the ovoid faeces contained therein.

The large intestine comprises the caecum, (Latin for blind) colon and rectum. The orderly arrangement of the large intestine of man as shown in textbooks of human anatomy: caecum and appendix low on the right, ascending colon rising on the right and lying against the posterior abdominal wall (retroperitoneal), the sagging transverse colon, the descending colon (again retroperitoneal) and the rectum low on the left side of abdomen, is not seen in the rat. Rowett (1952), for example, showed the caecum on the left towards the posterior end of the undisturbed abdominal cavity; Greene (1949) showed it well forward on the right. Hebel and Stromberg (1976) correctly pointed out that the caecum of the rat has a long mesentery and is variable in its position within the abdomen.

The thin-walled caecum of the rat is similar in size and shape to the stomach, except that the entry (ileum) and exit (ascending colon), are close together on its lesser curvature. The caecum has rounded end, the base, and a more pointed end. The more pointed end is sometimes called the caecal appendix, though the rat lacks the well-defined appendix found in man. The ascending colon runs forward from the caecum towards the liver, there is no well-defined transverse colon, and the rectum, a long narrow structure, is difficult to distinguish from the descending colon. Further description of the relationships between the colon and the duodenum and small intestine are probably unnecessary for the toxicologist, who will probably remove the gut and unravel it on a dissecting board.

Epithelium of Large Intestine

There are no villi in the large intestine of the adult rat. Picut and Coleman (2016), quoting Pacha (2000), reported that villi are found in the first part of the colon of very young rats.

Crypts and tubular glands lined by a columnar epithelium rich in mucous cells, extend from the surface to a

TABLE 13.2 The Large Intestine of the Rat

	Length (mm)	Diameter (mm)
Caecum	50–70	10
Colon	90–110	10–3 (narrowing distally)
Rectum	80	3– 10

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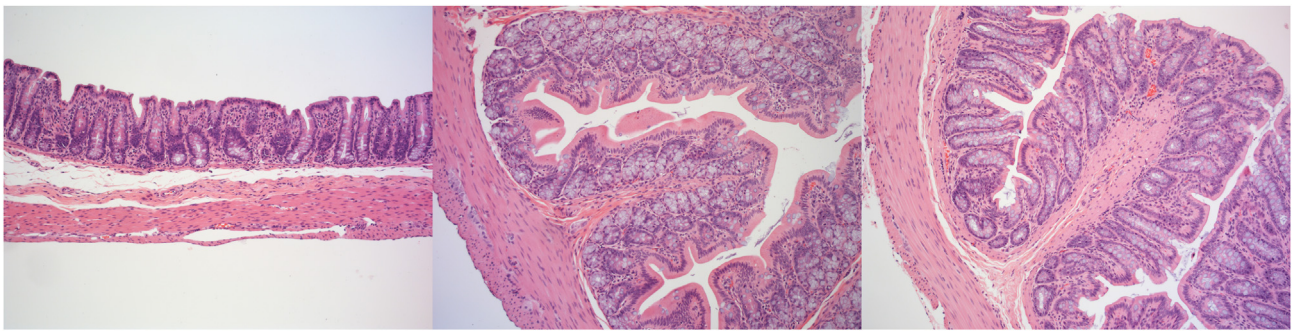


FIGURE 13.8 The large intestine. Caecum, colon and rectum.

well-defined muscularis mucosae: see Fig. 13.8. Mucus produced in the rat colon contains mainly sialomucins in the lower two thirds of the ascending colon, neutral and sulfomucins in the upper third, and sulfomucins in the descending colon (Pozharisski, 1990a,b).

The first part of the colon has mucosal folds that appear as parallel, oblique, striations which can be seen through the thin wall. The remainder of the colon has longitudinal folds.

The muscularis is complete: inner circular and outer longitudinal layers. In man the longitudinal layer occurs, mainly, as three longitudinally arranged bands, the taenia coli, which allow the large intestine to be easily recognised.

RECTUM AND ANAL CANAL

The rectum runs in the midline through the pelvis and leads to the anal canal. The anal canal is lined with skin characterised by apocrine sweat glands and well-developed sebaceous glands with extended acini, many of which open into single ducts (Hebel and Stromberg, 1976). The anal glands of the rat are less well developed than in some other mammals, for example the dog. In the dog, and in vestigial form in man, these glands are sometimes described as hepatoid, in that their histological structure looks a little like that of the liver. The anal canal is surrounded by the anal sphincter which includes both smooth and skeletal muscle.

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Chapter 14

Liver

The liver is of great interest to the toxicologist: it is invariably examined and removed at postmortem examination and then sampled for histological sectioning. Despite this, descriptions of the macroscopic anatomy of the rat liver vary widely, and the terminology or nomenclature applied to the various lobes and lobules of the rat liver is in a deplorable state. Authors use a variety of terms to identify different anatomical entities, and worse, a variety of terms to identify single entities. One reason for this muddle is the enthusiasm for forcing rat anatomy to conform to the descriptions used in human anatomy. In man the caudate lobe is a lobe 'with a tail', hence caudate, but in the rat the term is applied to a variety of structures, mostly without tails. Another reason for confusion is the lack of convention for describing the surfaces of the liver. Once the liver has been removed from the body and placed on a dissecting board, usually with the visceral surface uppermost, the terms anterior and posterior lose meaning. In the living animal, the liver is an arched three-dimensional structure that is curved rather like a helmet; when laid on dissecting board it becomes flat. Lastly, the macroscopic structure of the rat liver varies significantly from rat to rat. This is well illustrated in Komarek's account (2000), in which three variants are illustrated. In addition to these reasons, the mobility of the lobes of the liver after it has been removed from the body needs to be remembered. It is very easy to move lobes about, and they soon lose any close relationship to their position in the living animal. Of course, none of this would matter very much if all the parts of the liver responded in much the same way to a given toxicant. But it does matter a great deal if a study of lobar variations in response to toxicants is being undertaken, or if experimental surgery with specific lobes being either ligated or removed, is being performed.

DEVELOPMENT OF THE LIVER

The following short account is based on human embryology; it is appreciated that the development of the rat liver might differ in detail, but probably not as regards major features. The development of the liver in the mouse has been described in some detail by Kaufman and Bard (1999): it differs little from the development of the liver in man. In saying this we are thinking of macroscopic development; in terms of histological development, the differences are greater. Godlewski et al. (1997) have contributed a detailed comparative study of the development of the liver in rat and man, although some minor differences were noted, there is sufficient similarity between the two species to make the rat a good experimental model for human liver development. The account of Godlewski et al. (1997) provides information on the comparative timings of events in rat and man.

The Developing Liver and Its Blood Vessels

The liver develops from a diverticulum of the ventral surface of the fore-gut, just distal to the pylorus of the stomach. The diverticulum grows into the mesodermal tissue of the transverse septum (see Chapter 12: The Lung). As the diverticulum develops and branches. It comes into contact with a plexus of small veins formed between the left and right vitelline veins that drain blood from the yolk sac. As the connecting plexus between the vitelline veins develops the left vitelline vein disappears, leaving just the right vitelline vein, which in time drains the blood from the stomach and intestine and becomes the portal vein. Venous blood from the gut reaches the plexus of veins that have now become interwoven with the developing liver tissues, and leaves via what remains of the anterior end of the right vitelline vein, which becomes the part of the inferior cava that passes from the abdomen, through the central tendon of the diaphragm and into the thorax. There it continues to the sinus venosus of the developing heart. The anterior end of the right vitelline vein contributes only this small part of the inferior vena cava. The majority of the inferior vena cava, which runs down into the abdomen in company with the abdominal aorta, is formed by a complicated process of structural integration between the postcardinal, subcardinal and supra-cardinal veins of the abdomen. Readers who are interested in this process are referred to the excellent diagrams in Larsen's Text book of Human Embryology (1993, pp. 186–189).

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So much for the portal vein. The umbilical venous system also plays a critical part in the development of the liver. The paired umbilical veins, carrying oxygenated blood from the placenta, run to the venous arch feeding into the sinus venosus of the heart. The right umbilical vein disappears and the left shifts to feed into the plexus of veins within the liver. This hepatic plexus is actually a plexus of venous sinusoids with inputs from the portal vein and the left umbilical vein. Rearrangement of the sinusoids leads to the formation of a large channel running through the developing liver. This is the ductus venosus, which carries blood from the left umbilical vein to join that from the portal vein near its outflow from the developing liver. The flow of blood from the placenta stops with birth, and the left umbilical vein shrinks to a fibrous cord. The part of the ductus venosus within the liver also shrinks and forms the ligamentum venosum, but the pathway can still be traced from the umbilicus, via the round ligament (teres ligament: teres is Latin for round) in the free edge of the falciform ligament (see below), to the edge of the liver where the convex antero-ventral surface meets the concave visceral surface, and into the liver to join the ligamentum venosum that lies in a deep fissure in the adult liver. The teres ligament and the ligamentum venosum are all that remains of the left umbilical vein.

It will be clear by now that the developing liver receives venous blood from the portal vein and oxygenated blood from the left umbilical vein. The oxygenated blood passes straight through the liver and does not much mix with the venous blood delivered by the portal vein. The liver also has an arterial supply. The hepatic artery, a branch of the coeliac trunk (itself the first ventral branch of the abdominal aorta) grows into the developing liver in company with the developing system of bile ducts.

Formation of the Liver Parenchyma

The cells of the liver, the hepatocytes, are formed from a branching diverticulum of the gut. This branching system initially looks as if it might be about to form a gland with acini and ducts, but forms a series of plates of liver tissue. These plates, initially several cells in thickness, become perforated by the venous sinusoids of the plexus described above. Whether the plates form around the sinusoids, or the sinusoids drill through the plates, is not too important for us, what is important is that a labyrinth of tissue, the very parenchyma of the liver, is formed. The plates form what is called a muralium (Latin, muralis meaning a wall) that is one cell thick, a muralium simplex, in mammals (and, interestingly, in song birds) but several cells thick, a muralium duplex, in 'lower' vertebrates (Elias, 1963). Much controversy has surrounded the development of the duct system of the liver. Do the bile ducts and all their branches form directly from the primary diverticulum, or is there a secondary invasion of the developing parenchyma by a duct system? This need not detain us: the interested reader should refer to Rouiller's two volume treatise, The Liver (1963), in which the key players in the controversy have their say. One point about the duct system is important: the rat does not develop a gall bladder. The mouse does and so does the guinea pig. The horse and cetaceans do not. Why the rat does not develop a gall bladder appears to be quite unknown. Those readers interested in comparative anatomy will be interested to know that the occurrence of a gall bladder is very variable among birds.

THE RELATIONSHIP BETWEEN THE LIVER AND THE PERITONEUM

The complexities of the relationships between the liver and the peritoneum used to be one of the highlights of human topographical anatomy: anatomists revelled in *tour de force* descriptions that traced the path of the peritoneum around the abdominal cavity and the enclosed organs including the liver, in both sagittal and transverse planes: enthusiasm for such mental gymnastics has waned.

The embryonic gut is attached to the ventral and dorsal body walls by a mesentery that comprises two layers of peritoneum with blood vessels lying between the layers. The dorsal mesentery persists to provide the mesentery of the adult, which carries blood vessels and nerve fibres to and from the gut; the ventral mesentery disappears below the umbilicus, but above it carries the umbilical veins, later just the left umbilical vein, to the liver. As the developing gut expands it fills the abdominal cavity, but it is still surrounded by the visceral peritoneum that is continuous with the parietal peritoneum lining the body wall. The peritoneal cavity lies between the visceral and parietal layers of the peritoneum that is the direct descendant of the intra-embryonic coelom: see Chapter 12, The Lung. The peritoneal cavity contains nothing more than a little peritoneal fluid.

The double layer of peritoneum connecting the stomach and the first part of the duodenum to the ventral abdominal wall is described as the ventral mesogastrium (the mesentery of the gaster, Latin for stomach). As the developing liver grows into the ventral mesogastrium it forces apart the layers of this double layer of peritoneum. We can now visualise how the liver comes to be (A) entirely covered by visceral peritoneum and (B) how the liver comes to be attached to the ventral abdominal wall by what remains of the ventral mesogastrium, which is now called the falciform ligament. To be strictly accurate, the liver is not entirely covered by visceral peritoneum: over the convex surface the layers of peritoneum split

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apart and run to the posterior surface of the diaphragm leaving what is called 'the bare area' of liver adjacent to the tissue of the diaphragm.

A last complication. As the stomach develops it flops over to one side so that what was the left surface of the stomach becomes the ventral surface (anterior surface in man). This sideways flop causes a pocket of the peritoneal cavity to be enclosed behind the stomach. This pocket is the lesser peritoneal cavity or the omental bursa of human anatomy. We shall not pursue the delights of the omental bursa, but just note that in man, the caudate lobe of the liver, covered of course by visceral peritoneum, invaginates the right wall of the bursa. Why mention this obscure fact? The reason is that Hunt (1924) described the posterior lobule of the caudate or Spigelian lobe of the liver, see below, as 'lying in the lesser peritoneal cavity'. If any part of the rat liver is homologous with the caudate lobe of human anatomy this might well be it.

THE LIVER OF THE ADULT RAT

The liver is a large, soft, dark red and lobulated organ that lies at the anterior end of the abdominal cavity, that is moulded by its contact with the posterior surface of the diaphragm. It has two surfaces: a convex antero-ventral surface and a concave posterior or visceral surface. The visceral surface comes into contact with organs of the anterior abdominal cavity: the stomach, right kidney and right adrenal gland (see Chapter 25: Dissection of the Adult Rat for diagrams showing the liver lying in the abdominal cavity). The posterior vena cava runs along the dorsal abdominal wall with the abdominal aorta, then leaves the aorta to enter the liver on its visceral surface. It passes through the liver to emerge just posterior to the central tendon of the diaphragm, which it perforates. The aorta passes dorsal to the liver, penetrating the diaphragm close to the vertebral column.

If the liver is perfused with fixative prior to removal from the body it will retain its true shape, and the anatomical relationships between the lobes can easily be made out. If, on the other hand, it is removed unfixed it spreads out when placed on a dissecting board and all trace of the convex antero-ventral surface is lost.

The Lobes of the Liver

The rat liver comprises a large medial (median or middle) lobe that provides most of the convex surface of the organ. It is divided into two parts by the falciform ligament running from the ventral abdominal wall to the liver and carrying in its free margin the teres ligament. In addition, there are two lateral lobes: right and left. These lie behind the medial lobe and are best seen when the visceral surface of the liver is examined. The left lateral lobe is undivided but the right is partially divided by a deep cleft or fissure. Also visible on the visceral surface are two small disc-like lobes: the anterior and posterior lobules of the caudate lobe that wrap around the right side of the stomach and the abdominal oesophagus. The structure of the intact liver is shown in Fig. 14.1.

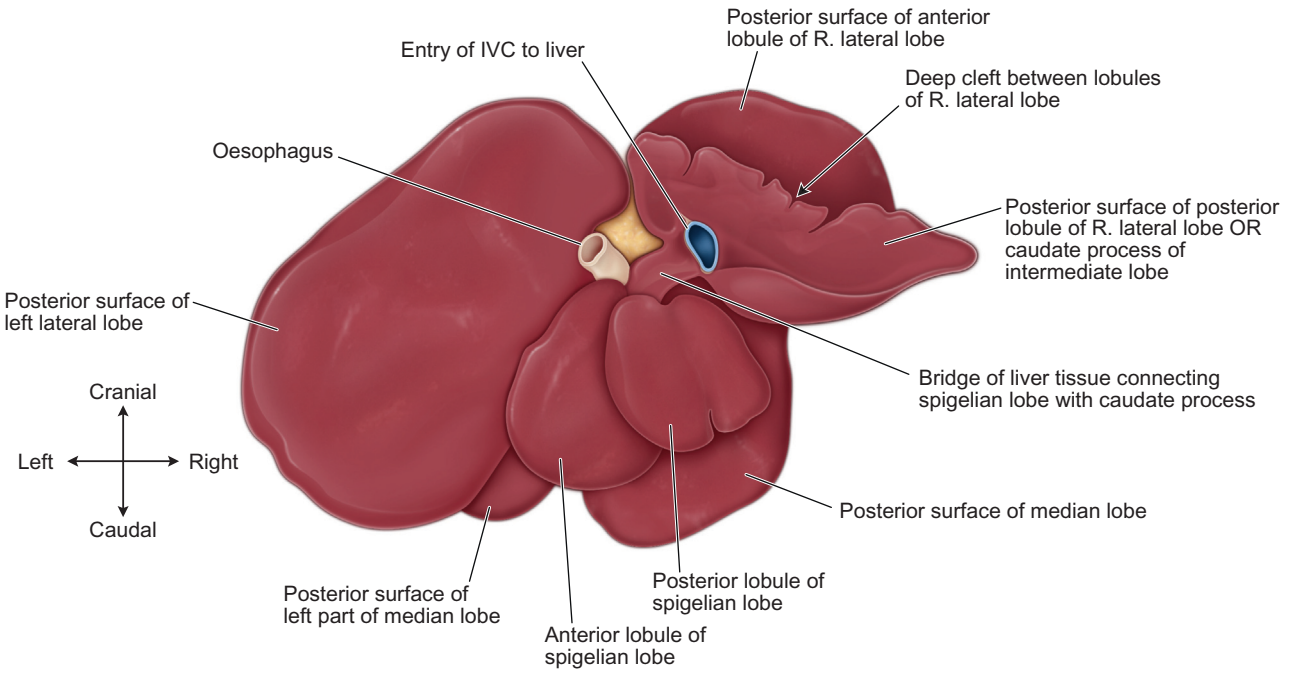


FIGURE 14.1 Macroscopic structure of the liver.

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TABLE 14.1 Terminologies Applied to the Lobes of the Liver

Hunt (1924)	Hebel and Stromberg (1976)	Martins and Neuhaus (2007)	Komarek (2000)
Median lobe[1] Right Central lobe .....[2] Left central lobe	Middle lobe	Middle or median lobe (ML[6]) Right medial lobe (RML) (IV, V and VIII)[4] .... Left medial lobe (LML) (III)	Lobus medialis dexter .... Lobus medialis sinister
Left lateral lobe	Left lobe Left medial lobe Great left lateral lobe	Left lateral lobe (LLL) (II)	Lobus lateralis sinister
Right lateral lobe Anterior right lateral lobe Posterior right lateral lobe	Right lobe	Right lobe (RL) (VI and VII) Inferior right lobe (IRL) or right anterior lobe (R1[5]) Superior right lobe (SRL) or right posterior lobe (R2[5])	Lobus lateralis dexter
Caudate or Spigelian lobe Anterior lobule Posterior lobule	Intermediate lobe Caudate process Ventral papillary process Dorsal papillary process	Caudate lobe (CL) (I and IX) Paracaval process (caudate process [3]) Spiegel lobe Anterior or superior portion (AC) Posterior or inferior portion (PC)	Lobus caudatus Processus caudatus Processus papillaris pars preventricularis Processus papillaris pars retroventricularis

1. Hunt describes the median lobe as being divided into "two lateral lobules, approximately equal in size, sometimes referred to as the right and left central lobes". Later he refers to the "right lobule" and to the "left lobule".
2. The dotted line represents the longitudinal fissure: the teres ligament enters the fissure at its posterior or dorsal end.
3. Note that this use of "caudate process" differs from that of Hebel and Stromberg and from that of Komarek.
4. Roman numerals indicate equivalent vascular segments of human liver. (Kogure et al., 1999, after Couinaud, 1981).
5. R1 and R2: abbreviations used by Kogure et al. (1999).
6. Abbreviation used by Martins and Neuhaus are shown immediately after the terms they used.

These lobes, which sound straightforward enough when described as earlier, have been given a variety of names. Table 14.1 compares the terminologies adopted by four authorities from 1924 to 2007. Names shown in the same colour refer to the same anatomical entities.

The Micro-Anatomy of the Liver

We use the term microanatomy here to describe the structural organisation of liver tissue; we shall use the term histology when we come to the liver cells themselves. Here, we are concerned with the structural organisation of liver tissue, with its arrangement into lobules, and the pattern of distribution of its blood vessels, lymphatic vessels and nerves.

Lobules and Acini

Histological sections of liver are easy to recognise, and all accounts of liver structure begin with the classical lobule. However, the reader should be aware that despite more than 150 years of intensive study the structure of the liver is still poorly understood, and great gaps remain in our knowledge. In addition, significant interspecies differences in structure exist, and the liver of the rat is by no

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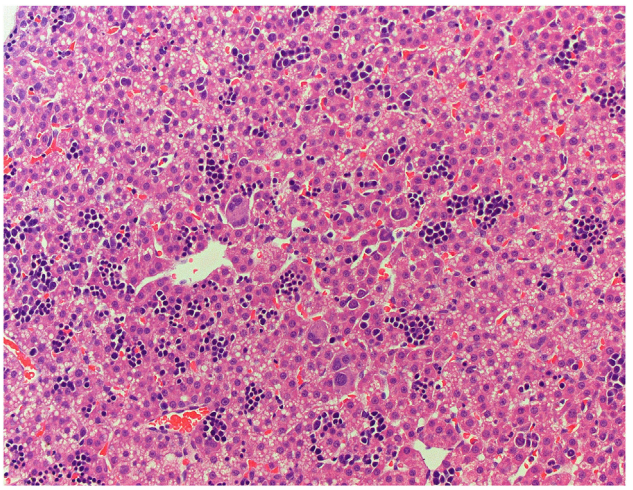


FIGURE 14.2 Rat liver at birth. At this early stage the liver is a haemopoietic organ. The small black dots are immature erythrocytes.

means identical in terms of its micro-anatomy to that of pig or man (Fig. 14.1).

Examination of a section of pig liver shows that the tissue is arranged in a series of roughly hexagonal areas. These are the classical lobules (or hepatic lobules) that were first described in detail by Kiernan (1833) in a 66 page paper, which is accessible via the Royal Society website. It provides an admirable review of the pre-1833 literature, and some fine drawings, in addition to an excellent introduction to liver structure. Each classical lobule has what is obviously a small tributary of the hepatic vein at its centre. At the periphery of the hexagon, at its corners, are small areas of connective tissue, the portal tracts or canals, containing a small vein, a branch (not a tributary of course) of the portal vein; a branch of the hepatic artery (with a thicker wall than the vein) and a small bile duct or ductule. Examination at higher magnification shows that the bile ductule is lined by cuboidal epithelium. The periphery of the hexagon is defined by thin septa of connective tissue. Between the central vein and the periphery run cords of cells, the hepatocytes. These are separated by sinusoids containing, but not usually filled with, blood cells. The cords appear to branch, but are seldom more than one cell in thickness. The cords are cross sections of plates of liver cells that are penetrated by the sinusoids from place to place: the sinusoidal labyrinth. Bile canaliculi run between the faces of the hepatocytes that do not abut onto the sinusoids. These are minute tubular spaces formed by the apposition of troughs in the surfaces of the hepatocytes. Bile is secreted into the canaliculi, and runs outwards to the periphery of the lobule where is enters very small connecting vessels that conduct the bile to the ductules of the portal tracts. Blood flows from the portal tracts to the central veins; bile flows in the opposite direction: along the canaliculi to the portal tracts. This counter-current flow of blood and bile occurs in all mammalian livers. What can only be clearly seen at electron microscopy is the space of Disse that lies between the hepatocytes and the endothelium of the sinusoids. Fluid and protein leak from the sinusoids into this space. This protein-rich lymph flows to the periphery of the lobule, and into another connective tissue space, of Mall, in the portal tracts. Here, it enters lymphatic capillaries that join to form small lymphatic vessels to conduct the lymph to the porta hepatis, and thence out of the liver and into the large thoracic duct that penetrates the diaphragm, into the thorax and on to the venous system at the root of the neck: see Chapter 7. Nerves, sympathetic and parasympathetic, travel along the portal tracts. These supply the vessels and ducts of the tracts and penetrate into the lobules.

If all that is required is an elementary account of liver structure then that given above is probably acceptable.

Classical Lobules and Others

Sections of pig liver cut in any plane always seem to reveal a system of hexagons, or at least multisided areas of about the same size. This might imply that the lobules are short prismatic structures with six sides to the mid-section, and perhaps pointed six facetted ends. Of course, one could cut such a structure so as to produce something other than a hexagon. If, for example, three planes meet at a point, then a section from near the tip of the solid enclosed by these planes will produce a triangle. Closer examination of sections of pig liver shows small areas that are not hexagonal, but might represent an angular section through the junction between the mid-section and the ends of, or through the ends of, classical lobules. But no morphologist would expect liver to be constructed with the regular symmetry of crystals or of a honey-comb! We should not be surprised to find areas of pig liver that are not hexagonal; these, it is assumed, come from random sectioning of the roughly prismatic classical lobules.

The classical lobules of pig liver are rather unusual, in that they are easy to recognise because of the connective tissue that outlines them. Connective tissue septa at the edges of classical lobules are found in the pig, the camel, bears (some authorities specify polar bears but say nothing of other bears) and raccoons (Fawcett, 1994). This is an odd assemblage of mammals. Classical lobules in the pig are usually shown with a portal tract at each corner. This is not always obvious, even in the pig, and is certainly not the case in man or the rat.

What Is a Lobule? What Is an Acinus?

When describing an exocrine gland, a lobule is typically defined as the smallest grouping of tissues that can, on a

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small scale, reproduce the functions of the gland. This usually comprises a collection of secretory cells (an acinus) arranged at the end of a duct. If several acini feed into one duct, then the lobule must be regarded as multiacinar. Clearly there are difficulties in transferring this concept to the liver, as the classical lobule is defined in terms of its blood supply rather than its secretions. If an acinus were to be defined in terms of its secretory activity, the smallest discrete volume of liver drained by the smallest branch of the biliary tree would qualify. If six bile ductules are arranged in portal tracts around the classical lobule: does this make the acinus equal to one sixth of a classical lobule? To complicate matters further, the bile ductule of a portal tract does not drain bile from just one classical lobule, but from three classical lobules, or more correctly from about a sixth of each of three classical lobules. Perhaps the acinus spans these three classical lobules, or perhaps we should think of a branch of the bile ductule of the portal tract, a branch that runs out into a septum at the periphery of a classical lobule and define the tissue that feeds bile to this septal ductule as an acinus.

The reason this has exercised physiologists for so long is that it is considered important to define the liver acinus in functional terms. Given that form follows function, deductions about function should be possible if form is properly understood. Pathologists seeking to understand disease processes affecting the liver expect these processes to appear first in the smallest functional units of the liver, and so also require a definition of the acinus. Knowing what an acinus actually is, and how it differs in structural terms from species to species, would make extrapolation from toxico-pathological findings in animals to expected effects in man a great deal stronger.

The Portal Lobule

Mall (1906) proposed a liver lobule that was triangular in outline in sections and centred on a portal tract with three 'central' veins at its corners. In three dimensions, this lobule might have had the shape of a triangular pyramid or that of two such pyramids placed base to base, a six sided polyhedron. The portal lobule fitted rather well with the idea of a lobule of an exocrine gland, the duct system was at its centre, but was not widely accepted as the fundamental unit of liver structure.

The Rappaport Acinus

Rappaport and his colleagues (1954) overturned thinking on liver structure with their proposal of an acinus, that was neither hexagonal nor triangular in section. What was proposed was a rugby ball shaped solid with septal branch of a portal venule as its long axis. In cross section, the

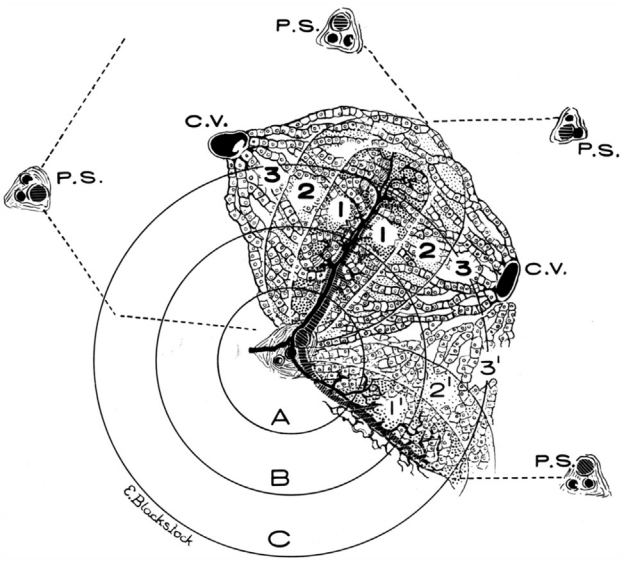


FIGURE 14.3 The blood supply of the hepatic structural unit: The structural unit occupies adjacent sectors of neighbouring hexagonal fields. Zones 1, 2 and 3 respectively represent areas supplied with blood of first, second and third quality with regard to oxygen and nutrients. These zones centre about the terminal afferent vascular twigs and extend into the periportal field from which these twigs orginate. Zones 1^1, 2^1 and 3^1 designate corresponding areas in a portion of an adjacent structural unit. In zones, 1 and 1^1, the afferent vascular twigs empty into the sinusoids.
The circles A, B and C delimit concentric bands of hepatic parenchyma arranged around a small portal field.

acinus might appear either circular or oval, depending on the plane of section. The distal ends of two acini met at corners of classical lobules not occupied by portal tracts: only three portal tracts were defined per hexagon (Fig. 14.3).

Rappaport developed his thinking in a series of papers and reviews (1954, 1963, 1973, 1976). The 1963 account is particularly detailed and wide-ranging. It is important to understand that he and his colleagues saw their model as three dimensional: they saw the septal venule arching up and away from the portal tract and supplying a berry or grape-shaped cluster of hepatocytes, which were themselves a discrete part of the great mass of hepatocytes arranged as plates. Not easy to visualise! Rappaport's illustrations from the 1954 paper shows the idea: see Fig. 14.4.

The model was further developed by dividing the acinus into three zones defined by their proximity to their blood supply. Zone 1 was closest to the septal venule, zone 3 was furthest away. The classical lobule has come to be regarded as the basis for structure and the Rappaport acinus as the basis for function. Pathologists noted that the pattern of fibrosis seen in some liver diseases conformed to the Rappaport model with bridges of fibrotic tissue radiating out from the central veins to the

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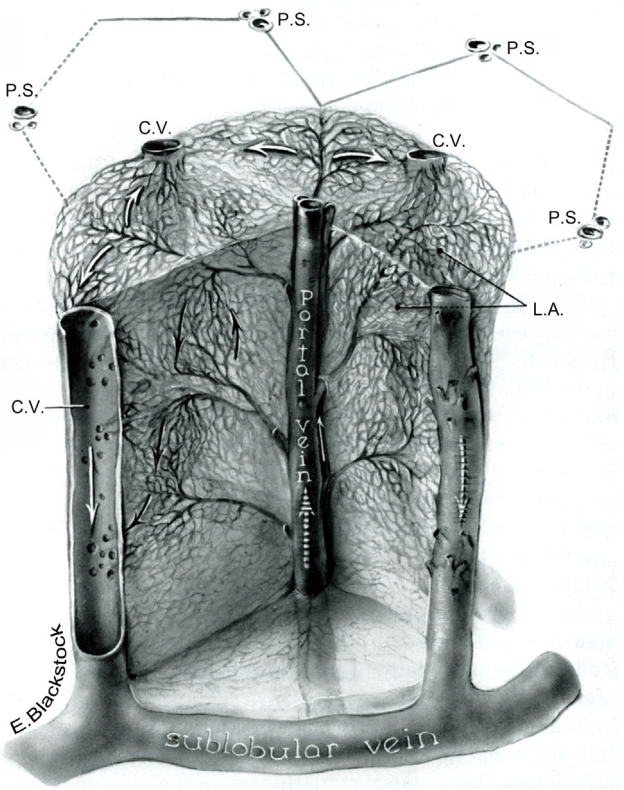


FIGURE 14.4 The vascular architecture of the liver in its relation to the hepatic structural units. The longitudinal aspect of a small portal space and its component structures is exposed. For simplification, only terminal portal venules branching out from the portal vein in this space are drawn. The blood coming up in the portal and arterial vessels supplies the hepatic units of several hexagonal fields. Note the arcuate courses of these terminal vessels which follow the axes of the irregularly arranged structural units. There are many anastomotic pathways permitting the flow of blood in all directions. LA = hepatic structural unit penetrating an hexagonal field which is situated well above the level of its origin. PS = small portal space or canal. CV = central vein.

portal tracts and apparently following the areas of zone 3 perfusion. The Rappaport model appeared in textbooks of pathology and hepatology (Sherlock and Dooley, 2002). One oddity of the Rappaport model was that the portal tracts appeared to be surrounded by a region of zone 3 perfusion: some would have expected the area around the portal tracts to be particularly well perfused.

Recent Developments, Focusing on the Rat

Studies of the hepatic micro-circulation (Matsumoto et al., 1979; Matsumoto and Kawakami, 1982), of the distribution of hepatocytes enzymes throughout the classical lobule (Lamers et al., 1989) and of the distribution of fibrosis induced in rats by injection of pig serum (Bhunchet and Wake, 1998) have cast doubt on the Rappaport model, at least as far as the rat is concerned (Malarkey et al., 2005; Saxena et al., 1999). Saxena listed five important features of the rat liver that differ from those of man.

1. In the rat, the sinusoids drain not only to central veins but also to much larger hepatic veins: sublobular veins and collecting veins.
2. In the rat, sinusoids are supplied with blood not only by the terminal branches of portal veins, but by a wide range of other branches of the portal veins including some that are large.
3. Rats lack septal branches of portal veins that radiate out along the imaginary edges of the classical lobules and form the axes of Rappaport acini.
4. Anastomoses between hepatic and portal veins are common in the rat, but rare in man
5. In the rat, a plexus of bile ductules surrounds even small portal veins, but in man, is found only in portal tracts containing large portal veins.

Noting the absence of septal veins in the rat: Bhunchet and Wake (1998) argued that the rat simply lacked classical lobules and Rappaport acini. They argued for a liver unit centred on the portal tracts, in fact for a unit rather similar to that proposed by Mall (1906). Teutsch et al. (1999) have also argued that the Rappaport acinus does not exist in the rat: their paper shows bewilderingly complicated three-dimensional reconstructions of rat liver. In their model a cone shaped secondary unit, subdivided into 14 primary units, is described. The primary units varied in shape from tri to heptahedral with branches of the hepatic veins, not portal veins, at their centres. Perfusion of tissue around portal tracts was relatively good compared with other area of tissue: a difference from the Rappaport model.

SUMMARY

It is difficult to disagree with Malarkey et al. (2005) when they said, 'The liver is a heterogeneous tissue whose functional unit of the lobule has continued to stump morphologists for over 300 years…'. As in so many areas of morphology, the closer we look, the more complex is the arrangement of tissue, and the more species differences appear to occur. With regard to the liver, it is clear that all mammals that have been studied have not only evolved a system involving the counter-current flow of blood and bile, but also that the structural details of the evolved systems vary from species to species. This is an important point for the toxicologist: though responses to chemicals may be similar across a range of species, we should not expect the morphological evidence of these responses to be entirely consistent.

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HISTOLOGY OF THE LIVER

Examination of paraffin wax sections of liver plays a large part in toxicological-pathology (Fig. 14.5). In the following short account, we shall focus on what can be seen in sections stained with H&E and add just a few notes on what has been discovered on electron microscopy. Detailed accounts of the histology of the liver are available in all textbooks of histology (Fawcett, 1994; Weiss, 1988). These accounts include outstanding electron-micrographs of liver tissue, but focus on human histology: the reader should be aware of possible species differences.

Liver is one of the easier tissues to study at the light microscopy (LM) level. Buffered formol-saline is an adequate fixative (many others can be used), standard dehydration and clearing, followed by infiltration with paraffin wax, blocking and cutting at 5 μm produces reliably good sections. Selection of blocks (blocking) should follow the guidelines set out by Ruehl-Fehlert et al. (2003). Staining with H&E is standard practice. Bile canaliculi can be demonstrated using classical techniques: Carleton (1957) recommended exposing tissue fixed in buffered formol-saline to 3% potassium dichromate for 3–7 days and then processing. The sections should be stained with Heidenhain's iron haematoxylin: canaliculi are stained black. Silver impregnation may be effective, but is capricious and expensive: see Carleton (1957) for details. Histochemical staining for alkaline phosphatase also demonstrates the canaliculi. For connective tissue staining, Masson's trichrome stain is excellent, and helpful for the identification of portal tracts. Sections should be lightly stained. Reticulin fibres can be demonstrated by silver impregnation, although this is not probably particularly useful as part of a short-term toxicology study.

As always, examination should begin with the lowest power objective lens available: the entire section should be scanned. It will be immediately obvious that rat liver is not arranged in the neat hexagonal lobules seen in a pig liver.

The second obvious feature is the presence of many large, apparently thin-walled veins. Some of these are central veins, with sinusoids opening into them; some are branches of the portal veins running in the portal tracts. Branches of portal veins can be identified by adjacent cross sections of small bile ducts (ductules): these are smaller in diameter than the veins and lined by a cuboidal epithelium. The clusters of blue- purple staining nuclei mark the epithelium. Large portal tracts often contain more than one bile ductule: a large tract should be found and examined: connective tissue staining helps in defining these tracts. Note that, the tract also contains a small artery: a branch of the hepatic artery. The combination of portal vein, bile duct and hepatic artery is described as a triad. Sherlock and Dooley (2002), describing human liver, pointed out that dyads were 'as frequent as triads': the branch of the portal vein was often missing. The connective tissue of the portal tracts is continuous at the hilum of the liver (the porta hepatis: the doorway to the liver) with the connective tissue capsule of the liver. This is Glisson's capsule and is a thin layer of connective tissue covered in most parts by the mesothelium of the visceral layer of the peritoneum.

Portal Tracts and Central Veins

A search for small portal tracts should be made. Each is surrounded by a continuous row of hepatocytes: formed from the limiting plates of three adjacent classical lobules. Higher magnification shows that the small terminal branches of the portal veins are thin-walled. The terminal branches of the hepatic arteries are also thin-walled, but a single layer of smooth muscle cells, with elongated nuclei, can usually be recognised. The cytoplasm of the

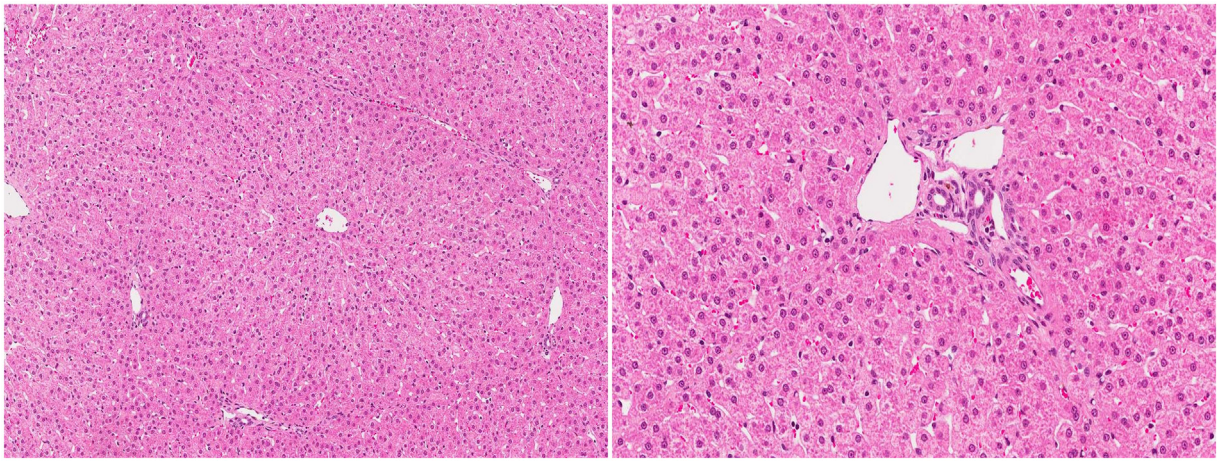


FIGURE 14.5 Low and medium power images of the rat liver. In the low power image on the left a central vein is shown surround by some portal tracts. The higher power image show detail of a portal tract.

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smooth muscle cells often stains a rather bright pink in H&E stained sections. The epithelium of the bile ductules should be examined under high power: it is a cuboidal epithelium. The wall of these little ductules is thin: in large ducts the wall contains smooth muscle, but there is no sign of this in the intrahepatic bile ducts of the rat and the epithelium is supported by a basement membrane (basal lamina) and connective tissue. The connective tissue of the portal tracts contains what look like small spaces. These may be the terminal vessels of the lymphatic system or fixation artefacts. Look for the nuclei of the endothelial cells that incompletely line the small lymphatic vessels. Cells with small dark nuclei are found in the connective tissue of the portal tracts. Lymphocytes and plasma cells are sometimes found in the connective tissue of the portal tracts. A small central vein should be located. It will be seen that this has hardly any real wall at all: the sinusoids (spaces between the columns of hepatocytes) open directly into the vein.

The Tissue Between the Portal Tracts and the Central Veins: Hepatocytes and Sinusoids

What appears to be rows of hepatocytes filling the space between the portal tracts and the central veins are the result of plates of cells being cut transversely. Note that the rows branch join with one another, as do the plates. The gaps between the rows are the sinusoids: some contain blood cells.

Hepatocytes

Examination of the hepatocytes at high magnification reveals them to be large polyhedral cells (sometimes described as hexagonal, but enough of hexagons) of about 20–30 μm diameter. The large pale nuclei are clearly defined by a very thin blue-purple staining margin of chromatin located close to the nuclear membrane. Large dark-staining nucleoli are obvious. Nuclei vary in size, the larger ones have been found to be polyploid. The liver is a 'medium turnover' organ so mitoses occur fairly regularly: in a young animal you will probably see 1–2 per section if you look carefully. Hepatocytes are said to have lives of about 150 days, after which they are removed by the process of apoptosis and replaced by the division of other hepatocytes.

The cytoplasm is granular, with spaces reflecting the removal of glycogen and fat during processing. Fawcett (1994) should be consulted for details of the organelles of the hepatocytes. Canaliculi cannot be seen in H&E sections. They run between the surfaces of hepatocytes that do not abut onto the sinusoids. Each canaliculus is formed by a trough-like indentation in the surface of the hepatocytes: the two indentations form the "walls" of the canaliculus. There are no walls as such, just the cell membranes of the hepatocytes. Microvilli project into the canaliculi. Each canaliculus is sealed by tight junctions where the cell membranes of the contiguous hepatocytes meet: no leak of bile from the canaliculi is permitted. The canaliculi form a chicken-wire mesh between the hepatocytes and drain into small ducts at the periphery of the classical lobules. These are the canals of Herring and are very difficult to make out in ordinary sections. Occasionally, a small cluster or short row of what look like the nuclei of cuboidal epithelial cells can be seen close to the portal tract: this may well be part of the wall of a canal of Herring. The surfaces of the hepatocytes that face the sinusoids have microvilli which project into the fluid-filled space of Disse between the hepatocytes and the walls of the sinusoids (Fig. 14.6).

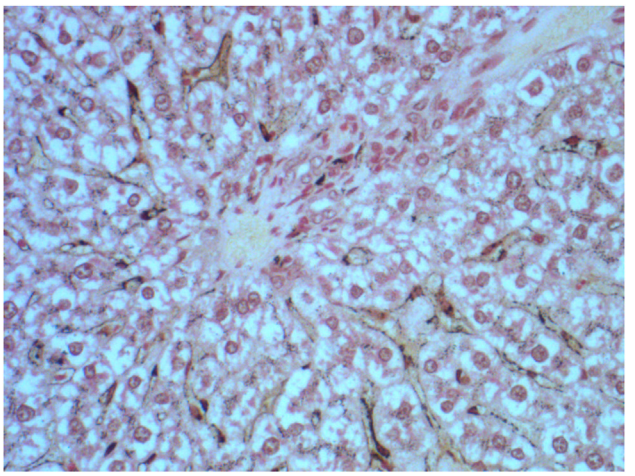


FIGURE 14.6 Space of Disse. Although difficult to see with standard staining, silver based staining techniques, where the particles collect in the space of Disse, permit visualisation.

Sinusoids

Hepatic sinusoids are about 10–15 μm in diameter. The endothelium is continuous but the endothelial cells are only loosely attached to one another allowing fluid and protein to leak through the intercellular junctions into the space of Disse. A further leak is allowed by the fenestrations of the sinusoid endothelium: fenestrae (one fenestra, two or more fenestrae) of about 100 nm diameter and not closed by diaphragms, occur as groups (sieve plates) and penetrate the endothelial cells. Braet and Wisse (2002) have provided a detailed and comparative review of the fenestrae of liver sinusoids. The endothelial cells of rat liver sinusoids lack a basal lamina; in some species, sheep, goat, cow, a basal lamina is found. Kupffer cells of the sinusoids have long been regarded as fixed macrophages of the reticuloendothelial system (Fig. 14.7). They lie, within the sinusoids, against the endothelial cells and send long cytoplasmic

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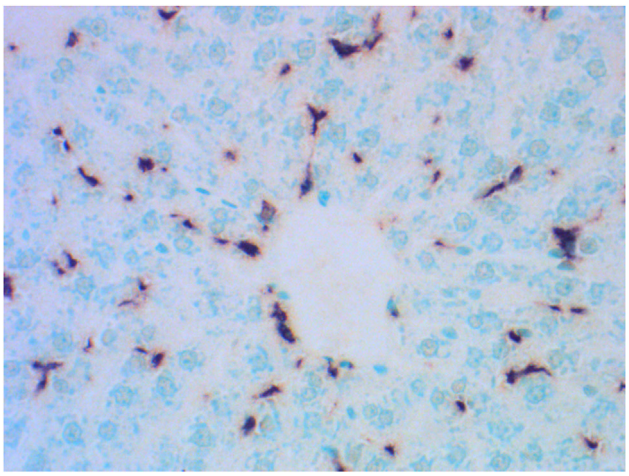


FIGURE 14.7 Kupffer cells. Revealing their origins this macrophage IHC stain demonstrates how Kupffer cells sit in the sinusoids.

processes between the endothelial cells and on into the space of Disse. Kupffer cells take up particulate material from the blood: see Wisse et al. (1996) and Bilzer and Roggel (2006) for detailed accounts of these cells. Kupffer cells can be recognised in H&E preparations by their dark angular nuclei. Fat storage cells (of Ito) which have a range of functions including production of collagen and a capacity for contraction, and pit cells (natural killer lymphocytes) are also associated with the sinusoids (Wisse et al., 1996) but cannot be distinguished in H&E stained sections.

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Chapter 15

Exocrine Glands

In this chapter, we deal with a number of exocrine glands of broadly similar histological structure: the lacrimal and Harderian glands, the salivary glands, the exocrine pancreas and the mammary gland. Exocrine glands release their secretions by means of ducts, in contrast to endocrine glands, which have no ducts. Exocrine glands can be classified according to the arrangements of their ducts and secretory tissue.

CLASSIFICATION OF EXOCRINE GLANDS

No classification of exocrine glands is perfect as they obstinately refuse to fit perfectly into man-made systems. The following classification is based on three questions:

1. Does the gland contain one, or more than one, cell? If the former, it is a unicellular gland, if the latter then it is a multicellular gland.
2. What is the nature of the duct that conducts its secretions to the point of discharge? If it is unbranched, then the gland is a simple exocrine gland, but if branched then the gland is a compound exocrine gland. Some exocrine glands, for example the lacrimal gland, have more than one duct leading to the point of discharge of secretions. In some glands, the duct system is far from being merely a conduit as the ductal cells can secrete substances into, or absorb substances from, the fluid secreted by the more distal parts of the gland.
3. Are the cells that produce the secretions arranged as tubules, acini or alveoli?

Unicellular Glands

The only example found in mammals is the individual mucous cell found in the respiratory and gastro-intestinal epithelium (Fig. 15.1).

Multicellular Glands

Simple Exocrine Glands (Unbranched Ducts)

1. In simple tubular glands, the secretory portion is a tubular structure. Secretions are led from the secretory tubules to the point of discharge by an unbranched duct. The secretory tubules may be straight (crypts of Lieberkühn), coiled (sweat glands) or branched like those of the gastric glands, the glands of the endometrium and Brunner's glands of the duodenum. The glands of the endometrium show the difficulty of fitting real glands into the classification, as there may be no nonsecretory portion present (Fig. 15.2).
2. Simple acinar glands have secretory cells arranged as expanded ovoids or spheres, which pour their secretion into a central lumen connected to the ductal system of the gland. More than one acinus may be connected with the terminal part of the duct.

Examples of a gland with a single acinus are rare in mammals, although examples occur in other vertebrates. The sebaceous glands of the hair follicles (Fig. 15.3) and the Meibomian glands of the eyelids provide the only examples in the rat or man.

Compound Exocrine Glands (Branched Ducts)

Compound exocrine glands have a branching duct system, and can be subclassified into either tubular or acinar, according to the nature of the secretory portion of the gland. In compound tubular glands, the secretory portion comprises secretory tubules. If the distal portion of the gland comprises a system of narrow, branching, ducts with many acini or alveoli opening into them, then the gland is described as a compound acinar gland. Larger salivary glands are in this category but are often described as compound tubulo-acinar glands. The lumina of the acini are small in the salivary glands, but somewhat larger in the mammary and prostate glands (Fig. 15.4): these glands are described as compound tubulo-alveolar glands. The distinction between an acinus and an alveolus is a little arbitrary but a useful guide is that if the lumen is wider than the height of the secretory cells then the secretory structure should be described as an alveolus.

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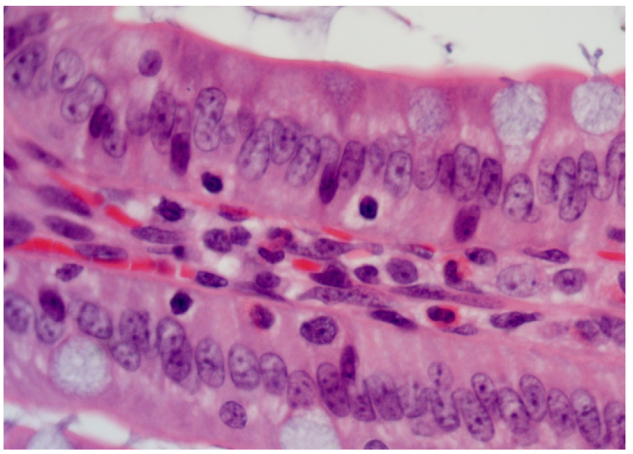


FIGURE 15.1 Unicellular mucous glands in the Ileum. In H and E mucous glands can be distinguished by a prominent vacuole with blue/grey staining contents.

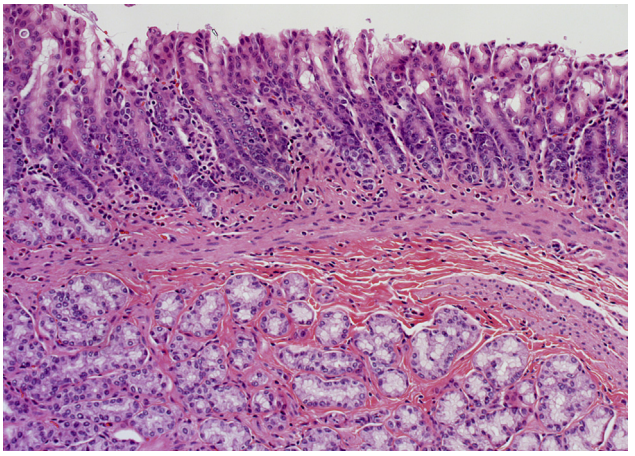


FIGURE 15.2 Brunner's glands.

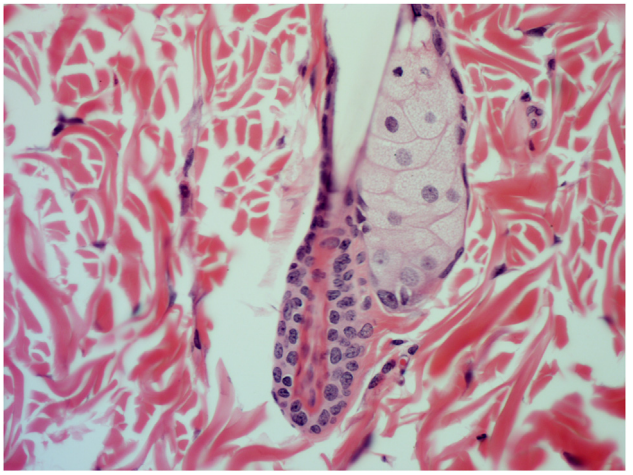


FIGURE 15.3 Sebaceous glands, with pale, eosinophilic cytoplasm and round central nuclei.

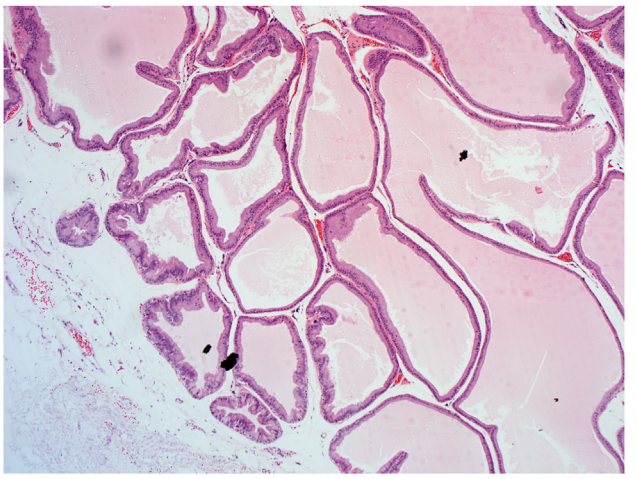


FIGURE 15.4 Prostate gland, with its large secretion filled lumina.

LACRIMAL GLANDS

The rat has two pairs of lacrimal glands on either side of the head. One gland lies within the orbit, the other just in front of the ear adjacent to the parotid salivary gland (Fig. 15.5). Man has only an intraorbital lacrimal gland located in the upper-lateral part of the orbit and extending into the upper eyelid. The extension into the eyelid seems not to occur in the rat. Accessory lacrimal glands (or foci of glandular tissue in the conjunctivae) have been described in man (Wolff, 1948).

Although the nomenclature of the rat lacrimal glands has been reasonably consistent over the years, there has been some variation as outlined below. Our preference is to use the terms intraorbital and extraorbital (Table 15.1).

General and Topographical Anatomy

Intraorbital Lacrimal Gland

The intraorbital lacrimal gland is roughly triangular measuring approximately $7.5 \times 7.5 \times 1$ mm and lies at the posterior angle of the orbit between the zygomatic arch and the eyeball, lateral to and partially behind the Harderian gland (Hebel and Stromberg, 1976; Greene, 1935). These glands fill the space behind the eyeball; a space largely filled by fatty tissue in man. The intraorbital gland gives rise to two ducts that join with the ducts from the extraorbital gland before emptying into the lateral corner (canthus) of the conjunctival sac.

Blood supply is via the lacrimal artery, which in man is a branch of the ophthalmic artery, but in the rat the origin is less clear-cut. Greene (1935) derived the lacrimal artery from the zygomatico-orbital artery, a branch of the superficial temporal artery that arises from the external carotid. Hebel and Stromberg (1976) derived the lacrimal

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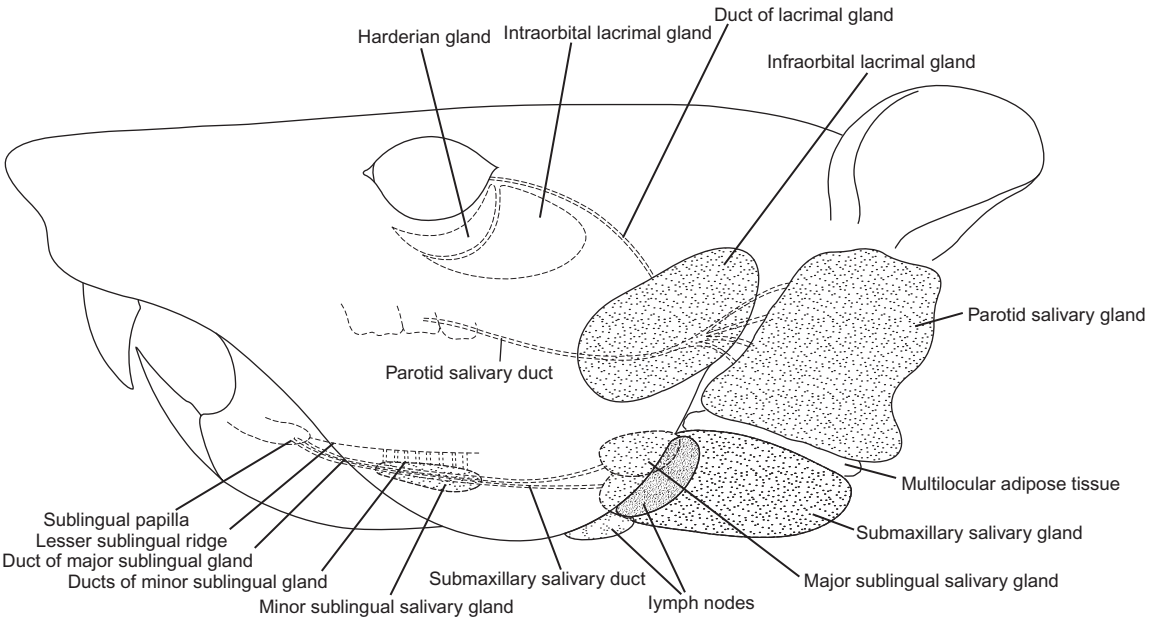


FIGURE 15.5 Salivary glands and associated structures.

TABLE 15.1 Terminologies Applied to Rat Lacrimal Glands

	Gland lying within the orbit, lateral and partly posterior to the Harderian gland	Gland lying outside the orbit, on masseter and temporalis muscles, rostral to the parotid gland
Hebel and Stromberg (1976)	Infraorbital gland	Extraorbital gland
Rowett (1960)	Intraorbital gland	Infraorbital gland
Greene (1935)	Intraorbital gland	Exorbital gland
Elwell and Leininger (1990)	Infraorbital gland	Extraorbital gland
Gancharova and Manskikh (2014)	Infraorbital gland or inferior gland	Extraorbital gland or superior gland

artery from the palatine branch of the pterygopalatine artery, itself a branch of the internal carotid artery.

Parasympathetic innervation is from the sphenopalatine ganglion, and sympathetic innervation probably accompanies arterial supply.

Extraorbital Lacrimal Gland

The extraorbital lacrimal gland is somewhat larger, measuring about $12 \times 8 \times 2$ mm. It lies rostral (anterior) to the parotid salivary gland, and is attached to the fascia overlying the masseter and temporalis muscles (Hebel and Stromberg, 1976). The extraglandular duct system is complicated, with six or more ducts leaving the gland and being bundled together as the lacrimal cord, which runs from the gland to the outer corner of the orbit. The ducts then combine to produce two ducts that join with those of the intraorbital gland (Lorber, 1993).

The gland receives its blood supply via a branch, or branches, of the temporal arteries that arise from the external carotid artery. Innervation of the gland does not seem to have been described in detail: it is assumed to be similar to that of the intraorbital gland.

Drainage of the Conjunctival Sac

The secretion of the lacrimal glands enter the lateral corner of the sac; fluid flows medially across the anterior surface of the eyeball and drains to the two small orifices (the puncta) of the lacrimal ducts on the edges of the

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upper and lower eyelids, about 1 mm or less from the medial ends of these structures. The lacrimal ducts join to form the nasolacrimal duct that passes through the infraorbital fissure, between the lacrimal bone and the maxilla and premaxilla, to turn medially and pass through the cartilage of the nose and enter the inferior nasal meatus or the vestibule of the nasal cavity (Chapter 10: Nasal Cavity). The nasolacrimal duct follows a similar course in man. For amusement, see Wolff (1948) for a description of the seven mucosal valves within the lacrimal duct of man, named for their discoverers, and thought, by Wolff, to be worth mentioning.

Histology

The intraorbital and extraorbital lacrimal glands have a similar histological appearance and will not be differentiated in this account. The glands can be described as a compound tubulo-acinar gland in the rat, but in man the acini should be called alveoli, as the lumina are comparatively large, in fact much larger than those of human salivary glands. In man the alveoli are not so closely packed, and fatty tissue can be seen between the lobules. These features make the lacrimal gland relatively easy to distinguish from the parotid salivary gland, another serous gland. Schechter et al. (2010) have provided a valuable description of the comparative histology of the rat, rabbit and human lacrimal glands.

The rat lacrimal gland is divided into lobules by septa of connective tissue that contain the vessels and nerve fibres of the gland. The interlobular ducts of the gland also run in these connective tissue septa. Lobular ducts enter the lobules and become intralobular ducts, which divide and become narrower. The last divisions of the intralobular ducts are the intercalated ducts (intercalary ducts) that lead into the acini that are arranged like bunches of grapes at the terminations of the intercalated ducts. The smaller ducts are lined by a simple cuboidal epithelium; the larger ducts by a compound cuboidal epithelium two or more cells thick. The extraglandular ducts may be lined with pseudostratified epithelium near their orifices.

The acini are tightly packed with barely discernible lumina. The cells of the acini produce a serous secretion containing a range of proteins, and the cytoplasm stains a deep purple-pink in H&E stained sections. The nuclei are clearly visible near the centres of the cells. The cells of the intercalated ducts stain less darkly than those of the acini so the ducts are easy to distinguish from acini. The nuclei of the cells of the acini vary in size, a feature that helps distinguish the lacrimal gland from other serous glands. This variation increases with age, especially in male rats. Changes associated with aging include increased pleomorphism of cells (cells of varying sizes and shapes), enlargement of nuclei (karyomegaly), areas of duct proliferation, Harderian gland metaplasia and fibrosis and lymphocytic infiltration of the gland (Gancharova and Manskikh, 2014).

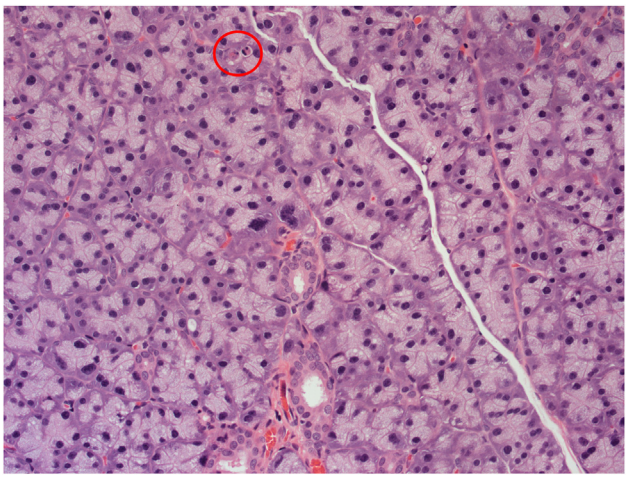


FIGURE 15.6 Lacrimal gland. Occasional apoptotic bodies (circled) can be seen at most ages.

Electron microscopy shows microvilli on the apical surfaces of the acinar cells and the baso-lateral cell membranes are deeply folded and interdigitated. Myoepithelial cells surround the acini and the first segments of the intercalated ducts. Many mast cells are found in the connective tissue of the rat gland, many more than are seen in human tissue. Plasma cells, a feature of the human gland interstitium, are rather rare in the rat (Schechter et al., 2010) (Fig. 15.6).

HARDERIAN GLAND

The Harderian gland was first described in deer in 1694 by Harder and is found in the majority of vertebrates, but not in fish, bats, cows and horses, terrestrial carnivores or higher primates (including man) (Payne, 1994). Despite this, the Harderian gland of the rat is of interest to toxicological pathologists because tumours of the gland can be induced by a number of carcinogenic compounds, and the presence of large numbers of oestrogen and androgen receptors may make it a target when endocrine disruption occurs. Examination of the Harderian gland forms an essential part of the histological review of tissues removed during postmortem examination of rats used in toxicological studies. There are two outstanding reviews of the histology of the Harderian gland: Payne (1994) and Sakai (1981). Payne's review is particularly well illustrated, and deals with the ultrastructure of the Harderian gland in detail. Specific details of the Harderian gland of the rat have also been provided by Djeridane (1994); this is perhaps the best paper available on the gland in the rat,

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and the following account is based on it. In addition, Reis et al. (2005) have provided a review of porphyrin production in the Harderian gland of the rat, Elwell and Boorman (1990) reviewed the structure of the gland with details of the histopathology of Harderian gland tumours, and Chieffi et al. (1996) have provided an extensive review of the cell biology and possible functions of the Harderian gland.

The possible functions that have been ascribed to the rat Harderian gland include lubrication of the conjunctiva (perhaps the most obvious function), the production of pheromones, a role in defending the retina against ultraviolet light, a role as a photoreceptor for the pineal gland, and the production of growth factors.

What unequivocal is that the rat Harderian gland produces and secretes porphyrins. As a result, the gland exhibits a red-pink fluorescence when examined under ultraviolet light, and concretions of porphyrin-based material may be found in the ducts of the gland. The production of large amounts of porphyrins by rodents is well known: Boyd (1970) noted that the bright red fluorescence of the bones and teeth of the ground squirrel was due to the level of porphyrins in these tissues.

General and Topographical Anatomy

The Harderian gland is conical in shape, light brown to grey in colour, and occupies most of the orbit posterior and medial to the eyeball. The intraorbital lacrimal gland lies lateral to, and rather behind, the Harderian gland. The gland lies against the orbital venous sinus and has a thin capsule of connective tissue, which has been described as the endothelium of the venous sinus; although other authors refer to intervening connective tissue (Elwell and Boorman, 1990).

The gland is lobulated and characterised by three clefts where the gland is wrapped around the optic nerve and extraocular muscles. A single excretory duct runs to the nictitating membrane at the medial side of the eye where it opens onto the anterior surface (Djeridane, 1994). In some mammals the duct opens onto the posterior, conjunctival surface of the nictitating membrane (Sakai, 1981). The gland is referred to by veterinary anatomists as the deep gland of the nictitating membrane (glandula palpebra tertia profundus), and distinguished from the main intraorbital lacrimal gland and the superficial lacrimal gland of the nictitating membrane (glandula palpebra tertia surperficialis).

Histology

The Harderian gland is an unusual exocrine gland in that it contains no intraglandular ductal system that can be differentiated from the secretory portion of the gland. This led Sakai to insist that the gland was a simple tubulo-alveolar gland, and to criticise those who classified it as a compound tubulo-alveolar gland. Having made his point with some force he wrote: 'However, it is of little significance to classify the glandular architecture; a full description is necessary to understand the histological architecture of the Harderian gland'. We agree, but have provided a less than 'full' description (Fig. 15.7).

The Secretory Portion of the Gland

The secretory alveoli (or tubules according to some workers) are lined by a cuboidal-columnar epithelium resting on a basement membrane. Eosinophilic A cells containing large lipid-filled vacuoles make up 75% of the cells of the epithelium; B cells which are characterised by a more basophilic cytoplasm and smaller lipid vacuoles, make up

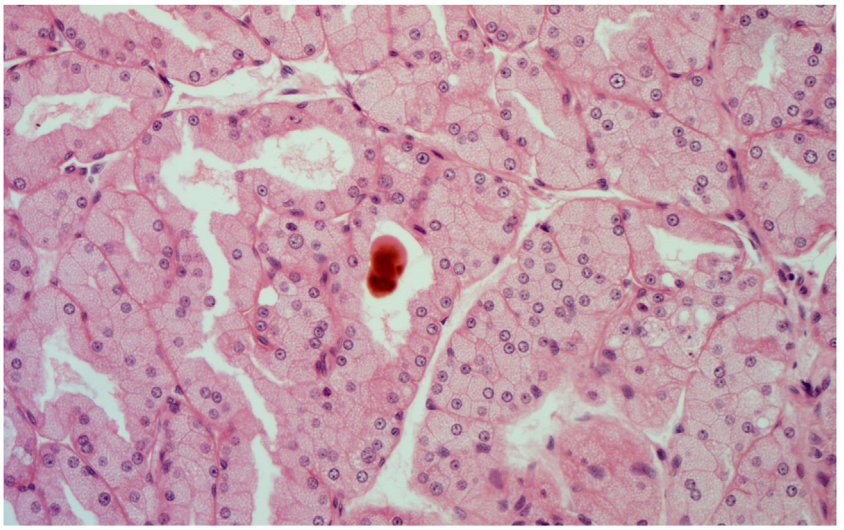


FIGURE 15.7 Harderian gland with a small porphyrin concretion in the lumen. The number and size of these tend to increase with age.

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the remainder. The lumina of the tubules contain occasional lamellated concretions that appear brown in H & E stained sections: see above. These concretions are rare in young animals but appear with increasing frequency as age advances. The walls of the secretory tubules are separated only by a thin layer of fine connective tissue, so epithelial cells often appear 'back to back'. Fenestrated capillaries and the usual cells of loose connective tissue, including mast cells and plasma cells, can be found in the connective tissue of the gland.

Electron microscopy of the secretory cells shows rather short apical microvilli, junctional complexes between the lateral cell membranes, and an infolded basal cell membrane; though this is not a marked feature of these cells. There are large basal nuclei, and plentiful cytoplasmic smooth endoplasmic reticulum; a characteristic of lipid producing cells. Unusually, the majority of lipid secretion appears to be by exocytosis, a merocrine process (in sebaceous glands, the mechanism is holocrine and in the mammary gland, it is apocrine), but the presence of occasional nuclei and other remnants of cells in the secretions suggests that some holocrine secretion takes place (Djeridane, 1994).

Myoepithelial cells are wrapped around the secretory tubules. These cells have elongated nuclei and their cytoplasm contains many bundles of microtubules. Myoepithelial cells, which are also found in the salivary and lacrimal glands but not the exocrine pancreas, expel the contents of the secretory tubules.

The Excretory Duct

The secretory elements of the gland discharge into a central tubule that is connected to an excretory duct, which has a sinuous part leading to an ampulla from which the secretion discharges to the anterior surface of the nictitating membrane. The excretory duct is lined by a stratified cuboidal epithelium in which two cell types can be identified at EM: C1 and C2 cells. Both contain membrane-bound granules and cytoplasm rich in a granular endoplasmic reticulum and probably secrete peptides. At the opening of the duct, the lining epithelium is stratified squamous and occasional mucous producing cells are also seen in the ductal epithelium (Djeridane, 1994).

SALIVARY GLANDS

As befits an animal with a mainly dry diet, the rat has large salivary glands. The major salivary glands are easily found at dissection, situated as they are beneath the skin and superficial fascia medial and posterior to the root of the auricle (external ear) on the ventral aspect of the neck: see Fig. 15.8.

There are three major glands: parotid, submandibular (which used to be called the submaxillary gland and is sometimes called the mandibular gland) and the major (greater) sublingual gland. Accessory glands are present in the floor of the mouth and the posterior part of the tongue. Collections of glandular tissue are also found in the substance of the soft palate (Cleaton-Jones, 1971) and elsewhere in the lining of the mouth. With the exception of the sublingual gland the major salivary glands are incompletely developed at birth.

General and Topographical Anatomy

Major Salivary Glands

The major salivary glands are easily identified when dissecting the ventral aspect of the neck and lower jaw. A midline incision allows reflection of the skin; the superficial fascia should then be removed. The key landmarks are the roots of the auricles (external ears) and the large masseter muscles that wrap round the inferior margins of the mandible. The arrangement of the glands is bilaterally symmetrical.

Parotid Gland

The parotid gland is a serous gland, pinkish in colour, divided into several lobes further subdivided into lobules, which can be seen as small discrete areas subdividing the surface of the gland. This subdivided surface allows the gland to be distinguished from the submandibular and major sublingual glands, which have a much smoother surface. The parotid gland forms an arc around the lateral edge of the submandibular gland, with part of the gland lying deep to the submandibular gland, the major sublingual gland, and the chain of four or five lymph nodes that lie rostral to and to the lateral side of the submandibular gland: see Fig. 15.8. The antero-lateral aspect of the parotid gland is in contact with extraorbital lacrimal gland, see above. The duct of the parotid gland (Stensen's duct: Stenson's duct is often given in textbooks but this is incorrect) arises from three or so smaller ducts that drain the lobes of the gland before running forwards on the surface of the masseter and turning inwards to enter the mouth just in front of the anterior edge of the muscle.

Submandibular Gland

The submandibular gland is a large, pale, oval gland that lies in the neck, mostly caudal to a line taken through the roots of the auricles. Each gland measures about $16 \times 12-15 \times 5$ mm: sufficiently large to bring the glands close together in the midline (Hebel and Stromberg, 1976). The gland is divided into four or five large lobes that can easily be distinguished, but what might seem to

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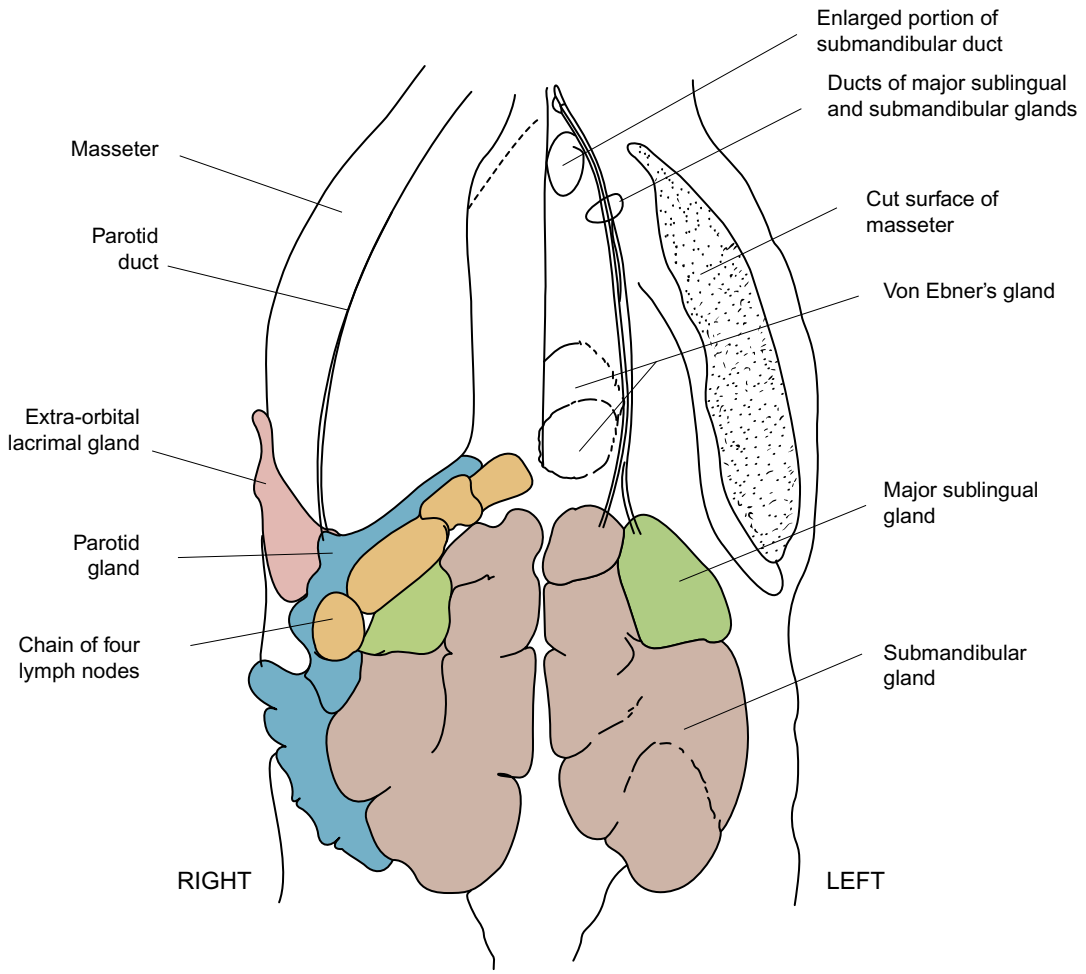


FIGURE 15.8 The salivary glands of the rat, from left to right the submandibular, the sublingual and the parotid.

be antero-lateral lobe is, in fact, the major sublingual gland. The ducts of the submandibular and major sublingual glands run deep to the mylohyoid muscle that forms the floor of the mouth. Once this muscle is removed the ducts can be seen running forward beneath the inferior surface of the tongue. The two ducts (on each side) run forwards and enter the floor of the mouth beneath the tongue, where they open on to the surface of the caruncle, a small mass of fleshy tissue. The submandibular duct (Wharton's duct) has a medial diverticulum just before its opening, presumably for storage of a small amount of saliva.

Major or Greater Sublingual Gland

The major sublingual gland is slightly darker than the submandibular gland. It lies antero-laterally on the ventral surface of the submandibular gland. It is important to distinguish this gland from the adjacent lymph nodes of similar size. Blocks of tissue taken for histological examination often contain lymph node as well as salivary gland: distinguishing these tissues by light microscopy is straightforward. For those who like eponyms, the duct of the major sublingual gland is called the duct of Bartholin or Rivini.

Minor Salivary Glands

Lesser or Minor Sublingual Gland

This gland measures $7 \times 3-4$ mm (Hebel and Stromberg, 1976) and lies laterally, between the mylohyoid and the intrinsic muscles of the tongue. It has a number of small ducts that open alongside the tongue in the floor of the mouth (the ducts of Walther in old fashioned human anatomy).

Glands of the Posterior Part of the Tongue

Two glands have been described, the serous glands of von Ebner that discharge around the circumvallate papilla, and mucous glands of Weber that discharge just in front of

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the epiglottis. The reader may be wondering how many more eponyms there are! The answer is that there are a few but eponyms are out of fashion and we shall not mention many more.

Blood and Lymphatic Supply of the Major Salivary Glands

All the major salivary glands are supplied by branches of the external carotid artery. The parotid gland is supplied by the posterior auricular artery and the superficial cervical artery, a branch of the cervical trunk; and the submandibular and major sublingual glands by glandular branches of the external carotid artery (Greene, 1935).

The venous drainage of the glands is likely to be of minor interest. Lymph from the salivary glands drains to adjacent lymph nodes: see Chapter 9, The Lymphatic System.

Innervation of the Major Salivary Glands

The salivary glands are innervated by the sympathetic and parasympathetic systems. Postganglionic sympathetic fibres reach the glands in the company of their arterial supply, but the parasympathetic supply is more complicated. The salivary (or salivatory) nuclei of the brain stem send preganglionic parasympathetic fibres to the facial (CN VII), glossopharyngeal (CN IX) and vagus (CN X) nerves. Preganglionic fibres leave the VII nerve on its course through the temporal bone to form both the chorda tympani and the greater superficial petrosal nerve. The chorda tympani journeys through the wall of the middle ear, leaves the temporal bone, and joins the inferior alveolar nerve a branch of the mandibular nerve (CN V3). The parasympathetic fibres leave this nerve to run to the submandibular ganglion where most of them synapse. Postganglionic fibres continue to the submandibular and major sublingual glands. Those fibres that depart via the greater superficial petrosal nerve leave the temporal bone, join with sympathetic fibres to form the nerve of the pterygoid canal, and run to the sphenopalatine ganglion from where fibres run to the glands of the soft palate (see Chapter 5, The Skull). Preganglionic fibres of the IX nerve leave that nerve as the tympanic nerve, enter the temporal bone and form the lesser superficial petrosal nerve. This tiny nerve leaves the temporal bone and finds its way to the otic ganglion, close to the parotid gland. There the fibres synapse, and postganglionic fibres run to the gland via the auriculo-temporal nerve, another branch of the mandibular nerve (CN V3). See Chapters 21(1) and 21(3) for further discussion.

Histology

The major salivary glands of the rat are compound tubulo-acinar glands that are surrounded by a connective tissue capsule, from which septa of connective tissue extend into the gland dividing lobe from lobe, lobule from lobule and acinus from acinus. The scant interacinar connective tissue contains the capillaries (blood and lymph) and nerve fibres that supply the acinar cells (Fig. 15.9).

The large lobar ducts that are surrounded by connective tissue containing the major blood vessels of the gland branch into interlobular and intralobular ducts. The intralobular ducts continue to branch, eventually becoming intercalated ducts with diameters less than those of the acini they drain. Several generations of intercalated ducts can be seen in the parotid and submandibular glands, rather fewer in the major sublingual gland. Segments of intralobular ducts show striations in the cytoplasm of their cuboidal epithelial cells: these are the striated ducts, and again are more often seen in the parotid and submandibular glands than in the major sublingual gland.

Acinar cells lie on a basement membrane that is visible at light microscopy. Myoepithelial cells are wrapped around the outer surfaces of the acini and the first parts of the intercalated ducts of all major salivary glands. These cells cannot easily be seen with a light microscope, but immuno-histochemical staining for smooth muscle

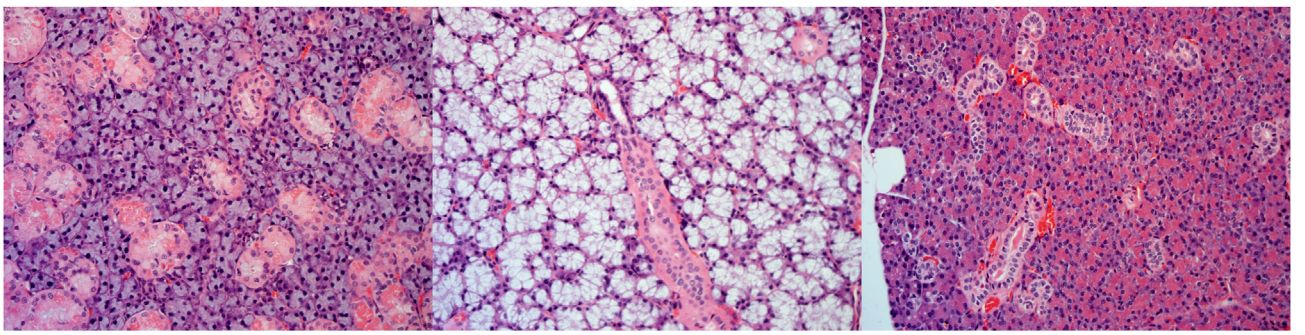


FIGURE 15.9 Composite of salivary glands. From left to right submandibular, sublingual and parotid.

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myosin allows them to be visualised. In H&E sections, the best that can be said is that some of the flattened, dark nuclei applied to the outer edges of transections of acini probably belong to myoepithelial cells.

Serous and Mucous Secretions

The acinar cells, the acini themselves, and sometime the glands, are divided into serous and mucous types. Serous cells produce a low viscosity, protein-rich secretion; mucous cells produce mucin. In H&E stained sections, the cytoplasm of serous acini stain a pink-purple colour, mucous acini stain much palely and more evenly. Specific stains for mucin, such as PAS or Alcian blue, stain the mucins of the mucous cells: reddish pink in the former case, pale blue in the latter. This is not always a clear distinction, as material that stains positively with PAS can often be found in what appear to be serous cells. The term sero-mucous is then appropriate.

The cytoplasm of serous cells is rich in granular endoplasmic reticulum and eosinophilic granules can be seen towards the apices of the cells. Attached and unattached ribosomes stain blue-purple with H&E, giving the cytoplasm its distinctive colour. In H&E stained sections, the basal cytoplasm of serous cells often stains a deeper pink than the apical cytoplasm, and the infolding of the basal cell membrane combined with the presence of many mitochondria may give a striated appearance. The nuclei of serous cells are round or oval, and lie in the lower halves of the cells. In mucous cells, the nuclei are dark staining and flattened against the basal cell membrane as though they has been pressed down by the large vesicles of mucin that fill the cells before discharge. At light microscopy, the apical surface of the cells often appears as a reddish line in H&E stained sections, but apical microvilli and intercellular canaliculi, lined with microvilli and running between the serous cells, can be seen with the electron microscope.

Ducts

Small ducts are lined by a single layer of cuboidal epithelium, but in larger ducts several layers may be present. Glands producing serous secretions are characterised by intralobular ducts with striated segments. The basal cytoplasm of the cuboidal cells of these ducts shows clear striations, caused by infolding of the basement membrane. Striated ducts play an important role in adjusting the composition of the secretions produced by serous acini. Sodium ions are absorbed from, and potassium ions added to, the secretion. The capacity to remove sodium ions depends on the rate of flow of secretion. At high flow rates, the sodium concentration of saliva rises, presumably because the time available for absorption of sodium ions is reduced.

Parotid Gland

The acini are arranged at the ends of a well-developed ductal system and many sections through intercalated ducts can be seen. Striated ducts are prominent.

Submandibular Gland

This is a mixed gland in man: both largely serous and largely mucous acini can be identified and serous demilunes capping mucous acini can be seen: see below. Amano et al. (2012) described the rat submandibular gland as a serous gland; Tucker (2007) described it as a mucous gland. Sero-mucous might be an appropriate term!

The most striking histological feature of the rat submandibular gland is the presence of many cross-sections of the intralobular ducts lined with an epithelium that stains very brightly with eosin. This is an androgen dependent feature that is, as would be expected, more striking in male rats, and unique to the submandibular gland. Hatakeyama et al. (1987) reported a greater number of cells producing acidic glycoproteins (Alcian blue, pH 2.5, positive) in the submandibular glands of male rats than in females. The cells of the granular ducts can be divided into two types: the principal cells that contain masses of eosinophilic granules, with the pillar cells appearing to be wedged in between them.

Pillar cells produce Fibroblast Growth Factor (FGF2). The principal cells produce a range of polypeptides and growth factors (Amano et al., 2012). Jacoby and Leeson (1959) provided a superb account of the development of the rat submandibular gland that focuses on the development of the granulated ducts: an example of classical histology at its best.

Major Sublingual Gland

The rat sublingual gland has fewer striated ducts than the other major salivary glands and contains mainly mucous acini, with collections of serous cells appearing as crescents at the edges of the mucous acini. These crescents have long been known as the crescents or demilunes of Gianuzzi (or of von Ebner or Heidenhain!), and are also seen in the human submandibular gland. It has been believed that the secretions of the serous cells flow between the mucous cells in intercellular canaliculi, and enter the mucous acini. Most textbooks of histology produce pictures of the demilunes. However, Yamashina et al. (1999) have shown by careful reconstruction of serial sections of the rat sublingual gland, that the demilunes are 'artefacts... produced by conventional fixation'; the mucous cells were distended by conventional fixatives

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and this distension displaced the serous cells from their positions between the mucous cells and forced them into crescents at the edges of the acini.

Development of the Salivary Glands

Salivary glands develop from the lining of the mouth, as solid buds of epithelial cells that grow down from surface epithelium. The buds extend to form solid cords that branch as they interact with the underlying mesenchymal tissue.

Three phases of development are defined. In the pseudoglandular phase, cords of cells and the expanded endings will become acini. Lumina appear in the canalicular phase, and in the final terminal bud stage, the acini develop into their adult form.

More work seems to have been undertaken on the development of the submandibular gland than other salivary glands. Jacoby and Leeson (1959) showed that at birth no acini were present, and the terminal units of the gland were tubular rather than acinar. Acinar development was found to be completed at 6 weeks, but the granular tubules were not fully developed at 4 months. Sexual dimorphism in the appearance of the granular ducts was not seen until 7 weeks after birth. Differentiation of the acini of the parotid gland of the rat also occurs after birth (Brown et al., 2016). The rat sublingual gland is better developed at birth than the other major glands: acini with serous demilunes are reportedly present at birth (Redman and Ball, 1978; Wolff et al., 2002).

Effects of Aging on the Rat Salivary Glands

Age-related changes are not a particularly dramatic feature in the salivary glands. Squamous metaplasia of duct epithelia and peri-ductal lymphocytic infiltration with decreased consolidated material in ducts have been reported in the rat sublingual gland at 24 months, and atrophy of the parotid glands occurs in less than 5% of rats. In affected rats ducts become dilated, and interacinar connective tissue increased with a 'mild mononuclear inflammatory response' (Mintz and Mooradian, 1987; Burek, 1978)

EXOCRINE PANCREAS

The exocrine part of the pancreas is a compound tubuloacinar gland. At light microscopy, there are similarities to the parotid gland, but the pancreas can, of course, be distinguished from the parotid by the presence of the islets of Langerhans. It also differs from the parotid in terms of the arrangement and structure of its ducts. Most textbook accounts of the pancreas treat the exocrine and endocrine functions of the organ as separate subjects. This view of no longer sustainable, as much of the evidence shows that the functions of the acini are affected by the secretions of the islets, and the concept of the insulo-acinar axis is now well established (Pandiri, 2014). For details of a number of aspects of the structure and function of the exocrine pancreas, the reader is referred to a group of reviews that were published in Microscopy Research and Technique in 1997. These provide an excellent starting point for further exploration of the literature. In particular, Motta et al. (general histology of the pancreas), Egerbacher and Böck (the duct system), Böck et al (development), Wayland (microcirculation) and O'Morchoe (lymphatic system) might be consulted: see Further Reading. The interrelationships of the exocrine and endocrine pancreas have been reviewed by Bertelli and Bendayan (2005). A large number of important papers on the ultrastructure of the pancreas have been published by Japanese authors, with Takahashi's paper on the ultrastructure as revealed by scanning electron microscopy is of outstanding interest. The monographs edited by Pour (2005) and by Foster (2009) provide comprehensive accounts. As always species differences may present a problem, but much of the work in the papers and reviews mentioned earlier has been done in the rat.

General and Topographical Anatomy

In man, the pancreas is described as a retro-peritoneal structure plastered, so to speak, to the posterior abdominal wall and covered by peritoneum on its anterior surface. In the rat, the gland is described as lying in the mesentery of the duodenum. It extends behind the stomach to reach the hilus of the spleen.

For descriptive purpose, the rat pancreas is divided into duodenal, gastric and splenic lobes. The splenic lobe, or tail, is a compact structure; the part lying close to the duodenum is rather more diffuse. The major ducts leaving the duodenal, gastric and splenic lobes combine and join the common bile duct before it enters the duodenum. This is a very different arrangement from that found in man. It might be noted that in the guinea pig, the pancreatic duct and the common bile duct enter the duodenum quite separately. Boyden (1965) has provided a discussion of the comparative anatomy of the entrance of the bile and pancreatic ducts into the duodenum. His account should be consulted for the many interspecies variations that have been recorded. It is chastening to note his comment that only the horse has an arrangement similar to that of man.

In man, the superior mesenteric artery and portal vein pass between parts of the pancreas to cross the anterior surface of the duodenum. In the rat, the pancreas receives its blood supply from the pancreaticoduodenal artery and the anterior mesenteric artery, which divides into a number of branches. These branches that include the arteries to the small intestine and the right side of the colon, cross the ventral surface of the duodenum (Hebel and Stromberg, 1976). Venous blood from the pancreas drains to the portal system.

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The lymphatic vessels of the pancreas drain to the posterior gastric lymph nodes (see Chapter 9: The Lymphatic System). A detailed description of the lymphatic system by O'Morchoe (1997) highlights its role in removing enzymes that leak from acinar tissue into the intercellular space. Blockage of the lymphatic drainage leads, rapidly, to breakdown of pancreatic tissue.

Development

The pancreas of the rat begins around the 11th day of gestation. Diverticuli appear on the dorsal and ventral aspects of the duodenum and extend into the mesentery as solid epithelial cords before branching (Pictet et al., 1972). The cords develop lumina, which first appear near the origin of the cords, and then extend outwards towards what will become the acini. The acini develop as expansions of the distal ends of the branches.

The islets also develop from the epithelial cords, and although most lose their connection with the anlagen of the duct system, they remain close to the acini. Small collections of endocrine cells may remain in the walls of the ducts, and single endocrine cells lying among the duct epithelium are well recognised. As in any organ that develops by branching, a complex interaction between epithelium and local mesenchyme is necessary for branching to occur in a normal pattern.

Histology

The lobes of the gland are drained by lobar ducts. Interlobular ducts run in the connective tissue septa between the lobules, collecting secretions from the intralobular ducts, which lie within the lobules. The majority of the intralobular ducts are narrow intercalated ducts, so fewer transections of large intralobular ducts are seen in the pancreas than in the parotid gland. No striated intralobular ducts are found in the pancreas. The connective tissue that surrounds the larger ducts is linked with the rather unimpressive connective tissue capsule of the lobes of the gland. Blood vessels, lymphatic vessels and nerves run in the connective tissue of the gland, which extends to the periacinar tissue. Here the connective tissue is little more than a network of fine collagen and reticulin fibres. Capillaries run in the interstices of this network, but do not penetrate the acini. The periacinar capillaries are non-fenestrated in contrast with those that penetrate the islets of Langerhans. The capillaries of the islets drain to venules, a proportion of which drain to the capillary network that surrounds the exocrine acini and the smaller intercalated ducts. These venules form a small portal venous system: a venous vessel with capillaries at both ends. No myoepithelial cells surround the acini, though stellate cells that may be a type of fibroblast have been described.

Acini

Acinar cells with wide bases and narrow apical surfaces rest on a basement membrane. Nuclei are rounded and lie rather below the midpoint of the cells. A pale area just above the nucleus reflects the position of the Golgi apparatus. Occasional binucleated cells may be seen. The cytoplasm contains membrane-bound zymogen granules of about 0.3−0.5 μm diameter in their supranuclear and apical cytoplasm. These granules, which stain bright pink in H&E sections, are released into the lumina of the acini by exocytosis. Cytoplasm below the nuclei stains purple due to the presence of plentiful granular endoplasmic reticulum and free ribosomes. Junctional complexes are found between the lateral margins of the cells, but do not seem to completely surround the cells. Intercellular canaliculi run between the cells of the acini. It has been suggested that these canaliculi are linked with a basal labyrinth

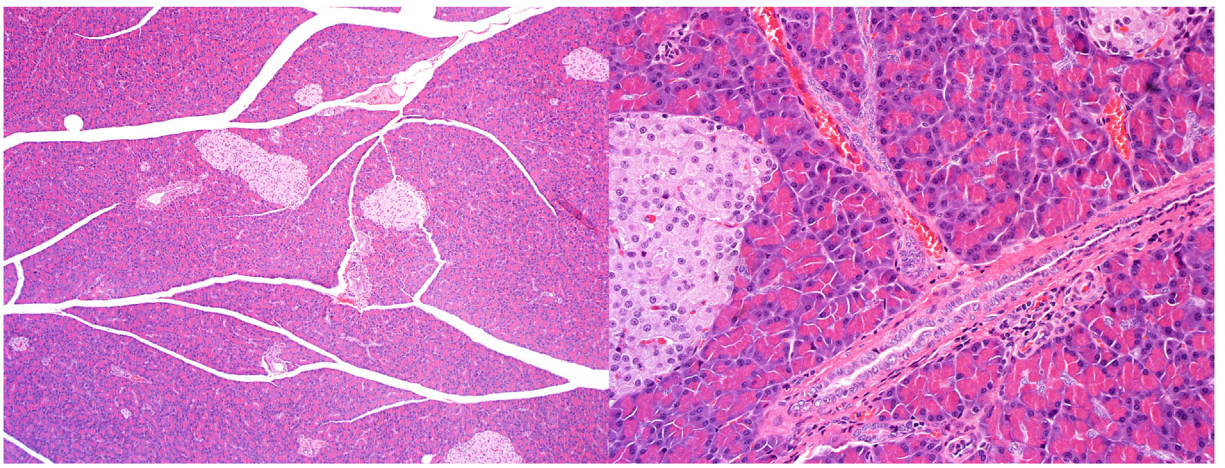


FIGURE 15.10 High and low power micrographs of pancreas. Note the narrow intra-lobular ducts draining into the larger interlobular duct.

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between the cells and the basement membrane (Fig. 15.10).

Ducts

The long branching intercalated ducts of the pancreas that run among the acini are less than half the diameter of an average acinus. The ducts are lined by a flattened epithelium that invaginates into the acini and provides the centro-acinar cells. The epithelial cells of the intercalated ducts can secrete water and bicarbonate ions, although in the rat this role is largely performed by the epithelium of the interlobular ducts. The cells of the intercalated ducts have been the focus of much attention for it is thought they might act as stem cells and progenitor cells for both islet and acinar tissue (Cleveland et al., 2012).

The columnar lining cells of the larger ducts are divided into principal cells (the great majority), goblet cells, basal cells, brush cells, argentaffin cells and endocrine cells, which produce all the hormones of the islets, but mainly insulin. Hormone production by duct cells and by the collections of endocrine cells sometimes seen along the ducts has not been thought to be important, but might play a role in controlling the functioning of gut epithelium. It has been noted that epithelial cells of the intestine express insulin receptors on their luminal surfaces (Buts et al., 1997; Pillion et al., 1985). Interest in exocrine secretion of hormones is expanding rapidly (Bertelli and Bendayan, 2005).

The lack of striated ducts in the pancreas might imply that absorption from the contents of the ducts is not as important in the pancreas as it is in the serous salivary glands. Interlobular ducts lack smooth muscle; the lining epithelium is surrounded by a layer of connective tissue. Towards the junction of the major ducts with the common bile duct a type of smooth muscle myosin, which may be produced by myofibroblasts, can be demonstrated.

MAMMARY GLAND

Gross Anatomy

The following account is based on that provided by Hebel and Stromberg (1976). The mammary gland is a diffuse subcutaneous gland that can be divided into six mammary complexes: three in the thoracic region (divided by Astwood et al. (1937) into one cervical and two thoracic groups), one in the abdominal region and two in the inguinal regions. The glandular tissue lies in the subcutis, the layer of adipose tissue immediately subjacent to the dermis to which the glandular tissue is attached. The cutaneous muscle (panniculus carnosus) lies between the gland and the surface, but in some areas may divide the glandular tissue into two layers. The glandular tissue proliferates during pregnancy in readiness for lactation, and at its greatest extends from the level of the parotid gland anteriorly to the medial aspects of the thighs posteriorly.

A total of 12 teats or nipples are found, 6 on the left and 6 on the right, following lines that curve towards the midline in the abdominal region, but drifting away from the midline both anteriorly and posteriorly. Each nipple is about 2 mm in length, increasing to 4 mm during lactation. The increase in length is due to the eversion of the usually inverted ring of epithelial tissue at the base of the nipple. Lactiferous ducts open into a sinus below the nipple. This sinus (the gland sinus) is linked with a second sinus within the nipple (teat sinus) that is connected to the surface by the papillary duct. No nipples develop in the male, though glandular tissue is found in the subcutaneous tissue.

Early Development

The mammary gland develops from islands of epithelium that thicken into clumps of cells resting on a basal membrane. The epithelial cells between these epithelial clumps atrophy and throughout foetal and early postnatal life the mammary glands remain as rudimentary buds of epithelium (Knight and Peaker, 1982). The development of the mammary gland is controlled by a number of hormones (Topper and Freeman, 1980). In the male, a testosterone induced condensation of the stroma promotes atrophy of the rudimentary buds, thus creating the male phenotype (Goldman et al., 1976). The male mammary gland morphology in the male rat is feminised in the absence of androgens, and androgens administered to female rat foetuses will cause virilisation (Goldman et al., 1976).

The roles of oestrogens and progesterone are less clear. Progesterone does not appear critical to embryonic development of mammary glands, and its absence in utero does not affect the potential for maturation in adulthood (Freeman and Topper, 1978). Although oestrogens promote mammary gland development in the mouse foetus, the opposite occurs in the rat foetus (Ceriani, 1970). The pituitary gland appears to be critical to mammary development with prepubescent female mammary growth being driven mainly by growth hormone and prolactin (Knight and Peaker, 1982). Normal mammary growth in both sexes is also dependent upon normal gonadal function, as demonstrated by the absence of development in ovariectomised or gonadectomised rats (Cowie and Folley, 1961).

To our knowledge, a detailed description of pubescent mammary gland development in males has not been published. In females, growth of the mammary gland during puberty is characterised by growth hormone driven differentiation of the epithelium into terminal end-bud units, followed by rapid expansion by elongation and branching of the ducts and hypertrophy of the fat pad (Cowie and Folley, 1961; Russo et al., 1989; Knight and Peaker,

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1982). The mammary fat pad surrounded surrounds the mammary tissue and contains a mixture of adipocytes, preadipocytes and fibroblasts (Hovey et al., 1999).

Sampling Mammary Gland

The neonatal rat has six paired of glands distributed along the milk line, one cervical pair, two thoracic, one abdominal and two inguinal (Astwood et al., 1937). A central lactiferous duct branches into secondary and tertiary ducts.

Sound scientific evaluation of the rat mammary gland requires a systematic approach to study design and sampling and processing of the mammary gland for histology, because the morphologic characteristics of the rat mammary gland are both site and age-specific. The investigator should use rats that are sexually mature at the start of the study, and collect the same mammary gland pair from each animal at necropsy. For regulatory studies most laboratories sample the inguinal mammary gland pair, which is collected with the overlying skin. Consistent sectioning is essential in the histological evaluation of the rat mammary gland, especially when it comes to evaluating atrophy and any subtle differences in histological structure.

Sex Differences in the Rat Mammary Gland

In most preclinical species, the appearance of the mammary tissue is broadly comparable between the male and nonlactating female, although there will generally be more tissue present in the females. In the rat there is significant sexual dimorphism. Although the morphological differences between female and male mammary gland morphology were first noted over 50 years ago (Ahren and Etienne, 1957), the significance of these differences were largely ignored in the literature until a review by Cardy (1991) and a subsequent publication by Rudmann et al. (2005).

Virgin female rat mammary gland has a basophilic tubular appearance with occasional branched tubular ducts which vary at different stages of the oestrous cycle. If the stage of the female cycle is not considered, it is possible that the variation could be interpreted as a treatment-related change (Lotz and Krause, 1978). Ducts, ductules and alveoli are lined by a one to two layers of cuboidal epithelial cells surrounded by myoepithelial cells, all resting on a basement membrane (Cardy, 1991; Masso-Welch et al., 2000). Mitoses and apoptotic cells are not observed. The alveolar buds appear as clusters of tubules with a central lumen. Terminal ducts are small and finger-shaped with thin walls and wide lumina (Fig. 15.11).

In the male, the mammary gland has an eosinophilic lobulo-alveolar appearance that is really quite florid in younger animals. The eosinophilic lobules of alveoli have few ducts, but as the animals age small areas of tubule and acini adjacent to the main bulk of the tissue take on a more female-like appearance. Both ducts and alveoli are lined by a pseudostratified or stratified epithelium. Cells are larger with abundant eosinophilic cytoplasm that is usually vacuolated (Cardy, 1991; Rudmann et al., 2005). Apoptotic cells are observed in the epithelial lining as well as in alveolar and ductal lumina. In many cases, alveolar and ductal epithelial cells fill the lumina of the ducts of the male gland.

The Endocrine Environment and Mammary Tissue

It is not just age that affects the appearance of the mammary tissue: the male gland is in particular is sensitive to changes indicative of endocrine disruption. The nature of the morphologic changes in the mammary gland can provide clues to the specific changes in the hormonal milieu created by a given xenobiotic. Oestrogens cause

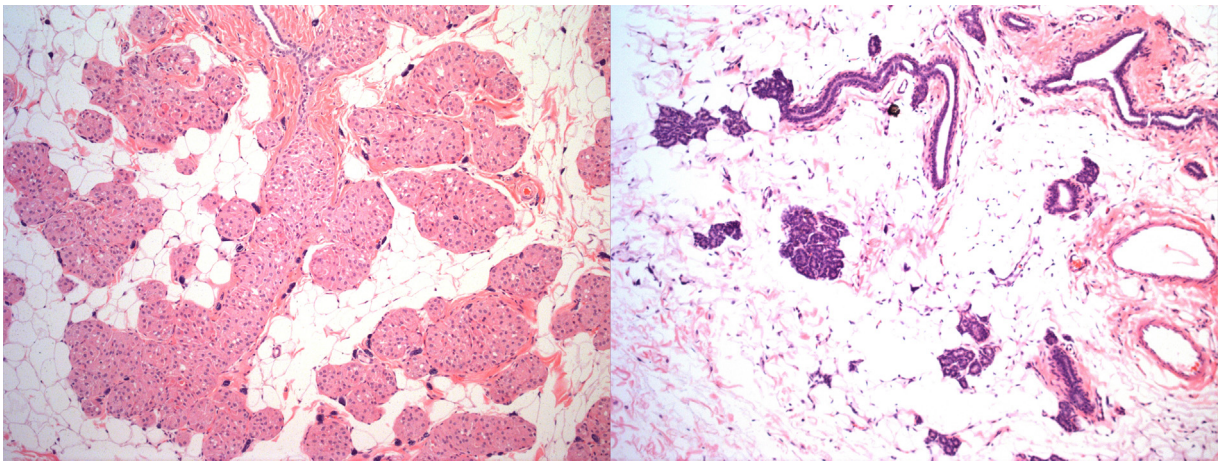


FIGURE 15.11 Sexual dimorphism in the rat mammary gland. Male tissue to the left and female to the right.

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feminisation of male rat mammary glands, and there may even be evidence of milk secretion in the ducts and alveoli of the feminised gland. Female rats typically present with multifocal or diffuse lobulo-alveolar hyperplasia (Biegel et al., 1998). In contrast, hypoestrogenism or oestrogen receptor antagonism in females causes atrophy of the both the ductular and alveolar components, and acinar atrophy in males. In females the epithelial cells of affected ducts and alveoli have a shrunken appearance with high nuclear to cytoplasmic ratios.

Unsurprisingly, the effects of testosterone and other androgen receptor agonists tend to have the opposite effect. Although effects on the male mammary tissue are not apparent, the female mammary tissue begins to take on the appearance of the male mammary tissue (Selye et al., 1936). However, these changes are not as simple as they might first appear, as hypophysectomy prevents the mammary gland changes, suggesting that pituitary hormones are probably necessary to mediate the effect (Laqueur and Fluhmann, 1942).

Prolactin is synthesised not only by the pituitary gland, but also at a number of extrapituitary sites, including the mammary gland (Ben-Jonathon et al., 1996). Pituitary prolactin promotes the alveolar development and initiation of milk production in pregnancy, which is thought to be sustained by the prolactin produced in the mammary tissue itself. (Lkhider et al., 1997). Prolactin mediated effects have been of particular interest to the toxicologist because of elevation of prolactin caused by dopaminergic receptor antagonists, a class of drugs that have been developed as antidepressants (Lotz and Krausse 1978; O'Connor et al., 1996). Prolactin effects typically result in feminisation in male rats accompanied by secretory activity in both sexes. This can lead to quite spectacular duct ectasia (Cardy, 1991). Very similar effects are seen in rats that are exposed to higher concentrations of growth hormone, which is thought to be a result of prolactin-like activity of growth hormone in the rat (Peckham et al., 1968).

Progesterone has important effects on prepubescent development as well as in pregnancy, acting in concert with other hormones to stimulate ductal development (Kamiya et al., 1998). Effects of blocking progesterone activity are comparatively few, but in females appear to be related to increases in serum prolactin secondary to pituitary effects.

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Chapter 16

Endocrine Glands

PITUITARY GLAND

The pituitary gland is found in all vertebrates and is perhaps the most interesting, certainly the most complicated, of all endocrine glands. The gland develops from two very different sources of ectoderm. The adenohypophysis develops from Rathke's pit, an evagination of ectoderm from the roof of the oral cavity. The neurohypophysis develops from the floor of the diencephalon, and may be considered as an extension of the hypothalamus. The hypothalamus plays an essential part in pituitary function as it controls the secretion of hormones from the adenohypophysis, and produces the secretions of the neurohypophysis.

Gross and Topographical Anatomy of the Pituitary Gland of the Rat

The pituitary gland lies on the upper surface of the basisphenoid bone just anterior to the joint with the basioccipital bone, occupying a shallow recess in the overlying brain. Above the gland, a thin layer of dura mater fuses with the thin connective tissue capsule of the gland.

The gland is triangular in sagittal section; the flat inferior surface of the gland is in contact with the basisphenoid bone. According to Hebel and Stromberg (1976), the anterior–posterior length is 3.0–3.5 mm, with a width of 4.5–5.5 mm, weighing about 8.9 mg in the male and 15.8 mg in the female. The gland is attached to the hypothalamus by a stalk that runs forward (rostrally) and expands to form the neurohypophysis, which lies in a groove in the upper surface of the adenohypophysis. Between the neurohypophysis and the adenohypophysis is a thin layer of tissue; the pars intermedia, and sometimes a fluid filled cleft. The adenohypophysis makes up about 80% of the volume of the gland; the pars intermedia and the neurohypophysis, each make up about 10% of the total volume. In man the pituitary lies in a cavity bounded anteriorly and posteriorly by upward projections from the dorsal surface of the sphenoid bone. This arrangement looks rather like a saddle; in fact it is called the sella turcica (Turkish saddle). No such structure is found in the rat. Hanström has provided an excellent review off the gross anatomy of the pituitary gland in mammals.

For sectioning, Kittel et al. (2004) recommended cutting across the decalcified skull from which the roof of the cranium and brain had been removed and preparing a block of tissue to include both the pituitary and underlying bone. Transverse sections prepared in this way show the internal carotid arteries that enter the skull and run alongside the gland indenting its infero-lateral aspects.

Nomenclature

The nomenclature of the pituitary gland is complicated by the several systems in use. The equivalence between the various terms used to describe parts of the gland is shown in Table 16.1.

TABLE 16.1 Nomenclature of the Pituitary Gland

Adenohypophysis (derived from oral ectoderm)	Pars distalis	Anterior lobe
	Pars tuberalis	
	Pars intermedia	Posterior lobe
Neuropophysis (derived from brain)	Pars nervosa	
	Infundibulum	Infundibular stem
		Medial eminence of the tuber cinereum

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To avoid confusion it is better, by far, to speak of the adenohypophysis (taken to be equivalent to the pars distalis), the pars intermedia and the neurohypophysis (taken to be equivalent to the pars nervosa), all which can be easily distinguished by light microscopy. The pars tuberalis of the rat is very small and of little importance to toxicologists. It is usually not visible in the standard histological section examined by a toxicological pathologist in a regulatory environment.

HISTOLOGY OF THE PITUITARY GLAND

Adenohypophysis

The parenchyma of the adenohypophysis comprises anatomising cords of cuboidal or polyhedral cells separated by wide-fenestrated sinusoidal capillaries. Routine haematoxylin and eosin staining shows the cells of the adenohypophysis to be divided into those that are clearly acidophilic (cytoplasm staining bright pink), and those that are not, the basophils and the pale staining chromophobes.

The secretory granules of the acidophils stain brightly with eosin, the source of the acidophilia, and basophils can demonstrated by staining with the Periodic Acid Schiff (PAS) technique In addition to a PAS stain, the secretory granules of the basophils stain brightly with aniline blue and are well demonstrated with a Masson's trichrome stain or with the Azan method (Drury and Wallington, 1980). The pale staining chromophobes are believed to represent acidophils and basophils that have discharged their granules, and as such do not stain positively with any of the methods indicated above (Fig. 16.1).

These classical histological techniques are detailed in Purves' exhaustive account of the comparative histology of the tissue (Purves, 1966). Today, immunocytochemical methods allow identification of cell types on the basis of function, and specific antibodies to all the secretory products of the cells are readily available (Lloyd, 1990). Lloyd provides details of the cell types and practical instructions for the avidin-biotin complex peroxidase immunostaining technique. The technique works well on material fixed in formalin and prepared as 4–6 μm paraffin wax sections. Application of these techniques allows for recognition of the cells listed in Table 16.2.

The releasing and inhibitory factors listed in the Table 16.2 are produced in the hypothalamus and carried to the adenohypophysis by the portal circulation of the gland (see below). The hormones of the adenohypophysis that act on endocrine glands, for example ACTH, require no hypothalamic inhibitory factors because negative feedback (inhibition) of the cells of the hypothalamus and pituitary is provided by the products of the target glands. In the cases of prolactin and growth hormone, which do not act specifically on endocrine glands, inhibitory factors are required.

The cells of the adenohypophysis are not randomly arranged, but carefully grouped with cell to cell connections controlling basal levels of secretion, and importantly, the ratio between stimulated or peak levels of secretion and basal levels. Le Tissier's review should be consulted for theories regarding the operation of 'memory' by the secretory cells of the adenohypophysis (Le Tissier et al., 2012).

Folliculostellate Cells of the Adenohypophysis

These stellate cells have long filamentous cytoplasmic processes somewhat similar to those of astrocytes, and are found throughout the adenohypophysis. The cytoplasmic processes extend between, and come into contact with, the secretory cells of the tissue. The term 'folliculo' refers

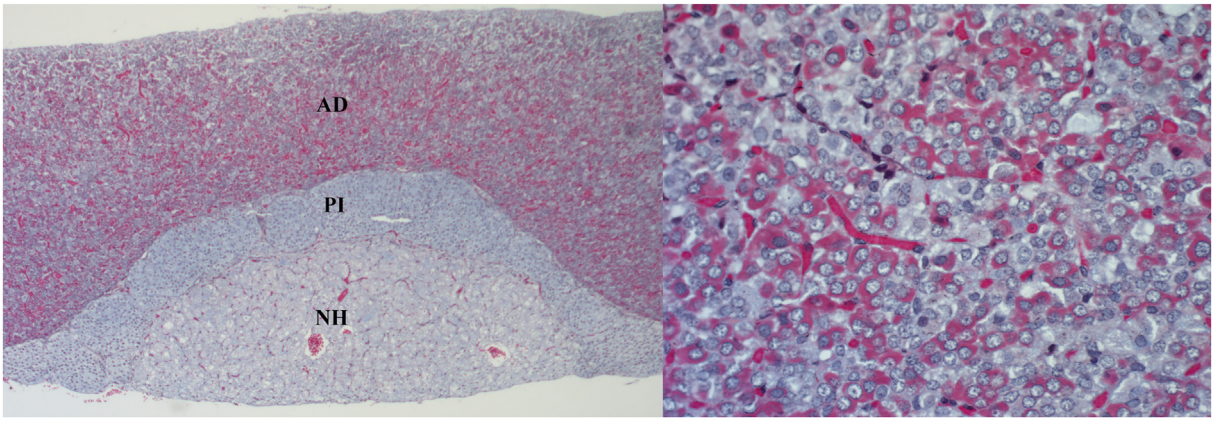


FIGURE 16.1 Pituitary gland (Masson's trichrome stain). Low power on the left. AD, adenohypophysis; PI, pars intermedia; NH, neurohypophysis. High power view showing acidophils and basophils.

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TABLE 16.2 Cells of the Adenohypophysis

Cell Type	Hormone(s)	Old Nomenclature	Releasing Factor	Inhibitory Factor	% of Cells in Male Rat	% of Cells in Female Rat
Mammotropic	Prolactin (PRL)	Acidophil	Vasoactive intestinal polypeptide (VIP)	Dopamine	17–52	25–50
Somatotropic	Growth hormone (GH)	Acidophil	Growth hormone releasing hormone (GHRH)	Somatotropic hormone inhibitory factor (SRIF) also known as somatostatin	20–35	28–35
Adrenocorticotropic	Adrenocorticotropic hormone (ACTH)	Basophil	Corticotropic hormone releasing hormone (CRH)		2–9	2–9
Thyrotropic	Thyroid stimulating hormone (TS)	Basophil	Thyrotropin releasing hormone (TRH) also stimulates prolactin producing cells		2–4	2–4
Gonadotropic	Luteinizing hormone (LH) in female, interstitial cell stimulating hormone (ICSH) in male	Basophil	Luteinizing hormone releasing hormone (LHRH)		LH: 5–11	ICSH: 5–9
	Follicle stimulating hormone (FSH)		Also known as Gonadotropin releasing hormone (GnRH)		FSH: 4–8	FSH: 4–12

Note: The terms tropic and trophic have both been used with reference to the hormones of the pituitary gland, for example, adrenocorticotropic or adrenocorticotrophic.

to the tendency of these cells to form follicles, at least in some species. These cells form an 'excitable network' with signals apparently transmitted via gap junctions between contiguous cells, and express receptors for hormones produced by the secretory cells (Fauquier et al., 2001). It seems likely that the folliculostellate cells coordinate the activity of the secretory cells via a feedback loop (Brokken et al., 2004).

Pars Intermedia

Species differences in the structure of the pars intermedia are marked, which in the rat consists of a thin layer of weakly basophilic cells. In transverse sections, it appears to line the trough in the adenohypophysis that supports the neurohypophysis. Howe and Maxwell's electron microscopy (EM) studies (1968) indicated 12 layers of cells to be present, but a light microscopy publication by Wingstrand (1966) showed only 4 layers of cells (also rat tissue). The surface in contact with the cleft that separates the pars intermedia from the adenohypophysis is arranged as a squamous or low cuboidal epithelium. The vascularity of this part of the pituitary gland is generally accepted to be low. Hypothalamic noradrenergic stimulation of the pars intermedia results in production of melatonin and beta endorphin.

Neuropophysis

The neurohypophysis has an entirely different structure to the adenohypophysis and pars intermedia. The neurohypophysis comprises a mass of unmyelinated nerve fibres

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that run into the gland from the supraoptic and paraventricular nuclei of the hypothalamus. These nerve fibres carry the neuronal secretory products, vasopressin/antidiuretic hormone (ADH) and oxytocin, which are released into the fenestrated sinusoidal capillaries of the neurohypophysis. The nerve fibres have periodic expansions known as Herring bodies along their length, which act as storage sites for the secretory products that are transported along the axons by means of the microtubule-based axonal transport system (Fawcett, 1994).

Blood Supply of the Pituitary Gland

The pituitary gland is supplied by superior and inferior hypophyseal arteries; the former arise from the Circle of Willis (see Chapter 7: The Cardiovascular System), the latter from the internal carotid arteries. The superior hypophyseal arteries supply the stalk of the gland and penetrate the median eminence (the lowest part of the hypothalamus) where they break up to form a primary capillary plexus. Axons from hypothalamic neurones secrete releasing and inhibitory factors that enter these capillaries. Long secondary capillaries or venules run from this plexus down the stalk of the gland and enter the adenohypophysis where they join the plexuses of sinusoidal capillaries around the secretory cells. Venous drainage is to local dural venous sinuses.

Pharyngeal Hypophysis

Tissue remnants from the developing adenohypophysis form the 'pharyngeal hypophysis' in man and other mammals, including the rat. It is not known to have any functional significance. The curious reader will find an excellent account in McGrath (1974).

THYROID GLAND

The thyroid gland has a long and interesting evolutionary history and is named for its shield-like shape in man: thyreos (Greek) = shield shaped.

A capacity for concentrating iodine is found in the mucin producing cells of the endostyle of Amphioxus (a cephalochordate) and the tunicates (Urochordata). Ammocoete larvae of the lampreys, which like Amphioxus feed by means of ciliated grooves in the pharynx, have a pouch in the floor of the pharynx containing ciliated cells, some of which are clearly glandular and secrete iodine containing substances (Romer, 1970; Barrington, 1975 and Gorbman et al., 1983). All vertebrates have a thyroid gland. In some fish, it is not found as a discrete gland, but as glandular tissue scattered alongside the aorta, however in all mammals the thyroid gland is found in the neck, just below the larynx.

Anatomy of the Rat Thyroid Gland

The thyroid gland of the rat is a small, pink and fleshy organ comprising two lobes arranged on either side of the trachea, just caudal to the cricoid cartilage of the larynx. Each lobe measures about 5 mm in length and 3 mm in width, weighing about 15 mg in male rats and about 20 mg in females (Hebel and Stromberg, 1976). The weight of the gland varies, enlarging under cold conditions. An isthmus, sometimes containing glandular tissue, connects the two lateral lobes in about 50% of rats.

The thyroid is surrounded by a thin connective tissue capsule from which septa extend into the parenchyma of the gland. In some species, the septa divide the gland into lobules, but this is not obvious in the rat. Two major arteries, the superior and inferior thyroid arteries, supply the gland (see Chapter 7: The Cardiovascular System). Venous drainage is via the left and right thyroid veins to the internal jugular veins, and by the inferior thyroid veins to the anterior venae cavae. Lymphatic drainage is to the internal jugular and posterior cervical lymph nodes (see Chapter 9: Histology of the Vascular System). The thyroid gland receives sympathetic innervation from the cervical sympathetic ganglia, and parasympathetic innervation from the superior and recurrent laryngeal nerves. The carotid sheaths lie lateral to the lateral lobes of the gland, and the recurrent laryngeal nerves run close to the posterodorsal aspects of the lateral lobes. The sympathetic supply is thought to be largely vasomotor; the function of the parasympathetic supply is not well understood.

Development of the Thyroid Gland

The thyroid gland develops from the thyroglossal duct, a diverticulum that appears in the floor of the pharynx near the posterior border of what will eventually become the tongue. In man the foramen caecum, a blind pit found towards the posterior margin of the tongue, marks the place where development of the thyroid gland begins (ectopic thyroid tissue can appear here). As the duct lengthens, it passes ventral to the hyoid bone and loses its lumen, before bifurcating to form the two lateral lobes of the thyroid gland. At first the glandular tissue comprises fenestrated sheets of epithelium, but these soon form the characteristic follicles of the gland.

The majority of the epithelial tissue of the gland, including all the cells that secrete the thyroid hormones, is derived from the endoderm of the pharynx. Endodermal cells from the fourth pharyngeal pouch migrate into the developing gland to form the calcitonin producing C cells (Johansson et al., 2015). C cells are only found in the thyroid gland in mammals. In other vertebrates these cells

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are located in the ultimobranchial bodies found close to the thyroid gland. The C cells are part of the Amine Precursor Uptake and Decarboxylation (APUD) series of cells that include the endocrine cells of the gut (Pearse and Polak, 1971). The endodermal origin of these cells is surprising. Evidence supporting the alternative neural crest origin comes largely from studies in quail-chick chimeras; if it comes to be agreed that endoderm can form APUD cells, doubt will be cast on the reliability of quail-chick chimeras for determining the likely origin of cell lines in mammals.

Histology

Though most textbooks of histology focus on the human thyroid gland, there are two accounts that deal specifically with the rat thyroid (Wissig, 1964 and Napalkov, 1990). The thyroid gland is unique amongst endocrine glands in that the main products of its cells are stored extracellularly in the follicles as a homogeneous material referred to as colloid. Follicular cells take up iodine from the blood and iodinate tyrosine residues of the glycoprotein thyroglobulin. Iodinated thyroglobulin is secreted by the follicular cells into the follicular lumina and stored as colloid. Colloid is taken back into the cells by micropinocytosis, and in active cells as larger droplets. Fusion of these droplets with lysosomes releases the thyroid hormones thyroxine (T4) and triiodothyronine (T3) from the basal surfaces of the cells. The mechanism of T3 and T4 release is not fully understood, but it does not involve the release of secretory granules.

The follicles are essentially spherical structures of between 15 μm and 100 μm diameter (see Fig. 16.2). Larger follicles tend to be found in the peripheral parts of the gland and may enlarge to form cysts in old rat. The epithelium of the follicles is a simple cuboidal epithelium, which may vary in height according to the activity of the cells. In active follicles it may look columnar, whereas when quiescent it may be close to squamous. The nuclei are centrally placed within the cuboidal cells but in active follicles appear closer to the bases of the columnar cells. Variations in the height of the cells can also occur within individual follicles (Lever, 1961).

The apical surfaces of the epithelial cells face the colloid of the follicles and are characterised by microvilli. Basal surfaces are flat and rest on a basal lamina that extends around the entire follicle. The cytoplasm is rich in granular endoplasmic reticulum, which increases in active cells. Junctional complexes between the lateral margins of the cells seal the epithelial surface.

The colloid of the follicles stains pink in H & E stained sections, and as the follicular cells become more active PAS positive vesicles of colloid can be seen in the apical cytoplasm. In sections stained with trichrome stains such as acid fuchsin, ponceau 2 R and aniline blue or Masson's trichrome stain, some colloid stains pink and some stains blue, even within single follicles. This rather striking pattern of staining was investigated by Mayer (1963) who found that it represented an artefact of fixation. Other artefacts include the fracturing of the colloid leaving linear clefts between areas of colloid, and the scalloped appearance of the edge of the colloid adjacent to the follicular cells. It is debatable whether this latter observation constitutes an artefact or if it represents active reabsorption of colloid by follicular cells.

Interfollicular and Parafollicular Cells

Examination of sections of thyroid shows groups of cells lying between the follicles. A long list of names has been applied to these cells since they were first discovered by Baber in 1877 (Ekholm and Ericosn, 1968; Hazard, 1977). On the basis of EM studies it was concluded that these so called parafollicular cells lay within the basal laminae of the follicles and that their interfollicular appearance was an artefact produced by tangential sectioning of follicles (Napalkov, 1990; Fawcett, 1994). For further discussion see Ekholm and Ericosn (1968) and Hazard (1977).

The pale staining parafollicular cells are larger than the epithelial cells of the follicles and lie against the basal

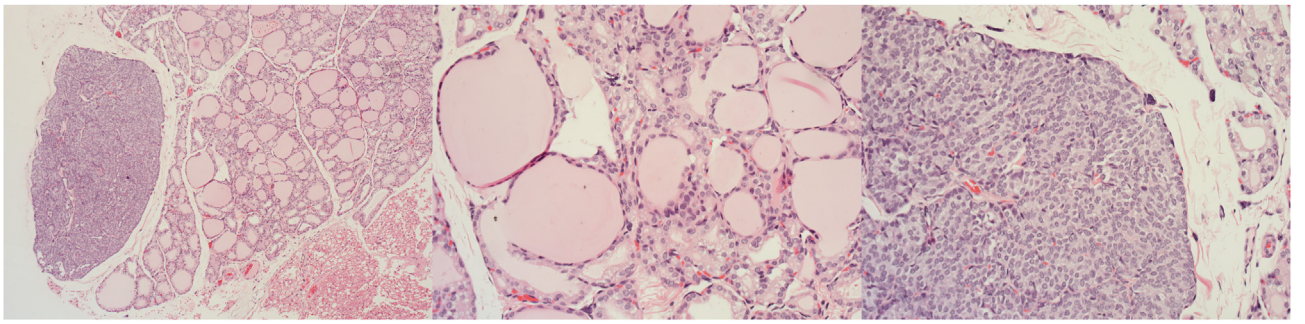


FIGURE 16.2 Thyroid and parathyroid glands.

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lamina either singly or as groups of two or three cells. They never extend to the colloid and are always within the follicular epithelium. Parafollicular cells contain membrane-bound secretory granules of 10–40 nm diameter containing calcitonin and somatostatin. In some species serotonin (5HT) is also present. The products of these cells are not stored in the colloid of the follicles, but are released from the basal cell membranes, and enter the capillaries adjacent to the parafollicular tissue. Occasional dark staining cells probably represent thyroid stem cells (Davies et al., 2011).

Follicular Capillaries

The follicles are surrounded by connective tissue that supports a dense network of anastomosing fenestrated capillaries, which could be described as sinusoidal capillaries (see Chapter 8: Histology of the Vascular System).

The capillaries lie close to the basal laminae of the follicles and indent the bases of the epithelial cells. An extensive network of lymphatic capillaries lies deep to the capillary network but cannot be seen in light microscopy (LM) sections.

PARATHYROID GLAND

Sandström is usually credited with discovering the parathyroid glands in 1880, although Owen had already described them in the rhinoceros (Kafetzis et al., 2011). No other organs that are essential to survival have been discovered since. These glands secrete parathyroid hormone (PTH), which regulates the concentrations of calcium and phosphate in the plasma through its actions on bone, kidney and gut. An increase in plasma calcium concentration leads to decreased production and secretion of PTH, an example of a direct negative feedback loop. PTH raises the concentration of calcium in the plasma, while calcitonin, produced by the C cells of the thyroid gland, acts as a physiological antagonist, and lowers the plasma calcium concentration. Parathyroid glands are not found in fish, but are present in all vertebrates that live on land. It seems that parathyroid glands evolved at the same time as vertebrates made the move from water to the land; perhaps the evolution of these small, but essential glands, facilitated that move. Marine (1932) provided an excellent account of early work on the parathyroid gland that is still worth consulting.

General and Topographical Anatomy

Most mammals have two pairs of parathyroid glands, but the rat has only one pair. Each gland lies at the anterior end of the dorsal surface of the thyroid gland, partially embedded within the gland, close to the trachea. Rat parathyroid glands are about 1.2–2.0 mm in length, 1.0–1.5 mm in width and 1–2 mg in weight; the glands of the female rat are about twice the size of those of the male (Hebel and Stromberg, 1976). The parathyroid glands are surrounded by a connective tissue capsule that sends fine septa containing the arteries and veins of the gland, and is continuous with that of the thyroid gland. Blood supply to the gland is via the hilum and comprises branches of the thyroid arteries; venous drainage is to the thyroid veins. Lymphatic drainage is as for the thyroid gland (see above).

Sympathetic fibres reaching the parathyroid gland are thought to be largely vasomotor in function.

Development

The parathyroid glands develop from the tissue of the third pharyngeal or branchial clefts, and thus are the anatomical, but not topographical, equivalents of the inferior parathyroid glands of man. In man these descend with the thymus leaving the fourth cleft to provide the superior parathyroid glands. It is thought that the cells of the parathyroid glands of the rat are derived from neural crest tissue associated with the third pharyngeal clefts.

Histology

The histological structure of the rat parathyroid gland has been reviewed by Capen and Rosol (1989) and by Chen et al. (2013). Murakami et al. (1987) provided a detailed account of the vasculature of the gland, and their paper should be consulted for details and for the authors' outstanding scanning electron micrographs of corrosion casts of the parathyroid blood vessels. The ultrastructure of the gland was described in detail by Lever (1957) and reviewed by Isono et al. (1990).

The predominant cell, the chief cell, is polyhedral in shape with a centrally placed nucleus. Chief cells are arranged in anastomosing cords. EM studies have shown that the cell membranes of these cells interdigitate and that the extent of interdigitation increases with the activity of the cells. The cytoplasm is rich in glycogen and stains more darkly in active cells as the amount of granular endoplasmic reticulum and the number of membrane-bound secretory granules increases. These membrane-bound secretory granules (150–200 nm diameter) contain the polypeptide PTH, but are surprisingly few in number and not much storage of PTH seems to occur. Movement of the secretory granules to the cell membrane has been shown to depend on the presence of microtubules. Colchicine, which inhibits formation of microtubules, inhibits secretion of PTH (Reaven and Reaven, 1975). There are no follicles in the rat parathyroid gland, though

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in some species they may occur as a developmental abnormality.

Much confusion has resulted from the interspecies differences in the types of cells of the parathyroid gland. Chief cells (or principal cells) and the oxyphil cells are recognised in man, but in the rat only chief cells are found, though these may take on a variety of appearances. Whether the oxyphil cells of man are a degenerate form of chief cells that increase in number with age is debatable, but these cells either do not occur in the rat, or if they do, are extremely rare (Tanaka et al., 1966; Ritter et al., 2012). Before leaving the oxyphil cells we shall just note that they are large pale cells, and that electron microscopy has shown that their cytoplasm is packed with mitochondria. These cells, sometimes referred to as oncocytes, are also found in the acini and ducts of the salivary glands.

Although Hebel and Stromberg (1976) listed four cells types in the rat parathyroid gland: light principal cells, dark cells, 'water-clear' cells and oxyphil cells, this view has since been challenged, and it has been suggested these are all derivatives of chief cells. Overstimulation of chief cells leads to conversion of these cells into water-clear cells, and the normal cyclical pattern of activity and comparative inactivity of chief cells is reflected by the dark and light chief cells respectively (Capen and Rosol, 1989). Water-clear cells have been shown to contain many vacuoles in their cytoplasm and are seen in some types of parathyroid adenoma. A multinucleated syncytial cell has been described in the rat and dog parathyroid. The cytoplasm of these cells stains deeply with eosin and the cells are characterised by many small dark nuclei. (Capen and Rosol, 1989; Meuten et al., 1984).

THE ADRENAL GLAND

The adrenal glands of the rat are embedded in fat at, or close to, the anterior poles of the kidneys. The cells of the cortex secrete a range of steroid hormones; the cells of the medulla may be regarded as modified sympathetic ganglion cells. They are innervated by preganglionic sympathetic fibres of the splanchnic nerve and produce adrenalin and noradrenalin.

General and Topographical Anatomy

The adrenal glands of the rat are small rounded structures, and although differences in shape between the right and left adrenal glands have been described, they are difficult to distinguish. Hebel and Stromberg (1976) gave the following dimensions.

Right adrenal: 4.0−5.5 mm in length, 3.0−4.5 mm in width and 2.8−3.0 mm in thickness, average weight 21.8 mg in the male, 25.7 mg in the female.

Left adrenal: 4.5−5.5 mm in length, 3.2−4.5 mm in width, 2.5−2.8 mm in thickness, average weight 20.5 mg in the male, 31.6 mg in the female.

The medulla occupies a much smaller volume than the cortex. In male rats, the medulla averages 1.8 mg, in the female 3.1 mg. The medulla is slightly irregular in shape with projections from the medulla entering the cortex, often appearing as isolated islands of medullary tissue in histological sections. The adrenal vein drains the medulla and the cortex and may be sheathed by medullary tissue at the point at which it leaves the gland.

Blood Supply of the Adrenal Glands of the Rat

In a detailed study of the vasculature of the rat adrenal gland, Lever (1955) described the arterial blood supply of the glands as follows.

Right adrenal gland: two arteries, arising as branches of a vessel that springs from the right renal artery, supply the gland from its inferior and medial aspects.

Left adrenal gland: a superior adrenal artery arises from the aorta just below the diaphragm, it divides into two branches; two middle adrenal arteries arise from a common stem that springs from the ventral surface of the aorta; a small inferior adrenal artery arises from the left renal artery. Lever noted that his findings differed from the description provided by Greene (1935).

One adrenal vein leaves each adrenal gland. The right vein drains to the vena cava and the left to the left renal vein via a diaphragmatic vein.

Development

In all mammals, the adrenal gland is made up of an outer cortex and an inner medulla that arise from quite different embryological tissues. The cortex arises from the coelomic epithelium (better described as endothelium as it is of mesodermal origin), and the medulla from neural crest tissue.

The foetal adrenal gland is relatively larger than the adult gland and differs in structure. The cells of the foetal adrenal cortex are larger than in the adult, and prior to birth, no zonal differentiation of the cortex is apparent. After birth, the foetal cortex undergoes apoptotic regression and begins to differentiate (Rosol et al., 2001; Walling et al., 2016). Lever (1955) described the zones of the adult gland as appearing in the following order: zona glomerulosa in the first postnatal week, zona fasciculata in the second week and zona reticularis in the third week. The zona intermedia (see below) appears on about the

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eighth postnatal day and the medulla also achieves its adult form shortly after birth with nests of medullary tissue among the cortical cells on the third postnatal day. These develop and create the medulla of the gland in a form comparable to the adult by the seventh postnatal day (Cater and Lever, 1954; Walling et al., 2016).

Histology of the Adrenal Cortex

The adrenal gland is enclosed by a thin connective tissue capsule pierced by the arteries that supply the gland. Beneath the capsule, the arteries form a network of arterioles that give rise to the sinusoidal fenestrated capillaries that supply the cortex. Blood from these capillaries flows into a plexus of venules located at the corticomedullary junction that continue into the medulla. There are also arterioles that run directly to the medulla. The medulla is thus supplied in two ways: directly and indirectly. For a detailed account of the vasculature of the rat adrenal gland see Lever (1952).

The adrenal cortex is divided into three zones each with a different arrangement of cells. These are, from the outer surface: the zona glomerulosa, the zona fasciculata and the zona reticularis: see Fig. 16.3. The cells of the zona glomerulosa are smaller than those of the zona fasciculata, contain fewer lipid droplets, and are arranged as arcades or arches, although they often resemble the acini of exocrine glands. The appearance of small balls of cells is the origin of the term glomerulosa.

Cells of the zona fasciculata contain many lipid droplets that disappear during standard histological processing giving the cells a vacuolated appearance, although frozen sections allow the demonstration of the lipid. The cells are described as sudanophilic because of the effective staining of lipid by Sudan Black or Red. The cells are arranged as long cords that run radially within the cortex separated from one another by a dense capillary bed.

Cells of the zona reticularis are arranged as short anastomosing cords and contain fewer lipid droplets than those of the zona fasciculata and stain more darkly in H & E sections.

The cells of the adrenal cortex produce steroid hormones. Aldosterone is produced by the cells of the zona glomerulosa, the glucocorticoids by the zona fasciculata and androgens by the zona reticularis, although in the rat the zona reticularis produces only small amounts of androgenic hormones (Van Weerden et al., 1992). EM examination shows that cells producing steroid hormones contain abundant agranular endoplasmic reticulum.

Mitochondria vary in size and structure from zone to zone. Those in the rat zona fasciculata are spherical in shape and contain not the usual cristae, but 60 nm diameter vesicles that appear to be unattached to the inner membrane of the mitochondria. The explanation for this very unusual configuration is unknown (Fawcett, 1994).

Two further zones of the adrenal cortex have been described in rodents: the zona intermedia between the zona glomerulosa and the zona fasciculata in the rat (Cater and Lever, 1954; Mitani et al., 1994), and the X zone between the zona reticularis and medulla in the mouse (Rosol et al., 2001). The zona intermedia is thought to contain the stem cells of the adrenal cortex and is characterised by mitoses of the cells of its inner and outer boundaries (Mitani et al., 1999, 2014). The function of the X zone of the mouse is unknown.

Histology of the Adrenal Medulla

The medulla is not separated from the cortex by a connective tissue boundary. Medullary cells are polygonal in shape and arranged as groups and short cords. These cells

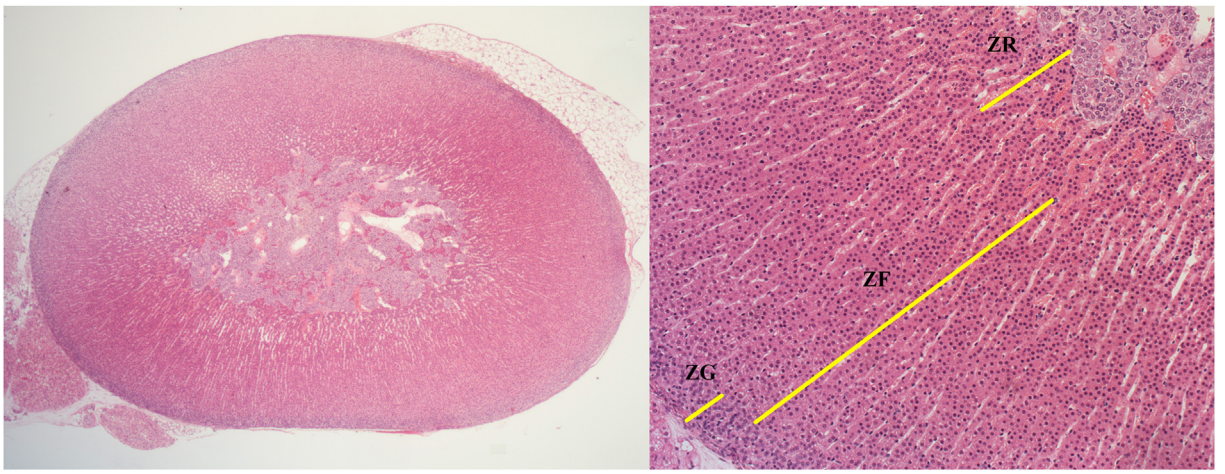


FIGURE 16.3 Adrenal gland low and high power. ZG, zona glomerulosa; ZF, fasciculata; ZR, reticularis.

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take on a brown colouration if the tissue if fixed with potassium dichromate, hence the name chromaffin cells for the cells of the adrenal medulla and similar cells found elsewhere. As noted above, these cells are modified sympathetic ganglion cells that produce adrenalin and noradrenalin, which are stored as membrane-bound granules in the cytoplasm of the cells, with single cells containing up to 30,000 granules (Fawcett, 1994) (Fig. 16.4).

Accessory Adrenocortical Glands of the Rat

Belloni et al. (1989) described a number of accessory adrenal glands that appeared to consist only of adrenal cortical tissue. These were reported to be less than 0.1 mm in diameter in the normal rat but are capable of enlargement following adrenalectomy. The authors reported that 60% of female rats survived bilateral adrenalectomy, and in 70% of these adrenocortical nodules developed in the retroperitoneal fat close to the kidneys. The nodules lacked any evidence of medullary tissue and the zona glomerulosa was not well defined.

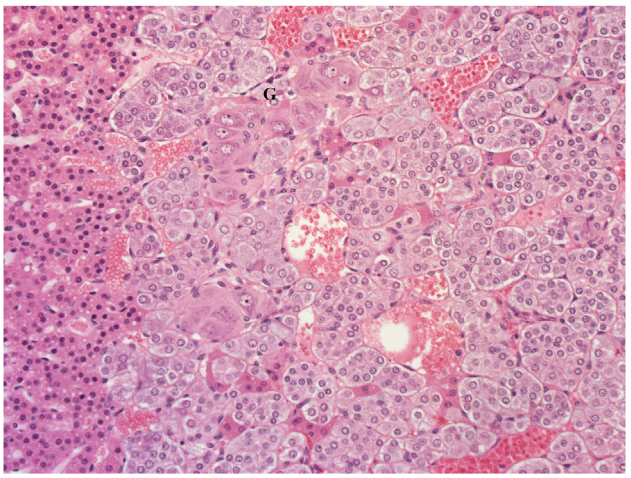


FIGURE 16.4 Adrenal medulla. Medullary cells interspersed with occasional ganglion cells (G).

ISLETS OF LANGERHANS: THE ENDOCRINE PANCREAS

'Where are the islets of Langerhans?' is the only question relating to histology likely to be heard in a general knowledge quiz. The answer is, of course, 'in the pancreas'. Langerhans first described these micro-organs in the rabbit in 1869 (Baskin, 2015), but they were soon identified in mammals and other vertebrates. Similar tissue occurs as endocrine follicles in the wall of the gut in cyclostomes (lampreys and hagfish), and as discrete organs in some teleost fish (Romer, 1970). The major products of the islets are insulin and glucagon, though a number of other products have been identified (see Fig. 16.5).

Development

The islets of Langerhans develop from the duct cells of the pancreas. The pancreas itself develops from two diverticuli of the duodenum that give rise to the ventral and dorsal pancreatic buds. In man, cells migrate from the pancreatic ducts and form isolated aggregates that develop into the islets, but in the rat the cells of the ducts proliferate and form cellular aggregates that remain in close contact with the ducts. Further differentiation within these aggregates forms the cells of the islets (Bouwens, 2004) (Fig. 16.5).

Histology

In the young rat islets are about 200 μm in diameter, and can be distinguished with the aid of a hand lens as small

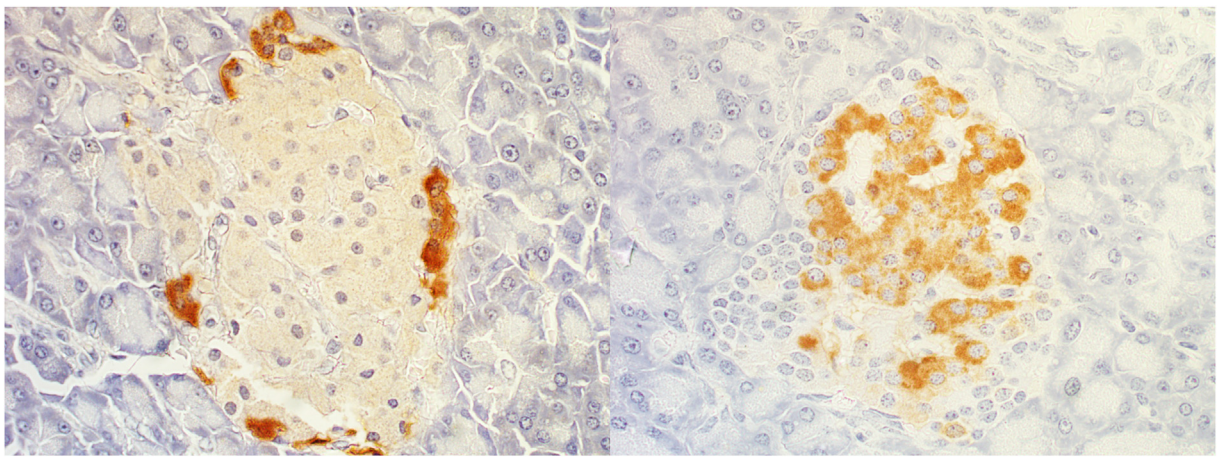


FIGURE 16.5 Islets of Langerhan stained for glucagon (left) and insulin (right).

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TABLE 16.3 Average Numbers of Cells of Different Types in the Islets of the Rat Pancreas

	Lower Duodenal	Upper Duodenal	Gastric	Splenic
Volume density of islets % (1)	2.56	2.28	2.87	3.28
Islet diameter μm (2)	191 (2.7)	157 (6.3)	210 (5.6)	223 (1.5)
Number of cells per islet (2)	11,294 (5091)	5559 (512)	15,285 (839)	18,835 (762)
Number of A cells per islet (2)	474 (37)	1272 (184)	4278 (227)	4887 (137)
Number of B cells per islet (2)	8807 (915)	3640 (620)	9905 (1161)	12,815 (710)
Number of D cells per islet (2)	487 (26)	329 (24)	595 (27)	712 (48)
Number of PP cells per islet (2)	1679 (168)	143 (20)	435 (32)	405 (30)

Notes:
Six adult male Wistar albino rats, aged 60 days and weighing 250–300 g were studied.
1292 islets were examined (lower duodenal: 328, upper duodenal: 245, gastric: 314 and splenic: 405). The part of the pancreas identified as lower duodenal develops from the ventral pancreatic bud, the other parts from the dorsal pancreatic bud
(1): Volume densities have been converted to percentages and the standard errors omitted.
(2): Standard errors shown in brackets.

pale areas in H & E stained sections of pancreas. The presence of islets allows the pancreas to be easily distinguished from other exocrine glands such as the parotid salivary glands. Islets are approximately spherical in shape, and random sections exhibit a range of diameters. The largest islets are found in the tail of the rat pancreas, the part closest to the spleen (Elayat et al., 1995). See Chapter 15, Exocrine Glands for a description of the general and topographical anatomy of the pancreas. Table 16.3 shows the distribution of cell types in the rat pancreas (data from Elayat et al., 1995).

It can be seen from Table 16.3 that the islets make up about 2%–3% of the volume of the pancreas. The adult rat pancreas contains up to about 20,000 islets, each containing several thousand cells. In man there may be more than 500,000 islets (Fawcett, 1994; Carter et al., 2009). Each islet is surrounded by a delicate meshwork of reticulin fibres containing a dense network of capillaries that extend into the interior of the islets. There is little connective tissue between the cells of the islets, and the capillaries lie in close proximity to the endocrine cells.

The cells of the islets cannot be differentiated in H&E stained sections. Classical staining techniques such as Mallory–Heidenhain azan and the Gomori aldehyde fuchsin do allow differentiation, but these have been replaced by specific immunocytochemical staining of the hormones produced by each cell type (see Fig. 16.5). Table 16.3 shows that the PP cells which produce pancreatic polypeptide are located mainly in that part of the pancreas that develops from the ventral duodenal diverticulum (referred to by the authors as the lower duodenal part of the pancreas) whereas the A cells which produce glucagon are rather uncommon in this region.

The nomenclature of the cells of the islets has changed over the years. Six cell types are found in the islets of the rat. These and their products are listed below. Alternative designations are shown in brackets.

A cells (α)	glucagon
B cells (β)	insulin

(C cells are not found in the rat, but have been described in the guinea pig pancreas and probably represent undifferentiated cells or cells without endocrine function. C originally stood for clear cells)

D cells (δ)	somatostatin
PP (F)	pancreatic polypeptide
Epsilon, ε	ghrelin

In both man and rat, the B cells are grouped in the centres of the islets, with the other cells in the periphery, although this distribution is not as clear in man as it is in the rat (Steiner et al., 2010). The ghrelin producing epsilon cells are seen in the foetal rat, but are relatively uncommon in the adult. They are a fairly recent discovery and it has been argued that ghrelin, which appears to control the production of insulin, is produced by A cells in man and by B cells in the rat (Wierup et al., 2014, Raghay et al., 2013).

All the hormones of the islets are polypeptides that are stored within the cells as membrane-bound granules. Morphologically, these granules appear to have greater or lesser densities when examined by electron microscopy, allowing the cells to be distinguished (Fawcett, 1994).

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Chapter 17

The Urinary Tract

The urinary tract comprises the kidneys, ureters, urinary bladder and urethra. In this chapter, we do not deal with either the male or female urethra which are described in Chapters 18 and 19.

THE KIDNEY

The rat has two bean-shaped kidneys. They are retroperitoneal structures located on the ventral surface of the dorsal abdominal wall, dorsal to the parietal peritoneum and towards the anterior end of the abdominal cavity. The right kidney lies above the right lobe of the liver; the left is close to the stomach.

The ventral surfaces of the kidneys are slightly convex; the dorsal surfaces are flat. It is generally understood that the main role of the kidney is the excretion of water and metabolic waste. Although this is the major function of the kidney, it has several other important functions: the regulation of electrolyte composition and acid−base balance, regulation of extracellular fluid volume, synthesis of hormones including erythropoietin, and metabolic functions. The kidney is also a target for several hormones including aldosterone and antidiuretic hormone (ADH).

Each kidney is connected to the urinary bladder by a ureter that is covered by parietal peritoneum running from the kidney to the dorsal wall of the pelvis and from there to the base of the bladder. The anterior end, or cranial pole, of the right kidney lies at the level of first lumbar vertebra (L1), its caudal pole lies at the level of L3, wholly under the cover of the lower ribs. The right kidney is about 20 mm in length and 10−15 mm in breadth. The slightly smaller left kidney lies a little more caudally.

The kidneys are readily identified after removal of the liver and gut (see Chapter 25: Dissection of the Adult Rat). Fat is found around the hilum of the kidney and its sides, but in the rat does not cover the dorsal or ventral surfaces (Hebel and Stromberg, 1976).

The kidney is of mesodermal origin, developing from the most caudal part of the nephrogenic ridge. It initially appears towards the posterior end of the abdomen, but moves forwards as the embryo develops. As it moves forwards its blood supply changes, so the definitive renal arteries are not formed until it reaches its final position. The ureters grow forward from the primitive bladder. The nephron has two embryological origins. The nephrogenic cap is derived from the mesoderm of the nephrogenic cord, and from this Bowman's capsule, proximal convoluted tubule, loop of Henle and distal convoluted tubule develop; and the ureter, from which the collecting duct and terminal distal tubule develop.

In man the kidney has a number of lobes that are not apparent from an external examination, but are immediately apparent in longitudinal sections. The rat kidney is unilobar, and can be considered as equivalent to one lobe of the human kidney. The long papilla of the rat kidney extends into the anterior end of the ureter, and is surrounded by a narrow space described as the fornix (a vault or arch).

The medulla is easily distinguished from the cortex, which is darker in colour and characterised by many small red dots distinguishable with a hand lens. These are the renal (Malpighian) corpuscles. The terminology of the layers of the medulla is complicated (see Fig. 17.1).

Cross sections of the interlobar arteries can be seen to the sides of the pelvis in histological sections of the entire rat kidney. Because the rat kidney is unilobular these cannot be interlobar vessels, their name is an example of inappropriate transfer of terminology from human anatomy. The interlobar arteries give rise to the arcuate arteries, which run between the cortex and the outer stripe of the medulla, defining the inner edge of the cortex. In the rat, the thin connective tissue capsule of the kidney is supplied by vessels that arise from the renal artery or the artery to the adrenal gland.

Examination of the cortex with a hand lens allows a number of radial striae to be identified. These striations are the medullary rays, containing collecting ducts and, according to Hebel and Stromberg (1976), the straight parts of the proximal and distal tubules of nephrons located in the outer part of the cortex (see below for

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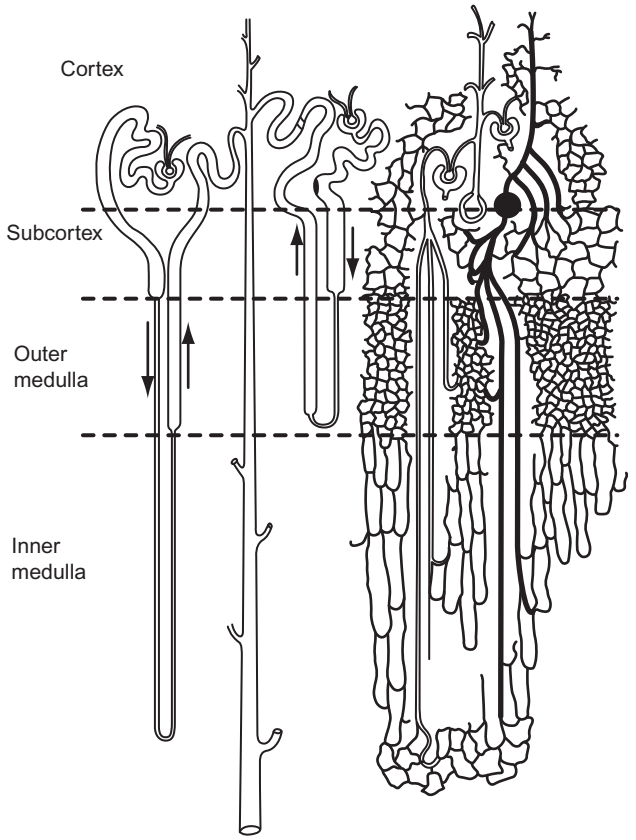


FIGURE 17.1 Blood vessels and tubules in the zones of the rat kidney. Diagram to illustrate the arrangement of vessels and tubules in the various zones of the kidneys (Fourman and Moffat, 1971).

further details). Striation of the cortex should not be confused with the clear striation of the inner medulla, which is caused by bundles of blood vessels running into and out from the inner medulla.

The pelvis of the kidney is the expanded anterior end of the ureter around which the kidney is wrapped, and into which the papilla is invaginated. The word pelvis means a basin (the official plural is pelves and not pelvises). It might better be described as a funnel, as it collects urine from the large papillary ducts (ducts of Bellini), and conducts it to the ureter. Although apparently simple, the pelvis is in fact complicated, and varies greatly between mammalian species. The key to this complexity lies in how far the epithelium that lines the ureter extends into the pelvis. The urothelium, (formerly called transitional epithelium because it could change its form from many layered to few layered as the bladder expanded) forms the epithelial lining of the ureter, bladder and first part of the urethra. In some mammals, including a rather odd group that includes primitive rodents (Aplodontia: mountain beavers, but not to be confused with true beavers), the beaver (Castor) and the pig, the urothelium extends over all the inner surface of the pelvis and over the sides of the papilla, but in the rat and many other mammals, it stops just short of the papilla, which is covered by a simple cuboidal epithelium. It has been suggested that passive diffusion may occur through this thin epithelium, but given the small surface area involved this is not thought to be significant. A second feature is the presence of secondary pyramids. These are projections into the pelvis, but are quite distinct from the papilla, indeed they have an entirely different structure. The secondary pyramids are flaps or opercula that project into the pelvis from the inner stripe of the outer medulla. Pairs of flaps at the anterior and posterior ends of the pelvis have been described in the rat; additional medial flaps have also been described. These flaps are shaped like pairs of buttresses and cover the interlobar blood vessels. The functional significance of the proximity of the flaps to the interlobar vessels is unknown. The secondary pyramids cause secondary fornices to appear. Pfeiffer (1968) has described the pelves of mammalian kidneys in some detail: he defined two types. The rat has a Type II pelvis; Type I pelves are found in the group mentioned above as including the Aplodontia; Moffat (1975) added a third group, Type III, but we shall not pursue this here.

Blood Vessels of the Kidney

The renal arteries are both branches of the abdominal aorta. The right renal artery passes dorsal to the posterior vena cava. On entering the kidney at the hilum, the renal arteries divide into three or four branches that run dorsally and ventrally around the pelvis of the kidney to reach the junction between the cortex and subcortex, where they form the arcuate arteries.

Interlobar arteries are end arteries that have no anastomotic connections with other arteries, either in the rat or in man. The arcuate arteries run at the junction of the cortex and the outer stripe of the outer medulla where they give rise to the interlobular arteries that run at right angles from the arcuate arteries towards the surface of the cortex.

The interlobular arteries give rise to the afferent arterioles of the glomeruli (diminutive form of glomus meaning a ball of blood vessels) that lie within the Bowman's capsules of the Malpighian corpuscles, and then recombine to produce the efferent arterioles. Afferent arterioles leaving interlobular arteries deep in the cortex point downwards towards the medulla; those leaving nearer the surface of the kidney point towards the surface.

The efferent arterioles soon divide to produce the capillaries of the dense capillary bed of the cortex and medulla. The capillaries surrounding the tubules of the cortex have fenestrations with an interesting substructure which at the EM level present a 'spoke and

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wheel' appearance (Fawcett, 1994). The hormone erythropoietin is thought to be produced by the endothelial cells of the capillaries surrounding the straight parts of the proximal and distal tubules. The vessels that descend into the medulla arise from the efferent arterioles of the juxtamedullary glomeruli (found close to the surface of the medulla), some of which pursue a straight course into the medulla, hence they are called the descending vasa recta (straight vessels). These vessels divide low in the medulla but their capillary branches soon recombine to form the ascending vasa recta. In this way, long vascular loops are formed. The ascending vasa recta deliver venous blood to the arcuate veins running just below (deep to) the arcuate arteries. The vessels that descend into the medulla also give off short branches that form a capillary plexus that surrounds all the tubules in the medulla. The capillary plexus of the inner stripe of the outer medulla is much more impressive than that of the inner medulla. In the rat the descending vasa recta are derived from the efferent arterioles of juxtaglomerular glomeruli, rather than the arcuate arteries.

Interlobular veins run alongside the interlobular arteries and collect venous blood from the capillary plexus of the cortex. As noted earlier, the venous return from the medulla runs to the arcuate veins. Arcuate veins deliver blood to the interlobar veins. Unlike their arterial counterparts, there is a good deal of anastomosis between the branches of interlobar veins. The renal veins pass from the kidneys to the posterior vena cava: the left renal vein passes ventral to the aorta.

The Renal Lobule

The kidney is an exocrine gland that secretes urine. As in all exocrine glands the functional unit of the kidney is the lobule, but unlike many other exocrine glands, the lobules are not separated by connective tissue septae. Combined with the fact that the lobule is not a discrete mass of secretory cells pouring their secretions into an intralobular duct, this makes the renal lobule singularly difficult to visualise and describe. The three-dimensional structure of the renal lobule is not much discussed in textbook accounts of the kidney.

A renal lobule may be defined as a number of nephrons that discharge urine into a single collecting duct. This duct begins near the surface of the cortex, runs through the cortex in a medullary ray and continues into the medulla where it joins other collecting ducts to form the large papillary ducts that open into the pelvis of the kidney at the surface of the papilla. Prior to this merging, the collecting duct (or tubule) may be regarded as the intralobular duct of the renal lobule, and as such can be considered to define the axis of the lobule.

Each lobule has a cortical and a medullary component. The cortical component comprises the glomeruli and their tubules (proximal convoluted tubules, loops of Henle and distal convoluted tubules) that feed into the collecting duct. These components form a transected cone of cortical tissue (or a frustum, in geometrical terms: a cone without its pointed end) that sits with its large end towards the surface of the cortex and its smaller end at the cortico-medullary junction. The surfaces of the frusta (or frustums) are defined by the interlobular vessels that supply blood to, and collect blood from, the nephrons of the lobule. The long axis of the frustum is defined by the medullary ray, which is conical with its point towards the surface of the kidney, and its base at the cortico-medullary junction. The medullary ray widens as it approaches the cortico-medullary junction because it contains, in addition to the widening collecting duct, straight parts of the proximal tubules of nephrons lying deep in the cortex, and straight parts of distal tubules returning from the medulla.

The medullary part of the lobule comprises the loops of Henle. The lobule also contains the blood vessels that the supply tissue within it. We must therefore include the dense capillary plexuses of the cortex and part of the medulla and the bundles of vessels that dip deep into the medulla and then return to the arcuate vessels at the cortico-medullary junction.

Although the cortical part of the lobule is tolerably well defined by the interlobular vessels and the medullary rays, the lobular structure in the medulla is impossible to distinguish. Little attention seems to have been paid to the three-dimensional shape of the renal lobule: the three-dimensional geometry of the distribution of interlobular vessels at the periphery of the cortical parts of the lobule seems not to have been explored in detail, at least not with the enthusiasm that has been lavished on the lobules of the liver (see Chapter 14: Liver). Fig. 17.1 shows the arrangement of the blood vessels and tubules in the several zones of the rat kidney.

THE NEPHRON

The nephron, of which there around 30–35,000 in the rat (Baines and de Rouffignac, 1969), is the functional unit of the kidney. The nephron can be separated into five parts: the glomerulus, the proximal tubule, the loop of Henle, the distal tubule and the collecting duct. Each part has a quite distinct function, which is reflected by quite different micro-anatomies. Other accounts use an alternative terminology for the segments of the nephron: proximal tubule, intermediate tubule, distal tubule and collecting system (Fawcett, 1994). Verlander (1998) has provided a detailed account of the ultrastructure of the

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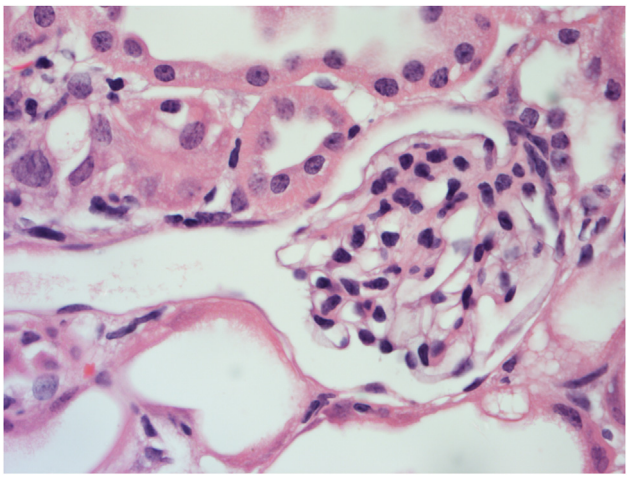


FIGURE 17.2 The glomerulus. This perfuse fixed glomerulus is surrounded by some rather damaged proximal tubules. But the loss of epithelium does allow us to see the continuous nature of the basement membrane.

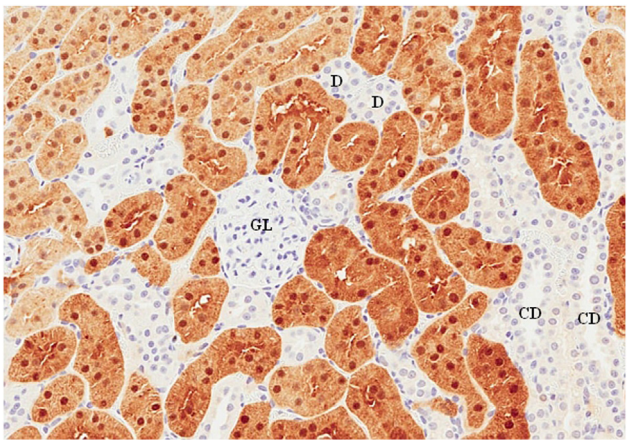


FIGURE 17.3 Kidney stained with α GST (alpha glutathione). The proximal tubules surrounding the glomerulus (G) are rich in αGST so stain brown, the distal tubules (D) have no positive staining. Also on the section are collecting ducts (CD).

nephron, but we shall focus mainly on the light microscopic appearances of the system (Figs. 17.2–17.4).

The Glomerulus

The term glomerulus, often used to imply the renal corpuscle, is a knot of capillaries that links the afferent and efferent arterioles within the renal corpuscle. These unusual capillaries receive blood at relatively high pressure (70 mmHg), and are both fed and drained by arterioles. The cytoplasm of the endothelial cells of the capillaries is extensively fenestrated, but unlike fenestrated capillaries in other locations, the fenestrations

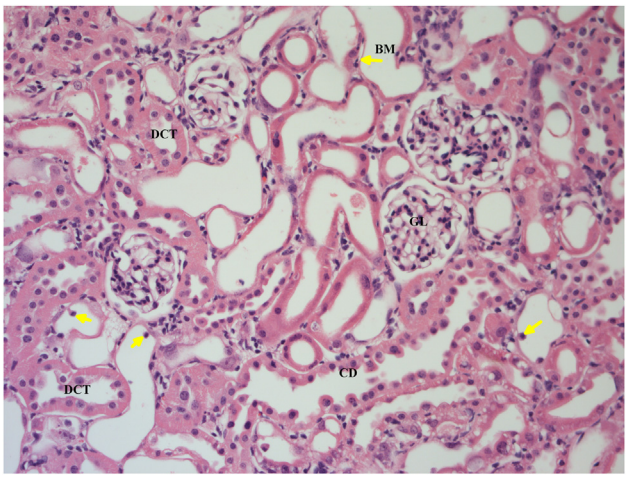


FIGURE 17.4 Toxicological changes in the proximal tubule. The distal tubule and collecting duct are spared but the proximal tubules can be seen at varying stages of regeneration. In some, the exposed basement membrane (BM) is clearly visible as are exfoliating epithelial cells (arrowed).

(70–90 nm diameter) are not closed by fine diaphragms (Fawcett, 1994). The capillaries are surrounded by specialised cells called podocytes (so named for their foot processes), which rather like pseudopodia, lie across the width of the capillaries and embrace the capillaries (see Fawcett, 1994, for outstanding scanning electron micrographs of the podocytes). The gaps between the foot processes, described as filtration slits, are spanned by fine diaphragms 4–6 nm in thickness that play an important part in controlling filtration, and prevent the passage into the urine of molecules of greater than 10 nm diameter. The podocytes are described as the visceral epithelium of Bowman's capsule and join the unspecialised parietal epithelium at the vascular pole of the renal corpuscle. Between the foot processes and the endothelial cells lies a thick, tri-laminate basement membrane that plays a large part in controlling the flow of materials from the capillaries and into the space (Bowman's space) surrounding the knot of capillaries. Bowman's space represents the expanded blind end of the nephron into which the knot of capillaries is invaginated. The combination of endothelial pores, basement membrane and slit pores preferentially holds back protein, but allows water and electrolytes to pass readily across. The glomerular filtrate is modified as it flows through the remainder of the nephron. Some substances are reabsorbed, at least 70% of the glomerular filtrate is reabsorbed in the proximal convoluted tubule, while others are excreted. The efficiency of the glomerular filter varies between species and sexes, that of the male rat being particularly leaky. Loss of selectivity of the filter leads to the appearance of protein in the urine, one of the more sensitive markers of renal disease.

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Between the glomerular capillaries, the mesangial cells provide support for the capillaries and also play a role in controlling the flow of blood through them. Mesangial cells are of monocyte or smooth muscle origin, and cover about 30% of surface of the glomerular capillaries. In addition to their supportive functions, it appears that mesangial cells may be involved in monitoring glucose levels in capillary blood via processes sent into the capillary lumen. It is also thought that mesangial cells are phagocytic and remove material from the glomerular filtrate (Fawcett, 1994).

The afferent and efferent arterioles that form the vascular part of the glomerular tuft are innervated by sympathetic nervous system but also contract in response to various vasoactive substances including angiotensin II, endothelin, adenosine and noradrenalin.

Proximal Tubule

As the filtrate leaves Bowman's capsule it enters the proximal tubule. The proximal tubules make up about 70% of the tubules seen in histological sections of the cortex and can be distinguished from distal tubules by their larger diameter and brush border. Initially, the proximal tubule is a highly convoluted tubule lined with a simple cuboidal epithelium with a well-developed brush border of microvilli. In poorly fixed sections, the microvilli appear to fill the lumen of the proximal tubules, an artefact that disappears when the tissue is fixed by perfusion. A thick, carbohydrate-rich glycocalyx that lies at the surface of the brush border can be stained with the Periodic Acid- Schiff (PAS) technique. Apical canaliculi penetrate the surface of the cells between the bases of the microvilli. The basal membrane of the cells is deeply infolded, and lateral membranes interdigitate with one another making the lateral borders of the cells impossible to define at light microscopy. The structure of the proximal tubules is indicative of their function. The brush borders of their epithelium greatly increases the surface area of the cells, thus improving their absorptive capacity. Looking at the ultrastructure, one is struck by the many mitochondria that lie mainly between the in-foldings of the basal cell membranes and support the high energy demand of this part of the nephron.

One of the energy dependent processes operating in the proximal tubule is the active transport of inorganic electrolytes from glomerular filtrate. Many transport systems are coupled to the transport of ions, possibly the most significant being the transport of glucose. Sodium and glucose share a transport system, with the creation of a sodium gradient facilitating the absorption of glucose by the proximal tubular epithelial cells (Mather and Pollock, 2010).

The proximal tubule, along with the liver, is a common site of drug induced toxicity. The susceptibility of the proximal tubule is a function of its high blood supply, allied to the large number of transporter systems present that can lead to selective accumulation of drugs. The high concentration of P450 enzymes and reliance on oxidative metabolism can then create a range of highly toxic reactive metabolites, even when the parent compound is relatively nontoxic.

The proximal tubule is divided into convoluted and straight (pars recta) parts. Recent work has divided the proximal tubule into segments designated as S1, S2 and S3 depending on the detailed ultra-structure of the lining cells (Verlander, 1998). S1 and S3 comprise, respectively, most of the convoluted and straight parts while S2 comprises parts of both. The straight parts are not confined to the cortex, but descend into the outer medulla where they terminate at a uniform level, which establishes the boundary between the inner and outer stripes of the medulla. As a general rule, the transport capacity of the proximal tubule decreases from S1 to S3, but there are also some transport specific mechanisms that are unique to the particular segments. For example, S2 is specifically where organic ions are secreted.

Loop of Henle

At the end of the proximal tubule the contents flow into the loop of Henle. Two types of loop can be defined. Long loops penetrate deep into the medulla, arising from nephrons with glomeruli near the cortico-medullary border, and short loops arising from those located nearer the outer surface of the cortex. Short loops have very short thin segments (see Fig. 17.1). The loop comprises a thin descending limb, and an ascending limb that has both thin and thick segments. Looking at a cross section of the kidney under a low power the regions of transition can be seen occurring in the outer medulla. The transition between the straight part of the proximal tubule and the thin descending limb of the loop occurs at the border between the outer and inner stripes of the outer medulla; the transition between the thin and thick parts of the ascending limb of the loop occurs at the border between the inner stripe of the outer medulla and the inner medulla.

The 'thick' and 'thin' terminology refers to the height of the epithelial cells of the tubules and not the diameter of the lumina. Thick parts of the loops have a diameter of about 60 μm, while the thin parts have a diameter of about 15 μm, and can look rather like venules. The function of the loop of Henle is to reabsorb salt and water, with about 25% of filtered ions and 20% of the filtered water being reabsorbed here. Pumping of sodium (or more accurately of chloride ions accompanied by sodium ions) from the thick part of the ascending loop creates a

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high osmolarity environment deep in the medulla. The system that creates this localised high osmolarity environment by an energy dependant process operating in one of a pair of tubules lying close to each other, but in which fluid flows in opposite directions, is described by physiologists as an example of a counter current multiplier. One might think that the gradient of osmolarity would be dispersed by blood flowing through the rather permeable blood vessels of the medulla, but this is not the case. The arrangement of the vasa recta allows them to act as a counter current exchange system, which although it has no part in creating the gradient, prevents that gradient being dispersed. It was once thought that sodium pumped out of the ascending limb of the loop would be reabsorbed by the descending limb, fitting neatly with the idea of a counter current multiplier. It is now known that the descending limb of the loop is permeable to water, but not to sodium ions. Urea actively absorbed from the collecting ducts as they pass through the medulla also helps to raise the osmolarity of the medullary interstitium. Though the idea of the counter-current multiplier has long been accepted, the exact mechanisms by which it works are still uncertain. What is certain, is that a high osmolarity environment exists deep in the medulla, and that urine reaching the collecting ducts is hypotonic. The high osmolarity of the inner medulla allows water to be withdrawn from collecting ducts, if and when the permeability of these tubules is raised by action of the ADH. It is also certain that mammals that live in dessert environments, for example desert rats, have long loops of Henle and can concentrate their urine to a much greater extent than those that live with free access to water, for example beavers, which have short loops of Henle. Further discussion of these processes may be found in the monographs of Moffat (1975) and Dicker (1970); Pallone et al. (2003), and Sands and Layton (2009) have provided detailed reviews of the concepts.

Thin Loop (or Limb of the Loop)

The thin part of the loop of Henle is lined by simple squamous epithelium. The thin limb is divided into two parts: the ascending and descending limbs. The descending limb is highly permeable to water, but not to salt. Permeability is largely dependent on the concentration of Aquaporin 1 in the epithelium. The descending thin loop is a primary site of water absorption. In contrast, the ascending loop is permeable to ions rather than water. Rats generally have a greater proportion of short loops than long loops, roughly in the ratio of 2:1.

Thick Loop (or Limb of the Loop)

The thick part of the ascending limb is lined by a simple cuboidal epithelium. These cells pump chloride ions from the urine; sodium moves with the chloride ions. In addition to the absorption of sodium, chloride and potassium ions; the thick limb is a major location of magnesium and calcium ion resorption. Given the impermeability of this segment to water, and the large amount of solute resorption that occurs, the tubular fluid becomes more dilute as it passages the thick ascending loop, which has given rise to the description to it as the 'Diluting Segment' of the nephron.

Given the requirement for active transport of Na^+ and Cl^- and other ions. The cells of the thick loop are rich in mitochondria and have a high ATP requirement, which makes them vulnerable to hypoxic damage due to high oxygen demand and a relatively poor blood supply.

Distal Tubule

After the loop of Henle, the filtrate enters the distal convoluted tubule. The distal tubule is lined by a cuboidal epithelium, somewhat higher than in the thick part of the ascending limb of the loop of Henle, but unlike that of the proximal tubule, it has no brush border. The lateral margins of the cells of the distal tubule are not interdigitated, so lateral cell boundaries can be seen at light microscopy. This, and the fact that distal tubules are a little narrower than proximal tubules, allows the distal tubules to be distinguished from proximal tubules. The distal tubules represent a relatively small proportion of the cortical tubules, and are always seen in close proximity to a glomerulus. The early distal tubule reabsorbs most of the remaining Na^+, K^+ and Cl^-, and is also the site of active calcium secretion. Like the thick part of the loop, it is relatively impermeable to water. It has a large number of mitochondria, but fewer per cell than the proximal tubule. The reason for the distal tubule lying in close proximity to the glomeruli is the presence of the macula densa, a group of specialised cells placed in close proximity to the afferent arteriole of the glomerulus.

Juxtaglomerular Apparatus

The juxtaglomerular apparatus lies between the glomerulus and the distal convoluted tubule of the same nephron. This location is critical to its function in regulating renal blood flow and thus the glomerular filtration rate (GFR). The juxtaglomerular apparatus consists of three cell types: the macula densa cells, the juxtaglomerular cells and the extraglomerular mesangial cells.

The Macula Densa

The macula densa cells of the distal convoluted tubule are smaller than the usual cuboidal cells of the tubule and packed rather closely together. They may be

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recognised by their close packing and dark staining nuclei. These cells appear to have a different morphological polarity from the adjacent cuboidal cells: the Golgi apparatus lies between the nuclei and the bases of the cells. Beneath the macula densa cells, the basal lamina is thin with extensions from the cells passing through it. It is thought that the macula densa cells are sensory cells that respond to the sodium concentration in the fluid within the distal tubule and, perhaps, to the rate of fluid flow past them. An increase in sodium concentration in the tubular fluid leads to a reduction in the production of renin by extraglomerular mesangial cells and juxtaglomerular cells.

Juxtaglomerular Cells

The juxtaglomerular cells secrete renin, and as specialised smooth muscle cells surrounding the afferent arteriole also have the capacity to affect the perfusion of the glomerulus. Although they are activated by prostaglandins released from the macula densa cells, they can also release renin independently of the macula densa. Baroreceptors found in the arterioles trigger renin secretion if there is a fall in blood pressure in the arterioles. Activation of the sympathetic nervous system can also stimulate renin release through activation of beta-1 receptors. Renin is a protease enzyme that catalyses the conversion of angiotensinogen to angiotensin I. Angiotensin I is converted into the active vasoconstrictor angiotensin II by Angiotensin Converting Enzyme (ACE) found in the kidney and largely in the lung.

Extraglomerular Mesangial Cells

Extraglomerular mesangial cells, also known as lacis cells or Goormaghtigh cells, are located in the space between the afferent and efferent arterioles, and the glomerular capillaries. These pale staining, renin containing cells are located just outside the glomerulus, near the vascular pole. They are a type of smooth muscle cell, and although their function is yet to be fully clarified, they play a role in autoregulation of blood flow to the kidney and regulation of systemic blood pressure through the renin-angiotensin system.

Role of the Juxtaglomerular Apparatus in the Control of Glomerulo-Tubular Balance, Blood Pressure and Fluid Volume Control

The key to the control by the juxtaglomerular apparatus of glomerular tubular balance (balancing of GFR and the reabsorption of water and sodium by the tubules) and of blood pressure lies in the inverse relationship between GFR and distal tubule sodium content. If GFR rises due to an increase in blood pressure, then the time available for sodium reabsorption by the tubules falls and the sodium concentration in the distal tubule increases. This stimulates the macula densa cells and leads to the release of adenosine, leading the glomerular vessels to constrict and reducing the GRF. It may be that this constriction of glomerular vessels is mediated by the glomerular mesangial cells, which are known to be contractile.

Conversely if blood pressure falls, due to some extrarenal mechanism, then GFR will also fall. This gives more time for sodium absorption and a decrease in sodium concentration in the distal tubule. Adenosine production is decreased and glomerular vessels dilate, increasing GRF. In addition renin production is increased, the increase probably being mediated by increased production of prostaglandins by macula densa cells. Angiotensin II production is increased and blood pressure rises leading to an increase in GFR.

The cells of the afferent arteriole act as baroreceptors, sensing blood pressure. If blood pressure falls then these cells are activated and renin production is increased. As a recipient of a large proportion of cardiac output, about 25%, the kidney is well placed to be a monitor of blood pressure.

Collecting Ducts

From the distal tubule the filtrate enters the collecting duct system, which represents the final opportunity for the kidney alter the body's electrolyte and fluid balance. In man, the average reabsorption by the collecting duct system is 4%–5% of the sodium and 5% of the water passing through the system, but at times of extreme dehydration more than a quarter of the filtered water may be reabsorbed here. We have not been able to find specific data with regard to the laboratory rat, but have no reason to believe that these numbers would be greatly different.

The variation in water reabsorption from the collecting duct system reflects the effects of hormones. The outer medullary and cortical collecting ducts are largely impermeable to water without the presence of ADH; so in the absence of ADH, water in the renal filtrate undergoes minimal alteration in the collecting ducts, thus promoting a diuresis. The collecting duct system also participates in the regulation of other electrolytes, including chloride, potassium, hydrogen ions, and bicarbonate. Although the collecting duct tree appears as a single system, it is subdivided into three major segments, which have three different functions in the urinary concentrating mechanism.

The first part, the cortical collecting tubule, raises the fractional solute concentration and absolute concentration of urea in the fluid it delivers to the outer medullary collecting duct. The outer medullary collecting duct further raises the absolute intraluminal urea

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concentration and the inner medullary collecting duct adds urea to the papillary interstitium, allowing the generation of maximally concentrated urine due to osmotic reabsorption of water.

Each component of the collecting duct system contains two cell types, intercalated cells and a segment-specific cell type. In the cortical collecting tubule this cell is rather unimaginatively named the connecting tubule cell, in the collecting ducts it is the principal cell and in the inner medullary collecting ducts is called the inner medullary collecting duct cell. Although they have been described as separate cell types, we have not been able to discover reports that clearly differentiate them either in terms of histology or function, and for most purposes it would seem reasonable to treat them as one cell type.

Intercalated Cells

The intercalated cells exist in two forms that are specialised for the transport of hydrogen and bicarbonate ions, respectively. Feeding animals an acid diet converts the bicarbonate secreting form to an acid secreting type, thus helping maintain urine pH in the face of variations in diet (Al-Awqati and Gao, 2011).

Principal Cells

In response to stimulation by aldosterone and ADH, the principal cells are mediators of sodium and potassium balance via sodium channels and potassium channels located in the apical membrane. Increased aldosterone increases Na^+/K^+-ATPase pumping, increasing sodium reabsorption and potassium secretion; increases in ADH lead to opening of aquaporin channels, providing a pathway for water to pass through the principal cells. The combination of aldosterone and vasopressin allows the principal cell control the amount of water and sodium that is reabsorbed (Schlatter and Schafer, 1987).

Histopathology of Renal Toxicity

The kidney, particularly the proximal tubule, is vulnerable to xenobiotic insult due to its generous blood supply, the presence of endogenous transporter mechanisms that can be used by xenobiotics, and its high metabolic capacity. The combination of these functions can result in high intracellular concentrations of toxic chemicals and/or their metabolites, that are many folds greater than found in the plasma (McDonough, 2010). The list of potential toxins is huge and growing; we suggest a review by Perazella (2009) as a good starting point for the reader interested in such compounds.

Histologically, the appearance of damage induced by toxic compounds in the proximal tubule follows a fairly predictable course. Necrotic epithelial cells become detached from the basement membrane and pass through the urinary system; they may be detected by microscopic analysis of the urine. The immediate response is for neighbouring cells to spread out to cover the exposed basement membrane, giving a dilated appearance of the tubules; the cells appear thinner than usual. It is important, although not always straightforward, to differentiate this from a genuine distension caused by increased back pressure from lower parts of the urinary tract.

Once the emergency patching, so to speak, is done, cell division occurs to replace the lost cells, which depending on the extent of the toxicity and the requirement for regeneration may give the cells a basophilic appearance, giving rise to the 'basophilic tubule' description that is often seen in toxicology reports. The kidney is remarkably capable in terms of dealing with toxic insults, and as long as the basement membrane remains intact the tubular epithelium will regenerate rapidly and completely, even if practically all the epithelium is lost. If the underlying basement membrane is damaged then repair with fibrosis and scarring will occur instead.

Damage to other parts of the nephron are comparatively less common. Although not a specific target for toxicity, the collecting ducts and loops of Henle may be extensively damaged in cases of papillary necrosis. This important prostaglandin mediated form of vascular toxicity is significant for certain classes of drug such as NSAIDs and tyrosine kinase inhibitors.

Monitoring kidney disease is an issue, as well-known markers such as serum creatinine and urea concentrations are problematic as regards their interpretation: changes in these variables occur only after a substantial time delay or significant kidney injury and newer markers are now beginning to gain traction (Vishal et al., 2008).

THE URETER

The more caudal position of the left kidney means that the right ureter of the adult rat is longer at 50 mm in length compared to the left 45 mm (Hebel and Stromberg, 1976). The ureter is narrow with an outside diameter of about 0.3 mm, and is lined by a transitional epithelium (urothelium) that is continuous with that of the renal pelvis. Beneath the epithelium is a wrinkled basal lamina and a thin, vascular lamina propria. Layers of smooth muscle cells surround the inner parts of the wall. The smooth muscle lacks the orderly arrangement of the smooth muscle of the gut, and is arranged as an inner longitudinal layer and an outer circular layer, the reverse of the arrangement in the gut. Contraction of the smooth muscle propels fluid to the bladder.

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The Urinary Bladder

The urinary bladder of the rat is roughly spherical in shape and is about 25 mm long and 15 mm wide when distended, and 10 mm long and 5 mm wide when empty (Hebel and Stromberg, 1976). It is located in the pelvic cavity anterior to the rectum and ventral to the reproductive organs. Lying horizontally, it projects forwards beyond the pubic bones that make up the ventral rim of the pelvic inlet and is slightly smaller in females than males.

The convoluted inner surface of the urinary bladder stretches as it fills with urine. The bladder is one of the most elastic organs of the body and is able to increase its volume greatly to accommodate urine (about 600–800 mL in man at maximum capacity and 1 mL in the rat). The transitional epithelium, elastic fibres and visceral (smooth) muscle tissue in the walls of the bladder allow it to stretch and then to return to its original size.

The ureters join the bladder on either side, towards the caudal end of its dorso-lateral surface. In man, the bladder is supported by a number of named ligaments, which do not seem to have been described in detail in the rat. The bladder can be divided into two main parts, the body, which is located above the ureteral orifices, and the base, which consists of the trigone, urethra-vesical junction, deep detrusor muscle and the anterior bladder wall.

The wall of the urinary bladder is made of several distinct tissue layers. The transitional epithelium that lines the organ is able to stretch significantly to accommodate large volumes of urine is a robust epithelium that provides protection from acidic or alkaline urine and other toxins. Deep to the mucosa, the submucosa consists of a layer of vascular connective tissue. Smooth muscle is found in the wall of the bladder; as in the ureter this is not arranged in neat layers. The smooth muscle of the wall runs into the wall of the urethra where it leaves the bladder. The muscular layer of the bladder wall is called the detrusor muscle. The detrusor muscle is structurally and functionally different from the trigonal and urethral smooth muscle, part of which forms the internal urethral sphincter that relaxes to allow urine to enter the urethra.

Urination occurs as a result of contraction of the detrusor muscle and the relaxation of the internal urethral sphincter. The external urethral sphincter, formed from skeletal muscle fibres, surrounds the urethra and allows voluntary control urination, at least in man. The control of the actions of the muscles that make up the muscularis is complex and has been the subject of many publications (Andersson and Arner, 2004).

The blood and nerve supply of the bladder are described in Chapter 7, The Cardiovascular System and Chapter 21(3), The Autonomic Nervous System.

Histology of the Urothelium

In the empty bladder, the urothelium comprises a number of layers and looks rather like a stratified epithelium, but as the bladder fills with urine the epithelium flattens out and the number of layers decreases. The surface cells of the epithelium of the empty bladder are rounded in shape with unique apical cell membranes containing plaques of thickened membrane interspersed with thinner areas. In addition, some membrane appears to be stored as vesicles within the cells. Because cell membranes cannot be stretched, this unique arrangement is required to allow access to more cell membrane as the cell flattens and expands its surface area. This 'extra' cell membrane is thus stored and ready for use. The cells of the epithelium are attached to one another by tight junctions and many desmosomes, ensuring the epithelium is water-tight. The epithelium which lines the lower urinary tract is normally a slowly proliferating tissue, but has great regenerative potential if injured by chemicals or the type of physical trauma caused by crystaluria.

Several specific agents have been identified as urinary bladder carcinogens in humans, and many of these have proven to be carcinogenic in various animal models, particularly in rodents (Cohen, 1998). Classes of chemical agents that are associated with bladder cancer include aromatic amines and amides, phosphoramide mustards, nitrosamines, arsenicals and various alkylating and cancer chemotherapeutic agents. In addition, chronic bacterial cystitis and schistosomiasis have been associated with an increased risk of bladder cancer, and there is some evidence that long-standing lower urinary tract calculi can lead to an increased risk of bladder cancer (Cohen and Johansson, 1992). Several occupational and environmental exposures have also been associated with an increased risk of bladder cancer as identified by a variety of epidemiologic investigations (Cohen et al., 2000).

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Chapter 18

Male Reproductive System

In this chapter we deal with the testis and epididymis, the ductus deferens (which used to be called the vas deferens), the several so called accessory glands and the penis (Fig. 18.1).

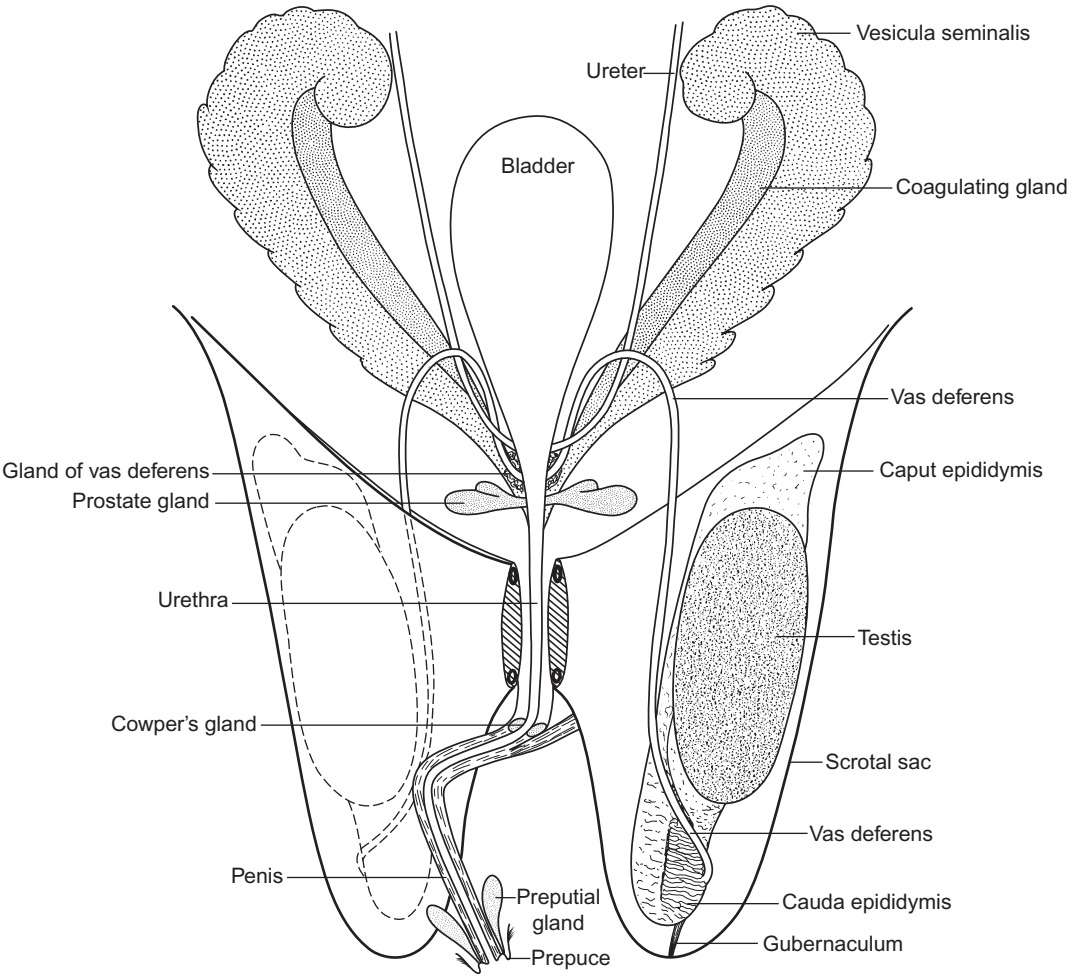


FIGURE 18.1 Male reproductive system.

EMBRYONIC DEVELOPMENT

The anatomy of the testis cannot be understood without some mention of its development and that of the kidney, as the testes develop close to the nephrogenic ridge (see Chapter 17: Urinary Tract). In mammals the complicated development of the kidney passes through three iterations, the pronephros, mesonephros and finally the metanephros, which becomes the adult kidney. All develop from the nephrogenic ridge (or cord) that runs along the dorsal abdominal wall above the parietal layer of the peritoneum.

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Each stage has glomeruli and an excretory duct; respectively the pronephric and mesonephric ducts and eventually the ureter. Like the pronephros, the pronephric duct does not last for long before becoming the mesonephric duct. The mesonephros persists for some time, but when it disappears its duct remains as the ductus deferens. The paramesonephric duct is formed alongside the mesonephric duct, but has nothing to do with the development of the kidneys. Although important in the female it appears and then almost disappears in the male leaving only a few remnants. The testes develop as a result of germ cells migrating from the yolk sac to the mesenchyme, alongside the mesonephros. These germ cells induce the formation of supporting cells that become the Sertoli cells of the testis. The developing testes expand and invaginate the parietal layer of the peritoneum that lines the abdominal cavity.

As the embryo develops the testes appear to migrate caudally. Although this is partially the result of the straightening of the embryo, genuine migration does occur, as the testes make their way from within the abdominal cavity to the scrotum. This complicated process seems to be guided by the gubernaculum, a bundle of connective tissue attached to the caudal end of the testis. It was once thought that the gubernaculum pulled the testes down into the scrotum during its migration, but this theory has now been discarded. The gubernaculum persists in adult life, attaching the testes and epididymides to the caudal end of the scrotum. Hebel and Stromberg (1976) described two ligaments: the ligamentum testis proprium and the ligamentum caudae epididymis (radiating into the cremaster muscle) in this region.

A tubular evagination of the parietal peritoneum forms at the posterior end of the ventral abdominal wall, which pushes into the abdominal wall and passes through its layers, creating an oblique passage through the wall that the testes follow. Indeed we could think of the testes sliding along above the tube of peritoneum.

The developing epididymides are attached to the testes and connected by the ductus deferens to the urinogenital sinus, and thence to the developing urethra.

As the tube-like evagination of the parietal peritoneum and the testes, followed by the ductus deferens, traverse the abdominal wall on their way to the developing scrotum, they pick up a series of layers of tissue. The first layer to be picked up comes from the transverse fascia that lines the abdominal cavity and lies immediately outside the parietal peritoneum. These origin and fate of subsequent layers are shown in the following table (Refer Table 18.1).

The external spermatic fascia is added at the external inguinal ring and the inner layers at the internal inguinal ring. By the time this process is complete the inguinal canal, which runs obliquely through the abdominal wall, encloses the spermatic cord, a thick walled tubular structure that runs from the inguinal canal into the scrotum. The spermatic cord contains the ductus deferens and the processus vaginalis, as well as the arteries that supply the testis and ductus deferens, a plexus of veins (the pampiniform plexus), lymphatic vessels and nerves. The wall is made up of the structures listed in the first four rows of the right hand column of the above table. The outermost layer, the external spermatic fascia, is surrounded by skin. The wall of the spermatic cord continues around the testis and epididymis as they lie in the scrotum. The rat spermatic cord also contains a large mass of fat near the testis.

In the rat the inguinal canal is short and wide, around 8−12 mm in width when distended (Hebel and Stromberg, 1976), but in man it is relatively narrow and long. Contraction of the cremaster muscle draws the testis and epididymis up towards the external inguinal ring. In the normal course of events, the testis is too big to pass through the external inguinal ring in man, but in the rat the testis can be retracted into the inguinal canal, indeed it can be pulled through the canal and back into the

TABLE 18.1 Relationship between layers of abdominal wall and coverings of the testis and ductus deferens

Origin of Layers	Final Form of Layers
Transverse abdominal fascia	Internal spermatic fascia
Transversus abdominis muscle	Contributes to the cremasteric fascia
Internal oblique muscle	Contributes to the cremaster muscle: skeletal muscle. This muscle is arranged in layers and has criss-crossing bundles of fibres
External oblique muscle	External spermatic fascia
Superficial fascia of ventral abdominal wall (called Camper's fascia in human anatomy)	Dartos muscle lying just beneath the skin of the scrotum. Rather oddly this is smooth muscle. Its function is to wrinkle the skin of the scrotum. Hebel and Stromberg (1976) described two layers: dermal dartos and dartos proper
Skin of the ventral abdominal wall	Skin of the scrotum

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abdominal cavity, despite the fact that the testes of adult male rat are not very much smaller than those of a man.

In the rat the processus vaginalis remains patent throughout life, meaning that the peritoneal cavity extends into the scrotum. In man the upper part of the processus vaginalis disappears, leaving a closed cavity around the testis. The walls of this cavity are closed together, rather like those of a collapsed balloon, and wrapped round the anterior surface of the testis. This two-walled structure is the tunica vaginalis, which contains a little fluid between its walls. It is also found in the rat, but in this species it remains connected to the peritoneal cavity.

The Testes

Although the testes are regarded as a retroperitoneal structure, some confusion and debate has arisen on this point (Pham et al. 2005). These authors asked the mildly heretical question, is the testis intraperitoneal? Generations of anatomists have taught that it is not. As is often the case, much depends on definitions, in this case – what precisely is meant by 'intraperitoneal'? Is the small intestine an intraperitoneal structure? Well, it is largely surrounded by visceral peritoneum, but it does not lie in the true peritoneal cavity. Actually nothing does except a small volume of peritoneal fluid. The peritoneal cavity is the potential space between the visceral and parietal layers of peritoneum, so under the strict definition this means that the testis is certainly not an intraperitoneal structure. We shall adhere to this conventional view.

The rat testis is an ovoid, about 20 mm in length and 14 mm in diameter, with a dense, collagenous outer coat: the tunica albuginea, which is whitish-blue in colour and 20–40 μm in thick. The visceral layer of the tunica vaginalis (the part of the processus vaginalis that is wrapped around the testis) is in contact with the tunica albuginea, but does not completely envelope the testis. On its dorsal aspect there is a connective tissue stalk called the mesorchium containing blood vessels and the efferent ducts leading from the seminiferous tubules to the epididymis. Beneath the tunica albuginea lies a vascular layer, the tunica vascularis.

In man, connective tissue septa radiates from the mesorchium to the tunica albuginea, dividing the testis into lobules, but in the rat just a few fine strands of connective tissue run between the tubules. Seminiferous tubules are hair-pin shaped, both ends being connected in the mesorchium to the rete testis. The epithelium of the seminiferous tubules comprises a basal layer of Sertoli, cells over which lies the layers of germ cells. The tubules are surrounded by loose connective tissue and a layer of flattened adventitial cells that look like flattened smooth muscle cells and are known to be contractile.

The Sertoli cells rest on the basement membrane (basal lamina) of the tubules and have oval nuclei, but their shape cannot be defined in ordinary sections. These cells are tall and stretch to the surface of the epithelium with long cytoplasmic processes that are wrapped around the cohorts of developing germ cells. It was once thought that the Sertoli cells formed a syncytium, but this is not so (Fawcett, 1994). Sertoli cells used to be called nurse cells, an appropriate term as they are essential to the development of the germ cells. Indeed, the spermatozoa develop from spermatids embedded in the cytoplasm of the Sertoli cells.

In the rat, a well-developed system of lymphatic sinusoids and blood vessels lies adjacent to the tubules. Large eosinophilic endocrine cells, Leydig cells, are found in the connective tissue between the tubules. The rat has relatively few Leydig cells, and they are often found to be surrounded by lymphatic spaces (Foley, 2001).

Spermatogenesis

Spermatogenesis is the process by which the germ cells develop from relatively undifferentiated spermatogonia into spermatozoa. Three descriptive terms can be found in the literature, to avoid confusion we define them here.

Spermatogenesis: the development of spermatozoa from spermatogonia.

Spermatocytogenesis: the development of spermatocytes from spermatogonia.

Spermiogenesis: the development of spermatozoa from spermatids.

The Germinal Epithelium

Spermatogonia

Spermatogonia are rounded cells that lie in contact with the basement membrane of the seminiferous tubules. They are the stem cells of the system, dividing to maintain their own numbers and to produce the cells that embark on the process of development into spermatozoa. Spermatogonia divide by mitosis into types A and B. Type A are the replacement cells and type B are the cells that develop into spermatocytes. Early type B spermatogonia cannot be distinguished from the spermatogonia in general, but they soon enlarge and begin the complex process of meiosis.

Meiosis is a two stage maturation process. The first division produces secondary spermatocytes and the second maturation division produces spermatids, which undergo no more division and develop into spermatozoa. Many readers will know that meiosis leads to a reduction in the number of chromosomes from the diploid number to the haploid number, which means a reduction from 42 to 21. Essentially the same process occurs in the development of ova in the female rat. By way of example, Table 18.2 explains the process in man.

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TABLE 18.2 Gamete Production in Man

Stage	Events, etc.	Male Cells	Female Cells	Ploidy	*n* Number	Number of Chromosomes
Resting stage of cells: oogonia and spermatogonia	Normal cellular metabolism	Spermatogonia	Oogonia	Diploid A, A′, Two A chromosomes are present, A and A′, hence the cell is diploid	2 Each chromosome contains one strand of DNA	46
Mitosis preparatory phase	DNA replication, centromere replication occurs	Spermatogonia	Oogonia	Diploid AA, A′A′, Two A chromosomes are present: AA and A′A′	4 The number of strands of DNA has doubled	46
Cell division		Spermatogonia	Oogonia	Diploid A, A′, Back to original state	2 Back to original state	46
Meiosis I. Preparatory stage with a long prophase	DNA replication	Primary spermatocytes The cells change from spermatogonia to primary spermatocytes on duplication of the DNA	Primary oocyte The cells change from oogonia to primary oocytes on duplication of the DNA. Long delay in women: process does not proceed until puberty	Diploid AA, A′A′,	4	46
	Crossing over occurs and the cells divide	Secondary spermatocytes produced	Secondary oocyte plus 1st polar body produced	Haploid AA, Only one A chromosome (AA) is present: hence the cell is haploid	2 This is a different sort of 2 from that seen above: A, A′,	23
Meiosis II	No DNA replication takes place, Cells divide	Spermatids produced	1st definitive oocyte produced plus further polar body.	Haploid A, Only one A chromosome is present: hence the cell is haploid	*n*	23

Notes: Ploidy refers to the number of copies of each chromosome present in the cell, the *n* number refers to the number of copies of each strand of DNA. Recall that humans have 46 chromosomes: 44 + XX or 44 + XY. The 44 non-sex chromosomes comprise two sets of 22: there are two number 15 s, etc. We could call these 15 and 15′. Let A and A′ be chromosomes and let us follow them through the process. If the amount of DNA in a chromosome doubles we shall show this by AA or A′A′.
Note the ploidy/*n* number combinations
Haploid: Both 2*n* and *n*
Diploid: Both 2*n* and 4*n*.

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In man, the first meiotic division begins with a 22 day prophase that is divided into five stages: leptotene, zygotene, pachytene, diplotene and diakinesis (mnemonic: let zoologists pet dangerous dingoes). Thread like chromosomes appear during leptotene and duplicate during zygotene, shorten and thicken during pachytene (pachyderms have thick skins), when crossing over occurs. Further shortening and preparation for the next stage occurs during diplotene and diakinesis. Once prophase is over, things move more quickly. The nuclear membrane disappears and the chromosomes line up along the equator of a recently formed spindle before moving apart to produce haploid secondary spermatocytes (see Table 18.2). This sequence is described as metaphase, anaphase and telophase.

Secondary spermatocytes exist for a brief interphase before a second meiotic division, during which no duplication of DNA occurs, and the secondary spermatocytes move quickly through prophase, metaphase, anaphase and telophase to produce the spermatids. Cell division now complete, each spermatid can develop into a spermatozoon.

An added complication is that the cells taking part in the sequence from primary spermatocytes to spermatids never actually separate when they divide, and the daughter cells of each 'division' remain connected by cytoplasmic bridges. Separation only occurs when the spermatids develop into spermatozoa, thus for a large part of their development the germ cells exist as a syncytium. This cannot be seen at light microscopy, but is very apparent at electron microscopy (Fawcett, 1994) (Fig. 18.2).

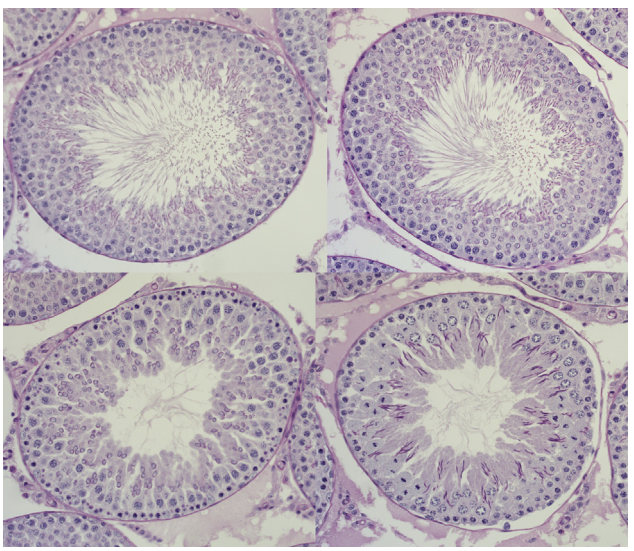


FIGURE 18.2 Testes composite – top left and right stages 4 and 8, bottom left and right stages 10 and 14.

The Cell Cycle of the Seminiferous Tubules of the Rat

Comparing cross sections of rat seminiferous tubule one will see a variety of combinations of cell types. In some sections the superficial cells are clearly spermatids that are about to become spermatozoa, in others, the superficial cells though certainly spermatids, are at an earlier stage of development. Early spermatids are rounded, becoming ever more elongated during the course of development. Leblond and Clermont (1952) noted that certain combinations of the cell types of the layers of the epithelium of the seminiferous tubules appeared again and again. Furthermore they noted that serial sections taken along the seminiferous tubules of rats and guinea pigs showed that there was a definite sequence of patterns, creating a wave that appeared to progress along the tubule repeating itself at intervals. This apparent wave occurs because at every point along the tubule cells are going through a series of stages of development and moving up from layer to layer, but that in each section through the tubule, the stages of development are slightly different. Clermont and Leblond defined a series of identifiable stages, each defined by a specific combination of cell types.

By way of understanding this concept, let us make some assumptions and create a model. Our conceptual testis has: layer 1 contains spermatogonia, layer 2 contains spermatocytes, layer 3 contains early round spermatids and layer 4 contains mature elongated spermatids. Let us also say that it takes 12 days for a spermatogonium to turn into a spermatocyte, for a spermatocyte to turn into a rounded spermatid and for a rounded spermatid to turn into an elongated spermatid; and that at the end of the final 12 days the elongated spermatid turns into a spermatozoon. Thus it takes 48 days for a spermatogonium to turn into a spermatozoon (Table 18.3).

We can set out our model, as in Table 18.3.

We can now follow a cell as it develops from a spermatogonium on day 01 to a spermatid on day 48 when it is about to turn into a spermatozoon. As our cell develops, it moves up through the layers of the epithelium as shown by the red highlighting. Our spermatogonium moves up on day 12 to become a spermatocyte on day 13, and a rounded spermatid on day 25, taking 12 steps to complete its journey in each layer of the epithelium. If we look at columns we can see that specific combinations occur again and again. For example, 44, 32, 19 and 07 means that a spermatogonium is in the 7th day of its development in the bottom layer of the epithelium, a spermatocyte (lying in the next layer) has been on its journey for 19 days, a rounded spermatid has been on its journey for 32 days and an elongated spermatid has been on its journey for 44 days. Note the regularity of the sequence: if a spermatogonium is at day 3 then an elongated spermatid

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TABLE 18.3 Rat Cell Cycle

Cell types	
D	37 38 39 40 41 42 43 44 45 46 47 48 37 38 39 40 41 42 43 44 45 46 47 48 37 38 39 40 41 42 43 44 45 46 7 48 37 38 39 40 41 42 43 44 45 46 47 48
C	25 26 27 28 29 30 31 32 33 34 35 36 25 26 27 28 29 30 31 32 33 34 35 36 25 26 27 28 29 30 31 32 33 34 35 36 25 26 27 28 29 30 31 32 33 34 35 36
B	13 14 15 16 17 18 19 20 21 22 23 24 13 14 15 16 17 18 19 20 21 22 23 24 13 14 15 16 17 18 19 20 21 22 23 24 13 14 15 16 17 18 19 20 21 22 23 24
A	01 02 03 04 05 06 07 08 09 10 11 12 01 02 03 04 05 06 07 08 09 10 11 12 01 02 03 04 05 06 07 08 09 10 11 12 01 02 03 04 05 06 07 08 09 10 11 12
Stages	8 9 10 11 12 1 2 3 4 5 6 7 8 9 10 11 12 1 2 3 4 5 6 7 8 9 10 11 12 1 2 3 4 5 6 7 8 9 10 11 12 1 2 3 4 5 6 7

Cell types: the 4 generations of cells

A spermatogonia

B spermatocytes

C rounded spermatids

D elongated spermatids

Numbers in the body of the table represent days

Small numbers in the lowest row are Stages

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is always at day 44. These vertical columns represent the appearance of cross sections of the seminiferous tubule in our model. We shall call these combinations, these columns, stages. 44, 32, 19, and 07 is a stage; 39, 27, 15, and 03 is another stage. Note that these stages appear every 12 days, so defining the cycle length of 12 days per cycle and 4 cycles in all.

We could name or number the stages, which is precisely what Clermont and Leblond did. The numbers in small type in the lowest row roughly represent their stages. Note, now, that the stage numbers should be shown in Roman numerals: V, VI, VII, etc. The numbering of the stages is arbitrary, but by looking at the cell types present we should be able to say whether a specific cross section represents stage IV or stage IX, etc.

So much for our model. Now let us change it to make it correspond to what happens in the rat. Mathematicians would call this reparameterisation of the model. We must make a lot of changes. The following modifications are based on Creasy (1997).

1. The repeat time or cycle duration, from the appearance of a specified stage to its next appearance, is 12.9 not 12 days.
2. Each cycle is divided into 14 rather than 12 stages (no longer a neat 1 day per stage).
3. The 14 stages vary in their duration. For example, in the rat, stage VII takes 58 hours but stages IX–XI take only 7 hours each. This variability has an important consequence: it means that if you look at random sections you are eight times as likely to see a section at stage VII that at, say, stage X.
4. The whole process takes 56 not 48 days.
5. The whole process involves 4.5 cycle not 4.
6. There are 19 steps in the rounded spermatid to spermatozoon sequence. This means that is a mismatch between the 14 stages and the 19 steps in the spermatid sequence. A few stages must contain more than one distinguishable type of spermatid.

With well-fixed tissue (modified Davidson's fixative is best), preferably stained with PAS, enthusiasm, expert tuition and lots of practice, most of the stages can be recognised. The key lies in the appearance of the spermatids.

The reader may be wondering how useful all this is. Creasy (1997) has pointed out that spermatid retention is an important toxicological response. She pointed out that the release of 'Step 19 spermatids' (the last step in their sequence) should occur at stage VIII. Note that in our simplified model stage VIII corresponds to mature spermatozoa being released and the development of elongated spermatids starting again. Now then, if spermatids are retained, due to damage to Sertoli cells, we shall see step 19 spermatids in stages IX–XIV. They should, of course, not be present in these stages.

In man, cross sections of human seminiferous tubule show more than one stage of the cycle. This led early workers to believe that the orderly cycle seen in rodents might not exist in man. In fact it does, but it is more difficult to recognise. Here are the parameters:

4 cycles, each of 16 days, 6 stages per cycle: total time from start to finish: 64 days. As the algebra textbooks used to say: constructing the model is left 'as an exercise for the reader'.

The Development of Spermatids Into Spermatozoa

The development of spermatids into spermatozoa takes place while the spermatids are partially enclosed by the Sertoli cells (Fawcett, 1994). As the spermatids elongate a dense acrosomal cap forms at the end of the spermatid buried deepest in the Sertoli cells, and the nucleus of the spermatid condenses into a mass of dark staining material. The other end of the elongating spermatid develops into the tail of the spermatozoon. This complicated process involves a pair of centrioles that control the formation of a long flagellum, a modified cilium with the usual $9 + 2$ arrangement of microtubules, but with another 9 thicker tubules arranged around the outside of the $9 + 2$ series. Mitochondria are arranged as a spiral around the first part of the flagellum. The cytoplasm that the spermatozoon does not use remains as a residual body (of Regnaud).

The fully developed spermatozoon comprises a head (acrosomal cap and nucleus) and tail. The tail is divided into a central connecting piece surrounded by the mitochondrial spiral, a long principal piece and a shorter tail piece. The rat spermatozoon is about 190 μm in length, and has a sharply hooked head. The shapes of the heads of spermatozoa vary widely across mammalian species. In man the head is a flattened ovoid. Interestingly, the spermatozoa of small rodents are much longer than those of man (53 um) and the elephant (50 um) (Bishop and Walton, 1960).

Although anatomical development of the spermatozoa is completed in the testis, acquisition of motility occurs in the epididymis and capacitation (the development of the capacity to penetrate ova and thus accomplish fertilisation) only takes place in the female reproductive tract after copulation.

The Pathway From the Seminiferous Tubules to the Ductus Deferens

Spermatozoa released from the surface of the epithelium of the seminiferous tubules pass via short, straight parts of the tubules (the tubuli recti) that are lined by Sertoli cells, but where no germ cells are found, into a labyrinth of short interconnected tubules (the rete testis: rete is

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Latin for a net), then out of the rete via series of spiralling efferent ducts (ductuli efferentes) and into the duct of the epididymis. The ductuli efferentes form small conical structures, which with their associated blood vessels, are called the coni vasculosi. The long coiled duct of the epididymis leads to the ductus deferens.

The epithelium of the tubuli recti and rete testis is a simple cuboidal epithelium, although microvilli and occasional flagella (long cilia) arise from the apical surfaces of the cells. In the ductuli efferentes, the lining cells bear cilia that are assumed to be motile and which play a part in moving the spermatozoa forwards.

EPIDIDYMIDES

The epididymides are a pair of long tubular structures attached to the dorsal surface of the testes: one epididymis per testis. Each is divided into a head (caput), body (corpus) and tail (cauda). The word epididymis has an interesting origin: didymi means twins, the testes are the twins and the epididymis lies upon the testis (Fig. 18.3).

The long convoluted duct of the epididymis is around 400 cm long in the rat and 600 cm in man (Hebel and Stromberg, 1976). The duct is lined by a pseudostratified columnar epithelium in which several cell types have been described. Tall principal cells with sterocilia (long microvilli) are interspersed with small basal cells. Another population of small cells with pale cytoplasm and dark rounded nuclei called halo cells are thought to be intraepithelial lymphocytes. The ductal wall contains smooth muscle that thickens as the duct approaches the ductus deferens. The duct of the epididymis is an absorptive duct, where some 90% of the fluid secreted by the seminiferous tubules is absorbed.

THE ACCESSORY GENITAL GLANDS OF THE MALE RAT

The accessory glands provide the bulk of the fluid content of the ejaculate. In the rat the fluid coagulates and forms a copulatory plug that seals the female reproductive tract after copulation.

The male rat has a well-developed complement of accessory genital or reproductive glands. These are the gland of the ductus deferens, the seminal vesicles, the prostate gland and the coagulating gland (regarded as a part of the prostate), the bulbourethral glands (of Cowper), the urethral glands and the preputial glands of the penis. The dimensions and shapes of these glands are summarised in Table 18.4. The data shown in the table are derived from Hebel and Stromberg (1976). The preputial glands are described with the penis.

Histological Structure of the Accessory Glands

Creasy et al. (2012) and Motohashi et al. (2015) have provided detailed descriptions of the histology of the accessory glands; here we provide only some brief notes.

Ductus Deferens

The ductus deferens is 5–6 cm in length, and runs in the spermatic cord from the tail of the epididymis to join the urethra as it passes through the prostate gland. As the ductus approaches the prostatic urethra it widens into an ampulla, where the ducts of the seminal vesicles join the ductus. The portion of the ductus that enters the prostatic urethra is described as the ejaculatory duct. Paired ejaculatory ducts open into the urethra as a papilla on either side of a ridge, the colliculus seminalis (Hebel and Stromberg, 1976). A small blind diverticulum, the utriculus masculinus, also opens into the colliculus. This male equivalent of the uterus is derived from the paramesonephic duct.

The epithelium of the ejaculatory ducts is folded and the mucosa surrounded by a mesh of elastic fibres resulting in the mucosa being thrown into a series of longitudinal folds. The wall of the ductus consists of three layers of smooth muscle arranged as inner and outer longitudinal layers and a middle circular layer. The epithelium is pseudostratified columnar with cells bearing sterocilia.

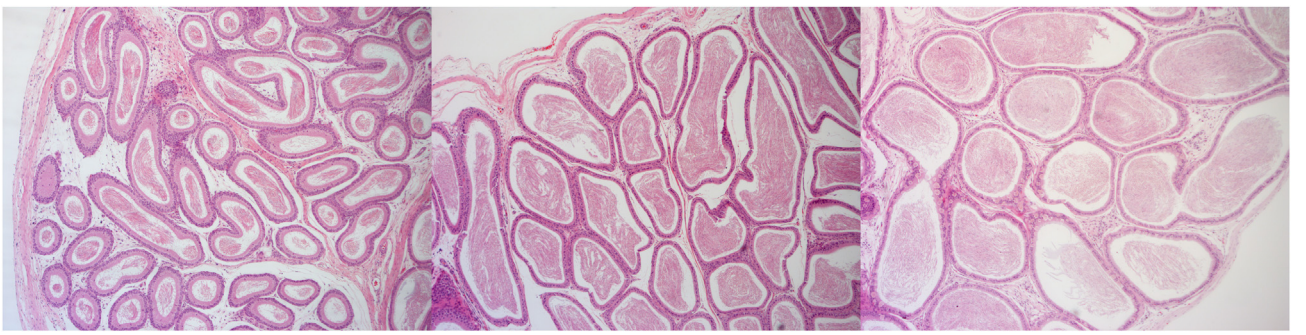


FIGURE 18.3 The epididymis. From left to right the initial segment of the head, the body and the tail.

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TABLE 18.4 Glands of the male reproductive tract

Gland	Shape	Length mm	Width mm	Weight
Gland of the ductus deferens	Two lobes lying along the terminal part of the ductus deferens	4	2	Each lobe: 25 mg
Seminal vesicles	Two large glands (R and L). Hook shaped with transverse ridges	17–25	8–11 5.6 in thickness	0.7–1.5 g (each gland)
Coagulation glands	Two lobes, tucked into the concavities of the seminal vesicles	3–6		40–110 mg (one lobe)
Ventral lobes of prostate gland	Two lobes, club shaped, projecting 5 mm cranial to pelvic brim	14–17	6–9 (max)	300 mg (one lobe)
Dorsolateral lobes of prostate gland	Two lobes wrapped around the proximal urethra and combined as a single mass		5–10	
Bulbourethral glands (Cowper's glands)	Two glands: R and L, each lateral to rectum, near root of penis	11–15	5 (max)	60–70 mg (one gland)
Urethral glands	Acini located in the wall of proximal urethra			

Gland of the Ductus Deferens (Ampullary Gland)

arge uniform acini are lined with a low cuboidal epithelium containing a secretion that stains pink in H&E sections. The acini are surrounded by connective tissue and smooth muscle fibres. These small glands have received very little attention from students of anatomy over the years (Fig. 18.4).

Prostate

The acini are lined with cuboidal/columnar epithelium that has a brush border in the lateral parts of the gland (Creasy 2012). The staining of the secretions is varied with the secretion in the lateral lobes staining more intensely with eosin than in the ventral parts of the gland. Connective tissue rich in smooth muscle surrounds the acini and encapsulates the gland. Other cells seen in addition to the secretory cells include basal cells and cells that resemble macrophages and lymphocytes.

The coagulating gland is regarded as a part of the prostate. The acini are lined by a mucosa that is infolded into the acini as papillary processes and covered by a columnar epithelium. The secretions of the gland stain pink with eosin (Fig. 18.4).

Seminal Vesicles

The saccular structure of the seminal vesicles is quite different from that of the other glands in this group. The intricate folding of the mucosa creates interconnected spaces which have the appearance of acini to the casual observer. The lining epithelium is columnar and the secretion stains deeply pink with eosin (Fig. 18.5).

Bulbourethral Glands

These are lobular mucous/sero-mucous glands with secretory cells that have small dark basal nuclei and are enclosed by myo-epithelial cells. The cytoplasm of the cuboidal/columnar epithelium stains pale blue in H&E sections and is filled with secretory granules which stain deeply with the PAS and Alcian blue techniques for mucin. The granules predominantly contain acid mucosubstances, mostly sulphated and carboxylated, but there are also some neutral mucosubstances. Electron microscopy shows some of the granules have a dense core while others are quite pale. A central duct drains the secretions of the lobules.

PENIS

The anatomy of the rat penis is similar to man, but contains a bone, the os penis or baculum, and has an anatomically more complicated glans penis. The os penis of the rat is about 7.5 mm in length and about 1 mm in diameter; sometimes two small pieces of bone are found, one in front of the other. The exact function of the os penis has been debated, but it appears to have a role in force transfer during copulation (Kelly, 2000).

There are three cylinders of cavernous tissue that contain large, endothelium lined, vascular spaces surrounded by a layer of connective tissue (tunica albuginea or tunica fibrosa). The ventral cylinder, the corpus spongiosum,

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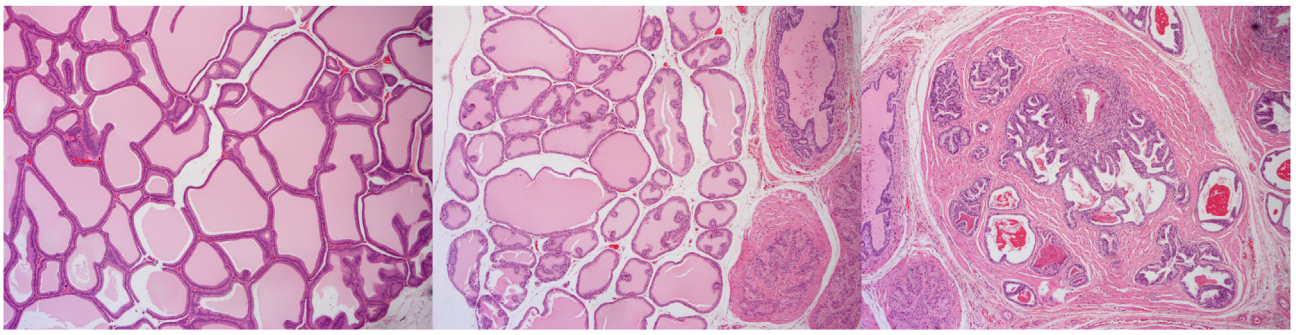


FIGURE 18.4 The accessory glands. From left to right the ventral prostate, dorso-lateral lobe and the ampullary gland.

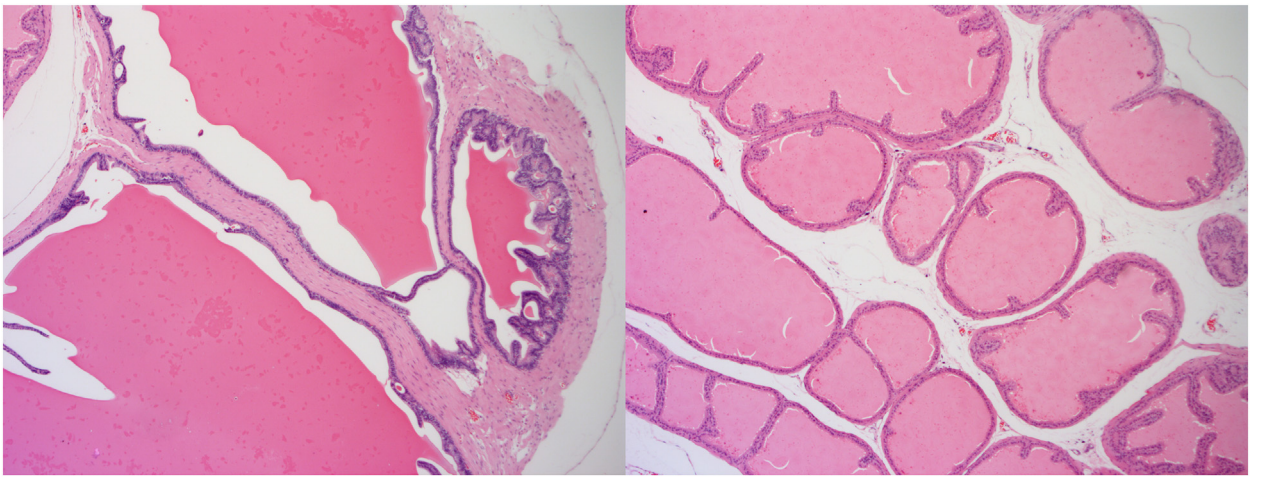


FIGURE 18.5 The seminal vesicles (left) and coagulating gland. The shrinkage of the contents is of course a processing artefact.

originates beneath the bladder at the bulb of the penis, where it is surrounded by the bulbo-spongiosus muscle. As it runs from the bladder to the orifice at the distal end of the penis, it is traversed by the urethra. The corpus spongiosum undergoes a complex expansion to form the glans penis, which includes the formation of a group of cavernous spaces. One surrounds the urethra, a pair lie on either side of the os penis, and a larger space lies beneath the surface of the glans. Sections through the glans show these spaces with the os penis in the middle and the urethra below. The glans is covered by hairless skin with short cornified spines that point towards the base of the penis. The prepuce contains the preputial glands and covers the glans.

The two dorsal cylinders, the corpora cavernosa, arise separately from the inner surface of the pelvis where they are covered by the ischio-cavernosus muscles. The paired corpora cavernosa fuse and form a single curved dorso-lateral cavernous space in the body of the penis. This mass of cavernous tissue stops well short of the distal end of the penis, and is attached to the proximal end of the os penis that runs forwards to the tip of the glans. The distal end of the os penis articulates with a short cartilaginous extension.

The flaccid rat penis is 20–28 mm in length, 3.6 mm in width and 2.8 mm in height with a caudal bend (Hebel and Stromberg, 1976). Proximal to the bend it is supported by the suspensory ligament of the penis, which is attached to the ventral abdominal wall.

The blood vessels of the penis, paired arteries and a single midline vein, run along the dorsal surface of the organ. Lymphatic vessels accompany the blood vessels. Inflow of blood into the spaces and obstruction of outflow, produces hardening of the tissue and erection of the penis that results in a straightening of the penis, but not to a marked increase in length, although the glans does swell.

The urethra is not a simple tube. It is flattened dorso-ventrally and has dorso-lateral and ventro-lateral horns. Small urethral glands, of Littré, open into the lumen of the urethra.

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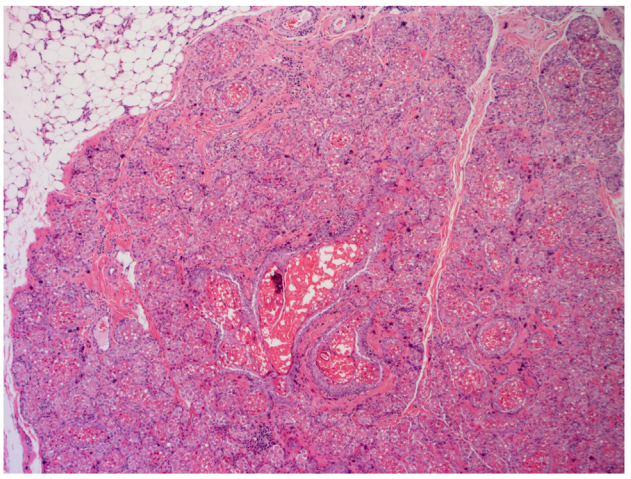


FIGURE 18.6 The preputial gland.

Histology of the Preputial Glands

The preputial glands are large sebaceous glands that deliver their secretions into the space between the inner surface of the prepuce and the glans penis (Fig. 18.6). Like the Zymbal's gland of the ear, it is of interest to the toxicologist because of its response to carcinogens. The gland comprises a mass of sebaceous acini comprised of cells containing large eosinophilic granules containing pheromones and is surrounded by a delicate web of connective tissue (Rudmann et al., 2012).

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Chapter 19

Female Reproductive Tract

The female reproductive tract comprises the ovaries, oviducts, the uterus and the vagina. Although the rat cervix is not comparable to that found in humans, the junction between the uterus and the vagina is sometimes referred to as the cervix.

DEVELOPMENT AND TOPOGRAPHY

The first stages of the development of the ovary are identical to those of the testis, and cannot be distinguished histologically; the later stages are very different. The ovary remains within the abdominal cavity, while the testis descends to the scrotum.

The oviducts and uterus are formed from the paramesonephric ducts (Müllerian ducts), which develop from the lining of the peritoneal cavity close to the mesonephric ducts (Wolffian ducts), but have nothing to do with the 'second kidney' or mesonephros (see Chapter 18: Male Reproductive System). The paramesonephric ducts are attached to the dorsal abdominal wall by a fold of parietal peritoneum, which develops into the broad ligament of the uterus. The ovary is attached to the surface of the anterior end of the broad ligament by a band of connective tissue known as the mesovarium. In humans, ova released from these surfaces enter the peritoneal cavity, but are quickly swept into the funnel-shaped mouths of the oviducts that run in the broad ligament and open into the peritoneal cavity, very close to the ovary. The oviduct is sometimes called the salpinx (Greek for tube), and the part of the broad ligament that enfolds the salpinx is the mesosalpinx. It will probably be obvious that there is a danger of infection making its way via the vagina, uterus and oviducts into the peritoneal cavity. Infection of the oviduct is referred to as salpingitis, and is one of the most common causes of infertility in women.

The anatomy of the rat is a little different, as the ovary is surrounded by a capsule of mesovarium, the ovarian bursa, pierced by the oviduct. The open end of the oviduct lies within it and the ova never actually enter the peritoneal cavity. This arrangement is unusual among mammals, and some authors maintain that the capsule around the rat ovary is not complete and that a slit connects the interior of the capsule with the peritoneal cavity.

The developing ovary, like the developing testis, is attached to the gubernaculum, which itself is attached to the developing uterus. The gubernaculum develops into the round ligament of the ovary, which connects the ovary with the tip of the adjacent uterine horn and the round ligament of the uterus, which connects the uterus with the abdominal wall at the internal inguinal ring. In Chapter 18, Male Reproductive System, we describe how the testis, accompanied by a projection of parietal peritoneum (the processus vaginalis), passes through the inguinal canal and into the scrotum. Things are not so very different in the female. The round ligament of the uterus, accompanied for some distance by a female equivalent of the processus vaginalis (human anatomists call this female equivalent the canal of Nuck), passes through the inguinal canal and into the labia majora of the external genitalia. Although this is what happens in female humans, it would seem that diverticuli of the peritoneum (the equivalents of the processus vaginalis) are not always present in female rats (Hunt, 1924). Hebel and Stromberg (1976) refer to the ligament of the ovary that is derived from the gubernaculum as the 'proper ligament of the ovary', placed it medial to the oviduct, and described it as blending with the mesosalpinx at the tip of the uterine horn. This ligament contains mainly smooth muscle fibres. They also describe a suspensory ligament that runs from the ovarian hilum to the crus of the diaphragm. This is presumably equivalent to the suspensory ligament of the human ovary (better described in human anatomy as the infundibulopelvic ligament) that forms that part of the broad ligament running from the ovary to the pelvic wall. Der Schoot and Emmen (1996) have provided an interesting discussion of the suspensory ligaments of mammalian gonads.

In humans, the ovary descends to the brim of the pelvis, but in the rat, the two horns of the uterus stretch forwards to the ovaries situated on the dorsal aspect of the abdominal wall.

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The Ovary

The ovaries of the rat lie caudal to the kidneys. Hebel and Stromberg (1976) placed the right ovary at the level of lumbar vertebrae 4–5 and the left at 5–6, but Rowett (1952) showed them a little further forward, with the left lying alongside the left kidney (Fig. 19.1).

In the mature adult, the ovary measures a $5 \times 4 \times 3$ mm^3 and weighs about 60 mg. Blood is supplied to the right ovary by a branch of the abdominal aorta, the right ovarian artery and returned via the right ovarian vein to the posterior vena cava. On the left, the vessels are connected with the renal vessels. The ovarian arteries run in the broad ligament and anastomose with the uterine arteries, which arise from the iliac arteries.

Oviduct

The oviduct is a narrow looped structure, 0.4–0.5 mm in diameter and 18–30 mm in length, lying adjacent to the ovary, with a funnel-shaped proximal end that pierces the ovarian bursa (Hebel and Stromberg, 1976). The distal end penetrates the tip of the uterus.

The mucosa of the proximal part of the oviduct is thrown into a series of branching longitudinal folds, which in cross section appear to almost fill the lumen, extending beyond the open cranial end of the oviduct as finger-like processes called fimbria. The folds become less prominent as the uterus is approached.

The mucosa is a simple columnar epithelium, with both ciliated and unciliated secretory cells. The ciliated cells are said to respond to oestrogen by increasing the frequency of ciliary beating at the time of ovulation. Beneath the epithelium a very vascular lamina propria contains large lymphatic capillaries. There is little evidence of a muscularis mucosae, though some smooth muscle fibres extend into the longitudinal folds. The wall of the oviduct contains a well-developed circular or spiral layer smooth muscle (the muscularis), and a less well-developed outer layer of longitudinally disposed smooth muscle. Rhythmic pumping of the tube aids transport of the fertilised ovum to the uterus. It is important to note that fertilisation, the entry of a spermatozoon into the ovum, occurs in the oviduct. The wall of the oviduct is very vascular with a well-developed layer of blood vessels between the circular and longitudinal layers of smooth muscle.

Uterus

The rat has a duplex uterus that is it is divided into two separate horns. The horns are 30–40 mm in length and join about 7–10 mm above the vagina, but each horn enters the vagina individually, so functionally the rat can

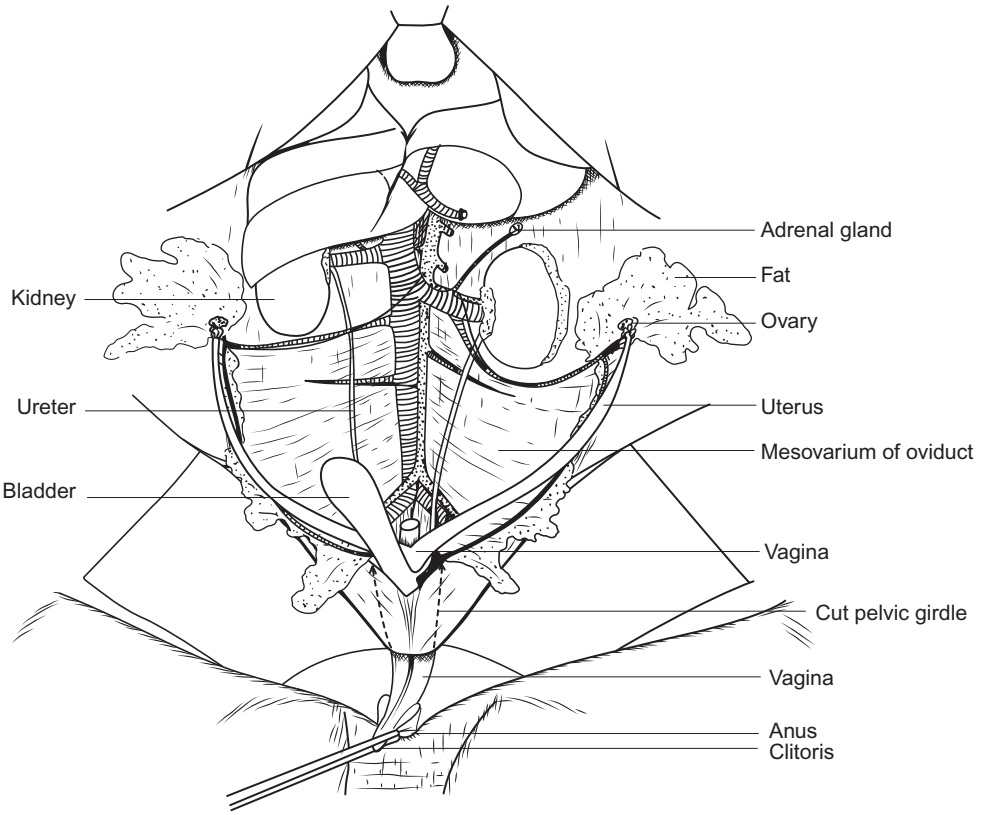


FIGURE 19.1 Dissection to show female reproductive system.

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be considered to have two uteri. Each opening of a uterine horn into the vagina is surrounded by a small ventral cushion, a large dorsal cushion and two lateral cushions of tissue. Hebel and Stromberg (1976) refer to this as the 'portio vaginalis uteri', the doorway from the uterus into the vagina. A short sagittal partition separates the entry points of the horns into the vagina.

Vagina

The rat vagina opens to the exterior just in front of the anus and behind the opening of the urethra. The vagina is thin walled, 15–20 mm in length and 3–5 mm in diameter, with a mucosa that is thrown into longitudinal folds.

HISTOLOGY

The surface of the ovary is covered by a simple epithelium, once called the germinal epithelium as it was thought that it was the source of the germ cells. This concept has long since been abandoned, and the epithelium is now called the ovarian surface epithelium (OSE). It is derived from the lining of the peritoneal cavity and is really modified parietal peritoneum. The cells of the epithelium vary from squamous to cuboidal, and play an important part in repairing the breach made in the surface of the ovary when ovulation occurs. The OSE has attracted much interest during the past 30 or so years because >80% of human ovarian cancers arise from this epithelium. The OSE has been reviewed in detail by Auersperg et al. (2001) and with regard to the rat by Gaytán et al. (2005).

Beneath the OSE lies the tunica albuginea, a layer of connective tissue containing many fibroblast-like cells. The collagen fibres of the tunica are thin and less well developed than those of the testes.

The remainder of the ovary can be divided into a cortex and a medulla. The cortex contains the follicles, the medulla and the large blood vessels running to and from the ovary at the hilum. Between the follicles of the cortex and the blood vessels of the medulla is the ovarian stroma, a fibroblast-rich connective tissue.

Follicles

Primordial Follicle

Immediately beneath the tunica albuginea lie the primordial follicles. These comprise a primary oocyte surrounded by a single layer of squamous follicular cells. The oocyte is about 25 μm in diameter with a well-defined nucleus, and a nucleolus that often stains pink in H&E stained sections. The development of the primary oocyte arrests during prophase of the first meiotic division (at diplotene: see Chapter 18: Male Reproductive System) so the cell is sometimes described as 'resting'. At puberty the rat ovary contains about 40,000 primordial follicles that are transformed into primary follicles throughout the reproductive life of the animal. This transformation seems not to be under hormonal control, nor is it related to the oestrous cycle. Most transformed follicles develop for variable periods before undergoing a form of degeneration called atresia, never reaching their final preovulation state. Atresia affects follicles at all stages of development up to the antral follicle.

Although it was also once thought that oogonia in the ovary gave rise to primary oocytes, this idea was discarded in the early 1950s when Zuckerman showed that the female mammal was born with a stock of primary oocytes that are used up during reproductive life and not replaced (Franchi et al., 1962; Zuckerman, 1970). Interestingly, this was the view put forward by Waldeyer as long ago as 1870, but later disputed and discarded. Zuckerman acknowledged that a few species of mammals, several prosimians in fact, did not seem to conform to his views, but these were dismissed as unimportant exceptions to the rule. The debate has been resumed in the last 20 or so years, and the question once more seems to be far from settled, though Zuckerman's views still prevail in most textbooks (Tilly et al., 2009; Esmaeilian et al., 2013).

Primary Follicle

Primary follicles can be distinguished from primordial follicles by the layer of cuboidal/columnar cells that surround the primary oocyte. These follicular cells develop further as the follicle develops.

Secondary Follicle

During follicular development the primary oocyte increases in diameter, often appearing to have no nucleus; a sectioning artefact created by the oocyte being 100 μm in diameter, so there is a fair chance that a 5 μm histological section will not pass through the nucleus of the cell. During development of the secondary follicle, the follicular cells multiply and form a multilayered avascular zona glomerulosa surrounding the oocyte. The zona pellucida (pellucid means transparent) starts to appear in primary follicle, and in the secondary follicle can be clearly seen between the zona glomerulosa and the plasma membrane of the oocyte. This layer of glycoprotein and acid mucopolysaccharide stains brightly with eosin, and deeply with connective tissue stains. The origin of the zona pellucida has been debated. Fawcett (1994) stated that it was produced by the oocyte, but that contributions from the zona glomerulosa could not be excluded. What is certain is that the oocyte and the surrounding glomerulosa cells remain

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in contact via gap junctions between cytoplasmic processes that pass through the zona pellucida.

Another development that begins in association with the primary follicle, but which becomes increasingly clear as the follicle develops, is the condensation of stromal tissue around the developing follicle. Two distinct layers of tissue, the theca interna (or theca folliculi) and the theca externa (theca: Greek for sheath), are formed. The theca interna comprises a vascular layer of polygonal secretory cells that will play an important part in the formation of the corpus luteum if the follicle proceeds to ovulation. These cells have all the ultrastructural characteristics of steroid producing cells and are rich in smooth endoplasmic reticulum. The theca externa is a cellular connective tissue. The theca interna is separated from the outer layer of glomerulosa cells by a thick basement membrane thought to be produced by the glomerulosa cells.

As the secondary follicle develops, fluid filled spaces appear among the cells of the zona glomerulosa. A follicle showing several well-developed fluid filled spaces is described as a vesicular follicle.

Tertiary or Antral Follicle

The tertiary or antral follicle is immediately identifiable. The fluid filled spaces of the glomerulosa coalesce to form a single cavity or antrum, while the oocyte, still surrounded by a few layers of glomerulosa cells, projects into the antrum. As the follicle develops the layers of glomerulosa cells around the oocyte decrease till just one or two layers, the corona radiate, remain. The oocyte continues to grow as the follicle develops, but has yet to progress beyond the prophase of the first meiotic division. Now things begin to move more rapidly. A number of tertiary follicles are 'selected' for further development into the follicles that will reach ovulation. The primary oocyte completes its first meiotic division, an unequal division producing a secondary oocyte and a smaller polar body. The secondary oocyte undergoes a second meiotic division without duplication of DNA, and then divides, producing a mature ovum and a second polar body. The first polar body can itself divide producing two further polar bodies. Polar bodies are not considered to be fertilisable and generally remain close to the oocyte, within the zona pellucida. In mammals they are short-lived, disappearing in man after 24–48 hours. Although in mammals they appear to be no more than waste bins for superfluous genetic material produced during meiosis, this is not the case for all animals. In many parthogenetic species the polar bodies are an essential part of the reproductive process.

The key to understanding this rather complicated process is to recall that prior to the second meiotic division is there no duplication of DNA; prior to the first meiotic division, and of course prior to all mitotic divisions, DNA is duplicated.

The Graafian or preovulatory follicle (named for the Dutch anatomist Regnier de Graaf, 1641–73).

The follicle continues to expand and is distended with follicular fluid, reaching a diameter of 600–700 μm. The oocyte is now 80–90 μm in diameter and surrounded by a corona of glomerulosa cells that project into the follicular fluid forming a promontory known as the cumulus oophorus or discus proligerous. The root of the promontory thins and detaches from the wall of the follicle, allowing the oocyte, surrounded by its corona, to float free in the antral fluid. All is now ready for ovulation. The Graafian follicle (in the rat there will be a number of these) distends the adjacent surface of the ovary in an area called the stigma. The tunica albuginea and the OSE become thin and rather avascular; rupture of the surface occurs, and the oocyte escapes. The rupture should not be thought of as the sudden bursting of a balloon; the oocyte is released, rather than fired, from the ovary.

The Corpus Luteum

Once the oocyte has been released, the ruptured follicle collapses in on itself. The centre fills with blood from the vascular theca interna, and the basement membrane between the theca interna and the glomerulosa breaks down. What were once the cells of the theca interna and the glomerulosa enlarge, accumulate lipid, and are transformed into plump, pale-staining polygonal cells: – 'lutein cells' (Fawcett, 1994). Fawcett distinguished glomerulosa lutein cells from theca lutein cells, the latter being more deeply staining. The tissue of the corpus luteum is invaded by sinusoidal capillaries, becoming an endocrine gland that produces progesterone. The corpus luteum is yellowish in colour because of the lipid it contains (luteum is Latin for yellow). The further development of the corpus luteum depends on whether mating has occurred, and whether fertilisation of the ova has resulted from the mating. If no mating occurs, the corpus luteum of the rat degenerates and plays little part in suspending the oestrous cycle. It persists longer if 'unsuccessful mating' has occurred (such as would be produced by mating the female rat with a vasectomised male), and for much longer if fertilisation has occurred. Sterile mating leads to a state of pseudopregnancy, whereby the oestrous cycle is suspended and the phase of dioestrus prolonged. If no mating occurs, the corpus luteum regresses over the following two or three cycles. When successful mating occurs, the corpus luteum (actually corpora lutea because the rat produces more than one ovum, often eight or so) persists until the feto-placental unit takes over hormone production.

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Complete degeneration of the corpus luteum leads to formation of a small, white, fibrous structure known as the corpora albicans, which persists in the human ovary, but disappears in the rat (Fig. 19.2).

The Oestrous Cycle of the Rat

Much confusion surrounds the words oestrus and oestrous (American authors use the terms estrus and estrous). Oestrus is a noun, and means the period of sexual receptivity or 'heat' experienced by female rats and other mammals, but not women. Oestrous is an adjective used to describe the cycle of events occurring in the ovary, uterus and vagina of the female animal that occur before and after ovulation. Many authors do not distinguish these terms.

The female rat ovulates every 4–5 days, so has a 4- to 5-day oestrous cycle. If female rats are prevented from mating this cycle will be repeated from puberty, which occurs at about 30–40 days (Porter stated 72 days but earlier opening of the vagina is usually seen) of postnatal life, until the end of reproductive life at about 450 days of postnatal life (Porter, 1957). The rat oestrous cycle has been studied in detail; see Mandl (1951). The following stages are defined:

Pro-oestrus: 18 hours

Oestrus: 25 hours

Metoestrus: 5 hours

Dioestrus: 59 hours

This indicated a cycle of 107 hours or 4.4 days.

Slightly different periods were described by Long and Evans (1922), the earliest workers in the field.

Defining where a female rat is in the cycle can be done by examining the cells by vaginal smears or by instilling and then removing fluid and examining it for cells. Five states or stages are usually defined. These are explained in Table 19.1.

For a more recent description of the stages of rat oestrous, see Paccola et al. (2013).

Perhaps, the most interesting aspect of the oestrous cycle: the development of the ovarian follicles and the times taken for the various stages of follicular development, is well shown in Fig. 19.3 by McGee and Hsueh (2000).

Fig. 19.3 shows the slow development from the primordial follicles to the antral follicles. Once the antral follicles are recruited for further development to Graafian follicles the process runs quickly: 2–3 days in the rat. The very slow transition from primordial to antral follicles in female humans is shown for comparison. It is essential to recall that the process of transformation of primordial follicles into antral follicles is continuous but that the transformation of antral follicles into Graafian

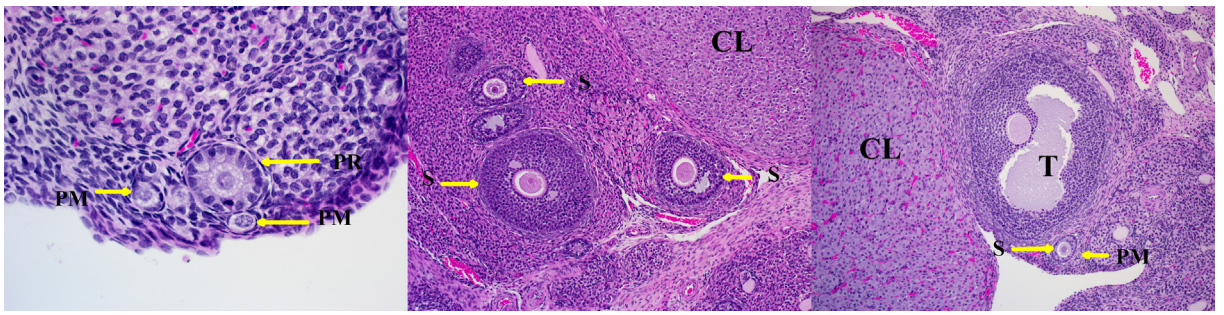


FIGURE 19.2 Ovarian follicles: On the left hand plate a primary follicle (PR) and two primordial follicles (PM). In the centre a collection of secondary follicles (S), the larger just about to transition to being tertiary follicle, the antrum can just be seen starting to emerge. A Corpora Lutea (CL) also present. On the left hand plate a large tertiary follicle (T), with an early secondary follicle, and just visible, a primordial follicle.

TABLE 19.1 Stages of the Rat Oestrous Cycle

Stage	Observations of Vagina and Cells Recovered on Swabbing
1: 12 h	Vaginal mucosa slightly dry, only epithelial cells in smear, vaginal lips a little swollen, animal coming into heat at end of stage
2:	Vaginal mucosa dry and lustreless, only cornified cells in smear, vaginal lips swollen, animal in heat
3: (stages 2 and 3: 27 h)	As in stage 2, cornified material abundant: cheesy material present. Animal not in heat. Ovulation occurs. Note that 'heat' is over before ovulation occurs
4: 6 h	Vaginal mucosa slightly moist, cornified cells and leucocytes in smear, swelling of vaginal lips gone
5: 57 h	Vaginal mucosa moist and glistening, leucocytes and epithelial cells in smear, variable amount of mucus present

Source: Modified from Parkes, A.S., 1929. The Internal Secretions of the Ovary. Longmans, Green and Co., London (Parkes, 1929).

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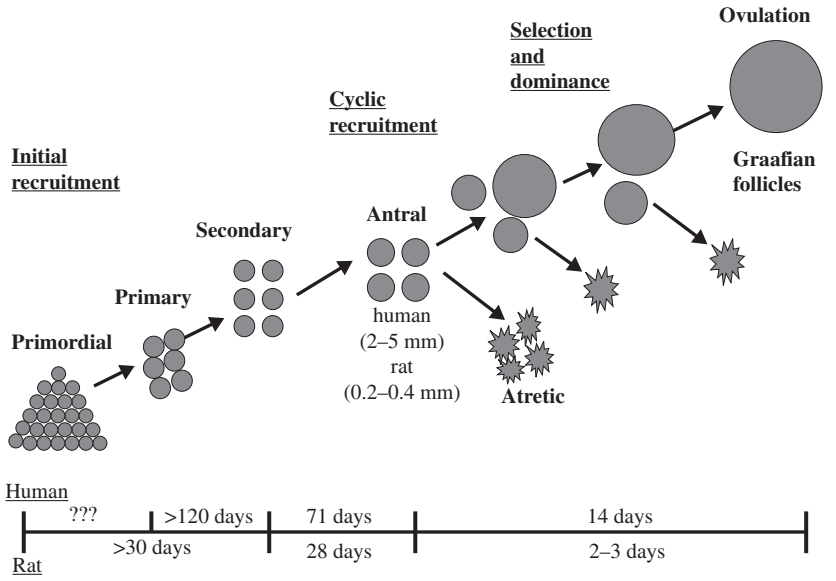


FIGURE 19.3 Duration of follicle recruitment and selection in human and rat ovaries. Primordial follicles undergo initial recruitment to enter the growing pool of primary follicles. Due to its protracted nature, the duration required for this step is unknown. In the human ovary, greater than 120 days are required for the primary follicles to reach the secondary follicle stage, whereas 71 days are needed to grow from the secondary to the early antral stage. During cyclic recruitment, increases in circulating FSH allow a cohort of antral follicles (2−5 mm in diameter) to escape apoptotic demise. Among this cohort, a leading follicle emerges as dominant by secreting high levels of estrogens and inhibins to suppress pituitary FSH release. The result is a negative selection of the remaining cohort, leading to its ultimate demise. Concomitantly, increases in local growth factors and vasculature allow a positive selection of the dominant follicle, thus ensuring its final growth and eventual ovulation. After cyclic recruitment, it takes only 2 weeks for an antral follicle to become a dominant Graafian follicle. In the rat, the duration of follicle development is much shorter than that needed for human follicles. The time required between the initial recruitment of a primordial follicle and its growth to the secondary stage is more than 30 days, whereas the time for a secondary follicle to reach the early antral stage is about 28 days. Once reaching the early antral stage (0.2−0.4 in diameter), the follicles are subjected to cyclic recruitment, and only 2−3 days needed for them to grow into preovulatory follicles. From McGee, E.A., Hsueh, A.J.W., 2000. Initial and cyclic recruitment of ovarian follicles. Endocr. Rev. 21 (2), 200−214.

follicles is cyclical and hormone-driven. Fig. 19.3 shows that some follicles, indeed most follicles, never make the full journey from primordial to Graafian, on the contrary most undergo a process of degeneration and form atretic follicles. The word atretic is derived from atresia meaning failure to penetrate or perforate; in the present case it means failure to complete development.

Atretic Follicles

Atresia can occur at any point in follicular development and is typified by the apoptosis of the cells of the zona glomerulosa. Small, dark, pyknotic, nuclei appear accompanied by the nuclear debris produced by karyorrhexis (the debris is described as karyorrhectic: the adjectival form). Cells from the theca interna persist and form masses of glandular tissue, a process that is particularly marked in rodents but not in humans. The zona pellucida seems to be resistant to degeneration and spaces containing the remnants of zonae may be seen.

Rete Ovarii

The remains of the mesonephric tubules sometimes appear as anastomosing tubules. Remnants of paramesonephric derivatives remain in the male and that remnants of mesonephric derivatives remain in the female. We have listed them, in Table 19.2, as they appear in humans because the reader may come across the terms in other accounts.

Functional parts of the males and female systems are shown in bold type. Textbooks of human anatomy and embryology should be consulted for details of the minor structures.

UTERUS

The wall of the uterus may be divided into the endometrium or mucosa, the myometrium, which is the muscular layer of the wall, and the perimetrium, a thin layer of connective tissue covered by a single layer of serosal cells. The endometrium contains tubular glands that extend into the lamina propria underlying the endometrial epithelium. The endometrial epithelium is a simple columnar epithelium that varies in height with the stages of the oestrous cycle. The lamina propria is notably vascular and contains large numbers of leucocytes. The myometrium comprises an inner circular layer (with respect to the long axis of the uterus) and an outer longitudinal layer. The structure of the endometrium varies with the phases of the oestrous cycle. These changes are summarised below.

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TABLE 19.2 Origins and Derivatives of the Male and Female Reproductive Systems

Origin	Male Product	Female Product
Genital ridge	Testis	Ovary
Genital ridge, lower part	Gubernaculum testis	Chorda uteroinguinalis or the round ligament of the uterus
Mesonephros	Epididymis	Epoöphoron
Mesonephric tubules	Ductuli efferentes	Ductuli epoöphori transversi
Other mesonephric tubules	Ductuli aberrantes (of Halleri)	
	Paradidymis (organ of Giraldes)	Paroöphoron
Mesonephric duct (Wolffian duct)	Ductus epididymis	Ductus epoöphori longitudinalis
	Ductus deferens	Ductus paraurethrales (of Gartner)
	Ductus ejaculatorius	
Cystic end of mesonephric duct (derivatives described as the stalked hydatids)	Appendix epididymis (or appendix epididymidis) Sometimes called the stalked hydatid of Morgagni	Appendix vesiculosa (several may be present and are sometimes called the hydatids of Morgagni)
Paramesonephric duct (Mullerian duct)	Appendix testis (sessile hydatid, sessile hydatid of Morgagni)	Uterine tubes
	Utriculus masculinus	Uterus
		Vagina

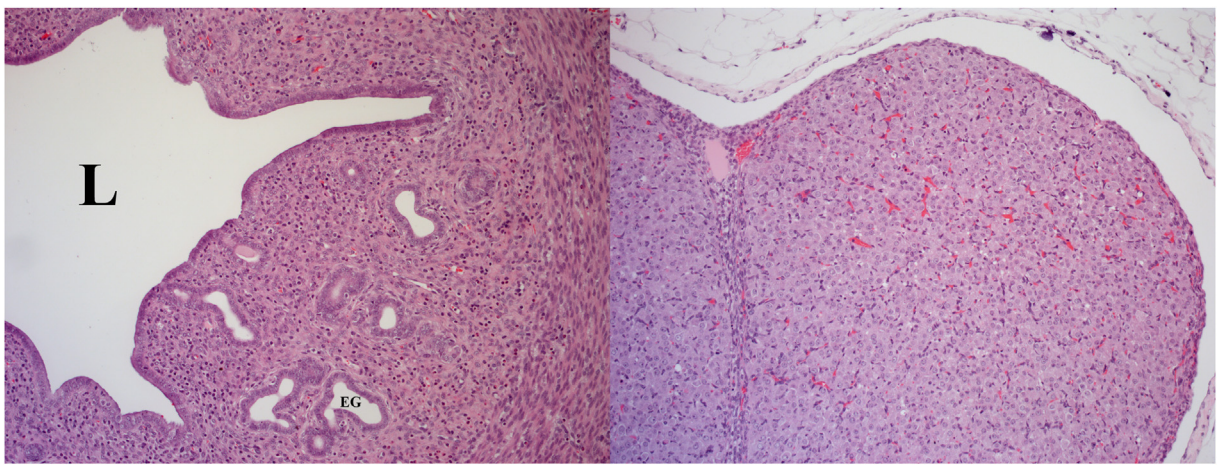


FIGURE 19.4 Lumen (L) is open and a mitosis can be seen in the endometrial gland marked, although they are not easy to see at this magnification (EG). The basophilic corpora lutea are large and lacking in mitoses.

Appearance of the Uterine Mucosa at Stages of the Oestrous Cycle

The appearance of the corpora lutea of the ovary is shown in brackets.

Pro-Oestrus

The epithelium comprises medium-sized columnar cells which increase in height as the stage progresses. The lumen starts to distend with fluid and occasional mitotic figures can be identified among the cells of the endometrium in the early part of the stage. The lamina propria contains many polymorph inflammatory cells, increasing in number in the later stages of pro-oestrus. The glands show dilatation of their lumina (relatively high proportion of basophilic (recent) corpora lutea) (Fig. 19.4).

Oestrus

The columnar cells of the epithelium reach their maximal height and as the stage progresses, apoptotic cells begin

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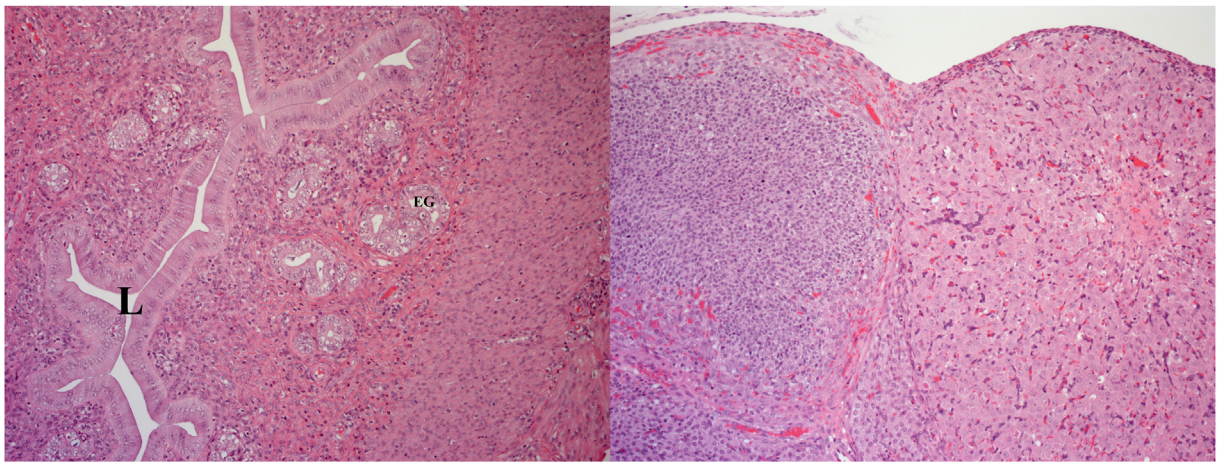


FIGURE 19.5 In this late stage of oestrus the lumen has already collapsed but the tall columnar cells are very evident and are the extensive apoptoses in the endometrial glands. The majority of the corpora lutea are eosinophilic and some very early developing corpora lutea with high mitotic rates may be seen.

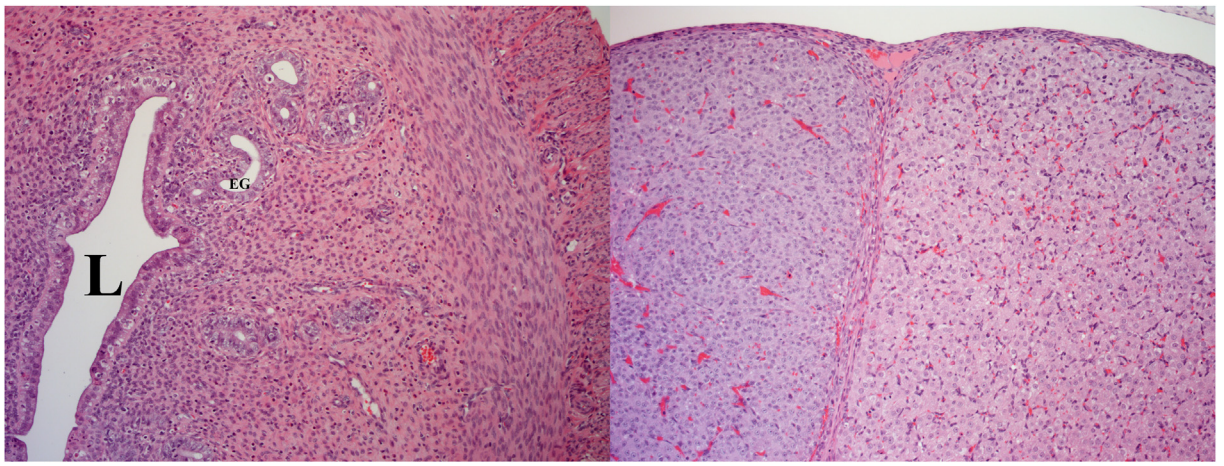


FIGURE 19.6 Apoptosis of the luminal epithelial cells now very clear to see, this generation of corpora lutea now quite large and still basophilic, with occasional mitotic figures that are quite difficult to see. The degeneration in the eosinophilic corpora lutea pictured is quite clear.

to appear in the epithelium, particularly in the endometrial glands. By the end of the stage apoptosis is significant. The lumen is fully distended with fluid. Many polymorph inflammatory cells can be seen in the lamina propria. Some endometrial glands remain dilated but others are beginning to collapse. (The ovary may have four sets of corpora lutea visible, many of which are large and eosinophilic) (Fig. 19.5).

Metoestrus

Initially the columnar cells are very tall but soon reduce in height during the stage. Apoptosis is predominantly in the luminal epithelial cells, there may be an occasion apoptotic cell in the endometrial gland epithelium early on. (Three sets of corpora lutea can usually be identified and large degenerating corpora lutea prominent.) (Fig. 19.6).

Dioestrus

The height of the columnar cells and that of the cells of the glandular epithelium is reduced to a minimum level. Fewer inflammatory cells are seen in the lamina propria but the fibroblasts of the lamina propria are plump as a result of activation by progesterone produced by the corpus luteum. Mitotic figures in endometrial cells begin to appear in early dioestrus and increase with time and are probably most common in the second half of the stage. (Corpora lutea become more eosinophilic as the cycle progresses so mostly eosinophilic corpora lutea as the ovary enters proestrus) (Fig. 19.7).

Though the changes just described can be identified at microscopy they are, of course, much less marked that those occurring in the female human in whom the lining of the uterus undergoes spectacular degeneration and is shed with the loss of a considerable volume of blood.

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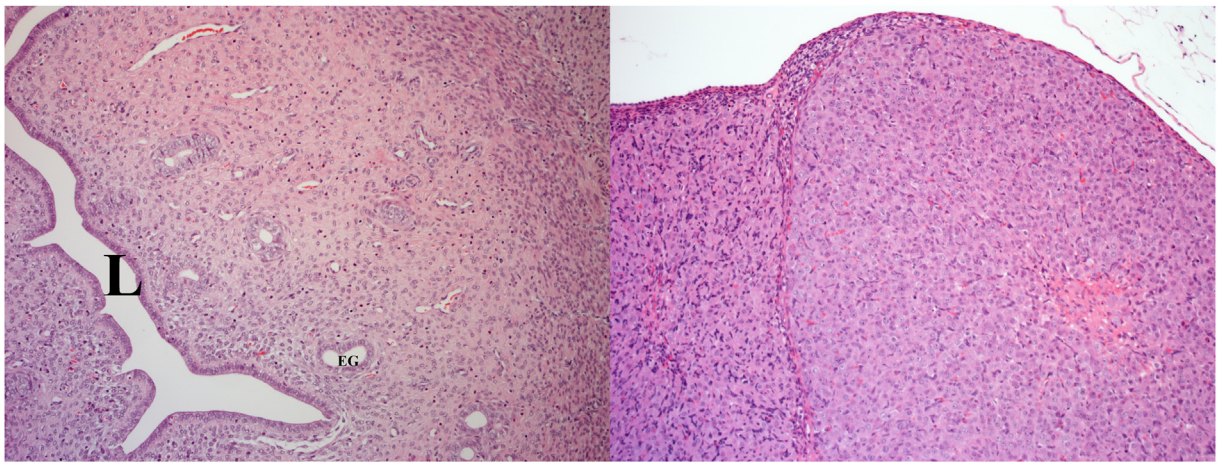


FIGURE 19.7 Quiescent appearance of the uterus in the main, although one or two mitotic figures can be seen in the luminal epithelium. The corpora luteal cells in this generation of corpora lutea are now quite plump and increasingly eosinophilic.

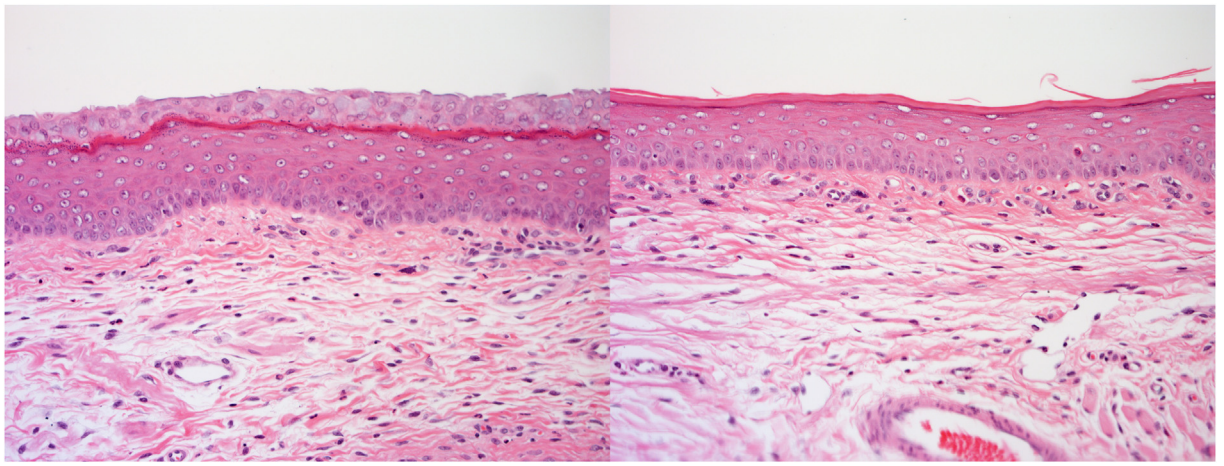


FIGURE 19.8 Proestrus to the left and oestrus to the right. Both stages are quite easy to differentiate.

These marked changes are the hallmark of the menstrual cycle and are seen only in primates.

VAGINA

The vagina of the adult rat is about 15−20 mm in length and about 3−5 mm in diameter when distended (Hebel and Stromberg, 1976). The wall has a mucosa and a muscular layer. The mucosa has a stratified squamous epithelium that varies in structure with the stage of the oestrous cycle. Rather unusually for a stratified squamous epithelium, the cells and the surface cells of the epithelium produce mucus at one stage of the oestrous cycle, but at another stage undergo cornification. During the production of mucus, the surface layers of the epithelium are described as the stratum mucification, and as the stratum corneum during the cornification stage. When fully developed, the cells of the stratum mucification lie on the stratum corneum. The deeper layers of the epithelium comprise the stratum germinativum, stratum spinosum and stratum granulosum, as seen in other stratified squamous epithelia of the keratinising type. Hebel and Stromberg (1976) noted that during the cornification stage a well-defined stratum lucidum could be identified. The muscle of the wall is largely longitudinal in arrangement. These changes are summarised below:

Appearance of the Epithelium at Stages of the Oestrous Cycle

Pro-Oestrus

Stratum mucification present. Stratum corneum appears at the surface during later pro-oestrus. Occasional polymorphs present in epithelium.

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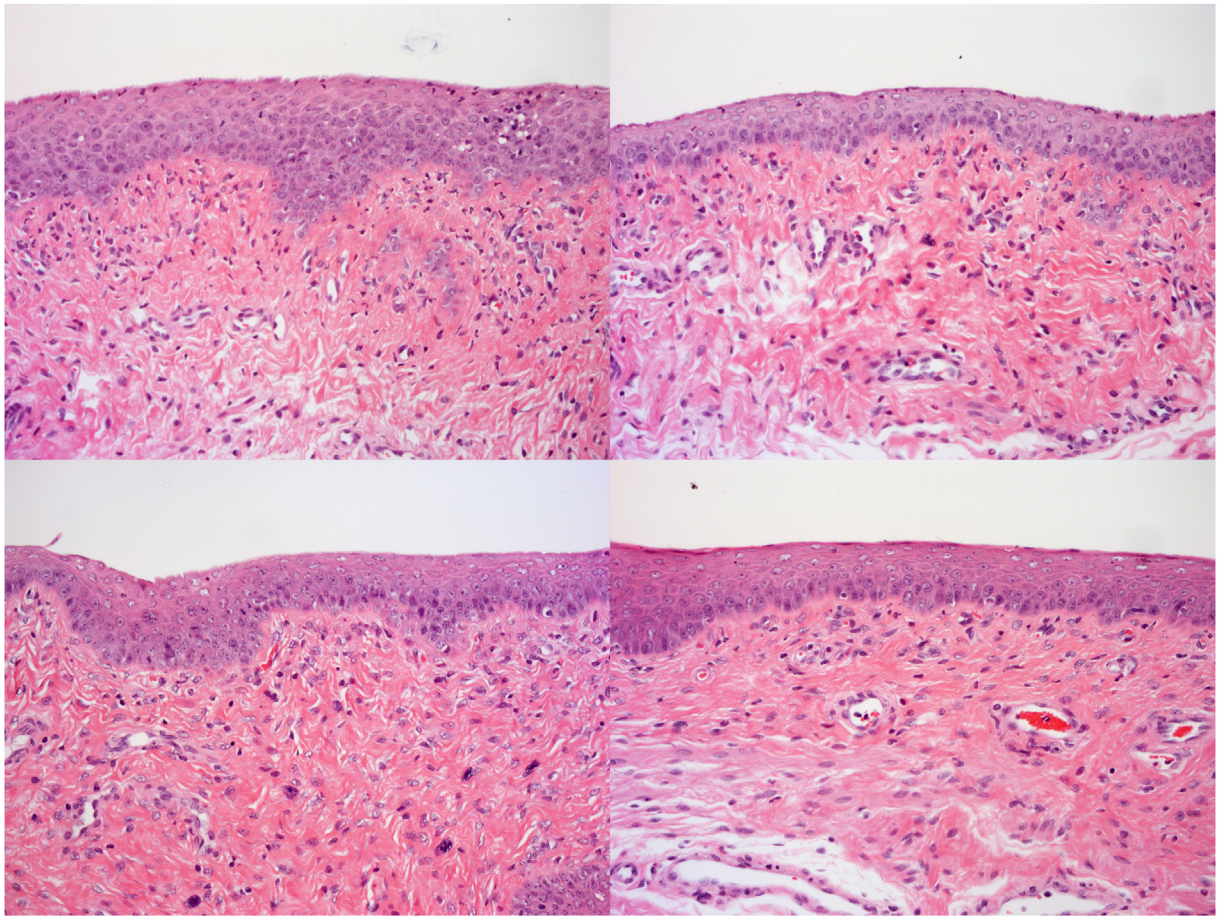


FIGURE 19.9 Metoestrus and dioestrus. Much more difficult to differentiate. Top left is early metoestrus and top right late metoestrus. Bottom frames early and late dioestrus.

Oestrus

Stratum mucification not present. Stratum corneum well developed and cornified cells appear in the lumen of the vagina. Polymorphs increasing in number (Fig. 19.8).

Metoestrus

Stratum mucification, stratum corneum and stratum granulosum absent. Stratum germinativum reduced to four to six layers of cells. The many polymorphs present early in metoestrus later decline in number.

Dioestrus

Stratum germinativum shows plump, polygonal cells: this change is described as early mucification of the stratum germinativum. Few polymorphs present (Fig. 19.9).

Notes on Cycle Staging

Staging the oestrus cycle is considerably more straightforward than staging spermatogenic cycles in the testes. There are however a few recommendations that are worthy of consideration before beginning.

1. Take longitudinal sections of the vagina, the changes in the female reproductive tract pass though in waves and not all parts will be the same at any one time.
2. Be wary of cage effects, females in the same cage tend to synchronise their cycling.
3. Given the small numbers of animals normally used in most studies (10 or less), and the variation you see as background, it is unlikely you will see an effect in terms of the numbers of animals in different stages of the cycle, but asynchronicity of cycling (vagina, uterus and ovaries appear to be indifferent stages) is a very sensitive indicator of endocrine disruption.

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4. Always remember that it is a continuous cycle and there will be grey areas.
5. Your counts should be roughly in line with the length of each stage. If they are not, you are probably making a mistake. Most common error is to over report metoestrus.

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Chapter 20

The Brain and Spinal Cord

INTRODUCTION

The acquisition of a working knowledge of the anatomy of the rat central nervous system (CNS) presents a formidable challenge to anybody who has not undertaken some training in mammalian or human anatomy, and specifically in either mammalian or human neuroanatomy. Even a brief glance at a textbook of neuroanatomy will show how complicated the subject is and how many structures have been discovered, described and named within the spinal cord and brain. In this chapter we have not attempted a detailed description of the anatomy of the rat spinal cord and brain; instead we have provided what we hope will be a useful introduction to the subject and an introduction to the relevant literature. We have listed fewer original papers in this chapter than in some others but have provided a fairly long and annotated list of textbooks and atlases that should help the reader in need of detailed information. The primary literature is vast, dating from the 19th century and beyond, though it is still probably fair to say that more is known about the neuroanatomy of primates and the cat, which has long been a popular model for neurophysiologists, than of the rat. Human neuroanatomy has been studied in great detail, and good accounts at both elementary and more detailed levels are available, but the rat brain is very different from that of man, with a number of structures that are either proportionally larger in the rat than in man or vice versa. For example, the piriform cortex, which is associated with olfaction, is a large and important part of the cerebral cortex of the rat, but a rather minor component of that of man. Similarly, the hippocampus of the rat is very well developed and again proportionally much larger than that of man. The cerebellum, however, is less impressive in the rat than it is in man.

There are two very good ways of learning about neuroanatomy. The first is to make a study of the development of the brain: the embryological approach. This approach is relatively easy if the worker is interested in human neuroanatomy because introductory accounts are available, but less so if the neuroanatomy of the rat is the focus of attention. Paxinos, of whom more later, has published an atlas of the developing rat brain (Paxinos and Ashwell, 2008). This work runs to more than 500 pages and is the leading work in the field. It is not intended to be an easy introduction to rat neuroembryology. Paxinos' 'The Rat Nervous System' (2014), of which the 4th edition is available via Science Direct, contains three chapters on development: again these chapters are excellent, but not easy reading for the beginner. The second approach is comparative. Following the structure of the brain from fish to man is an enlightening study, but not one to be undertaken lightly. Books on comparative neuroanatomy tend to be large and off-putting to the beginner. Several are listed in the annotated bibliography to this chapter.

The way that is likely to appeal most to the reader is to plunge in and see what can be made of the adult rat brain. A very useful short account has been provided in 'The Brain, An Introduction to Functional Neuroanatomy' by Watson et al. (2010): The authors have provided a chapter on the rat brain and have included a 'mini-atlas' with short descriptions of each of a short series of sections through the brain. Their chapter, combined with a selective examination of Paxinos and Watson's The Rat Brain in Stereotaxic Coordinates (6th edition, 2007), would provide a valuable introduction to the subject. Craigie's Neuroanatomy of the Rat (Zeman and Innes, 1963) is a classic; Paxinos' book (Paxinos and Watson, 2007) provides a more up to date and more detailed account.

We have mentioned the works of Dr. George Paxinos several times. All that remains to be said is that his contribution to neuroanatomy has been most impressive: his books are uniquely valuable and should be available to all those who seek a detailed knowledge of the rat brain.

HOW THE BRAIN WORKS

How indeed! The simplest way of looking at how the brain works is shown in Fig. 20.1.

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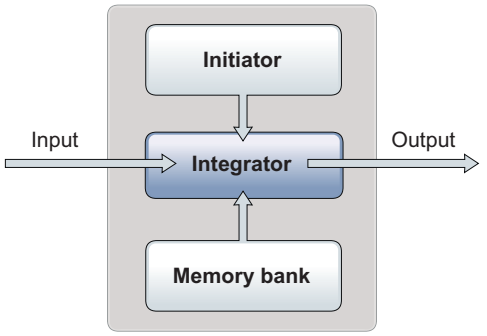


FIGURE 20.1 Diagram showing essential components of central nervous system.

Here the brain is shown as a black box with an input and an output: afferent and efferent connections. Within the black box, just three sub-boxes are defined: an initiator, a memory bank and an integrator. It is assumed that the system can initiate output without the need for input. We know that this is true for man, because thoughts and reflections can lead to action, but whether this is the case in the rat and other animals is an open question. Whether cats reflect on the ways of mice, or mice on the ways of cats, is unknown.

Setting these speculations aside, it is easy to think in terms of integration of input and output. Patterns of stimuli created by interactions between the environment and the body are sensed by receptor systems and converted into action potentials that travel along afferent nerve fibres to the CNS. The reflex arc is the simplest of circuits. The terminals of the incoming fibres synapse with the dendrites of motor neurons and trigger action potentials travelling outwards from the CNS to effector structures such as muscles and glands. Although simple reflexes, such as the 'knee-jerk' and other 'tendon reflexes' are well known, they are probably quite rare. More often interneurons, interposed between the terminals of afferent fibres and the cell bodies of efferent fibres, allow a measure of integration. Note that integration takes place in the spinal cord as well as in the brain.

Afferent signals from the spinal cord travel to the brain in bundles of nerve fibres (tracts). In a classic sensory pathway, a painful stimulus is converted into a signal that travels to the spinal cord via nerve fibres arising from neurons of a dorsal root ganglion. Fibres of the dorsal root synapse in the cord with the dendrites of neurons in the dorsal horn and axons from these secondary neurons cross the midline and travel to the brain in the spinothalamic tracts. The spinothalamic tracts also convey information on touch, pressure and temperature. On reaching the thalamus, they synapse with neurons that send axons to the sensory cortex. Spinocerebellar tracts and the dorsal columns of the spinal cord carry information from joint and tendon receptors to the cerebellar and cerebral cortices.

Efferent output relies upon activation of neurons running to effector organs in the periphery, for example, muscles and glands. Neurons that control skeletal muscle are found in the ventral horn of the spinal cord and the nuclei of cranial nerves including those that innervate muscle derived from the branchial arches. These nuclei are activated by nerve fibres travelling from several sources including the cerebral cortex, the red nucleus, the vestibular nuclei and the superior colliculi.

Connections with the superior and inferior colliculi allow reflex movements to be triggered by visual and vestibular input. Motor and sensory areas of the cerebral cortex, the basal ganglia, the red nucleus and the cerebellum are all involved in the coordination of movement. The complex process required to produce well-adjusted, coordinated, and smooth movements requires a great deal of cooperation between centres within the CNS.

DEVELOPMENT OF THE BRAIN AND SPINAL CORD

The development of the brain is formidably complicated: the briefest outline is presented here as a means of introducing key terminology and giving the reader who is not familiar with the CNS an introduction to the system.

The spinal cord and brain of all vertebrates develop, early in the development of the embryo, from the neural tube, which is formed by a longitudinal infolding of the neural plate that forms from the dorsal ectoderm (neuroectoderm). We should note that the most posterior part of the system develops from the mesoderm, which forms the caudal eminence, a solid rod that develops a lumen and fuses with the posterior end of the developing neural tube. At least this is the case in man (Larsen, 1993). The ectoderm forms a pseudostratified columnar epithelium around the central canal of the neural tube and then proliferates and differentiates into neurons, astrocytes, oligodendrocytes and ependymal cells.

The Brain

Expansion of the anterior end of the neural tube creates a linear series of three interconnected vesicles, the prosencephalon (forebrain), metencephalon (midbrain) and rhombencephalon (hindbrain). The expanded central canal of the rhombencephalon has a rhomboid shape, hence the name.

Two lateral expansions of the central canal of the prosencephalon become the lateral ventricles, around which the cerebral hemispheres develop. This leads to the prosencephalon being subdivided into a central part (the diencephalon) and two cerebral hemispheres (the telencephalon). The olfactory bulbs develop from the cerebral hemispheres.

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The walls of the developing brain thicken, and the shape of the central canal changes. The two lateral ventricles are connected with the midline third ventricle via the foramina of Monro. The third ventricle, which is a narrow slit (actually a hollow ring in the rat: see below) surrounded by the diencephalon, is connected by a narrow passageway (the aqueduct of Sylvius) with the diamond-shaped fourth ventricle of the medulla and pons: see below for further details.

The development of the choroid plexuses of the lateral ventricles provides a severe test of the understanding of human neuroembryology and is not well described in the rat. Broadly speaking, a sheet of meningeal tissue is tucked in under the developing hemispheres above the roof of the third ventricle which it invaginates and also extends laterally over the thalamus to invaginate the medial wall of the curving lateral ventricles.

The Forebrain or Prosencephalon

The Telencephalon

The telencephalon has undergone more evolutionary changes than any other part of the brain. The cortex can be thought of in terms of three 'ages'. The paleopallium is the oldest and is involved with olfaction. In the rat, it is represented by the prepyriform and pyriform lobes of the cerebral hemispheres, an area of the brain much better developed in the rat than in man. The next oldest, the archipallium, represented by the hippocampus, has functions related to olfaction and memory, amongst others. The neopallium, the newest part, is represented in both man and rat by the bulk of the cerebral cortex (Romer, 1970).

The basal ganglia that include the caudate nucleus, putamen and globus pallidus develop from the tissue of the telencephalon. The caudate nucleus and the putamen are part of one great nucleus known as the corpus striatum. The basal ganglia have an important role in the control of muscular activity and are part of what used to be known as the extrapyramidal system, so called to distinguish it from the pyramidal system.

The pyramidal system itself comprises the nerve fibres (axons) of the pyramidal tracts that run from the cerebral cortex to the ventral horn neurons of the spinal cord and the nuclei of cranial nerves in the brainstem. These tracts create linear bulges on each side of the ventral surface of the medulla. In the cross section, these bulges look a little like pyramids, hence the name of the tracts. Note that the tracts are not named for the pyramidal cells of the cortex that give rise to some of the fibres of the tracts.

In man, the great expansion of the cerebral hemispheres follows a curve that starts near the connection between the third and lateral ventricles and then sweeps back and down to form the posterior and temporal lobes of the hemispheres. The lateral ventricles and basal ganglia follow this curve. In the rat the development of the cerebral hemispheres is less dramatic, and the relationship between the hippocampus and the lateral ventricles is quite different from that seen in the human brain. The olfactory bulbs of the rat are larger than in man, and the anterior horns of the lateral ventricles extend forwards into these bulbs. The cerebral hemispheres are connected with each other via the corpus callosum, and the anterior and posterior commissures. Commissures of the hippocampus and habenular nuclei also exist.

The Diencephalon

The diencephalon is the part of the forebrain, which develops from the foremost primary cerebral vesicle. The diencephalon consists of structures that are lateral to the third ventricle, and includes the thalamus, the hypothalamus, the epithalamus and the subthalamus. The hypothalamus, lying ventral to the thalamus, controls visceral functions including appetite, thirst, sexual behaviour and large parts of the autonomic and neuro-endocrine systems.

The Midbrain or Mesencephalon

The midbrain comprises the tectum, tegmentum, the cerebral aqueduct and the cerebral peduncles, as well as several nuclei and fasciculi (bundles of nerve fibres, tracts). Caudally, the midbrain adjoins the metencephalon (pons and cerebellum), while rostrally it adjoins the diencephalon (thalamus, hypothalamus).

Two pairs of dorsal expansions of the midbrain produce the superior and inferior colliculi (a colliculus is a mound): important centres for reflexes involving visual and auditory inputs to the brain. Well-defined bilateral nuclei, the left and right red nuclei, appear in the midbrain but are more pale pink than fire engine red! Many tracts pass through the midbrain, going up to the forebrain, or down to the hindbrain and spinal cord. The major outflow from the cerebral cortex consists of two ventral bundles that leave the forebrain as the cerebral peduncles. The midbrain also contains the substantia nigra, which can be thought of as a posterior extension of the deep nuclei of the hemispheres: the basal ganglia. The substantia nigra is named for its black neuromelanin pigment. Despite this name the substantia nigra of the albino rat is pale.

The Hindbrain or Rhombencephalon

Development of the rhombencephalon leads to formation of the medulla oblongata (myelencephalon: the surface is white), the pons and cerebellum (metencephalon). The word pons means a bridge, and it does indeed provide a bridge of nerve fibres connecting the two halves of the

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cerebellum via the middle cerebellar peduncles. The medulla, pons and mesencephalon make up the brainstem. The medulla is sometimes referred to as the bulb because it appears as an anterior expansion of the spinal cord.

The central canal widens during early development. The roof of the fourth ventricle becomes very thin and rises at its centre like a rather flat tent. The cerebellum grows from the dorsal edges of the rhombencephalon and forms two hemispheres that fuse in the midline over the roof of the fourth ventricle. The pons, like the cerebellum, is much less well developed in the rat than in man. The cerebellum is also connected on each side to the brainstem by superior and inferior peduncles. The superior mainly carries fibres leaving the cerebellum; the inferior carries fibres running to the cerebellum from the spinal cord and nuclei of the medulla.

The Spinal Cord

The developing neurons form columns in the dorsal and ventral parts of the lateral walls of the neural tube. Some in the ventral columns develop long axons that run out from the developing cord as the ventral roots of the spinal nerves. Some in the dorsal columns also develop long axons, which remain in the cord and form the ascending tracts that connect the spinal cord with the brain. The brain is also connected with the cord by tracts running in the opposite direction. The majority of cells form shorter axons and remain as interneurons of the cord. Development of the columns of neurons produces the grey matter, while the long axons become bundled together as tracts and form the white matter. The grey matter lies deep to the white matter throughout the CNS, except in the cerebral and cerebellar cortices, where a secondary differentiation and migration of neurons brings them to the surface.

Further development of the grey matter of the spinal cord causes it to take up a butterfly shape with projections called the dorsal and ventral horns. The cells of the grey matter begin to cluster into nuclei ('nucleus' is used in neuroanatomy to describe a cluster of neurons). The cells of the dorsal horns tend to form layers or laminae. Axons from cells of the dorsal root ganglia, which are derived from neural crest tissue and not from the neural tube, run to the dorsal horns as the dorsal afferent roots of the spinal nerves: many synapse with cells of the dorsal horns.

The cells of the dorsal columns form the alar (alar means resembling a wing) plates or laminae of the developing spinal cord, and those of the ventral columns form the basal plates. The neurons concerned with the great functional systems of the CNS, the somatic and visceral afferent and efferent systems, are arranged in an orderly fashion in the alar and basal laminae. Although the topography of the system changes as the spinal cord merges into the medulla, these arrangements are largely maintained: see Chapter 21(3), The Autonomic Nervous System.

THE EXTERNAL ANATOMY OF THE RAT BRAIN

Fig. 20.2 show the major features of the surface of the rat brain. Only the larger features have been labelled. The reader is directed to an atlas of rat neuroanatomy for details of other structures that can be observed. In the figures shown here the olfactory bulbs are labelled as the olfactory lobes; the term olfactory bulbs is used today.

In contrast to man, the rat has large olfactory bulbs at the anterior pole of the brain, and the surface of the cerebral hemispheres is not thrown into a mass of ridges (gyri) separated by grooves (sulci). Only the horizontal rhinal fissure, which separates the older parts of the cerebrum from the newer parts, is obvious (older and newer in an evolutionary context, of course). This fissure can be better seen from the ventral or lateral aspects of the brain: see Fig. 20.2B and C. The posterior poles of the hemispheres are rounded and wider than the anterior poles but do not overlap the cerebellum as they do in man. The surface of the cerebellum is fissured. The pineal gland lies between the posterior poles of the hemispheres and can be seen by separating the poles and removing meningeal tissue. The dorsal surface of the brainstem is visible posterior to the cerebellum. As the brainstem approaches the cerebellum, it expands and a median sulcus or groove runs along its dorsal surface. A few dorsal roots of the anterior, cervical and spinal nerves may be visible on either side if some spinal cord has been removed along with the brain.

The lateral and ventral surfaces are more complicated than the dorsal surface in that more structures are visible.

The pyriform sulcus can be seen on the lateral aspect of the cerebral hemisphere: towards its lower edge.

More Detailed Anatomy and Histology of the Rat Central Nervous System

The Telencephalon

The major parts of the telencephalon are the cerebral cortex (of various types), the hippocampus and the basal ganglia. Commissures (bundles of nerve fibres) connect the left and right halves of the brain. The largest of these, the corpus callosum, is found in all higher mammals, but not marsupials. There are a few easy to recognise structures in the telencephalon, but hundreds more have been described, most of which require expert knowledge to distinguish and name. A series of coronal sections of the rat brain are provided in Fig. 20.3. These identify some of the major structures and should be examined in association with the text.

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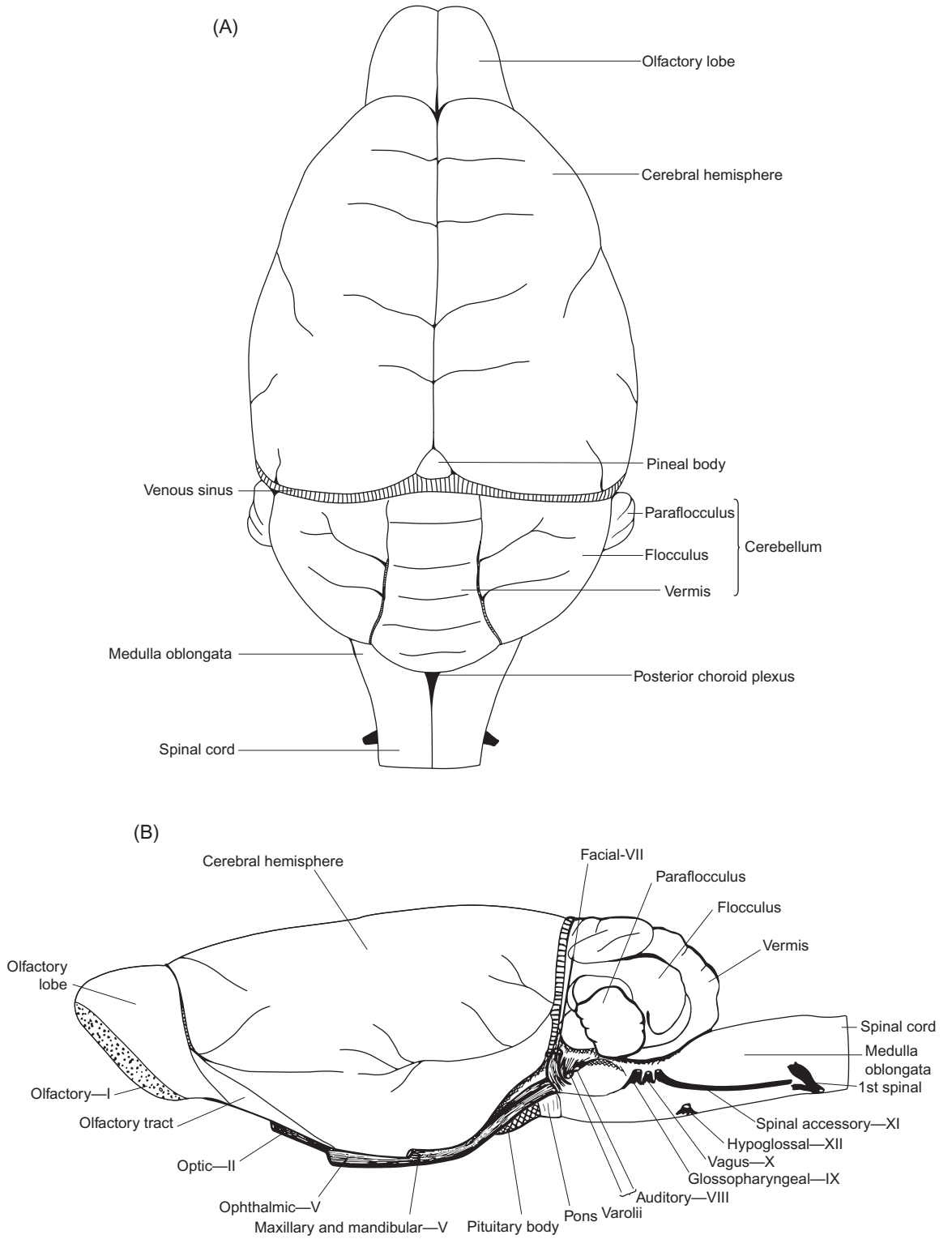


FIGURE 20.2 (A) Dorsal view; (B) ventral surface; (C) lateral surface.

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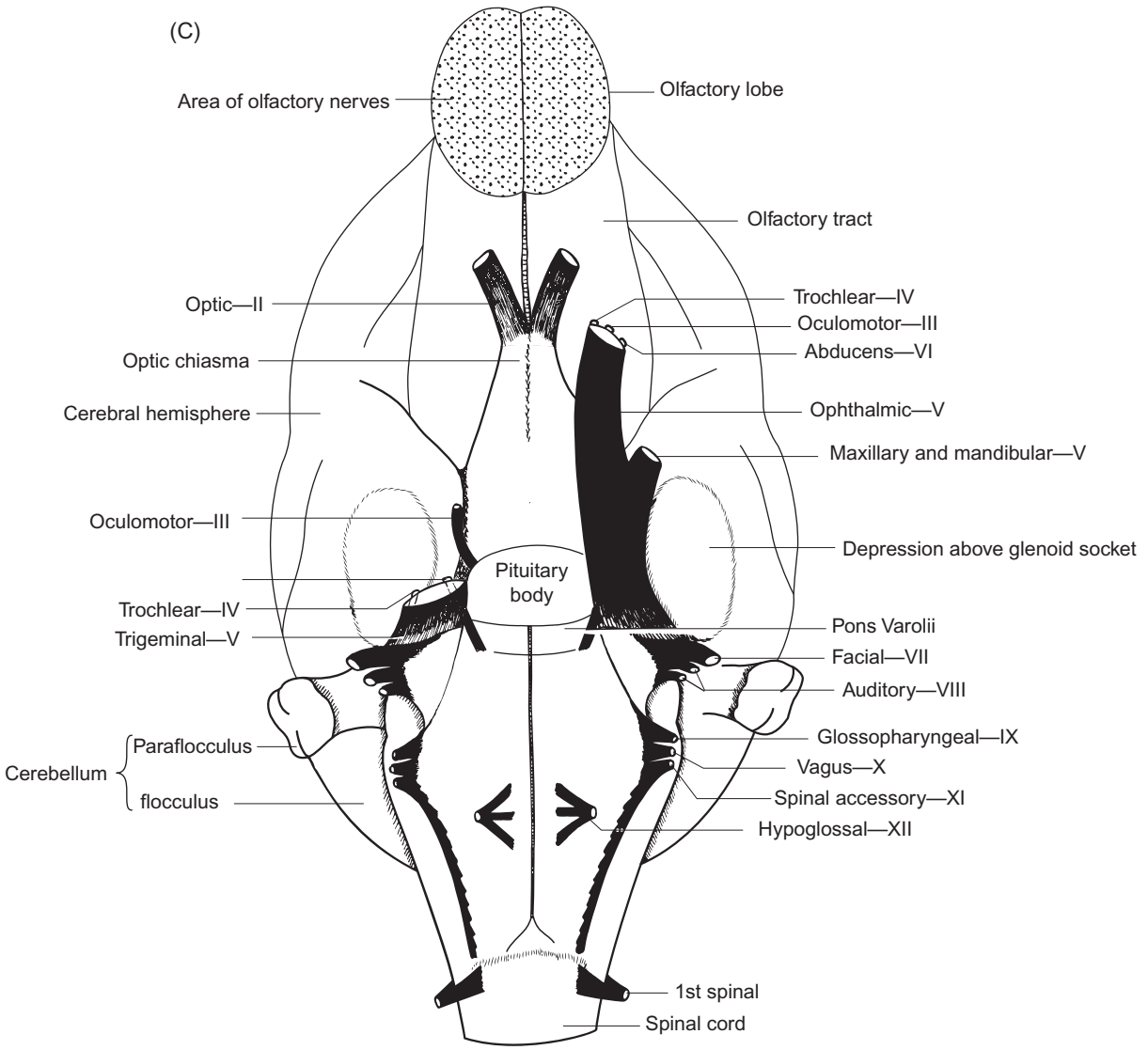


FIGURE 20.2 Continued.

Cerebral Cortex

The cerebral cortex comprises the neopallium, phylogenetically the youngest part of the brain, and the phylogenetically older rhinencephalon.

The Neopallium

Functional Divisions of the Neopallium

Almost every biologist has seen a drawing of an odd-looking little man with a large mouth, huge hands and spindly legs superimposed upside down on a drawing of the human brain. This purports to show the distribution of areas of the body represented in the sensory cortex. Almost as well-known are the maps of the human cerebral cortex produced by Brodmann dividing the cortex into more than 50 areas, although recent work by Glasser et al. (2016) has greatly expanded this number by characterising 97 new areas, in addition to the 83 previously reported. The 180 defined areas of each hemisphere were identified using a semiautomated MRI technique; we are not aware of equivalent studies in the rat.

The subtlety of the cyto-architectonic differences between different areas of cortex is remarkable, and to the non-specialist all but undetectable. This led Crosby et al. (1962), one of the greatest neuroanatomists of the 20th century, to write, 'The study of cortical lamination requires special preparations, special training and infinite patience. It is not a field for the novice to attempt. Indeed it is not even a study in which those with training in other branches of neuroanatomy can feel confident to offer an opinion'. As one might expect in such a field a specialised terminology has grown up that is bewildering to the

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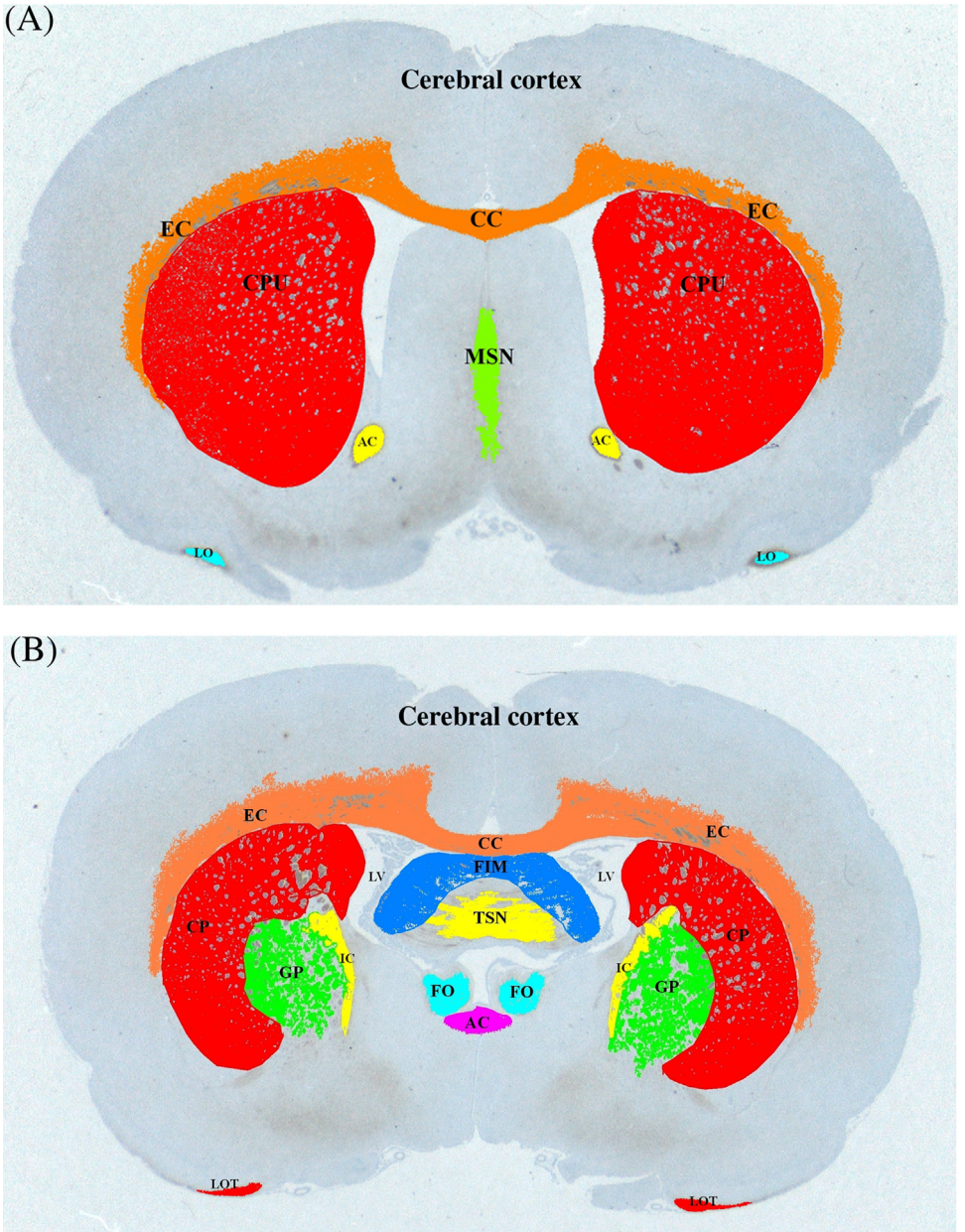


FIGURE 20.3 Cross sections of selected regions of the brain showing some of the major structures. (A) Forebrain towards the rostral end of the corpus callosum. *CC*, corpus callosum; *EC*, external capsule; *CPU*, caudate-putamen; *MSN*, medial septate nuclei; *AC*, anterior commissure; *LO*, lateral olfactory tract. (B) Forebrain at the level of the anterior commissure. *CC*, corpus callosum; *EC*, external capsule; *CP*, caudate-putamen; *GP*, globus pallidum; *IC*, internal capsule; *AC*, anterior commissure; *FO*, fornix; *FIM*, fimbria; *TSN*, transverse septal nuclei; *LV*, lateral ventricle; *LOT*, lateral olfactory tract. (C) Forebrain at the level of the hippocampus. *CP*, caudate-putamen; *CC*, corpus callosum; *EC*, external capsule; *IC*, internal capsule; *FIM*, fimbria; *DCW*, deep cerebral white matter; *HN*, habenular nuclei; *AMYN*, nuclei of the amygdala; *OPT*, optic tract. (D) Midbrain and pons. *CP*, cerebellar peduncle; *PDG*, periaqueductal grey matter; *SC*, superior colliculus; *RN*, reticular nuclei; *MODG*, molecular layer of the dentate gyrus; *SN*, substantia nigra; *PN*, pons; *TFP*, transverse fibre of the pons; *DHC*, dorsal hippocampal commissure; *DCWM*, deep cerebral white matter. (E) Hind brain in the area of the fourth ventricle. *GI*, giganto-reticular nuclei; *RN*, raphe nuclei; *TRIN*, spinal trigeminal nuclei; *TRIT*, spinal trigeminal tracts; *OC*, oliviocerebellar tract; *FAC*, facial nerve nuclei; *PYR*, pyramidal tracts; *VSC*, ventral spinocerebellar tract.

beginner. Some aspects of this terminology are set out in Table 20.1.

Another method of classification of human cerebral cortical structure is that introduced by Economo (see Crosby et al., 1962, for a detailed discussion and references to the primary literature). This classification that overlaps with that shown in Table 20.1 is set out in Table 20.2.

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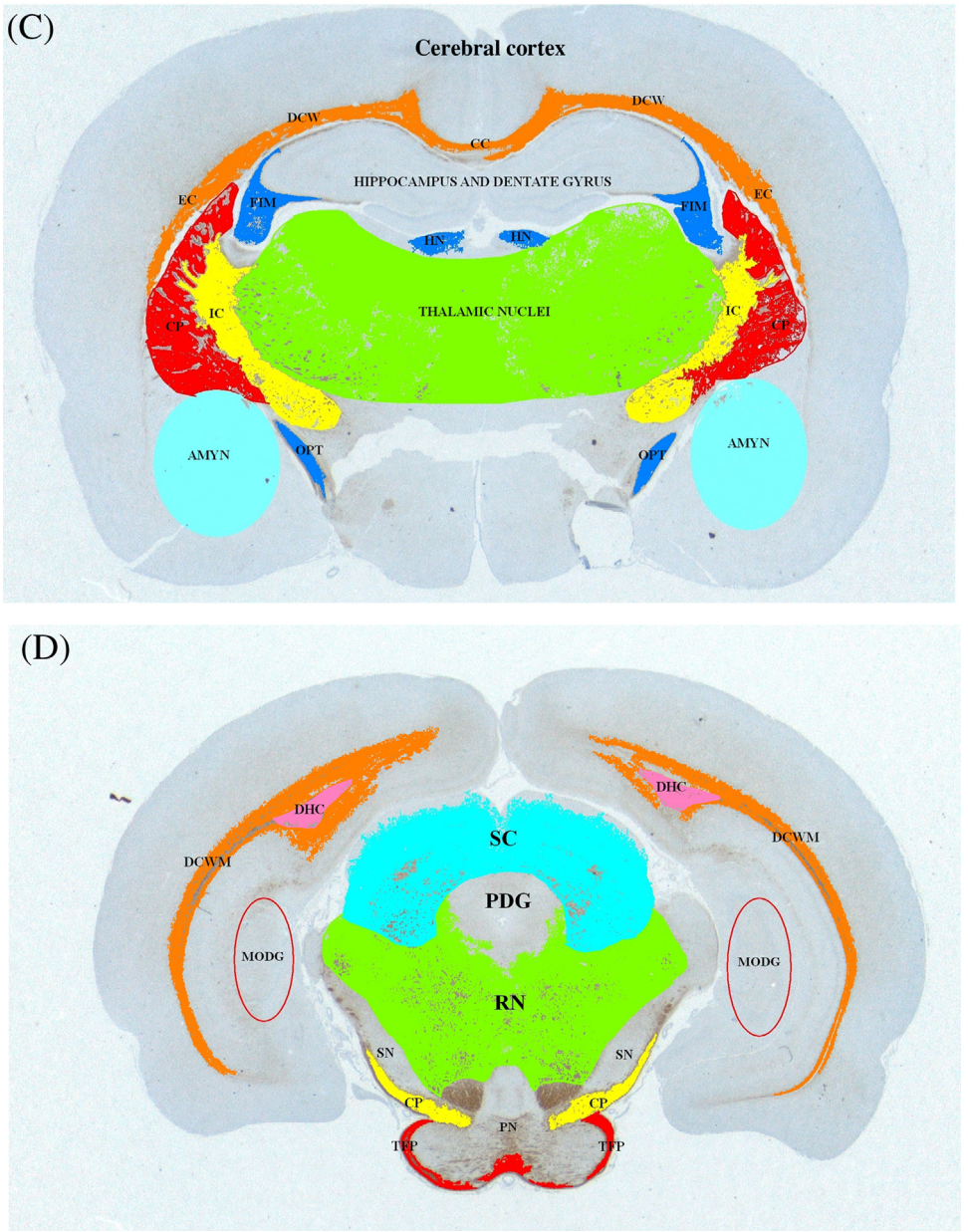


FIGURE 20.3 Continued.

Kreig (1946) applied Brodmann's cytotectonic classification of areas of cortex in man to the rat and produced another map (see Fig. 20.4). A few of the Brodmann areas shown in Fig. 20.4 are listed below.

Area 2: main sensory area
Area 4: main motor area
Areas 17 and 18: visual cortex
Area 51: prepyriform cortex

A list of all the Brodmann areas is provided in Lockard's book: Desk Reference for Neuroscience (1992).

Histology of the Cerebral Cortex

The great difficulty met in describing the histology of the cerebral cortex is the variety of neurons that have been described. Such descriptions date from the introduction of silver impregnation techniques by Golgi, and their extensive use by Santiago Ramon y Cajal. Cajal, indisputably the greatest of classical neuro-histologists, produced two large volumes on the Histology of the Nervous System. These were published in Spanish and translated by the author into French, but the first complete English edition was not published until 1995 (Swanson and Swanson, 1995). Cajal's

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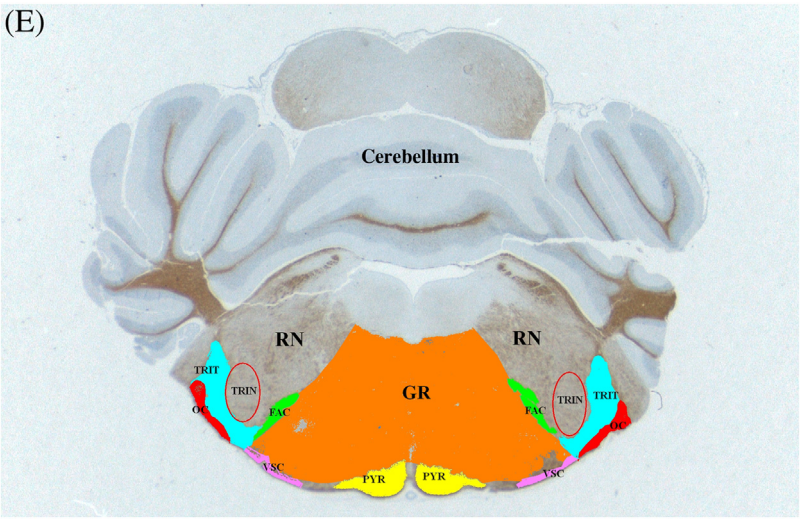


FIGURE 20.3 Continued.

TABLE 20.1 Terminology Used in Describing the Cytoarchitecture of Human Cerebral Cortex and Also Sometimes Used in Describing Rat Cortex

Pallium	Status During Development	Adult Status	Subdivisions	Alternative Name
Neopallium	Homogenetic cortex: or isocortex: six layers present at least at some stage of development	Homotypic cortex: six layers present in adult cortex, also known as eulaminate or granular cortex. Typical of general sensory cortex		Koniocortex
		Heterotypic cortex: six layers not present, or at least obscured, in adult cortex	Modified granular cortex, as found in the visual cortex	Koniocortex
			Agranular cortex: as found in the motor cortex	
Archipallium and paleopallium	Heterogenetic cortex or allocortex: six layers never present, more usually three			

Koniocortex: cortex containing small granular cells that resemble dust (Pneumokoniosis was the old name for lung disease induced by coal dust).
Source: Based on Crosby, E.C., Humphrey, T., Lauer, E.W., 1962. Correlative Anatomy of the Nervous System. The Macmillan Company, New York (Perhaps the best human neuroanatomy textbook of the 20th century).

drawings of networks of neurons are unrivalled and may be found in many modern textbooks of neuroanatomy. He was intrigued by the shapes of cells, the arrangements of their cytoplasmic processes and their interconnections with other cells. This approach, combined with a lifetime devoted to producing exact pen and ink drawings of histological sections, seems a little dated today, but Cajal's findings and thinking underlie much of our current understanding of how the neurons of the brain are arranged. To ignore his work would be sheer folly. The reader is encouraged to find a copy of his work and to marvel.

Cajal's work led to the concept of layering of cells of the cerebral cortex, a concept that still survives, although more recent work has shown that although layering is obviously present, the functional arrangement of neurons in the cortex may be columnar (Mountcastle, 1997).

Major Cell Types of the Cerebral Cortex

The features of the major cell types are summarised in Table 20.3.

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TABLE 20.2 Economo's Classification of Cerebral Cortex in Man

Classification in Terms of Number and Type as Defined by Economo	Classification: Heterotypic or Homotypic	Location	Thick or Thin	Granular Cells Layers II and IV	Pyramidal Cells Layers III and V
1. Agranular cortex	Heterotypic	Precentral gyrus, primary motor cortex	Thick	Granular cells poorly developed, even in the granular layers many small pyramidal cells are found	Large cells
2. Frontal cortex	Homotypic	Frontal lobe	Relatively thick	Granular layers distinct, loosely arranged cells	Large cells
3. Parietal cortex	Homotypic	Parietal lobe	Thick	Dense granular layers	Pyramidal cells rather irregularly arranged and layers III and V are thin
4. Polar cortex	Homotypic	Near frontal and occipital poles	Thin	Well-developed granular layers	Pyramidal cells rather sparse near occipital pole, present near frontal pole
5. Granulous cortex or koniocortex	Heterotypic	Calcarine sulcus: primary optic cortex	Very thin	Densely packed granular cells in layers II and IV; also in layer III. Layer IV may be duplicated	Reduced

Again, to save space we have summarised the layers of the cerebral cortex in Table 20.4.

It is important to note that the disposition of the layers varies markedly from area to area within the cortex and that much of what we know of the histology of the cortex comes from studies undertaken in primates and cats. Reference to Paxinos (2014) will provide further details of the rat cerebral cortex.

Hippocampus

The hippocampus is named for its perceived similarity in shape to a sea horse. The hippocampus is small in man, but an easily recognisable structure, found in the floor of the inferior horn of the lateral ventricle. In the rat, it is a much more splendid structure, sweeping downwards and laterally in a great arc inside the neopallium and pushing forwards beneath the corpus callosum. It is one of the easiest structures to recognise in a coronal section of the rat brain. The great downwards sweep of the hippocampus is mirrored by the development of the inferior horn of the rat lateral ventricle. The cortex of the hippocampus undergoes some complex rolling during its development.

The three-dimensional structure of the hippocampus is difficult to visualise. One might think of it as a sheet of rather primitive cortex that has been rolled and then bent. Cappaert et al. (2014) have provided a detailed and up-to-date account of the rat hippocampus. Here we try to orientate the reader with a simplified account of the cytoarchitecture of this phylogenetically ancient part of the brain. Persisting with the concept of the hippocampus as a rolled sheet of cortex, then the free end of the roll forms the dentate gyrus. The part of the roll immediately adjacent to the dentate gyrus is the cornua ammonis (CA) (Ammon's Horn: named for a ram-headed Egyptian God). Adjacent to the cornua is the subiculum that joins the general cerebral cortex. The CA has been subdivided into parts: CA1-3 (Fig. 20.5).

Dentate Gyrus

The dentate gyrus is sometimes considered as related to, rather than part of, the hippocampus proper, though both

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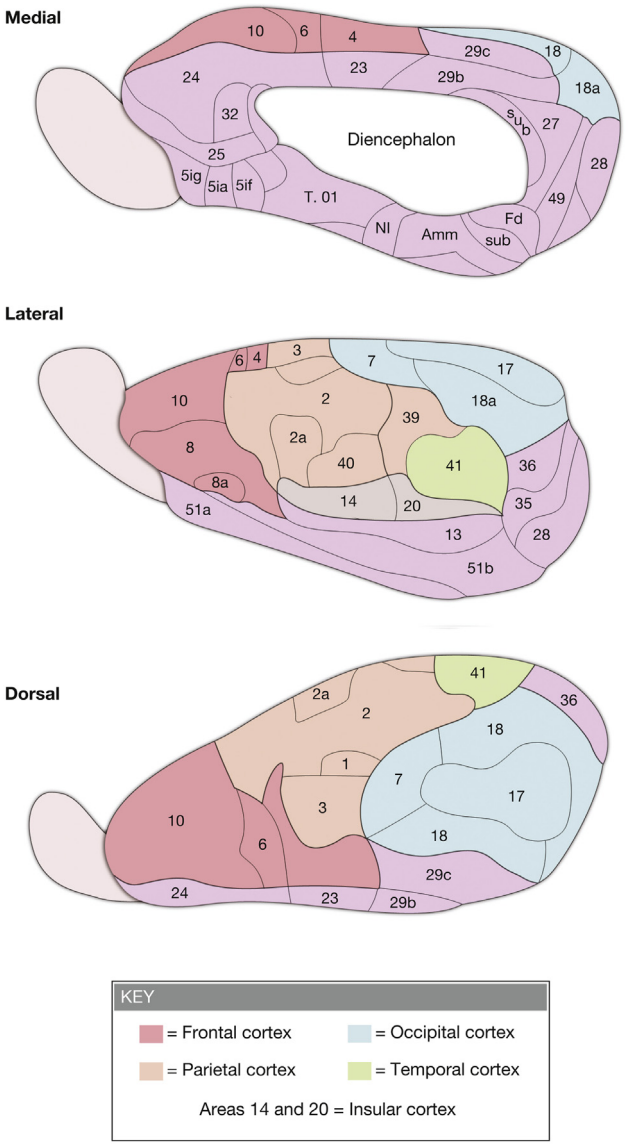


FIGURE 20.4 Division of rat cortex into lobes and 'Brodmann areas'.

belong to the archipallium. The cortex of the dentate gyrus has three layers. The superficial molecular layer comprises mainly nerve fibres sweeping in from the adjacent subiculum. The granular cell layer can be thought of as comprising modified pyramidal cells. Ranson and Clark (1959) commented: 'Originally this layer of pyramidal cells (the pyramidal cells of the hippocampus proper) was continuous with the granule layer of the fascia dentate (another name for the dentate gyrus), but in all higher mammals a break in the cellular stratum has occurred at the point of transition between the two divisions of the archipallium'. The layer of granule cells is not difficult to recognise, though its shape varies from section to section: the cell bodies stain a deep blue-purple in H&E-stained sections. Granule cells have branching dendrites that ramify in the overlying molecular layer, and long axons that run via the subjacent layer of polymorphic cells to the layer of pyramidal cells of the CA are noted. In man, the granule cells of the dentate gyrus appear to be able to increase in numbers during adult life: a rare characteristic among neurons of the CNS (Kiernan and Rajakumar, 2014). Lastly, there is a layer of polymorphic neurons that comprises mainly nerve fibres and scattered interneurons.

Cornua Ammonis

This area abutting the dentate gyrus at one end and the subiculum at the other is divided into three (CA1, CA2 and CA3). The surface is covered by the layer of nerve fibres described as the alveus (Latin for trough, a rather poor term for this surface). Nerve fibres run through the alveus on their way to the fimbria (Latin for fibres), and thereafter to the fornices, the main outflow from the hippocampus. The axons of the pyramidal cells of the CA run to the alveus. Five layers have been described in the CA, but we shall mention just three: the superficial molecular layer, the layer of pyramidal cells and the deeper layer of polymorphic cells. For further details of individual cell types, for example, basket cells and chandelier cells, the reader is referred to Cappaert et al. (2014).

The Basal Ganglia

The basal ganglia are a group of large nuclei located in the substance of the cerebral hemispheres, anterior to the main bulk of the hippocampus. Also found beneath the corpus callosum, on each side, are the fornices (fornixis Latin for an arch), the efferent tracts of the hippocampus. The two fornices approach each other as they run forwards, join to form a commissure and then part to turn down in front of the interventricular foramina before curving back and heading for the mammillary bodies at the posterior end of the hypothalamus.

Three major nuclei are described in man: the caudate nucleus, the putamen and the phylogenetically older pallidum or globus pallidus, which some consider to be derived from the diencephalon rather than from the telencephalon. In man the caudate and putamen are parts of a single nucleus that have become partly separated from each other by the internal capsule. The grouping of the putamen with the pallidum as the lentiform (lens-shaped) nucleus is misleading, as they are quite distinct in origin and function, albeit topographically close. The caudate nucleus of man is well named; it has a long tail, not seen in the rat, that curves in a C shape around the thalamus, ending in the amygdala near the anterior pole of the temporal lobe. The pale globus pallidus (pale due to the presence of myelinated fibres) lies medial to the putamen.

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TABLE 20.3 Major Cell Types of Cerebral Cortex

Cell type	Major location. Few cell types are restricted to one layer, or even to a few layers, only	Cell body: shape	Axons	Dendrites	Golgi type I: axons leaving cerebral cortex II: axon remaining in cortex; interneurons
Pyramidal cell	Mainly in layers III and V but also found in other layers	Pyramidal, 10–15 μm long; Betz cells of layer V of motor cortex: 'giant pyramids': 100 μm in man	Basal, long, leave cortex via subjacent white matter	Long, much branching, run to surface layer, many dendritic spines	I
Fusiform	VI (deepest layer)	Spindle-shaped /elliptical nuclei	Long, leave cortex via subjacent white matter	Local dendritic bush formed (arborisation), largely located in layer V	I
Retzius–Cajal cell	I	Small cell bodies, elliptical, long axes often horizontal	Lengthy but confined to layer I, run horizontally	Local branching, confined to layer I, run horizontally	II
Basket cell	IV	Angulated	Branching to form 'baskets' around cell bodies of adjacent pyramidal cells	Local dense bush formed (arborisation)	II
Stellate cell	III	Angulated	Short, interact with dendritic trees of pyramidal cells	Local dense bush formed (arborisation)	II
Cells of Martinotti	V/VI	Angulated	Long, project to surface of cortex to run, horizontally, in layer I	Local arborisation	II

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TABLE 20.4 Layers of the Cerebral Cortex

Layer Number	Layer Name	Descriptive Notes	Horizontal/Tangential Bundles of Nerve Fibres
I	Molecular layer	Largely horizontal fibres: dendrites of pyramidal cells, axons of cells of Martinotti and both of cells of Retzius–Cajal	
II	External granular layer	Many small pyramidal cells (pyramidal cells are not confined to the pyramidal layers!) and interneurons	
III	External pyramidal layer	Typical pyramidal cells, cell bodies tending to become larger towards the inner aspect of the layer. Axons project to other parts of the cerebral cortex as association fibres and fibres of commissures	
IV	Internal granular layer	Mainly stellate cells, other types of interneurons and some pyramidal cells present	Outer line of Baillarger: line of Gennari in primate visual cortex
V	Internal pyramidal layer	Largest pyramidal cells present, largest of all (Betz cells) in primary motor cortex. Axons project to extra-cortical locations: basal ganglia, brainstem and spinal cord	Inner line of Baillarger
VI	Multiform layer	Fusiform cells, small pyramidal cells, interneurons. Axons of pyramidal cells project to thalamus	

Note that the width of the layers varies from place to place in the cortex and that the width of the rows in the table does not correspond to the width of the actual layers.

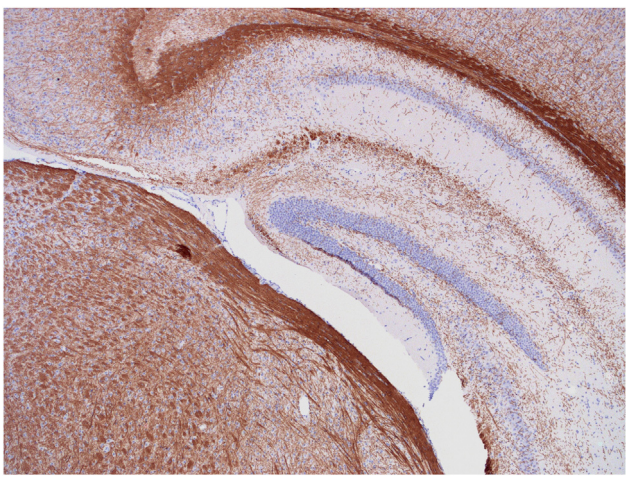


FIGURE 20.5 Myelin basic protein staining in the area of the hippocampus. Note the almost complete absence of myelin in the hippocampus.

The basal ganglia lie lateral to the anterior parts of thalamus and below the forward extension of the hippocampus, which itself lies beneath the corpus callosum.

In the rat the caudate nucleus and putamen form one large rounded nucleus that fills the space between the medial surface of the cortex and the lateral surface of septal structures at the level of the optic chiasma. In cross section the pallidum appears as a small triangular area towards the ventromedial border of the caudate-putamen. The caudate-putamen is crossed by myelinated fibres making their way to and from the cortex, giving it a striated appearance: hence the term corpus striatum that is sometimes applied to the basal ganglia as a group.

For many years the basal ganglia, substantia nigra, subthalamus and red nucleus were regarded as the central parts of the extrapyramidal system. It was generally believed that this system 'looked after' movements of the body that could be regarded as reflex in nature. Basal ganglia are well developed in reptiles and birds, animals whose movements were considered to be more 'reflex' than 'willed'. It was also noted that diseases that affected the basal ganglia and related nuclei produced effects rather different from those that affected the motor cortex of man. A number of interesting syndromes that include odd writhing movements (chorea or athetosis) and occasional out-flinging of limbs (hemiballismus) have been shown to be associated with lesions of this group of nuclei. All of these effects are different from the paralysis, initially flaccid but later spastic, which follows damage to the motor cortex and pyramidal tracts. Thus the idea that the extrapyramidal system

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represented a primitive movement-control system that had been superseded in man by the pyramidal system was formed. These concepts have now been discarded, and the terms pyramidal and extrapyramidal are considered misleading (Brodal, 1969). Current thinking is that the basal ganglia, thalamus and cortex are involved in a complicated series of feedback loops that control movement, with the pallidum being the efferent centre of the basal ganglia system, with projections to the thalamus and brainstem centres. The reader is referred to Lanciego et al. (2012) for a recent account of the basal ganglia.

The Rhinencephalon

The rhinencephalon includes the olfactory and limbic systems. Although the limbic system is not thought to be primarily involved in odour discrimination, it certainly receives input from the olfactory bulbs and takes behavioural cues from that input. The limbic system in man is involved with emotion, linking closely with the hypothalamus, and in the rat includes a good deal of the medial surface of the cerebral hemisphere, the hippocampus and the entorhinal cortex at the lower posterior ends of the hemispheres. Hamilton (1976) has provided a valuable, albeit short, account of the rat limbic system.

Histology of the Prepyriform and Pyriform Cortices

Although detailed accounts of the cytoarchitecture of the rhinencephalon are available, we shall describe just two areas (Burns, 1982).

The cortex of the prepyriform area has three layers: a superficial molecular layer of nerve fibres and occasional cell bodies, a pyramidal cell layer of small pyramidal and stellate cells, and a granular layer of small round-bodied neurons.

The cortex of the prepyriform area cortex has four layers. A peripheral layer of densely packed nerve fibres of the lateral olfactory tract synapse with dendrites rising to the surface from the pyramidal cells of the deeper layers. Deep to the layer of nerve fibres, there is a layer of small pyramidal cells with dendrites that rise to surface layer. A layer of medium-sized pyramidal cells also send dendrites to the surface, forming the efferent pathway from this part of the cortex. Axons from the anterior end of the pyriform cortex run to the medial forebrain bundle; from the posterior end they run to the amygdala.

All textbooks of neuroanatomy show complicated diagrams of the interconnections of the structures of the limbic system, most of which are derived from the work of Gloor (see Gloor, 1997).

The Olfactory System

The olfactory system includes all structures on the pathway from the olfactory epithelium to the cerebral cortex, where the olfactory tubercle, prepyriform and pyriform cortex are involved in the interpretation of that input. The olfactory bulbs of the rat are large and project from the anterior ends of the cerebral hemispheres. The bulb has a laminated structure with an important layer of mitral cells that send their axons back to the brain as the olfactory tracts.

The axons streaming back from the bulb can be grouped as lateral, medial and intermediate olfactory tracts. The lateral tract is the largest, carrying the majority of the information that will be interpreted in terms of odour. It runs to the prepyriform and pyriform cortex. The medial and intermediate tracts link with the limbic system, especially with that part of the hippocampus that lies anterior to the anterior end of the corpus callosum, and the many nuclei of the septal region at the anterior end of the forebrain.

A small secondary bulb, embedded in the main bulb, receives input from the vomeronasal organ.

Limbic System

In addition to those areas of cortex assigned to the limbic system, a number of subcortical masses of grey matter, or nuclei, form part of the system. Of these the most important are the amygdala and a group of nuclei in the septal area at the front of the forebrain that include the nucleus accumbens, which lies below the anterior end of the combined caudate and putamen. There are many connecting pathways to the limbic system. Four important pathways are listed below:

The fornix arches forwards from the hippocampus before turning down towards the hypothalamus and then back to the conjoined mammillary bodies.

The medial forebrain bundle is a large bundle of fibres that links the olfactory system with the hypothalamus and continues into the midbrain.

The diagonal band of Broca runs from the medial olfactory area to the preoptic region of the hypothalamus.

The cingulum (Latin: meaning band or girdle, and interestingly the origin of the word shingles: a viral disease of peripheral nerves) runs back over the cingulate gyrus (another part of the limbic system) to the entorhinal cortex and hippocampus.

The Diencephalon

The diencephalon lies centrally, rather low down between the two cerebral hemispheres, in front of the midbrain. The roof of the third ventricle provides the roof of the diencephalon: its floor lies above the central part of the hypothalamus. The diencephalon terminates anteriorly at the appropriately named lamina terminalis, which is derived from the original anterior end of the neural tube. The lateral walls of the third ventricle are much thickened forming the thalamus and epithalamus above, and parts of

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the hypothalamus below. Though the thalamus is usually referred to in singular, it should be remembered that there are right and left thalami. The right and left thalami are linked by a mass of tissue that runs across the midline through the 'hole in the doughnut' formed by the circular canal that comprises the third ventricle of the rat. The lateral wall of the thalamus (the old lateral wall of the diencephalon) fuses with the medial walls of the cerebral hemispheres, and a free exchange of nerve fibres occurs across this obliterated boundary. The cerebral cortex is connected with lower centres by the corona radiata, a great curved fan of nerve fibres that narrows ventrally to form the internal capsule which passes between the thalamus and the basal ganglia to form the cerebral peduncle. The internal capsule contains myelinated fibres running in both directions between the thalamus and the cerebral cortex, as well as the corticospinal and corticopontine fibres.

The Thalamus

The thalamus is a large mass of grey matter in the dorsal part of the diencephalon that acts as a major relay centre for sensory information, other than olfactory input, on its way to the cerebral cortex, and has a role in the regulation of consciousness, sleep and alertness.

The thalamus is not an isolated structure. The reticular formation of the midbrain, for example, runs directly into the thalamus and links with its intralaminar nuclei. Galen used the Greek term thalamos to suggest an inner room, or bedroom, within the brain. Each thalamus is roughly ovoid in shape, swelling at its posterior end to form the pulvinar (meaning cushion), which overlaps the medial and lateral geniculate nuclei (or bodies). The anterior pole of the thalamus lies just behind the interventricular foramen. The thalamus contains a bewildering number of nuclei; the terminology applied to these nuclei is regrettably inconsistent. Homologies between rat and man are arguable, and we suggest readers needing topographical details of the individual nuclei to consult Paxinos and Watson (2007).

Nuclei Concerned With the Relaying of Specific Sensory Information to the Cerebral Cortex

This ventral group of thalamic nuclei receives input from the medial lemnisci and the trigeminal lemnisci, and then projects efferent fibres to the cerebral cortex, ending in the outer stripe of Baillarger (see below). These are described as 'specific,' because they convey information about specific sensory modalities. The ventral group of nuclei also includes the medial and lateral geniculate nuclei (or bodies), key parts of the auditory and visual systems.

The Hypothalamus

One of the most important functions of the hypothalamus is to link the nervous system with the endocrine system via the pituitary gland. Anatomically close to the pituitary gland, the hypothalamus is well placed as a centre for coordinating responses to olfactory stimuli in the rat, and as a controller of what is sometimes rather disparagingly called 'vegetative physiology' or the 'internal environment'. The hypothalamus also contains sensory systems for monitoring the osmotic pressure and temperature of the blood. More importantly in the rat than in man is the role of the hypothalamus in coordinating behavioural responses to olfactory stimuli. Much rat behaviour is focused on those essentials of life, feeding and reproduction. The large rhinencephalon and the hypothalamus coordinate such activities on the basis of olfactory information.

The hypothalamus forms the floor of the diencephalon, creating a shallow trough joining the mesencephalon behind to the lamina terminalis in front. The anterior columns of the fornix arise from the hippocampus and run down behind the lamina terminalis, before curving back on each side to reach the mammillary bodies at the posterior end of the hypothalamus. The side walls of the hypothalamus curve up to meet the thalamus. The external surface of the hypothalamus can be seen on the ventral surface of the brain. As with the thalamus, many discrete nuclei have been described in the hypothalamus. Details can be found in textbooks of rat neuroanatomy.

Afferent Connections of the Hypothalamus

The medial forebrain bundle, containing both incoming and outgoing fibres, is a well-defined tract linking the anterior nuclei of the hypothalamus with the mesencephalon. The fornix brings output from the hippocampus to the mammillary bodies and other hypothalamic nuclei. Fibres from the amygdala (part of the rhinencephalon) arrive at the nuclei of the ventromedial part of the hypothalamus. Fibres from the cerebral cortex also run to the hypothalamus.

Efferent Connections of the Hypothalamus

We have noted that the medial forebrain bundle contains efferent, as well as afferent, fibres. The dorsal longitudinal fasciculus provides a link between hypothalamic nuclei and autonomic centres in the lower brainstem. Other fibres include some running close to the walls of the third ventricle and into the thalamus. A large mammillothalamic tract, the bundle of Vicq d'Azur, links the mammillary bodies with the thalamus, and the mammillotegmental tract links the mammillary bodies with the reticular formation of the brainstem.

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The Epithalamus

The epithalamus includes the habenular nuclei and their interconnecting fibres (the habenular commissure), the pineal gland (pineal: shaped like a pine cone), well known for its production of melatonin, and two strands of white matter, the striae medullaris, which run along the upper, inner edges of the thalamus (in the sides of the roof of the upper part of the third ventricle) connecting anteriorly with olfactory structures in the septum and posteriorly with the habenular nuclei and the commissure that runs from side to side at the posterior end of the diencephalon. Habenula is derived from habena: Latin for a bridle.

Receiving input from the olfactory tubercles, thalamus and hypothalamus, the habenula nuclei send efferent fibres via the fasciculus retroflexus to the interpeduncular nuclei of the midbrain that lie between the cerebral peduncles. The habenular nuclei are also linked on each side to the amygdala by nerve fibres that run back from the septal area via the striae medullaris. All this suggests that the habenular system is another linking and integrating system between olfactory input and behavioural and autonomic activity.

The Subthalamus

The subthalamus lies beneath the posterior end of the thalamus, behind the hypothalamus and above the cerebral peduncles, and links the midbrain to the diencephalon. It is separated from the thalamus by the lateral lemniscus (carrying auditory input) and contains collections of nerve cell bodies from the zona incerta and the subthalamic nuclei. The subthalamic nuclei receive a major input from the globus pallidus. The zona incerta receives input from a large number of centres and may represent a part of the reticular formation – intralaminar nuclei of the thalamus complex. For details of this and of the fields of Forel (H1 and H2 areas), which have been defined in this part of the brain, the reader is referred to Paxinos and Watson (2007) and to Zeman and Innes (1963).

THE MIDBRAIN OR METENCEPHALON

Lying between the pons and the diencephalon, the midbrain contains the cerebral aqueduct connecting the third and fourth ventricles. The nuclei of the oculomotor (CN III) and trochlear (CN IV) nerves and the red nucleus are all found in the midbrain. The tectum forms a roof over the aqueduct and supports the anterior and posterior colliculi. The tegmentum lies between the tectum and the cerebral peduncles. The anterior colliculus consists of layers of neurons separated by layers of white matter. In primitive vertebrates, it is a vital part of the visual system, but in mammals, this role is performed by the lateral geniculate body of the thalamus and the visual cortex. Both the anterior colliculus and the lateral geniculate body have a layered structure, which is presumably important in sorting out input from the retina. In mammals the anterior colliculus receives input from the retina, acting as a relay to the nuclei of the cranial nerves that control movement of the eye, and to the Edinger–Westphal nucleus (also of the midbrain), which provides the parasympathetic supply to the eyeball. The pretectal nuclei located at the anterior end of the tectum are also involved in the intricate control of eye movement and the tracking of moving objects.

The posterior colliculus is concerned with auditory input. Fibres from the cochlear ganglia travel via the lateral lemniscus to the posterior colliculus where they relay. Efferent fibres from the posterior colliculi run to the medial geniculate nucleus (or body) of the thalamus. Efferent fibres from the medial geniculate body run to the auditory cortex. The posterior colliculus lies on the mainline of the auditory system, unlike the anterior colliculus, which no longer lies on the mainline of the visual system in mammals.

The anterior end of the tectum leads to the large posterior commissure of the rat brain. This commissure has its own nucleus and is linked with the pretectal nucleus. The epithelial lining of the junction between the aqueduct and the third ventricle is thickened, and forms the subcommissural organ of the rat. Exactly what this intriguing structure does is obscure, but in all vertebrates it gives rise to a very odd structure: Reissner's fibre. This fibre seems not to contain nerve fibres and runs back through the aqueduct and fourth ventricle and all the way along the central canal of the spinal cord (Woollam and Collins, 1980).

A number of tracts run in the tegmentum. The medial lemniscus is in a lateral position, the medial longitudinal bundle is present and so is the dorsal longitudinal fasciculus, which connects the dorsal and ventral tegmental nuclei with the mammillary bodies.

The red nucleus bulges forwards over the posterior part of the ventral diencephalon, where it is a major feature of the midbrain that stands out clearly in histological sections, because bundles of myelinated fibres (largely coming from the cerebellum) sweep around it. Afferent inputs to the red nucleus arrive from the superior cerebellar peduncles, the basal ganglia and the cerebral cortex. Efferent connections include the decussating rubrospinal tracts (more important in the rat than in man) and the fibres that run in the central tegmental tract to the inferior olive (more important in man than the rat).

The nuclei of the fourth cranial nerves lie in the dorsal part of the tegmentum. The roots of the nerves decussate above the aqueduct and then emerge from the dorsal surface of the midbrain. The fourth cranial nerve is the only one to emerge from the dorsal surface of the brainstem, just why it should do so is unknown. The third nerve nuclei form a complex group, as one might expect, given that four of the extra-ocular muscles are supplied by this

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nerve. The efferent nerve fibres from this group of nuclei emerge from the ventral surface of the midbrain. The neural linkages between the nuclei of the cranial nerves that supply the extra-ocular muscles, and their controlling centres, are complicated. Over indulgence in alcohol upsets this complex system and may produce double vision!

The pale substantia nigra is part of the extrapyramidal system and receives a major input from the basal ganglia. Dopamine is the most important neuro-transmitter in the substantia nigra, a lack of which leads to Parkinson's disease, a disease characterised by muscular rigidity and tremor, all effects that one might expect as a result of interference with the feedback loops controlling the activity of skeletal muscles. The corticospinal and corticopontine tracts run in the basis pedunculi (a compound plural noun meaning both cerebral peduncles) ventral to the substantia nigra.

THE HINDBRAIN OR RHOMBENCEPHALON

The hindbrain or rhombencephalon includes the medulla, pons and cerebellum.

Pons

The pons of the rat is proportionally narrower and less well developed than that of man. The fibres that give the pons its name are efferent fibres running across the midline from the pontine nuclei to the central part of the cerebellar cortex. Fibres from the cerebral cortex travel to the pontine nuclei in the corticopontine tracts, passing through the internal capsule on their way to the pons. The corticospinal (pyramidal) tracts lie just dorsal to the area of pontine nuclei and corticopontine fibres. In man the corticospinal tracts form bundles that lie between the pontine nuclei. Corticobulbar fibres run with the corticospinal fibres and divert to cranial nerve nuclei. These fibres are called corticobulbar fibres because they run to the cranial nerve nuclei of the bulb.

The remainder of the pons, the tegmentum (Latin for roof), contains a number of tracts running anteriorly and posteriorly and some cranial nerve nuclei. The trigeminal nerve has a significant presence in the pons, with its motor, and a large part of its sensory nuclei, occurring at this level of the brainstem.

Routine sections of the rat brain are cut at approximately 90 degrees to the long axis of the brain, but the slight curvature of brainstem and standard sections often show part of the dorsal midbrain above part of the ventral pons. The human brainstem is naturally much larger than that of the rat and is often removed from the rest of the brain and sectioned at 90 degrees to *its* long axis.

Cerebellum

The cerebellum and pons make up the metencephalon. Connected to the brainstem by the superior, middle and inferior peduncles, the cerebellum lies above the pons and is relatively narrower from front to back than the large, rounded, cerebellum of man. The central function of the cerebellum is the adjustment of the control of movement so that it is smooth and well coordinated. In order to discharge this function the cerebellum receives input from the spinocerebellar tracts (carrying information from proprioceptors), the vestibular nuclei, the cerebral cortex, basal ganglia and red nucleus. The output of the cerebellum arises in its deep nuclei, for example the dentate nucleus, and runs in the superior cerebellar peduncle to the red nucleus and thalamus. A sagittal section of the cerebellum shows it shaped like an inverted horseshoe. The cerebellum is divided into right and left halves by the vermis, a band that runs round the cerebellum, beginning just behind the pineal gland and ending above the posterior end of the roof of the fourth ventricle. In man the vermis looks a little like a worm. To each side of the vermis lie the cerebellar hemispheres that are subdivided into lobes and lobules. The parafloccular lobes arise from the posterior end of the vermis. Details of the very complicated terminology that have been applied to the lobes and lobules can be found in textbooks of neuroanatomy: see bibliography. The most anterior and posterior parts of the cerebellum are concerned with the vestibular system, the middle part is concerned with the cerebral cortex, and the parts in between (at front and back) are concerned with the spinal cord. The middle part, like the middle cerebellar peduncle, is less well developed in the rat than in man, reflecting the poorer development of the cerebral cortex in the rat.

Buried deep in the white matter in the centre of the cerebellum are three pairs of nuclei, the most important of which is the dentate nucleus (so called because it appears toothed in sections). These nuclei are the relays between efferent fibres from the cortical Purkinje cells and the efferent fibres that flow from the cerebellum in the superior cerebellar peduncle. Other efferent fibres from the cerebellum run to the reticular nuclei in the brainstem and to the complex of vestibular nuclei.

Histological Structure of the Cerebellum

The histological structure of the cerebellum is fascinating; it is also one of the easier parts of the brain to recognise in a histological section. Coronal sections lie in the long axis of the lobes of cerebellum but sagittal sections cut across the lobes and show the classic 'cauliflower' appearance rather better (Fig. 20.6).

It is obvious, even on low power examination of histological sections, that the cerebellum can be divided into a

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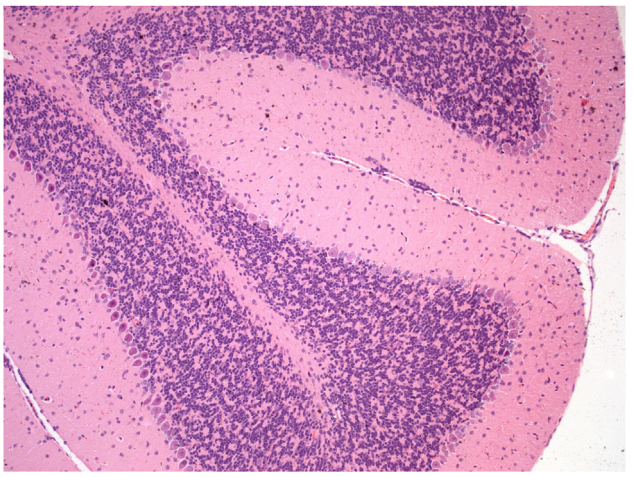


FIGURE 20.6 Cerebellum H&E stain.

medulla and cortex. The medulla comprises the central white matter containing the myelinated incoming and outgoing fibres of the cerebellum, and the deep nuclei of the cerebellum. The occasional cell bodies present are mostly astrocytes and oligodendrocytes.

The grey matter of the cortex is divided into three layers: a superficial, molecular layer (so called because there are only a few scattered nuclei present), a clearly defined single layer of large cells with cells bodies shaped like wine flasks (the Purkinje cell layer), and an inner, thick, densely staining layer of cells that resemble lymphocytes (the granular cell layer).

Purkinje Cells

Purkinje cells are unique to the cerebellum. Purkinje cells are large neurons, with large, eosinophilic cell bodies that contain some Nissl substance, and like large neurons elsewhere in the CNS, appear particularly sensitive to toxicological insult: trimethyl tin is a classic example. Long axons run through the granular cell layer to the deep nuclei of the cerebellum.

The outstanding feature of Purkinje cells is their superb, two-dimensional, dendritic fan that extends upwards from the cells across the axis of the lobules of the cerebellum. In primates the lobules are subdivided into folia (leaves), with the dendritic fans arranged at 90 degrees to the axes of the folia. In the monkey the dendritic fan may carry 60,000 dendritic spines that can interact with the horizontal branching axons of the granular cell layer running along the long axes of the lobules. Each dendritic fan may be traversed by 200,000 horizontal fibres, and each granule cell may be in contact with 500 dendritic fans. Even a moment's thought will suggest that a vastly complex process of integration is possible.

Granule Cells of the Granular Layer

Granule cells are small with nuclei that stain darkly, and little cytoplasm can be seen in ordinary histological sections. Nor can much be seen of their axons and dendrites, unless silver staining techniques are used. Their short dendrites cluster around the terminals of incoming fibres, forming little islands that stand out as pale pink-stained spaces in H&E-stained sections. These spaces between the packed nuclei of the granule cells are the cerebellar glomeruli or islands. The incoming fibres are described as mossy fibres because their terminals look a bit like the ends of fronds of club moss, at least that is said to be the case in embryo. Mossy fibres come from many sources including the spinal cord, pontine nuclei and the vestibular nuclei. In the glomeruli synapses between incoming fibres and granule cells occur. The axons of the granular cells run to the molecular layer before branching as T junctions to produce the horizontal, unmyelinated fibres that we have described as running through the dendritic fans of the Purkinje cells. Another group of incoming axons, described as climbing fibres, climb the dendritic fans of the Purkinje cells. These fibres are thought to originate in the inferior olivary nuclei.

Other Neurons

Three other types of neurons have been described. The stellate cells of the molecular layer are few and far between, but send axons and dendrites horizontally to interact with the dendritic fans of the Purkinje cells. Basket cells lie deeper in the molecular layer and provide a network of fibres around the bodies of the Purkinje cells. Golgi Type II cells are present in the Purkinje cell layer, and their three-dimensional dendritic trees extend into the molecular layer.

An enormous amount of work has been done of the circuitry of the cerebellum; indeed it was the first example of a so-called 'neuronal machine' (Eccles et al., 1967). There are probably three main points that the reader should try to take away:

1. Input from mossy and climbing fibres is excitatory to the deep nuclei of the cerebellum, granule cells and Purkinje cells (glutamergic synapses are involved).
2. The Purkinje cell axons have an inhibitory effect on the neurons of the deep cerebellar nuclei, granule cells and on Golgi Type II cells (GABA-ergic synapses are involved).
3. Granular cell input to Purkinje cells is excitatory (glutamergic synapses are involved).

Textbooks of neurophysiology should be consulted for further details.

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The Medulla Oblongata

The medulla oblongata is the most posterior part of the brainstem and merges with the spinal cord at, or just posterior to, the foramen magnum. The internal structure of the medulla differs from the spinal cord, partly as a result of the decussation of the pyramidal tracts. These large tracts begin in the cerebral hemispheres and travel via the internal capsule to the cerebral peduncles along the ventral surface of the midbrain and pons, before suddenly abandoning their ventral position and diving into the substance of the medulla. The right and left pyramidal tracts cross each other in the medulla and take up their positions in the dorsal columns of the spinal cord. This great decussation is one of the most easily recognised features of the medulla.

A second feature that is easy to recognise is the dorsal displacement and widening of the central canal to form the fourth ventricle. The fourth ventricle widens as it approaches the pons before narrowing to form the aqueduct of the midbrain. The displacement and widening of the central canal causes the grey matter of what were the dorsal and ventral horns of the spinal cord to spread out and form an arc beneath the fourth ventricle. The columns of neurons of the grey matter of the spinal cord continue forwards in their relative positions. The General Somatic Efferent (GSE) columns, which are located in the ventral horn of the spinal cord, take up a medial position in the arc, and the General Somatic Afferent (GSA) columns, which are located in the dorsal horn of the spinal cord, take up a lateral position. The terminology is explained in Chapter 21 (1) where we discuss the cranial nerves in some detail.

The sequence from lateral to medial in the grey matter of the medulla runs: GSA, SSA, GVA, SVA, SVE, GVE and GSE (see Chapter 21.1 for abbreviations). All this is rather confusing and a few examples will help. The motor nucleus of the 12th cranial nerve is a GSE nucleus and lies on either side close to the midline; the 5th cranial nerve (trigeminal) contains sensory fibres from the face and its major sensory nucleus lies on the lateral side of the grey matter. The nuclei of the cranial nerves do not follow this plan as precisely as they might do. The nucleus ambiguus, for example, is found ventral to the arc of grey matter we mentioned a moment ago. The nucleus of the facial nerve (CN VII) has also taken up a more ventral position in the pons than the nucleus of abducens nerve (CN VI). The nuclei of the cranial nerves can best be recognised by their position; details of the variations in their cellular structure are beyond this account.

In addition to the nuclei of cranial nerves, the medulla oblongata contains nuclei of the reticular formation. This large and important series of nuclei runs through the medulla oblongata to the anterior end of the midbrain. They lie in the grey matter around the axis of the brainstem and are bewilderingly numerous.

The white matter of the medulla contains many tracts (also known as fascicles or funiculi), which run forwards and backwards in the fasciculi brainstem. The spinothalamic and spinocerebellar tracts originate in the spinal cord, whereas some tracts, for example, the medial lemnisci, arise in the medulla. The medial lemnisci arise from the nuclei of the dorsal tracts and lie near the midline, dorsal to the pyramids, before decussating as the internal arcuate fibres.

Decussations, of which there are many in the brainstem, are one of the bug-bears of neuroanatomy. There is no obvious reason why so many bundles cross the neuroaxis, and one could be forgiven for thinking that decussation is an example of neuroanatomical cussedness!

In addition to the long ascending and descending tracts running to and from the spinal cord, shorter tracts connect structures within the brainstem. The medial longitudinal bundle (or fasciculus) connects the vestibular nuclei with those of the cranial nerves that control movements of the eye. The dorsal longitudinal bundle connects the hypothalamus with important centres in the reticular formation and with cranial nerve nuclei involved with the autonomic nervous system. The central tegmental tract links the red nucleus with the cerebellum via the inferior olivary nucleus. The inferior olivary nucleus is readily recognisable in sections of the human medulla, but in the rat it is smaller and less obvious. Efferent fibres from the inferior olivary nucleus decussate and enter the cerebellum via the inferior cerebellar peduncles.

The spinal nucleus and tract of the trigeminal nerve (CN V) is one of the largest structures in the rat medulla oblongata. The sensory fibres enter the pons and run forwards and backwards in the brainstem creating a large, well-defined tract of myelinated nerve fibres that travels back as far as the cervical spinal cord. Fibres from the tract synapse with neurons in the adjacent, and equally long trigeminal main sensory nucleus, which can be thought of as an extension of the dorsal horn of the spinal cord. The majority of fibres mediating touch, pain and temperature cross the midline to form the trigeminal lemniscus, which can be thought of as the equivalent of the medial lemniscus. The fibres of the trigeminal nerve that mediate proprioception from structures of the head (the equivalents of the fibres of the dorsal columns of the spinal cord) have cell bodies located in the mesencephalic nucleus of the trigeminal nerve.

The abducent (CN VI), facial (CN VII) and auditory (CN VIII) cranial nerves appear just behind the pons. The abducent nerve arises from its GSE nucleus near the dorsal midline, in line with the nucleus of the 12th cranial nerve. The nucleus of the facial nerve is displaced ventrally, so as a result the root of the nerve hooks round the abducent nucleus before turning back and leaving the ventrolateral aspect of the brainstem. The trapezoid body contains fibres from the 8th cranial nerve nuclei that cross

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the midline and then turn forwards as the well-defined lateral lemniscus running to the posterior colliculus and medial geniculate body of the thalamus. The vestibular fibres of the 8th cranial nerve run to a complex of vestibular nuclei below the floor of the fourth ventricle, from where efferent fibres run back to the spinal cord as the vestibulospinal tracts to the cerebellum and medial longitudinal bundle that links the vestibular system with the nuclei of cranial nerves controlling movement of the eyes.

The Reticular Formation

The reticular formation is a matrix of nerve fibres and nerve cell bodies that form much of the core of the brainstem, extending from the medulla, indeed from the spinal cord, to the intra-laminate nuclei of the thalamus. At the first sight it looks a little like neurological packing that fills the gaps between the better defined tracts and other nuclei of the system, but it is much more than that.

The nuclei of the reticular system have been classified according to a number of criteria:

1. By location in sections of the brainstem: the series of raphe (raphe means seam) nuclei located near the midline of the brainstem.
2. By level within the brainstem: the caudal pontine reticular nucleus.
3. By cell type: the nucleus gigantocellularis with large cell bodies or the parvocellular reticular area with small cell bodies.
4. By connection: those receiving a major afferent input tend to be located lateral to those producing a major efferent output, running, for example to the spinal cord via the reticulospinal tracts.
5. By the transmitter substances found in the nuclei: catecholaminergic, including noradrenalin and dopamine for example the locus coeruleus (named for its blue colour); cholinergic and serotonergic, including those of the raphe series.
6. By function: those of the parabrachial area (near the superior cerebellar peduncle or brachium conjunctivum) that are involved in the control of respiration.

The reticular formation is connected to all parts of the brain. We have not listed the many nuclei of the reticular formation, which are best identified by reference to a stereological atlas of the rat brain for example Paxinos and Watson (2007).

The Ventricular System of the Rat Brain

Good descriptions of the rat ventricular system have been provided by Levinger (1971) and Ozdemir et al. (2005). Levinger's paper is particularly helpful as it contains photographs of resin casts of the ventricular system that give a three-dimensional perspective.

Lateral Ventricles

The lateral ventricles are connected to the third ventricle at the interventricular foramina of Monro. The main parts of the lateral ventricles run anterior–posterior, splaying away from the midline posteriorly. A narrow extension of the anterior end of the lateral ventricle (of the anterior horn to be precise) enters the olfactory bulb, and from the posterior part a wide, curved, hollow plate called the inferior horn sweeps downwards following the outer surface of the hippocampus (Levinger, 1971). Unlike man the rat has no posterior horn to its lateral ventricle.

The Third Ventricle

In man this is a slit, in the ox a series of tubes, and in the rat a ring-shaped canal that looks like a doughnut standing on edge. A connection between the right and left thalami passes through the 'hole in the doughnut'. The third ventricle has anterior and posterior diverticuli that extend down into the hypothalamus, and a small diverticulum that extends upwards into the stalk of the pineal gland.

The Aqueduct

The aqueduct is narrow and has an upward running diverticulum that passes into the posterior colliculus.

The Fourth Ventricle

From the above the fourth ventricle is seen to be rhomboid in shape. Cerebrospinal fluid (CSF) escapes into the subarachnoid space via the lateral foramina of Luschka. In man there is a further foramen, located in the midline in the roof of the fourth ventricle: the foramen of Magendie, which according to Levinger (1971) does not occur in the rat.

Cerebrospinal Fluid

The choroid plexus, which invaginates the roofs the third ventricle and fourth ventricles and the lateral ventricles, secretes CSF into the ventricles. The vascular folds of the choroid plexus are separated from the CSF that fills the ventricles by ependymal cells, a layer of specialised, cilated cells that overlie a dense capillary plexus and play an important role in the production and regulation of the composition of the CSF. Tumours of the ependymal cells and the choroid plexus may be seen occasionally in ageing rats. Zheng (2001) has reviewed the toxicological effects of substances on the choroid plexus: metals may have significant effects.

CSF formed in the lateral ventricles flows through the foramina of Monro into the third ventricle and on through

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the aqueduct to the fourth ventricle from which it escapes to the subarachnoid space around the brainstem via the lateral foramina of Luschka. Most CSF is absorbed by the veins of the pia and arachnoid mater, but some is also absorbed by lymphatic vessels running in the meninges. In man absorption also occurs via the arachnoid granulations that project through the dura into the superior sagittal sinus. Very little CSF actually finds its way down into the central canal of the spinal cord. Blockage of the flow of CSF along any part of this pathway from the ventricles to the subarachnoid space leads to hydrocephalus.

SPINAL CORD

The spinal cord comprises a fluted central column of grey matter surrounded by white matter.

The position of ascending and descending tracts in the spinal cord is shown in Fig. 20.7A and B.

Incoming fibres enter the cord via the dorsal roots of the spinal nerves and run in to the dorsal horns of the grey matter where many synapse with interneurons. Some turn back to the white matter without synapsing to form the dorsal tracts (dorsal funiculi or columns) of the cord, while others synapse, and either turn to the white matter of their own side of the cord before running forwards to the brain, or cross the midline to join tracts running in the white matter there. Many of these tracts have been identified and named for their destinations, for example, the spinocerebellar and spinothalamic tracts. The tracts of the dorsal columns may also be named for their shape, gracile (thin) and cuneate (wedge-shaped). Furthermore, running in the white matter are the descending tracts that run from the brain to the cord, which are named for their origins, for example, the corticospinal tract and the rubrospinal tract that run from the cerebral cortex and red nucleus, respectively. Although many textbooks show diagrams illustrating the position of the tracts in the white matter of the cord, these are usually based on human or cat neuroanatomical studies; detailed accounts of the arrangement of the tracts in the rat are rather sparse. Recent studies by Sengul (2014) and by Sengul and Watson (2014) that focus on the rat and mouse summarise current thinking. The most noteworthy difference in the arrangement between the rat and primates is the position of the corticospinal tract. In primates it runs in the lateral white matter, but in the rat it runs in the dorsal white matter (Fig. 20.3).

The grey matter of the cord is arranged as a series of laminae, which were first described in the cat by Rexed (1952), and subsequently became known as the Rexed laminae (Sengul, 2014; Sengul and Watson 2014). The laminae are thought to represent functional groupings of neurons, some of which have been identified as having specific activities. The grey matter is a column, and the groups of neurons lying within it form lesser columns that can be seen as nuclei in cross section. Some have been named; Clarke's column (or nucleus) is the origin of the dorsal spinocerebellar tract, and the intermediolateral column is the origin of the preganglionic sympathetic outflow from the thoracolumbar segments of the cord.

Another striking development of recent years is the identification of a multitude of presumed neurotransmitters in the grey matter of the cord. Only a specialist would wish to remember all these substances, but to show how complicated things have become we list a selection: acetylcholine, calcitonin-gene-related peptide, calmodulin, dopamine, cholecystokinin, enkephalin, GABA, glutamate, glycine, neuropeptide Y, serotonin (5 hydroxytryptamine), somatostatin, substance P, vasoactive intestinal polypeptide, calretinin, nitric oxide, neurotensin and parvalbumin. Details of the publications that provided evidence of the presence of these compounds may be found in Sengul (2014). Clearly a complicated system, and any expectation that a simple set of circuit diagrams will explain what is going on in the grey matter of the cord, is unlikely to be met.

The Tracts

The tracts of the rat spinal cord are discussed in detail by Sengul and Watson (2014), but even in their account evidence is quoted from studies in mammals other than the rat. Fig. 20.7A and B show the positions of the major tracts. The major features of selected tracts are summarised in Tables 20.5 and 20.6.

Further ascending tracts: spinocervicothalamic pathway, spinoreticular tract, spinomesencephalic tract, spinoparabrachial tract, spino-olivary tract, spinovestibular tract and spinohypothalamic tract. See Sengul and Watson (2014) for details of these tracts.

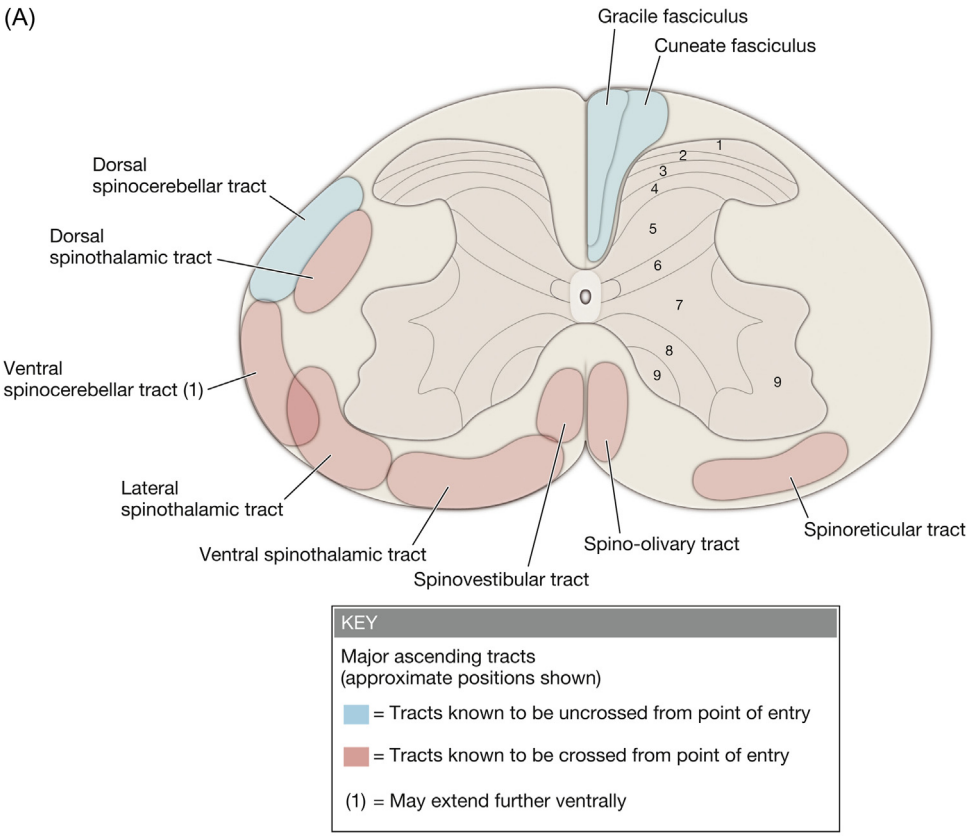
There are numerous other descending tracts, including the hypothalamic and diencephalic projections to cord, cerebellospinal tracts, reticulospinal tracts (see section on reticular formation, below), descending projections from trigeminal and dorsal column nuclei, raphespinal and coerulospinal tracts, solitariospinal tract and projection from nucleus retroambiguus to cord (see Sengul and Watson (2014) for further details).

Fig. 20.3 show only the approximate positions of the major tracts of the rat spinal cord. It is important to remember that there is considerable overlap of fibres between tracts.

IDENTIFICATION OF TOXICOLOGICAL CHANGES IN THE BRAIN

It will be obvious that recognition of site-specific toxicological effects requires that the affected sites are present in histological sections, and that those sites can be recognised and examined in detail. In tissues such as the liver this is a

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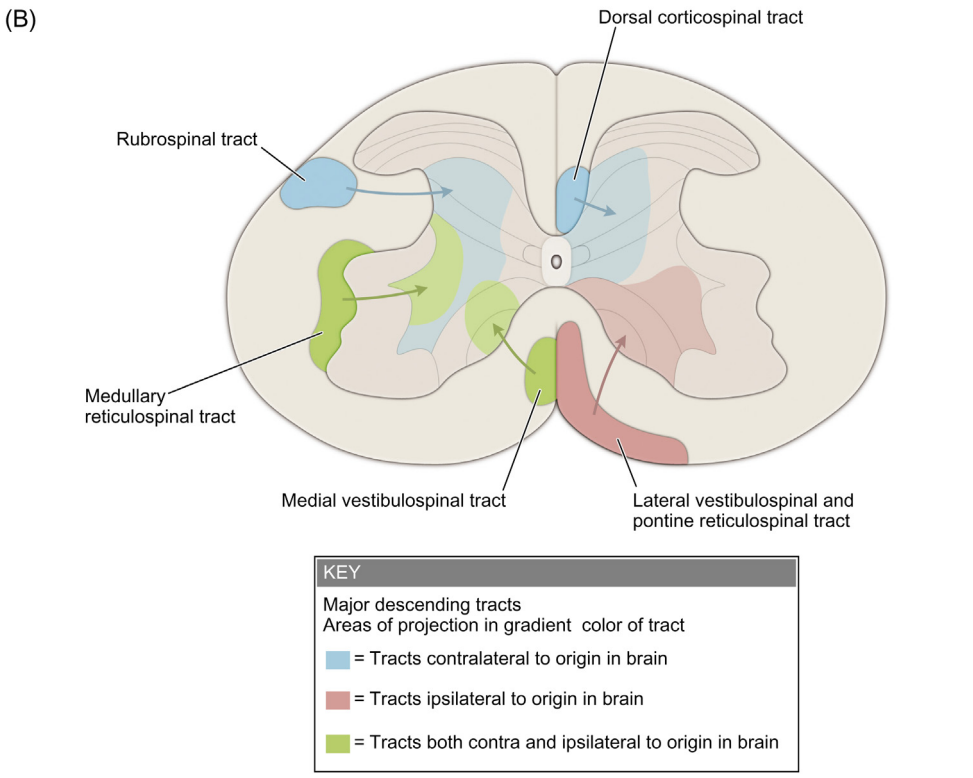


FIGURE 20.7 Major ascending and descending tracts of the rat spinal cord.

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TABLE 20.5 Ascending Tracts (From Spinal Cord to Brain)

Tract	Function/Modality	Laminae of Major Origin	Crossed/Uncrossed in Cord	Brainstem Route	Myelinated/Unmyelinated
Direct[a] dorsal column pathway: fasciculi gracilis and cuneatus	Proprioception,[b] touch and vibration	No synapses in dorsal laminae	Uncrossed	To gracile and cunneate nuclei, cross in internal arcuate fibres, medial lemniscus to thalamus	Myelinated
Spinothalamic tracts	Ventral: crude touch and pressure	1, 3–7 and 10	Mainly crossed	Follows medial lemniscus to thalamus	Myelinated and unmyelinated
	Lateral: pain and temperature	In cervical and thoracic segments: 4 and 5			
	Dorsolateral: pain				
Spinocerebellar tracts: dorsal and ventral. Ventral from lower thoracic segments and further back; dorsal from cervical and thoracic segments	Proprioception and cutaneous information[c]	4, 5, 7 and 8. Clarke's column	Ventral: crossed	Superior cerebellar peduncle	Myelinated and unmyelinated (?)
			Dorsal: mainly uncrossed	Inferior cerebellar peduncle	

[a] *There is also an indirect pathway: synapsis occurs before fibres enter dorsal columns (postsynaptic dorsal column pathway).*
[b] *Usually called conscious proprioception or position sense; applicable in man, possibly not in rat.*
[c] *Usually called unconscious proprioception.*

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TABLE 20.6 Descending Tracts (From Brain to Spinal Cord)

Tract	Origin	Crossed or Uncrossed	Function	Target Laminae of Cord	Myelinated/Unmyelinated
Corticospinal tracts[a]	Motor and sensory neocortex. Often assumed to be mainly from motor cortex but this is untrue in rodents	Crossed: crosses in the decussation of the pyramids in the medulla	Motor control in primates, debatable in rat	7 in primates; 3, 4, 5 and 6 in rat. Not direct to ventral horn motor neurons, perhaps not very important in control of limb movement in rat	Myelinated
Rubrospinal tract	Red nucleus	Crossed in ventral tegmental decussation	Major function in control of motor function. More important in rat than in primates	5, 6 and 7	?
Tectospinal tract	Anterior colliculus	Crossed in dorsal tegmental decussation	Control of head turning in response to visual stimuli	5, 7 and 8 mainly cervical segments	?
Vestibulospinal tracts: lateral and medial	Vestibular nuclei	Uncrossed	Major coordination of extensor activity in limbs and trunk	7 and 8	?

[a]*Often called the pyramidal tracts but this name is falling into disuse and the separation between the pyramidal and extrapyramidal systems is now outdated. It seems to be clear that the corticospinal tracts, so important in man, are perhaps rather unimportant in the rat. The rat relies on 'lower' centres to control its motor activity: this is in line with the lesser development of the neocortex in the rat as compared with that of primates.*

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relatively straightforward process. Toxicological effects may be largely confined to either portal or centrilobular areas, and although there is evidence of some minor lobar differences in toxicity, by and large any reasonable sampling regime will allow the investigator to assess both the extent and nature of the toxicity. This is not the case for toxicological effects on the brain, where many toxic effects are of a highly site-specific nature. Under some circumstances it is necessary to know here to look before toxicological effects can be recognised. In practice, when the effects of a new compound are being studied, it may not be known where the effects will occur. Finding the effects may require very extensive sampling of the brain. The examination of serial sections of the entire brain would be an ideal approach, but this is not possible in practice. Our approach is to cut 10 sections of the rat brain and to mount these on two slides. This scheme gives a reasonable sample of all the major areas of the brain and is manageable in terms of the number of sections that have to be examined.

This approach allows the following areas of the brain to be examined:

- The frontal, parietal and piriform cortex, including the basal forebrain, septal nuclei and anterior portion of the caudate-putamen.
- The frontal, parietal and temporal cortex, including an anterior level of the thalamus/hypothalamus, amygdala and either the fimbria/fornix or the anterior (dorsal) hippocampus.
- The parietal, temporal and entorhinal cortex, including a posterior level of the thalamus and/or the anterior midbrain and posterior (dorsal plus ventral) hippocampus.
- The occipital lobes including the middle to posterior portion of the midbrain.
- The middle of the cerebellum with underlying pons.
- The posterior portion of the cerebellum with underlying medulla oblongata.

These sections include all the major structures including those listed below.

Piriform cortex, amygdala, anterior commissure, basal forebrain, caudate-putamen, cerebellar cortex, cerebellar white matter, cerebral peduncle, cingulate cortex, corpus callosum, dentate gyrus, entorhinal cortex, external capsule, fornix, frontal cortex, globus pallidus, hippocampus CA1-3, hypothalamus, internal capsule, medulla oblongata, midbrain, occipital cortex, parietal cortex, pons, pyramidal tracts, reticular nuclei, retrosplenial cortex, septal nuclei, temporal cortex, thalamus, trapezoid body, trigeminothalamic tract and olfactory bulbs.

The areas examined are representative of about 70% of the brain: the caudal and rostral extremes are not examined. This approach is likely to identify toxicological effects in the major structures in the brain, although it is always possible that some highly site-specific effect in a small nucleus may be overlooked.

THE CELLS OF THE CENTRAL NERVOUS SYSTEM

Detailed accounts of the histology of the CNS have been provided by Crosby et al. (1962) and may be found in textbooks of histology (e.g., Fawcett, 1994). In essence, there are two functional cells types in the CNS: neurons and the neuroglia. In addition, there are other supporting cells that include the ependymal cells, the cells of the choroid plexus and the meninges, and the cells that comprise the vasculature.

Neurons

Neurons are the essential cells of the nervous system. All neurons comprise a cell body (perikaryon) and cytoplasmic processes, one axon and usually many dendrites (Fig. 20.8). Exceptions occur, for instance, the cells of the dorsal root ganglia have a single process that sends one long process into the spinal cord and another to the periphery. Both processes have the functional characteristics of axons. Dendrites are characterised by their branching, with each branch carrying a multitude of short dendritic spines, so providing a vast surface area for synapse with the processes of other nerve cells. Axons also give off branches but far fewer than do dendrites.

The cell bodies of the neurons of the CNS are most frequently angular in shape but may be spherical or spindle shaped. The large pyramidal cells of the motor cortex are instantly recognisable: the axon springs from the base of the pyramid and the dendrites from the apex. The Purkinje cells of the cerebellar cortex are another easily

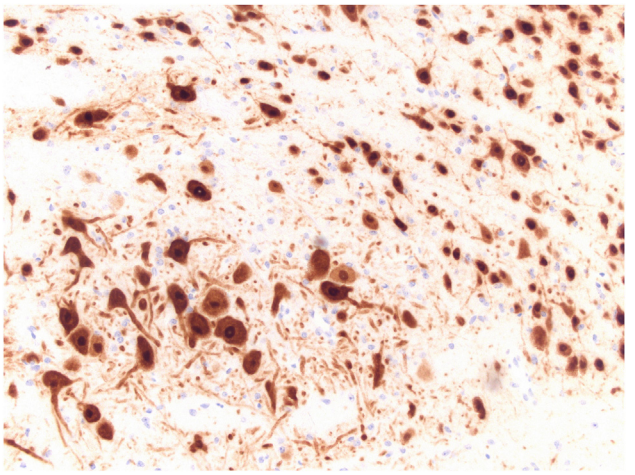


FIGURE 20.8 Neurones stained with Neun-1, an immunohistochemical stain for neurons.

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recognisable type: flask-shaped cell bodies with an axon springing from the base of the flask, while dendrites arise from the apex of the cell represented by the neck of the flask.

Neurons have been divided into two groups: Golgi I cells with long cell processes and Golgi II cells with short cell processes. The former provide the neurons that send their axons through the tracts of the CNS and the latter provide the local circuit neurons or interneurons of the system.

The cell bodies of neurons contain large nuclei that stain rather palely in neurons that are actively synthesising material or darkly in those that seem not to be doing so. The small dark staining nuclei of the granular layer of the cerebellar cortex provides a good example of the latter type. A large nucleolus can usually be identified. The cytoplasm of the cell body contains granular material called Nissl substance that stains deeply with aniline dyes. Nissl substance has been shown to be granular endoplasmic reticulum; the deep staining being due to the presence of ribosomes. Nissl substance occurs throughout the cytoplasm of the neuron cell body, extending into the dendrites, but is absent from the axon and the part of the cell body, the axon hillock, where the axon arises. The Nissl substance contains the essential subcellular machinery for protein synthesis, and neuropeptides that cannot be synthesised at axon terminals must be transported from the cell bodies along the axon. Transport of material along axons occurs both away from (antegrade), and towards (retrograde), the cell body. Both fast and slow transport processes have been defined. Other neurotransmitters, such as acetylcholine, can be synthesised at the axon terminals. Nissl substance is also interesting in that it disappears from the cell bodies of neurons that have undergone section of their axons, a form of retrograde degeneration.

The cell bodies contain a multitude of neurofibrils that include microtubules, microfilaments and neurofilaments which can be stained with silver impregnation techniques. Much has been discovered about the composition of these structures [see Fawcett (1994) for details].

The axons of nerve cells are described as either myelinated or unmyelinated. Myelinated axons are wrapped in many layers of myelin that provide an insulating layer around the axon. Myelin is produced by Schwann cells in the peripheral nervous system and by oligodendrocytes in the CNS. In the peripheral nervous system, the myelin sheath is interrupted at intervals where one Schwann cells hands over to the next, creating short segments where the axon is effectively naked. These gaps are known as the nodes of Ranvier, and there are similar gaps in the myelin sheaths of axons in the CNS. Unmyelinated axons occur as groups of axons wholly enveloped by one Schwann cell or oligodendrocyte. The thick multilayered myelin sheaths of myelinated fibres are absent.

Glial Cells

The word glia is derived from the Greek word for glue and was coined because glial cells were seen as the glue that held the CNS together. It is difficult to think of a more inadequate explanation of the function of glial cells, given that their functions include myelination of the CNS, contribution to the blood–brain barrier, removal, recycling and storage of transmitter substances, control of the concentration of potassium ions in the perineuronal milieu and storage of glycogen. Some glue!

Glial cells are divided into astrocytes, typified by their stellate cell bodies and ramifying cytoplasmic processes, the more rounded oligodendrocytes, which have few, if any, cytoplasmic processes and the microglia.

Astrocytes

Astrocytes have been subdivided into three forms: protoplasmic with rather thick cytoplasmic branches, fibrous with longer, branching processes that contain many fibrils (that can be stained using special silver impregnation techniques) and velate with veil like protoplasmic extensions. Fibrous astrocytes occur mainly in the white matter of the CNS and the protoplasmic astrocytes mainly in the grey matter. All astrocytes are attached to neurons by foot processes or pedicels and often to the walls of capillaries. It might reasonably be said that the surface of a neuron of the CNS is never naked, as areas not involved in synapses are in contact with astrocyte foot processes. Specialised forms of astrocytes have been described in many parts of the brain (Fig. 20.9).

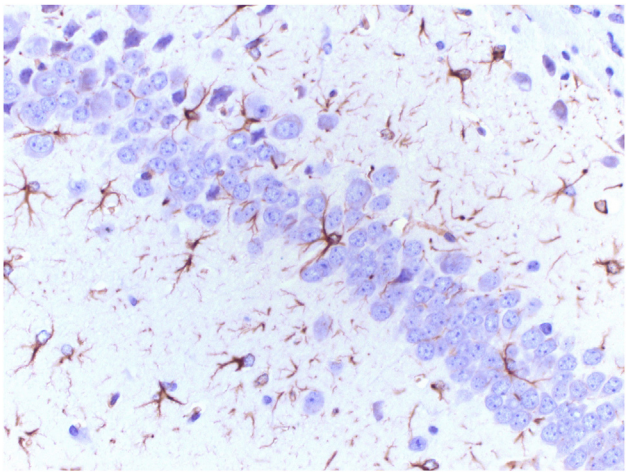


FIGURE 20.9 Astrocytes stained with glial fibrillary acidic protein (GFAP) the standard marker for astrocytes.

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Oligodendrocytes

The oligodendrocyte is the CNS equivalent of the Schwann cell of the peripheral nervous system. In early life the oligodendrocytes are responsible for the production of the myelin that forms myelin sheaths, so during this early stage of CNS development, the oligodendrocyte is highly active and probably at its most vulnerable. It has been estimated that during the peak of the myelination process, an oligodendrocyte can elaborate about three times its weight in membrane per day. For the rat the period of the greatest activity is probably represented by the immediate postnatal period, from around postnatal days (PND) 10–35. The ensheathment of multiple axons by a single oligodendrocyte is a highly coordinated event: oligodendrocytes do not ensheath different axons sequentially at different time points, but the process is completed within a brief time window. In the rat, this is typically within 12–18 hours (Watkins et al., 2008). Oligodendrocytes do not simple wrap any adjacent neuronal process but preferentially select axons with diameters above 0.2 μm (Simons and Trajkovic, 2006).

The mature oligodendrocyte may support membrane up to 100 times the weight of its cell body. It is self-evident that cells with this level of synthetic activity may be vulnerable at several levels: the high metabolic rates required to sustain this level of activity necessitate consumption of large amounts of oxygen and ATP. (McTigue and Tripathi, 2008; Mi et al., 2005; Bradl and Lassmann, 2010). Each oligodendrocyte can myelinate up to 40 axons, so the destruction of even only a few oligodendrocytes can have an extensive demyelination effect.

Microglia

Microglia (used as a plural noun meaning microglial cells) are an exception to the general rule that both the neurons and glia of the CNS develop from the neuroectoderm. Microglia were first described by Rio-Hortega in 1920. At that time, the function and ontology of microglia was unknown and the term microglia were purely descriptive based on their size and shape of the cells, and their obviously nonneuronal characteristics.

Microglia are derived from macrophages of mesodermal origin. Microglia are only found in the brain and spinal cord, but betray their origins in that their primary function is protection of the CNS by immune surveillance and phagocytosis. For the majority of the time, microglia are in an inactive state. Activation typically occurs in neuroinflammatory and neurodegenerative disease or injury.

The activated microglia have an amoeboid morphology and revert to type, inasmuch as they behave very much as one might expect given their macrophage lineage, demonstrating antigen-presenting, phagocytic and cytotoxic capabilities. They may also play a role in maintaining the blood–brain barrier (Kofler and Wiley, 2011). The assumed amoeboid morphology is important as it permits the activated microglia to interact with neurons and astrocytes and to migrate to sites of injury. Of particular importance is their role at sites of necrosis to which they migrate, proliferate and ingest debris resulting from the necrotic events.

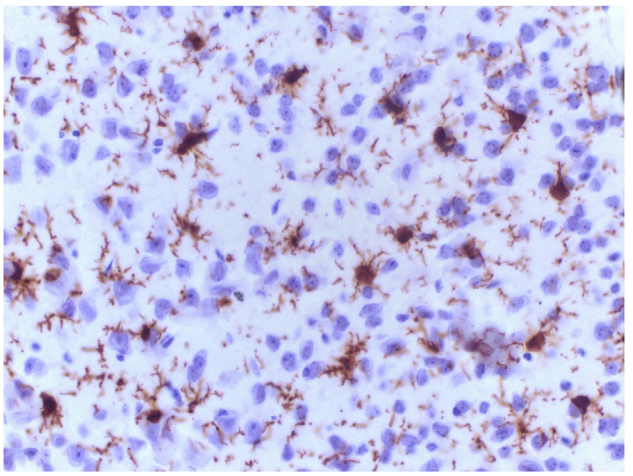


FIGURE 20.10 Microglia stained with insulin binding antibody (IBA-1).

Microglia also have a physiological role in the young animal during the period of neurodevelopment when they remove cells have undergone programmed cell death (Xavier et al., 2014). For the young rat, this will largely be in the first 4–5 weeks of life, so evidence of apoptosis in adult rats can usually be considered likely to be the result of some toxicological insult (Fig. 20.10).

White and Grey Matter

At first sight under the microscope, the brain tissue appears to be a fairly amorphous eosinophilic mass, with only a few readily identified structures, such as the hippocampus and the granular cell layers of the cerebellum. Looking at an H&E section of the relatively amorphous mass that constitutes the majority of the brain, one can differentiate areas that are slightly darker and lighter. The grey matter that constitutes the cerebral cortex is, rather counterintuitively, very slightly paler than the other parts of the brain that are predominantly composed of white matter. The white matter is so called because of the appearance in life, when it has a white sheen to it due to the high level of myelination. Looking at the grey matter under the microscope, it is apparent that it is more cellular than areas that are predominantly white matter. The cellular constituents of the grey matter include astrocytes, microglia and neurone cell bodies. In addition, the white matter contains large numbers of oligodendrocytes.

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Myelin and Myelination in More Detail

As noted above, myelin is white, making parts of the brain filled exclusively with nerve fibres appear as lighter coloured white matter, in contrast to the darker coloured grey matter that marks areas with high densities of neuron cell bodies. Myelin is an electrically insulating phospholipid layer that surrounds the axons of many neurons; it comprises around 80% lipid fat and 20% protein. The predominant function of the myelin layer is to increase in the speed at which impulses propagate along the myelinated fibre and prevent electrical current from leaving the axon. The increase in speed of impulse transmission is a result of saltatory conduction: ion flow across the membrane occurs only at the nodes of Ranvier (Woodbury, 1965). Imperfections in myelination may result in significant functional defects, and demyelinating diseases are responsible for some of the most distressing diseases in man. The best known of these diseases is undoubtedly multiple sclerosis, but there are several other diseases such as acute disseminated encephalomyelitis, neuromyelitisoptica, transverse myelitis, chronic inflammatory demyelinating polyneuropathy and Guillain-Barré syndrome.

The CNS is at its most vulnerable when it is still plastic and only partially protected by a blood–brain barrier that is immature and permeable. For man we are not absolutely certain of the chronology of these milestones, but in the rat we have a sound idea of the critical time points. Although there is some myelination areas of the pons and medulla oblongata between PND 4 and 8, there is little myelin in the midbrain and forebrain in the early days of life, and extensive myelination of the rat brain begins around PND 10.

Myelination starts during the fifth foetal month in man; so at birth, there are several areas that are already myelinated: these include the brainstem, cerebellum and the posterior limb of internal capsule. In all mammals, myelination of the hindbrain occurs first and thereafter the process moves rostrally (Bjelke and Seiger, 1989). Myelination is well regulated but does not seem to be the result of any intrinsic hard wiring, rather the onset of CNS myelination appears determined by the degree of neuronal differentiation, the electrical activity of neurons seemingly the signal for the onset of myelination (Brinkmann et al., 2008; Groves et al., 1993). There is evidence that, to some extent, myelination occurs in response to a functional demand, with the areas related to early nursing, such as suckling motoneurons myelinating first (Yang et al., 1995), followed by other motor and sensory regions, and lastly learning areas. Although evaluation of myelination in purely anatomical terms is certainly valid, we may well be doing the evolutionary process a disservice in that it has probably ensured that these areas are strategically positioned to permit efficient myelination of the juvenile brain. Delayed myelination appears largely recoverable, but there is evidence that the extent of disturbed myelination may be less than that produced by an undisturbed process. Severe damage to oligodendrocytes by toxic substances results in permanent demyelination and debilitating disease.

Artefacts

In closing this chapter, it is worth considering the problem of histological artefacts. Artefacts are seen from time to time in just about all histological sections, but artefacts in sections of the brain, spinal cord and peripheral nerves are particularly troublesome. There are three common artefacts of which the less experienced observer needs to be aware.

The first of these is one that is seen in the brain and is known as the dark neuron artefact. Depending on the quality of fixation, variable numbers of nerve cell bodies with shrunken nuclei and cytoplasm, often with tortuous dendrites radiating out from the neuron, may be seen. Post-mortem ischaemia is doubtless a factor, and in the opinion of the authors, the major factor, as this change is far less commonly seen in tissue that is perfused fixed (Cammermeyer, 1978), although poor handling of the tissues at dissection has also been considered a factor (Garman, 1990). This artefact can be seen almost anywhere in the brain, but the cerebral cortex, hippocampus (areas CA1 and CA3 in particular), cerebellar cortex (Purkinje cells) and large neurons generally appear to be particularly susceptible.

This artefact is important in diagnostic terms, as some consider early stages of ischaemic change to be indistinguishable from the artefactual change. However, the true change tends to be characterised by the presence of neurons in different stages of degeneration rather than by clusters of dark neurons with a similar appearance that typify the artefactual change. More chronic, extensive, lesions tend to be accompanied by glial changes, which are never seen with the artefact.

A second artefact that may be seen in all myelinated tissues is the presence of myelin bubbles. This artefact appears as a space surrounding axons and is most obvious in longitudinal sections of peripheral nerves and spinal cord, especially larger peripheral nerves (Yao et al., 1994). This artefact is most often seen in paraffin-embedded tissue and can be largely avoided by plastic embedding. Although the precise cause of this artefact has not been the subject of much research, the general consensus is that the effect of alcohol in the dehydration process is a primary cause of the problem; sections for plastic embedding are usually dehydrated with acetone. Rough handling of tissue at necropsy may also contribute.

From time to time, this artefact is confused with both axonal degeneration and intramyelinic oedema. It is

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relatively straightforward to differentiate between the artefact and axonal degeneration, as true pathological change includes degeneration of the axon within vacuoles described as digestion chambers. In early intramyelin oedema, it is more difficult to differentiate between artefact and pathologically important changes as the vacuoles tend to be empty. Typically, however, there will be a fairly distinctive topographical distribution of pathological changes that is toxicant specific. In some cases, the investigator will be forced to resort to ultrastructural investigations to distinguish the separation of myelin lamellae that occurs in the genuine lesion.

A third artefact, white matter vacuolation of the brain, is another myelin-related artefact. This is quite variable and can be widespread in some preparations. Vacuoles tend to be well rounded and of variable size; quite naturally they will be confined to areas of myelination. Areas most affected include the corpus callosum, internal capsule, the cerebellar white matter and the brainstem. Again there is sound evidence that this is related to the processing, and most laboratories plan their processing of the brain to ensure that samples are not held in various grades of alcohol for extended period of time (Wells and Wells, 1989). As described above, the genuine lesions tend to occur in specific locations, usually bilaterally, whereas the distribution of the artefactual lesions tends to be fairly random.

BIBLIOGRAPHY

As was mentioned at the start of this long chapter, we have provided an annotated bibliography that contains mainly references to books rather than to the primary literature. Some of the texts mentioned below fall into the category of 'classic accounts'; some are rather old and, in part, dated. Despite this we list them: much information on the anatomy of the rat brain can be found in such accounts.

Books on the Comparative Anatomy of the Vertebrate Central Nervous System

AriënsKappers, C.U., Huber, G.C., Crosby, E.C., 1936. The Comparative Anatomy of the Nervous System of Vertebrates Including Man, 2 volumes. The Macmillan Company, New York (A monumental work, regarded in its day as the last word on the subject. Contains a vast amount of useful information).

Crosby, E.C., Schnitzlein, H.N., 1982. Comparative Correlative Neuroanatomy of the Vertebrate Telencephalon. Macmillan Publishing Co., Inc, New York (Elizabeth Crosby's last book: uniquely valuable).

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Chapter 21

Peripheral Nervous System

Chapter 21.1

Cranial Nerves

MAKING SENSE OF THE CRANIAL NERVES

Various cranial nerves are mentioned in the other chapters of this book; in this chapter, we treat them as a group and try to discover some underlying principles that explain their complexity. The search for such principles has been underway since the late 19th century. Balfour, in 1885, demonstrated that the head of vertebrates was segmented in the same way as the rest of the body. Once this was established it seemed safe to assume that the cranial nerves should follow a pattern similar to that of the spinal nerves, with each cranial nerve associated with a particular segment. The segments were named from premandibular at the front of the skull, to the obvious occipital segments at the back. Thinking along these lines can be traced through the works of Goodrich (1930), De Beer (1951) and Young (1962), and represents an important aspect of classical comparative anatomy.

This linking of cranial nerves with segments might be regarded as the first of four lines of attack on the problem of making sense of the cranial nerves, although recent work has shown that segmentation of the head is more complex than first thought.

A second line of attack involves examination of the cranial nerves in terms of whether they represent dorsal or ventral roots of segmental nerves. Each spinal nerve has a dorsal and a ventral root which join just as the nerve leaves the vertebral column. The dorsal root is distinguished by the presence of a dorsal root ganglion where the cell bodies of the afferent neurones reside. These neurones carry sensory information from the periphery to the spinal cord. The ventral roots carry the motor fibres that travel in the opposite direction.

Casual examination of the cranial nerves shows some are entirely motor, for example, the VIth cranial nerve (the abducent nerve): some are almost entirely sensory, for example the VIIIth cranial nerve (vestibulo-auditory); and some are decidedly mixed, for example the Vth or trigeminal nerve. In primitive chordates, such as amphioxus and the lampreys, the dorsal and ventral roots of the spinal nerves do not combine to form the spinal nerves but remain quite separate. Members of the English school of comparative anatomy suggested that this was the case regarding the cranial nerves, some representing dorsal roots, and others ventral roots (Goodrich, 1930; De Beer, 1951; Young, 1962).

A third approach relates to the neurophysiological or functional components of the cranial nerves. It was appreciated that neurons of the ventral horn of the spinal cord were grouped and formed columns running the length of the cord. It was also appreciated that these columns could be categorised by the tissues supplied by the fibres arising from the neurons of the columns. The general somatic efferent (GSE) column is found in the ventral horn of the cord, and contains the cell bodies of neurones sending fibres to skeletal muscle. The general visceral efferent (GVE) column is found towards the ventral side of the lateral horn in the thoracic region of the cord, and from here efferent fibres of the sympathetic nervous system take their origin. Afferent systems are treated in the same way, and both general somatic afferent (GSA) and general visceral afferent (GVA) columns have been described: the input handled by these columns will be described shortly.

Although this approach worked well for the spinal cord, when the brainstem was considered, it soon became clear that other columns would need to be defined. These were the special visceral efferent (SVE) column that contained the cell bodies of neurones supplying skeletal muscle associated with the pharyngeal or branchial arches, and the special visceral afferent (SVA) column handling the incoming information from the taste buds. Some confusion was created by writers using the term special somatic efferent (SSE) to describe the column containing the cell bodies of neurones supplying skeletal muscle associated with the branchial arches. This confusion has largely disappeared, though some authors prefer the term ‘branchiomotor’ and do not use the term SVE at all. The term special somatic afferent (SSA) has been applied to the column handling input from the ear (vestibular and

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auditory). Thus, three columns on the efferent side: GSE, GVE and SVE and four on the afferent side: GSA, GVA, SVA and SSA are now defined: seven in all.

All that remains are the nerves supplying the extraocular muscles, which have nothing to do with the branchial arches. It was agreed that GSE was the correct term for the column that contained the cell bodies of the neurones that supplied these muscles. The neurophysiological components are set out in Table 21.1.1.

Once all this had been accomplished, and the neurophysiological components of the cranial nerves defined, two-way tables could be produced. Some took the cranial nerves and defined their components [e.g. Vagus nerve: GVE (e.g. parasympathetic supply to the heart), SVE (motor supply to muscles of the larynx), GSA (a minor component as regards input from skin, some fibres come in from the skin of the external auditory meatus, but an important input from mucous membrane of the larynx), GVA (e.g. from the airways, gut and the chemoreceptors in the aortic bodies) and SVA (input from taste buds of base of tongue)]. The vagus nerve has nothing to do with GSE and SSA functions.

Others took a component and defined the cranial nerves that carried this component. In the case of the GVE, this would include the oculomotor nerve (carrying parasympathetic fibres that are on their rather complicated way to the ciliary ganglion), facial nerve (carrying parasympathetic fibres on their way to the sphenopalatine ganglion via the greater superficial petrosal nerve and to the submandibular ganglion via the chorda tympani), glossopharyngeal nerve (carrying parasympathetic fibres on their way to the otic ganglion via the lesser superficial petrosal nerve) and the vagus nerve (carrying parasympathetic fibres to the heart, lung and gut).

There is yet another approach that considers which cranial nerves are associated with each branchial arch. Elsewhere in this book, we describe the trigeminal nerve as the nerve of the first arch, and the facial nerve as the nerve of the second arch. From studies of comparative anatomy, we know that a nerve running to a given branchial arch usually sent a branch round the gill slit in front of that arch. This led to the distinction of post-trematic (behind the hole, i.e. the gill slit) and pretrematic (in front of the hole) branches. Further study showed, for example in cartilaginous fish, that each nerve belonging to a given branchial arch has four branches: a dorsal sensory branch to the skin (GSA), a pharyngeal branch (GVA and SVA), a pretrematic branch (GVA and SVA) and a post-trematic branch (GVE, SVE, GVA and SVA). The challenge to comparative anatomists was to take each cranial nerve in a range of animals and define, in terms of the foregoing classification, each of the existing branches of that nerve. The problems associated with answering questions such as, 'where is the pre-trematic branch of the facial nerve?' resulted in the blunting of enthusiasm, especially when it was discovered that some branches simply did not exist in some species and that any given branch did not always contain all the components defined earlier.

An example illustrates the problem. The chorda tympani is a branch of the facial nerve, the facial nerve is the nerve of the second arch. The chorda tympani carries taste fibres from the front part of tongue, a first arch derivative, therefore the chorda tympani is the pretrematic branch of the facial nerve. But if that is so, what are the GVE

TABLE 21.1.1 Functional Components of the Cranial Nerves

Component	Abbreviation	Function
General somatic afferent	GSA	Sensation from skin and mucous membrane: for example touch, pain, temperature
Special somatic afferent	SSA	Input from vestibular and auditory apparatus: from the ear
General Visceral Afferent	GVA	Input, for example, from gut, from carotid and aortic bodies and carotid sinus
Special visceral afferent	SVA	Input from taste receptors
General somatic efferent	GSE	Motor supply to skeletal muscle derived from myotomes
General visceral efferent	GVE	All the efferent parasympathetic system running in cranial nerves: to the intrinsic muscles of the eye, the salivary glands and all the organs supplied by the vagus nerve
Special visceral efferent	SVE	Motor supply to skeletal muscle derived from tissue associated with the pharyngeal arches: muscles of mastication (1st arch), facial muscles (2nd arch), muscles of pharynx and larynx

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efferent fibres that run to the submandibular ganglion doing in the chorda tympani? Perhaps we are wrong. Marshall (1962) wrote:

> *The chorda tympani branch of mammals may represent the pretrematic branch (of the facial nerve), but it is more probably representative of the internal mandibular nerve of fishes which is post-trematic in position. It supplies the mucous membrane of the tongue with taste or special visceral sensory fibres and in tetrapods, visceral efferent fibres to the salivary glands. The true pretrematic branch is probably the prespiracular nerve of fishes and is reduced or absent in tetrapods.*

He continued:

> *The pharyngeal branch is represented by the palatine nerve of fishes, which runs medially above the palato-quadrate bar; it is the greater superficial petrosal nerve of mammalian anatomy, and contains visceral afferent and efferent fibres.*

The parasympathetic supply emanating from the brainstem presents certain problems for those who seek to establish some underlying principles for the organisation of the cranial nerves. One might think that GVE fibres would be found in nerves that correspond with ventral roots such as the oculomotor nerve, but in fact GVE fibres are also found in the facial, glossopharyngeal and vagus nerves, and these correspond with dorsal roots. Romer (1970) took the view that in the most primitive condition the efferent autonomic fibres left via the dorsal root, in later forms by both the dorsal and ventral root, and in mammals, via the ventral root. Here again, the cranial nerves of mammals seem to reflect an earlier state of organisation than the spinal nerves.

Opinions vary as to whether comparative anatomy is of any real help in unravelling the complexity of the cranial nerves. Comparative anatomists with an interest in the cranial nerves of all chordates are probably more enthusiastic than mammalian or human anatomists, who might take some convincing.

Thus far the olfactory nerve and the optic nerve have not been considered. The optic nerve is a special case as it is not actually a nerve in the usual sense of the word, but a tract of the central nervous system (CNS) that has taken up an unusual position, and thus it is not easily classified according to the criteria outlined earlier. The olfactory nerve is a true nerve and might be classified as containing SVA fibres: after all the sense of smell is as 'special' as the sense of taste.

TABLE 21.1.2 Analysis of Cranial Nerves by Neurophysiological Components of Their Fibres

Number	Name	GSA	SSA	GVA	SVA	SVE	GVE	GSE
0	Terminalis	(X)						
I	Olfactory		(X)		(X)			
II	Optic		(X)					
III	Oculomotor						X	X
IV	Trochlear							X
V(1)	Trigeminal (Nasociliary branch of ophthalmic division of trigeminal[a])	X						
V(1, 2 and 3)	Trigeminal[b] (Ophthalmic division minus nasociliary Maxillary division Mandibular division)	X				X		
VI	Abducent							X
VII	Facial	X		X	X	X	X	
VIII	Acoustic/vestibular		X					
IX	Glossopharyngeal	X		X	X	X	X	
X	Vagus	X		X	X	X	X	
XI	Accessory	X		X	X	X	X	
XII	Hypoglossal							X

Brackets (X) indicate arbitrary or debatable allocations.
[a]*This represents the profundus branch of the ophthalmic division of the trigeminal nerve in cartilaginous fish. It represents the dorsal root corresponding to the oculomotor nerve (ventral root): see Table 21.1.3. In mammals, it is represented by the nasociliary branch of the ophthalmic division of the trigeminal nerve.*
[b]*This represents the remainder of the trigeminal nerve.*

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There are two further nerves to consider, the terminal nerve has no known function, and runs alongside the olfactory nerve, but seems to have nothing to do with the sense of smell, (Vilensky, 2014; Winkelmann, 2014), and the nerve from the vomeronasal organ (Jacobson's organ, not to be confused with 'his nerve', which is the tympanic branch of the glossopharyngeal nerve) and is more important in rats than in man, sending fibres to the accessory olfactory bulb (see Chapter 10: Nasal Cavity).

The cranial nerves listed in Table 21.1.2 a analysed by components. Shaw has provided a most interesting account of the history of the numbering of the cranial nerves (Shaw, 1992).

The table can be read from left to right in terms of the components of the cranial nerves, or from top to bottom in term of the components and the nerves in which they feature.

The cranial nerves are analysed by segments and arches in Table 21.1.3.

GANGLIA OF THE CRANIAL NERVES

Two types of ganglion are associated with cranial nerves:

1. The equivalents of the dorsal root ganglia of the spinal nerves are found along the path of those cranial nerves that convey afferent fibres to the CNS. In the spinal

TABLE 21.1.3 Analysis of Cranial Nerves by Classical Segments, Somitomeres and Visceral Arches

Classical Segment	Somitomeres	Rhombomeres	Visceral Arch[a]	Gill Arch[b]	Visceral Slit	Gill Slit	Ventral Root	Dorsal Root
1 Premandibular	4	1					III	V(1) profundus (nasociliary)
2 Mandibular	5	2, 3	1				IV	Remainder of V
					1			
3 Hyoid	6	4, 5	2				VI	VII, VIII
					2	1		
4	7	6, 7	3	1			–	IX
					3	2		
5	?	8	4	2			–	X
					4	3		
6	Occipital somites 1	8	5	3			XII	X
					5	4		
7	2	8	6	4			XII	X, XI
					6	5		
8	3	8	7	5			XII	X, XI
9	4							

Note: The terms visceral, pharyngeal and branchial are used to describe the arches with which we are concerned here. Mammalian and human anatomists use the terms pharyngeal and branchial interchangeably in this context. Comparative anatomists reserve the term branchial for the gill arches of fish. The terminology and numbering of segments, arches and slits almost inevitably leads to confusion or, at least, to mental indigestion. The classical numbering of segments seems unhelpful today when we have the numbering of somitomeres and rhombomeres. The reader is referred to Larsen (1993) for details of the somitomeres of the developing head and the rhombomeres of the developing brainstem.

[a]*Visceral arches: often referred to by human anatomists as branchial or pharyngeal arches.*
[b]*Gill arches: referred to by comparative anatomists as branchial arches.*

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TABLE 21.1.4 Analysis of Ganglia by Type (See Above), the Cranial Nerves and Their Origins

Cranial Nerve	Ganglion and Type	Alternative Anatomical Names for Ganglia	Origin of Neurones
I Olfactory	Primary neurones of the olfactory system located in the olfactory epithelium		Nasal placode
III Oculomotor	Ciliary ganglion GVE (Type 2)		Neural crest
V Trigeminal	Trigeminal ganglion GSA (Type 1)	Semilunar ganglion	Neural crest and trigeminal placode
VII Facial	Superior geniculate ganglion (Type 1)[a] GSA,	Geniculate ganglion not divided into superior and inferior parts	Neural crest and 1st epibranchial placode
	Inferior geniculate ganglion (Type 1) GVA, SVA (taste)		1st epibranchial placode
	Sphenopalatine ganglion (Type 2) GVE and SVE		Neural crest
	Submandibular ganglion (Type 2) GVE and SVE		Neural crest
VIII Vestibulo-cochlear (auditory or vestibulo-acoustic)	Acoustic ganglion (Type 1): SSA		Otic placode
	Vestibular ganglion (Type 1): SSA		Otic placode and neural crest
IX Glossopharyngeal	Superior ganglion (Type 1) GSA	Petrosal ganglion	Neural crest
	Inferior ganglion (Type 1) GVA, SVA (taste)		2nd epibranchial placode
	Otic ganglion (Type 2) GVE and SVE		Neural crest
X Vagus	Superior ganglion (Type 1) GSA	Jugular ganglion	Neural crest
	Inferior ganglion (Type 1) GVA, SVA (taste)	Nodose ganglion	3rd and 4th epibranchial placodes
	Vagal parasympathetic ganglia (Type 2) found in target organs: respiratory system, heart and gut		Neural crest

Table contains information from Larsen, W.J., 1993. Human Embryology. Churchill Livingstone, London.
[a]*So named because it occurs at a right angle or knee shaped bend of the facial nerve and genu is Latin for knee.*

nerves, the cell bodies of the neurones serving the GSA and GVA systems are found in the single ganglion of each dorsal root. This is not the case for the cranial nerves. For example, the vagus nerve has a small superior ganglion (the jugular ganglion) serving the GSA system, and a larger inferior ganglion (the nodose ganglion) serving the GVA and SVA (taste) systems. Where there are two ganglia, the one serving the GSA system always lies above the one serving the GVA and SVA (taste) systems (Brodal, 1959).

2. Ganglia of the parasympathetic nervous system. The ciliary, sphenopalatine, submandibular and otic ganglia contain the handover from preganglionic to postganglionic parasympathetic nerve fibres. The cell bodies of the preganglionic fibres are in the CNS, the cell bodies of the postganglionic fibres are in the ganglia. These ganglia are rather odd in that (1) each is associated with a branch of the trigeminal nerve (CN V), (2) none of the preganglionic fibres leave the brain in the trigeminal nerve but (3) all postganglionic fibres travel in branches of the trigeminal nerve. No sympathetic ganglia are found in the head.

The ganglia of the cranial nerves develop from neural crest tissue and from the ectodermal and epibranchial placodes. There are three ectodermal placodes: the nasal, lens and otic placodes; and four epibranchial placodes, which are associated with pharyngeal clefts and a trigeminal placode that Larsen (1993) recorded as developing between the epibranchial placodes and the otic placode.

The details of the ganglia of the cranial nerves are set out in Table 21.1.4.

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The ganglion cells of the optic system are found in the retina.

THE PARASYMPATHETIC GANGLIA OF THE HEAD AND THEIR ROOTS

Confusion often results from the description of the roots of the parasympathetic ganglia of the head. For example, the ciliary ganglion is described as having a sensory root, a sympathetic root and a parasympathetic root. Here the word 'root' is used in a very different sense from when describing dorsal and ventral roots of spinal nerves.

CILIARY GANGLION

This small ganglion is found behind the eyeball attached to the nasociliary nerve, a branch of the ophthalmic division of the trigeminal nerve. This attachment is described as the sensory root of the ganglion, because afferent fibres passing through the ganglion (but not synapsing in the ganglion) run to the ganglion via the short ciliary nerves, and out of the ganglion with the nasociliary nerve on their way to the trigeminal ganglion. The parasympathetic root is the connection between the ganglion and the branch of the oculomotor nerve that supplies the inferior rectus muscle of the eyeball. Efferent parasympathetic fibres that begin in the Edinger–Westphal nucleus of the midbrain travel in the oculomotor nerve and leave it for the ciliary ganglion, where they synapse. Postganglionic fibres leave the ganglion in the short ciliary nerves and run to the eye, where they supply the muscles of accommodation and the constrictor pupillae.

The sympathetic supply to the head travels, as postganglionic fibres (the ganglion is the superior cervical ganglion), in association with arteries and their plexuses. Sympathetic fibres can leave 'their' periarterial plexuses to form a nerve of their own. Sympathetic fibres travelling with the ophthalmic artery leave it for the nasociliary nerve, before passing on to the ciliary ganglion, in the sensory root of that ganglion (the sensory root is not entirely sensory), and onwards via the short ciliary nerves. They run in these nerves to the eyeball and supply its blood vessels. Sympathetic fibres to the dilator papillae run in the long ciliary nerves that leave the nasociliary nerve, but have nothing to do with the ciliary ganglion. The long ciliary nerves run to the eyeball and supply the dilator papillae muscle with postganglionic sympathetic fibres; and the iris, ciliary body and cornea with sensory fibres (GSA). The sympathetic supply to the levator palpebrae muscle hitchhikes from the plexus around the ophthalmic artery to the branch of the oculomotor nerve that supplies the muscle.

SPHENOPALATINE GANGLION

The sphenopalatine ganglion lies in the pterygopalatine fossa, which in the in the rat is only a shallow groove towards the lower, posterior end of the orbito-temporal fossa (see Chapter 5: The Skull). The ganglion is attached to the maxillary branch of the trigeminal nerve which reaches the fossa via the foramen orbito-rotundum (a combination of the superior orbital fissure and the foramen rotundum of man). The sensory root of the ganglion is provided by the maxillary nerve. Fibres of the parasympathetic root takes a rather tortuous course, with preganglionic fibres travelling in the facial nerve, before leaving close to the geniculate ganglion to form the greater superficial petrosal nerve, which climbs out of the middle ear to enter the cranial cavity. It continues, having met and combined with the deep petrosal nerve (postganglionic sympathetic), to form the nerve of the pterygoid canal, passing through the pterygoid canal (as the Vidian nerve), into the sphenoid bone and thence to the sphenopalatine fossa. The sympathetic root and the parasympathetic root of the ganglion thus arrive as one nerve. There are many distal connections of the ganglion. GSA, parasympathetic and sympathetic fibres (GVE) innervating the small salivary glands of the palate are carried in all the nerves leaving the ganglion, including the sphenopalatine and palatine nerves and the nerves to the teeth of the upper jaw. GSA fibres to the skin run in the infraorbital nerve, which emerges on the face. GVE fibres reach the lacrimal gland (at least in man) via series of 'jumps' from the zygomatic branch of the maxillary nerve to the lacrimal branch of the ophthalmic nerve.

OTIC GANGLION

This ganglion, tiny in the rat, is found attached to the mandibular branch of the trigeminal nerve just below the foramen ovale by which the branch, or division, leaves the cranial cavity. Again the parasympathetic supply to the ganglion follows a tortuous path along the lesser superficial petrosal nerve, a branch of the glossopharyngeal nerve. In man, this nerve arises in the middle ear from the tympanic branch (Jacobson's nerve) of the glossopharyngeal nerve. Postganglionic parasympathetic fibres leave the ganglion to join the auriculotemporal nerve, which innervates the parotid gland. The tympanic branch of the CN IX may not exist in the rat: for further information on the otic ganglion of the rat see Chapter 21 (3), The Autonomic Nervous System.

SUBMANDIBULAR GANGLION

Another ganglion attached to a branch of the trigeminal nerve, in this case the lingual branch of the mandibular nerve. Parasympathetic fibres run in the lingual nerve

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TABLE 21.1.5 Analysis of Cranial Nerves by Brain Stem Nuclei and Neurophysiological Components and These Are Summarised in the Following Table

Components	Supplying or Receiving Input From (Examples)	Midbrain	Pons	Medulla	Cranial Nerves
Efferent					
GSE	Motor supply to skeletal muscle derived from myotome: extra-ocular muscles and muscles of tongue	Motor nuclei of oculomotor and trochlear nerves	Motor nucleus of abducent nerve	Hypoglossal nucleus	III IV VI XII
SVE (Branchiomotor)	Motor supply to skeletal muscle derived from tissue associated with pharyngeal arches		Motor nucleus of trigeminal nerve Motor nucleus of facial nerve	Nucleus ambiguus[a]	V VII IX and X
GVE	Parasympathetic supply to intrinsic muscles of the eye, salivary glands, heart and smooth muscle of gut	Edinger–Westphal nucleus	Superior salivatory nucleus Inferior salivatory nucleus	Dorsal motor nuclei of glossopharyngeal and vagus	III VII IX IX, X
Afferent					
GVA	Visceral pain, distension, input from carotid and aortic bodies and carotid sinus			Dorsal sensory nuclei of IX and X	IX, X
SVA	Taste			Nucleus of tractus solitarius	VII, IX and X
GSA	Proprioception Other sensation, for example pain and touch	Mesencephalic nucleus of trigeminal nerve	Main sensory nucleus of trigeminal[b]		V V (Fibres travelling in VII, IX and X also carry GSA input to the trigeminal nucleus)
SSA	Vestibular input Auditory input		Vestibular nucleus Cochlear nucleus		VIII VIII

[a] *Note spelling Nucleus ambiguus not ambiguous.*
[b] *Note that CN VII, IX and X carry input to this nucleus.*

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having joined this nerve when it was joined by the chorda tympani, a branch of the facial nerve. The chorda tympani passes through the middle ear, across the upper part of the tympanic membrane, and leaves via the petro-tympanic fissure (the 'busy fissure' described in Chapter 23: The Ear). Postganglionic fibres from the submandibular ganglion rejoin the lingual nerve to innervate the submandibular gland and the tongue. Some preganglionic parasympathetic fibres do not synapse in the ganglion, but run to the sublingual gland where they find the cell bodies of their postganglionic partners.

All the ganglia are associated with branches of the trigeminal nerve and get their preganglionic parasympathetic fibres from the oculomotor, facial and glossopharyngeal nerves. All postganglionic sympathetic fibres reaching the ganglia do so from arterial plexuses, and leave the ganglia via branches of the trigeminal nerve. The only synapses found in the ganglia are part of the parasympathetic system.

ODDITIES OF THE GENERAL SOMATIC AFFERENT SUPPLY TO THE HEAD

The facial, glossopharyngeal and vagus nerves all contain GSA fibres from the skin and mucous membrane. All the afferent fibres running in to the CNS via these nerves make their way to the sensory nucleus of the trigeminal nerve.

The cranial nerve nuclei of the brainstem are analysed in Table 21.1.5.

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Chapter 21.2

The Peripheral Nervous System

THE SPINAL NERVES

Introduction

As in the chapter of the cranial nerves, we have not sought to follow each nerve, providing, rather, an overview of the system. For the reader needing details of the individual nerves, we would suggest that they consult Greene (1963): an admirably detailed and illustrated account. One of the drawbacks of a nerve by nerve account is that the reader, absorbed in the examination of the 'trees' (nerves), may lose sight of the 'wood' (spinal nerves as a whole). Once the system is understood, the details of the individual nerves can be added later if necessary.

In the rat, the spinal cord runs in the vertebral canal from the foramen magnum of the skull to the level of the fourth lumbar vertebra (L4), but in man, reaches only as far as the junction between the first and second lumbar vertebrae (L1 and L2). The spinal cord is characterised by cervical and lumbar expansions: the former at the level of the posterior cervical vertebrae, and the latter at that of the posterior thoracic vertebrae. The expansions accommodate the increased inflow and outflow of nerve fibres from and to the limbs.

The posterior end of the cord tapers to the conus medullaris. The vertebral canal posterior to the origin of the conus contains the roots of the spinal nerves that leave the canal through intervertebral or sacral foramina posterior to the fourth or fifth lumbar vertebrae. This bundle of nerve roots is called the cauda equina, supposedly because it looks like a horse's tail. Given that the spinal cord is shorter than the vertebral canal, it follows that the segmental levels of the cord do not match the vertebrae. In the cervical region, there is a good match, but the lumbar segments of the cord lie adjacent to the lower thoracic vertebrae, and all the sacral segments lie opposite the first four lumbar vertebrae.

The vertebral canal is lined by dura mater that is continuous with the inner layer of the intra-cranial dura. The outer layer of the intra-cranial dura forms the periosteum of the inner surface of the bones of the skull and is continuous with the periosteum of the outer surfaces of these bones. The cord itself is closely covered by pia, separated from the arachnoid mater by the subarachnoid space that contains cerebrospinal fluid.

There are 34 pairs of spinal nerves: 8 cervical, 13 thoracic, 6 lumbar, 4 sacral and 3 caudal or coccygeal nerves. The first cervical nerve emerges anterior to the

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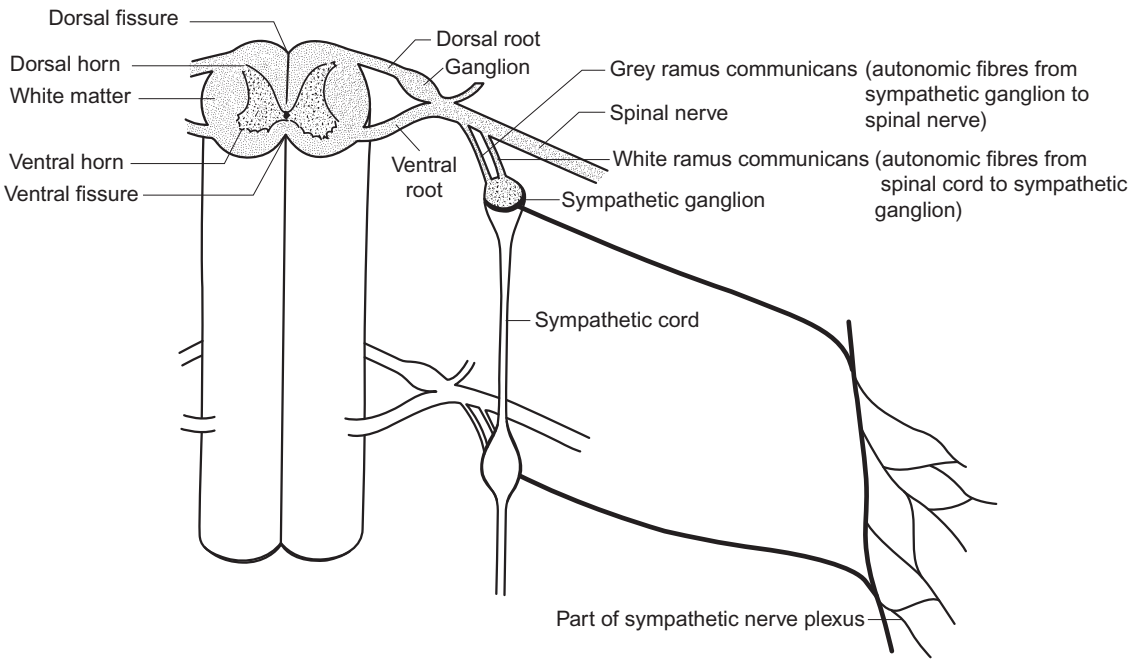


FIGURE 21.2.1 Part of spinal cord and sympathetic cord with ganglia and their connections (highly diagrammatic).

first cervical vertebra. Each subsequent cervical nerve emerges anterior to the corresponding vertebra, with the exception of the eighth, which emerges between C7 and T1. The first thoracic nerve emerges between T1 and T2. All subsequent spinal nerves emerge posterior to the corresponding vertebra.

Each spinal nerve is attached to the cord by an afferent dorsal root and an efferent ventral root: see Fig. 21.2.1.

In the rat, all the dorsal roots bear ganglia, but in man, the dorsal root ganglion of the first cervical nerve may be missing. Each dorsal root ganglion is found close to, or in, the intervertebral foramen whence the corresponding spinal nerve leaves the vertebral canal. As a result, the long roots of the posterior spinal nerves are found in the posterior part of the vertebral canal.

Each spinal nerve is formed by fusion of its dorsal and ventral roots, and soon divides to produce an anterior primary ramus and a posterior primary ramus. The posterior primary rami are the source of the nerve supply to the skin of the dorsal aspect of the head and body, and the longitudinal muscles that run along the dorsum of the body and forwards to the head. The anterior primary rami are more complicated and interesting. Although the terms anterior and posterior are more appropriate to human than to rat anatomy, we have retained them to prevent confusion with the dorsal and ventral roots of the spinal nerves.

The anterior primary ramus of each thoracic spinal nerve is attached by two small nerves to the sympathetic chain that lies adjacent to the ventral surface of the vertebral column; see Fig. 21.2.1. These connections are the white and grey rami communicantes. The white rami carry preganglionic sympathetic fibres from cell bodies in the lateral horn of the spinal cord to the ganglia of the sympathetic chain. The grey rami carry postganglionic fibres back to the anterior primary rami of the spinal nerves. The white rami are myelinated, the grey rami are not (or hardly so): this explains their names: myelin is whitish in colour. Only the anterior primary rami of the thoracic spinal nerves (and the first lumbar) give off white rami communicantes, whereas every anterior primary ramus receives a grey ramus. Thus, the anterior primary rami of the cervical nerves receive grey rami communicates from the sympathetic ganglia of the neck. See Chapter 21(3), The Autonomic Nervous System, for further details.

The anterior primary rami are generally larger than the posterior primary rami, and all bar those from the thoracic segments of the cord form plexuses. Typically cervical, brachial and lumbo-sacral plexuses are recognised, but some authors define a lumbar plexus and a sacral plexus. The anterior primary rami of the thoracic spinal nerves form the intercostal nerves that run around the chest wall between the ribs. The more posterior intercostal nerves continue and supply the muscles and skin of the ventral abdominal walls.

Generally, the posterior primary rami do not form plexuses, but a posterior cervical plexus has been described in man (Greene, 1963).

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The Cervical Nerves and the Cervical Plexus

The cervical plexus comprises a series of loops, or anastomoses, between the anterior primary rami of the cervical nerves that link with the hypoglossal nerve (CN XII). Branches arising from the plexus supply muscles, including sternomastoid, cleidomastoid, clzvotrapezius levator scapulae, infra-hyoid and the scalene muscles; as well as carrying sensory fibres that run to the skin.

THE BRACHIAL PLEXUS

'Brachial' refers to the arm or forelimb, and the brachial plexus gives rise to the nerves that supply the muscles and associated structures (joints, ligaments, etc.), and skin of the forelimb. It also innervates the muscles around the shoulder. The brachial plexus is complicated. In human anatomy, a special terminology of roots, trunks, cords and branches of the brachial plexus has developed, but even with the aid of a well-established nomenclature it is not easy to commit to memory. In the rat, the plexus is even more complicated and can not easily be described in the terminology of human anatomy. Given this book is about the anatomy of the rat, it might seem odd to provide a description of the human brachial plexus before describing that of the rat, but we think that starting with the human brachial plexus helps in understanding the rat plexus.

Perhaps the best classical description of the comparative anatomy of the mammalian brachial plexus has been provided by Paterson in 1887 (this account is accessible in the archive of the *Journal of Anatomy*; in 1887 it was the *Journal of Anatomy and Physiology*). This is referred to by Greene (1963) who provided a short description of the plexus, and a splendidly complicated illustration with detailed descriptions of the nerves arising from the plexus. Hebel and Stromberg (1976) describe the rat brachial plexus only very briefly, but Martinez-Periera and Zancan (2015) have provided a concise description and comparison of the brachial plexus of the rat with that of

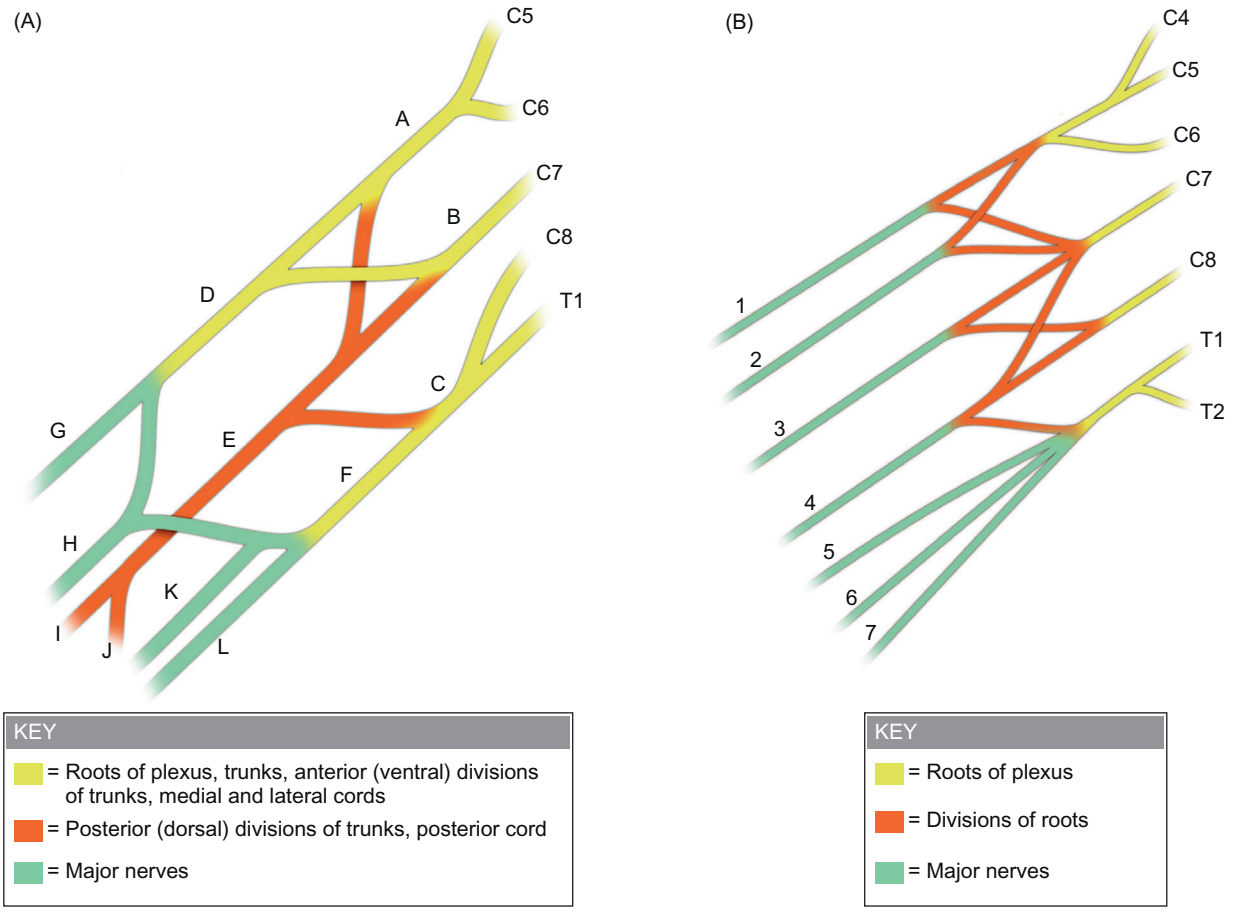


FIGURE 21.2.2 Classification of nerve fibres. A—A, B, C: upper, middle and lower trunks. D, E, F: lateral, posterior and medial cords. G: musculocutaneous nerve, H: median nerve, I: radial nerve, J: axillary nerve, K: medial cutaneous nerve of forearm, L: ulnar nerve. Labelling to appear beneath B—1: axillary nerve, 2: musculocutaneous nerve, 3: radial nerve, 4: median nerve, 5: ulnar nerve, 6: medial brachial cutaneous nerve (medial cutaneous nerve of the arm in man), 7: medial antebrachial cutaneous nerve (medial cutaneous nerve of the forearm in man).

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man and other mammals. The similarity of the organisation of the brachial plexuses in rats and humans led them to choosing rats for experimental surgery and microsurgery studies.

The Human Brachial Plexus

The human brachial plexus is shown, diagrammatically, in Fig. 21.2.2(A).

The reader will see that it is complicated. We shall begin by defining a few 'rules':

1. The brachial plexus arises from a series of roots, which should not be confused with the dorsal and ventral roots of spinal nerves. Each root is the anterior primary ramus of a spinal nerve. The anterior primary rami give off branches before they settle down, so to speak, as the roots of the plexus. The roots of the brachial plexus come from spinal nerves: C5, C6, C7, C8 and T1 (variations will not be discussed here).
2. There are three trunks, the roots from C5 and C6 combine to form the upper trunk, the root from C7 forms the middle trunk, and the roots from C8 and T1 combine to form the lower trunk.
3. Each trunk divides and gives rise to an anterior and a posterior division (sometimes called ventral and dorsal divisions).
4. There are also three cords, the three posterior divisions combine to form the posterior cord, while the anterior divisions from the upper two trunks combine to form the lateral cord, and the anterior division of the lower trunk forms the medial cord.
5. Branches of the medial and lateral cords join to form the median nerve. The radial and axillary nerves spring from the posterior cord, the ulnar nerve from the medial cord.
6. A number of other nerves also arise from the plexus.

One is left wondering how such complexity has arisen. The answer is that the muscles of the limbs have, at least in part, migrated from the body wall to the limbs during the evolution of land dwelling creatures, but are not built up from muscle from single segments, but from contributions from several segments of the body. As a result, the nerves to individual muscles contain fibres from more than one segmental level of the spinal cord. To further complicate matters flexors draw their nerve supply from the anterior or ventral divisions of the anterior primary rami, while extensors draw theirs from the posterior or dorsal divisions. These complex requirements have been met by the development of plexuses that allow the nerve fibres from various segmental levels to be combined appropriately. There seems to be no obvious explanation as to why the evolution of the rat has led to one solution to the problem and that of man to another, suffice to say that both solutions work, as do the many other solutions that have evolved in other groups.

Before moving on to the rat, a few examples of the segmental innervation of human arm muscles are given below:

Biceps: C5, C6
Coracobrachialis: C5, C6, C7
Triceps: C6, C7, C8
Flexor pollicis longus: C6, C7, C8
The interossi of the hand: C8, T1.

The Brachial Plexus of the Rat

The brachial plexus of the rat as described in detail by Angélica-Almeida et al. (2013) differs from the description provided by Özbag et al. (2009). The latter authors defined three trunks (cf human brachial plexus); the former did not mention trunks. Both groups, and earlier writers (Greene, 1963, following Paterson, 1887), noted that it was not possible to define the cords described by human anatomists. Fig. 21.2.2(B) is adapted from Angélica-Almeida et al. (2013). What is immediately obvious is that there is an extensive exchange of nerve fibres, and that the major nerves draw on more than one spinal segment. Many smaller nerves have been omitted from both diagrams. Note that the diagrams are stylised representations, more like the diagrams of Underground Railway systems than proper maps. Angélica-Almeida et al. (2013) include photographs of their dissections in their paper, which may be examined for further details of anatomical relationships.

The contributions made by spinal segments to four major nerves are shown in the following table.

Nerve	Man	Rat
Median	C6–T1	C7–T1
Ulnar	C8–T1	C7–T1
Radial	C5–T1	C5–T1 (also given as C6–C8)
Musculocutaneous	C5–C7	C5–C7

It is clear from the table that the major nerves of the forelimb of man and the rat arise from similar spinal segments.

Nerves of the Forelimb

Although Greene (1963) provided the only detailed and well-illustrated account of the nerves of the rat, it is less detailed than that found in the larger textbooks of human and veterinary anatomy. This is not a synopsis of

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Greene's work, but only short notes on a few of the more important nerves and some general principles. Readers involved in experimental surgery of the nerves of the arm or brachial plexus should consult Greene's book.

The muscles of the forelimb can be divided into those of the extensor compartments and flexor compartments of the first, second and third segments of the limb. The extensor muscles of the first and second segments, that is the arm and forearm in man, and brachium and antebrachium in the rat, are supplied by the radial nerve, which enters the limb from the axilla with the axillary artery and the median and ulnar nerves. The radial nerve spirals around the humerus to reach the extensor compartment where it supplies triceps and brachioradialis, before emerging on the radial side of the flexor aspect of the upper end of the second segment. From here, a large branch spirals around the radius before heading for the extensor muscles of wrist and hand (paw). Smaller branches innervate the skin over the extensor surface of the second segment and paw. In the rat, but not in man, the radial nerve innervates the short head of biceps in the first segment of the limb, and sends a branch to coracobrachialis (Greene 1963).

The ulnar nerve, initially close to the median nerve on the inner side of the first segment, passes back into the limb and down to enter the second segment from behind the medial epicondyle of the humerus. In man, it curls round the epicondyle and can be knocked by accident causing sudden pain, the origin of the 'funny bone'. It gives off no branches in the first segment of the limb, but in the second segment, innervates flexor muscles on the ulnar side of the limb, before entering the paw and innervating the small muscles, with the exception of those associated with the first digit (thumb or Pollux).

The median nerve stays close to the brachial artery, entering the second segment in front of the elbow joint to innervate the flexor muscles of the forearm not innervated by the ulnar nerve, and the muscles of the first digit.

These nerves also innervate the skin, augmented in the forelimb by two long nerves arising from the brachial plexus: the cutaneous nerves of the arm and forearm in man and of the brachium and antebrachium in the rat.

Innervation of the muscles of the flexor compartment of the first segment of the limb is from a dedicated nerve, the musculocutaneous nerve, which supplies biceps, brachialis and coracobrachialis and the overlying skin. These three muscles lead to the nerve being called the BBC nerve by medical students.

The skin of the limbs is supplied, of course, by spinal nerves, we have mentioned some above. Areas supplied by specified spinal levels (or segments) are called dermatomes. No dermatome is supplied from only one spinal level, and the overlap that occurs explains why cutting a single dorsal root does not lead to complete denervation of any one area of skin. The dermatomes of the rat fore and hind limbs have been worked out in detail by Takahashi and Nakajima (1996).

THE LUMBO-SACRAL PLEXUS

At first glance the lumbo-sacral plexus appears more complicated that the brachial plexus, but this is not so. Greene (1963) pointed out that Paterson's description of the lumbo-sacral plexus of the porcupine (Paterson took the porcupine as a typical mammal as regards the spinal plexuses) was not closely applicable to that of the rat. Greene also noted that the rat lumbo-sacral plexus was similar to the human plexus, and that the account given in Cunningham's Textbook of Anatomy could be used as a basis for describing the lumbo-sacral plexus of the rat.

The lumbo-sacral plexus predominantly comprises of the anterior primary rami of spinal nerves L1–S6, but caudal nerve elements are present towards the sacral end. The anterior primary rami divide into anterior and posterior divisions, which then recombine to form nerves. For example, the femoral nerve is composed of the anterior primary rami of nerves L2, 3 and 4. Although Greene (1963) used anterior and posterior when describing the divisions of the anterior primary rami, the terms ventral and dorsal may be substituted.

Important nerves arising from the plexus include the sciatic and femoral nerves, the obturator nerve; and the ilio-hypogastric and ilio-inguinal nerves. The root values of these nerves, that is the spinal segments from which they arise, are listed below. Root values for the tibial and common peroneal nerves of man are shown in brackets.

Anterior Divisions of the Anterior Primary Rami

Ilio-inguinal nerve	L1, L2
Ilio-hypogastric nerve	T13, L1
Obturator nerve	L2, L3, L4
Tibial nerve[a]	L4, L5, L6 (L4, L5, S1, S2, S3)[a]*The tibial and common peroneal nerves are the two parts of the sciatic nerve.*

Posterior Divisions of the Anterior Primary Rami

Femoral nerve	L2, L3, L4
Common peroneal nerve[a]	L4, L5, L6 (L4, L5, S1, S2)[a]*The tibial and common peroneal nerves are the two parts of the sciatic nerve.*

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The femoral and common peroneal nerves innervate the extensor muscles of the knee and ankle; and the tibial nerve innervates the flexor muscles of the knee and ankle.

Topography of the Lumbo-Sacral Plexus

The nerves (ilio-inguinal and ilio-hypogastric) of the upper part of the lumbar plexus arise within the psoas muscle, ventral to the transverse processes of the lumbar vertebrae, and leave under the lateral edge of psoas to enter the body wall. The femoral nerve, which arises further posterior, sweeps posteriorly and ventrally to emerge beneath the inguinal ligament and enter the thigh. The lateral cutaneous nerve of the thigh passes beneath the lateral part of the inguinal ligament. The sciatic and obturator nerves pass posteriorly and dorsally within the pelvis to escape via the greater sciatic foramen and the obturator foramen, and appear dorsal to the hip joint.

The sciatic nerve is the most important of these. It comprises the tibial and common peroneal nerves in the rat, which in man are combined in the upper part of the sciatic nerve and separate just above the knee joint. The tibial nerve runs between the muscles on the posterior aspect of the thigh, behind the knee joint and on between the two heads of the gastrocnemius muscle, giving off branches to muscles, skin and the knee joint on the way. It ends by dividing into the lateral and medial plantar nerves, which pass into the sole of the foot to innervate the small muscles of the foot (hind paw). These nerves are comparable with the ulnar and median nerves of the forelimb. The common peroneal nerve divides just distal to the knee to form the superficial peroneal nerve, which travels down the lateral aspect of the lower leg, and the deep peroneal nerve, passing round the head of the fibula to enter the anterior compartment and supply the muscles therein.

Hilton's Law

This is a useful law that states that joints receive nerve fibres from the nerves that supply the muscles that act across the joints. Thus the elbow joint receives nerve fibres from for example triceps, biceps and brachialis.

Shorter Nerves Around the Hip Joint

Other nerves arising from the sacral part of the lumbo-sacral plexus include the nerves to the glutei (gluteus medius, minimus and maximus), obturator internus, quadratus femoris and piriformis and the associated gemelli. The superior gluteal nerve innervates gluteus medius and minimus and the inferior gluteal nerve gluteus maximus. The origins of these nerves are as follows.

From Anterior Divisions of Anterior Primary Rami of Sacral Nerves

Nerves to obturator internus, quadratus femoris and the gemelli muscles.

From Posterior Divisions of Anterior Primary Rami of Sacral Nerves

Nerves to the glutei and piriformis.

Nerve Supply to the Skin of the Hindlimb

As in the case of the forelimb, the long cutaneous nerve arises from the spinal plexus and runs down the antero-lateral aspect of the thigh. The sural nerve leaves the common peroneal nerve high in the thigh and runs down the lateral aspect of the leg. Greene (1963) provides a detailed comparison of the sural nerve of the rat and that of man. The posterior cutaneous nerve of the thigh arises from anterior divisions of anterior primary rami of sacral nerves and runs on the posterior aspect of the thigh. For details of the dermatomes of the hindlimb see Takahashi and Nakajima (1996).

Nerve Supply of the Tail

The posterior part of the sacral plexus (L6, S1−4 and Caudal 1−2) provides the pudendal nerves, nerves to the perineum and the inferior caudal trunk that supplies branches to the tail. See Chapter 21(3), The Autonomic Nervous System, for an account of the sacral parasympathetic outflow.

THE HISTOLOGICAL CLASSIFICATION OF NERVE FIBRES

Peripheral nerves are made up of bundles of nerve fibres. The fibres vary in diameter and conduction velocity and have been classified. Two systems of classification of nerve fibres are in use. One system divides nerve fibres into three types: A, B and C, and subclassifies those in group A as Aα Aβ, Aγ and Aδ. The system is based on classification by descending conduction velocity, from A to C. Conduction velocity is related to fibre diameter: A fibres are larger and conduct more rapidly than C fibres. The second system deals only with afferent fibres and classifies them into four groups: Groups I, II, III and IV. Here too, classification is in terms of descending conduction velocity. Unfortunately, there is no exact read across from one system to the other, at least not at all points. Textbooks of neurophysiology should be consulted for further details.

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The following table combines the two systems.

Class	Destination or Origin	Diameter (μm)	Conduction Velocity (m/s)
Motor fibres Aα Myelinated	Extrafusal muscle fibres	12–18	60–100
Motor fibres Aγ Myelinated	Intrafusal muscle fibres: to contractile elements of muscle spindles	2–8	10–30
Deep afferents Group I Myelinated	Primary muscle and tendon receptors	12–22	70–120
Deep afferents Group II Myelinated	Secondary muscle receptors	6–12	30–70
Deep afferents Group III Myelinated	Aponeuroses, joints, and 'deep' pain receptors	2–6	12–30
Cutaneous afferents Group II, (Aα, Aβ)	Tactile receptors	6–12	30–70
Cutaneous afferents Group III (Aγ, Aδ)	Heat, cold and 'fast' pain	2–6	12–30
C fibres, Group IV, Afferents serving 'slow' pain receptors	'Slow' pain receptors	1–2	0.7–2.0
Also: postganglionic autonomic fibres Unmyelinated	Postganglionic autonomic fibres to glands, smooth and cardiac muscle		
B fibres, preganglionic autonomic fibres Myelinated	Autonomic ganglia	3	3–5

Modified from Bowsher (1970).

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Chapter 21.3

The Autonomic Nervous System

INTRODUCTION

The autonomic nervous system (ANS), sometimes known as the involuntary nervous system, is familiar to all biologists, but not easy to define. It comprises those parts of central and peripheral nervous systems that control functions of the body not under voluntary control. For example, heart rate, smooth muscle activity and secretions of the salivary glands are all under autonomic control. Smooth muscle under the control of the ANS is found in the vasculature, gut, urinary tract and iris. Although the muscle of the oesophagus is largely striated in the rat, it too is under the control of the ANS.

The literature of the ANS is extensive. Beginning with Gaskell (1916) and Langley (1921), further monographs by Kuntz (1946) and by Mitchell (1953) carried the story into the mid-20th century. Mitchell's monograph contains a detailed description of the ANS in man, and a historical review of the development of thinking about the ANS. More recently Gabella (1976) and Jänig (2006) have updated our knowledge base.

Furness (2014) described the peripheral ANS with special reference to the rat, and the development of the ANS has been reviewed by Young et al. (2011) as part of a series of papers on the comparative anatomy of the system (Holmgren and Olsson, 2011; Nilsson, 2011). Blessing (1997) provided a provocative argument calling for the abandonment of the terms ANS, reticular formation and limbic system. His advice has not been followed, but his arguments are worth reading.

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Respiratory muscle such as the diaphragm usually functions without conscious control, but as it can be brought under conscious control it is classified as a function of the voluntary nervous system. The division of the ANS into the sympathetic and parasympathetic nervous systems is well established, but can be misleading, as it suggests that they are opposites of each other (Furness, 2014). This perception arose from early work that showed that stimulation of the sympathetic system led to acceleration of the heartbeat, whereas stimulation of the vagus led to slowing. Most elementary textbooks speak of the flight and fright response for the sympathetic system, with the implication that the opposing activity of the parasympathetic system confers a calmer, more reflective, status. There is some value in all these concepts, but they should not be carried too far.

The ANS has a central and a peripheral component. The central component has been described in Chapter 20, The Brain and Spinal Cord, here we describe only the peripheral ANS.

The peripheral ANS comprises three parts:

1. an efferent component,
2. an afferent component and
3. the enteric nervous system.

The efferent component is usually divided into sympathetic and parasympathetic components. The former leaves the central nervous system (CNS) via the ventral roots of the spinal nerves of spinal segments T1 to L2; the latter leaves the CNS via cranial nerves III, VII, IX and X and the ventral roots of the spinal nerves of spinal segments L6–S1.

The afferent component runs in all the nerves carrying autonomic fibres to the periphery, and in the spinal nerves and plexuses surrounding blood vessels. It cannot be easily divided into sympathetic and parasympathetic. As in the case of the somatic afferent fibres of spinal nerves, the cell bodies of the afferent autonomic neurones are located in ganglia, often in the dorsal root ganglia of the spinal nerves. Afferent fibres running in the splanchnic nerves travel through the white rami communicantes (going the other way to that of the efferent fibres heading for the sympathetic ganglia) and pass via the dorsal roots of the spinal nerves to enter the cord.

The enteric nervous system can be seen as a peripheral brain, controlling the activities of the gut and a range of functions similar to those of the spinal cord. Afferent input can be integrated, with local reflex arcs involving interneurones, and efferent fibres running to target structures. The enteric nervous system could control the activities of the gut without reference to the CNS, but is usually influenced by, if not under the control of, the CNS.

THE EFFERENT COMPONENT OF THE AUTONOMIC NERVOUS SYSTEM

The efferent component of the ANS includes the outflow of preganglionic fibres from the CNS, the autonomic ganglia to which the preganglionic fibres run, and the postganglionic fibres that run to target organs. Because preganglionic fibres synapse with a number of postganglionic neurones, the latter greatly outnumber the former. The outflow of preganglionic fibres from the CNS can be divided into three parts:

1. Outflow from the CNS
   a. the cranial parasympathetic outflow,
   b. the thoracolumbar sympathetic outflow and
   c. the sacral parasympathetic outflow.
2. Ganglia

   A ganglion is a discrete and identifiable collection of the cell bodies of neurones, usually enclosed by a capsule of connective tissue. They can be found throughout the body. There are dorsal root ganglia of the spinal nerves, ciliary and sphenopalatine ganglia, ganglia of the sympathetic chains, coeliac ganglia and pelvic ganglia, to name but a few. Some confusion may arise by referring to the coeliac ganglion, and ignoring the fact that what may be intended, is the plexus of nerve fibres with ganglia embedded in that plexus.

A fundamental difference between the ganglia of the dorsal roots of the spinal nerves, and the ganglia of the efferent arm of the ANS, is that in the former no synapses occur, in the latter they do.

The ganglia of the ANS can be thought of in groups:

The parasympathetic ganglia associated with cranial nerves, the ciliary, sphenopalatine and otic ganglia.

The sympathetic ganglia of the sympathetic chains. These chains run ventral to the vertebral column and comprise a series of ganglia and connecting nerve fibres.

In addition to the ganglia of the sympathetic chains, there are plexuses and ganglia in the thorax, and prevertebral plexuses and ganglia in the abdomen. The thoracic ganglia include the cardiac, pulmonary and oesophageal plexuses, all containing ganglion cells and a meshwork of nerve fibres. In the abdomen there are the coeliac, superior (or anterior) mesenteric and inferior (or posterior) mesenteric plexuses and ganglia. Other ganglia, including those associated with the kidneys, are also present. The pelvic ganglia are mixed ganglia connected to the spinal cord by the pelvic splanchnic nerves and can be found in the pelvic plexus. Both sympathetic and parasympathetic fibres synapse here.

The ganglia of the target organs are parasympathetic ganglia, mostly microscopic in size. These small collections of cell bodies of postganglionic neurones can be

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found in the heart, the respiratory system and the walls of the gut and associated glands.

Distribution of Postganglionic Fibres

Parasympathetic Outflows

The Cranial Parasympathetic Outflow

The cranial parasympathetic outflow involves the oculomotor, facial, glossopharyngeal and vagus nerves (CN III, VII, IX and X), and represents the general visceral efferent (GVE) outflow from the brainstem. There is also a special visceral efferent (SVE) outflow that innervates the skeletal muscles associated with the branchial or pharyngeal arches, and has nothing to do with the ANS (see this chapter (20)].

The cranial GVE outflow supplies the iris and ciliary muscles of the eye, the salivary, lacrimal and Harderian glands, and via the vagus nerves, the heart, respiratory system and the smooth muscles and glands of much of the gastrointestinal canal and its associated glands, including the pancreas and liver. The vagus has a very wide distribution, and thus a long and complicated course. The cranial parasympathetic outflow originates in the neurones of the Edinger–Westphal nucleus, the salivatory nuclei and the motor nucleus of the vagus. Preganglionic fibres leave the brainstem in cranial nerves III, VII and IX and run, often by complicated paths involving other cranial nerves, to the ciliary, sphenopalatine and otic ganglia. These ganglia have been described in Chapter 21(1); here we provide a further discussion of the otic ganglion of the rat.

The Otic Ganglion of the Rat

The otic ganglion of the rat differs from that of man in terms of its nervous connections. In man preganglionic parasympathetic fibres reach the otic ganglion via the lesser superficial petrosal nerve, which arises from the tympanic plexus found in the mucosa over the promontory of the medial wall of the middle ear. The plexus is connected with the glossopharyngeal nerve (CN IX) by the tympanic nerve (nerve of Jacobson), and via a small communicating branch with the geniculate ganglion of the facial nerve (CN VII). It should be said that the functions of this communicating branch are uncertain. The lesser superficial petrosal nerve runs through the tegmen tympani to emerge lateral to the greater superficial petrosal nerve on the upper, intracranial, surface of the temporal bone. From here, it runs forwards and leaves the middle cranial fossa via a small foramen adjacent to the foramen ovale, before running a short distance to the otic ganglion. Further small twigs from the otic ganglion communicate with the nerve of the pterygoid canal and the chorda tympani nerve (Durward, 1951).

There is no tympanic nerve in the rat, so if we assume that the lesser superficial petrosal nerve arises from the tympanic plexus, then it can only be carrying forwards the preganglionic fibres that arrived at the plexus from the facial nerve. Such would be a surprising finding, as it is generally accepted that the otic ganglion receives preganglionic fibres that originate in the inferior salivatory nucleus of the brainstem, and leave in the glossopharyngeal nerve. A communicating nerve links the facial and glossopharyngeal nerves near their origins, and may well play the same role as the tympanic nerve in man (Al-Hadithi and Mitchell, 1987), so perhaps the lesser superficial petrosal nerve does not exist in the rat.

A nerve fascicle links the otic ganglion with a branch emanating from the superior cervical ganglion, which may represent the lesser superficial petrosal nerve in the rat. If so it would appear to have a very different function from that of the lesser superficial petrosal nerve of man, as this would mean that the equivalent of the lesser superficial petrosal nerve carries postganglionic sympathetic fibres from the superior cervical ganglion to the otic ganglion. (Al-Hadithi and Mitchell, 1987). Although some postganglionic sympathetic fibres reach the ganglion in man, it is not via the lesser superficial petrosal nerve.

Suzuki and Hardebo (1991) have also studied the lesser superficial petrosal nerve of the rat. In their description the lesser superficial petrosal nerve is linked with the greater superficial petrosal nerve close to the origin of the latter from the geniculate ganglion. They describe the lesser superficial petrosal nerve as leaving the tympanic bulla via the petro-tympanic fissure and reaching the posterior margin of the otic ganglion. These authors also showed a branch of the tympanic plexus running to the otic ganglion. It may be that the nerve described by these authors as the lesser superficial petrosal nerve is the fine fibre linking the facial nerve and the otic ganglion described by Al-Hadithi and Mitchell (1987). The problem is further complicated by Suzuki and Hardebo describing the lesser superficial petrosal nerve as carrying efferent, postganglionic fibres, from the otic ganglion to the greater superficial petrosal nerve, and on via the deep petrosal nerve to the plexus around the internal carotid artery to the blood vessels of the intracranial pia mater.

Distilling all this we suggest the following:

The otic ganglion receives preganglionic parasympathetic fibres that leave the brainstem in the glossopharyngeal nerve.

The route followed by these fibres is uncertain, though it seems possible that they transfer to the facial nerve, and from there reach the otic ganglion via a fine extratympanic branch of the facial nerve.

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There is no tympanic nerve in the rat.

There is uncertainty as to which nerve in the rat should be regarded as the equivalent of the lesser superficial petrosal nerve of man.

Further comparative studies are needed.

This highlights the dangers of extrapolating from human anatomy to the anatomy of the rat.

The Vagus Nerve (CN X)

The vagus is by far the most far-reaching of the cranial nerves, indeed its name is derived from the Latin word vago, to wander. The vagus contains both afferent (GVA and special visceral afferent (SVA)) fibres and efferent fibres (GVE and SVE).

Afferent fibres far outnumber the efferent, and the SVA component is minor, comprising fibres from taste buds at the most posterior part of the tongue, epiglottis and upper larynx. An even less significant general somatic afferent (GSA) component includes sensory fibres from part of the external auditory meatus.

Efferent fibres originate in the dorsal motor nucleus of the vagus (GVE) and the nucleus ambiguus (SVE) of the brainstem. The GVE component innervates smooth muscle, glands and cardiac muscle, while the SVE component supplies skeletal muscle derived from the posterior branchial or pharyngeal arches.

The vagus nerves leave the skull via the jugular foramina in company with the glossopharyngeal and accessory nerves (CN IX and XI), passing through the afferent jugular and nodose ganglia, which are the equivalent of dorsal root ganglia of the spinal nerves and lie within or just behind the foramina (Greene, 1935; Hebel and Stromberg, 1976). Branches of the vagus nerve arising close to the nodose ganglion run to the pharyngeal plexus, while the cranial (or superior) laryngeal nerve, also arising close to the nodose ganglion, runs to the larynx. There seems to be some doubt as to whether the cardiac branches of the vagus arise in the neck or/and in the thorax (Hebel and Stromberg, 1976; Greene, 1935).

Traversing the neck, the vagus nerve travels close to the common carotid artery and enters the thorax to run in the dorsal mediastinum. Just before entering the thorax the right vagus nerve gives off the right recurrent laryngeal nerve, which hooks around the right subclavian artery and turns towards the larynx. As the left vagus nerve crosses the arch of the aorta it gives off the left recurrent laryngeal nerve, which hooks around the arch and the ligamentum arteriosum, before turning dorsally to run forwards close to the left side of the trachea, heading for the larynx. Many small branches run to the autonomic plexus around the oesophagus, the pulmonary plexuses at the roots of the lungs and, via the cardiac nerves, to the cardiac plexus around the great vessels as they enter and leave the heart. Branches to the cardiac plexus arise from the vagus nerves, and perhaps from the recurrent laryngeal nerves in the neck, but the latter source is debatable. These plexuses also receive sympathetic fibres from the cervical sympathetic ganglia. Some preganglionic fibres running in the vagus synapse in the cardiac ganglia of the cardiac plexus, others run on to synapse in the ganglia of the heart itself. As the heart and diaphragm move back during development (they descend in man) the nerves lengthen, and as a consequence the autonomic nerves that run to the cardiac plexus arise well forward of the heart. For example, the phrenic nerve, which innervates the diaphragm, arises from spinal segment C4 in the rat and C3, C4 and C5 in man.

Textbooks of physiology often refer to the depressor nerve. This nerve, appears as a distinct nerve in the rabbit, but not in man, and is not described in the rat. It is an afferent nerve that runs from pressor-receptors in the arch of the aorta to the cranial, or superior, laryngeal nerve. If the nerve is divided, and the effects of stimulating the distal and proximal segments compared, it will be found that stimulation of the distal segment has no effect on the heart rate, but that stimulation of the proximal segment slows the heart. The slowing is mediated, as a reflex, by efferent fibres running in the vagus nerves.

The vagus nerves cross the diaphragm and enter the abdomen in company with the oesophagus. Before piercing the diaphragm, the vagus nerves form the truncus dorsalis (and truncus ventralis) (Hebel and Stromberg, 1976; Niederhäusen, 1953). The fibres of the dorsal trunk contain 80% from the right vagus nerve and 20% from the left.

As a result of rotation of the stomach during development, the left vagus nerve lies on the ventral aspect of the stomach, and the right vagus nerve on its dorsal aspect (anterior and posterior in man). Branches supply the stomach, and the coeliac, anterior mesenteric (and perhaps posterior mesenteric) plexuses of the abdomen. The parasympathetic fibres that are distributed via the branches of the plexuses cannot be distinguished visually from sympathetic fibres, and like the sympathetic fibres, are distributed with the arterial supply to the target organs. Although the vagus innervates all the small intestine and much of the colon in man, the parts of the gut supplied by the vagus nerves in the rat are restricted to those supplied by the cranial mesenteric artery (Hebel and Stromberg, 1976).

The Thoracolumbar Sympathetic Outflow

All preganglionic fibres of the sympathetic system arise from GVE neurones in the lateral horns of spinal segments T1 to L2 or L3. Preganglionic fibres leave the

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spinal cord via the ventral roots of the spinal nerves, and pass to the spinal nerves. The ganglia of the sympathetic chain are connected to the anterior primary rami of the spinal nerves by two short nerves, the white and grey rami communicantes (the white rami contain the GVE component). The myelination of the white rami is poorly developed, and the colours of the rami are too similar to allow differentiation by colour alone. The white rami carry GVE fibres from the anterior primary rami to the ganglia; the grey rami carry postganglionic sympathetic fibres back to the anterior primary rami for further distribution. In total, there are about 15 white rami communicantes on each side. In man every spinal nerve receives a grey ramus, but in the rat this seems to be less certain. Durward, 1951; Hebel and Stromberg, 1976; refer to grey rami reaching the lower cervical spinal nerves, but say nothing about grey rami of the anterior cervical spinal nerves.

The Sacral Parasympathetic Outflow

Preganglionic parasympathetic fibres arise from cell bodies in the lateral horns of the sacral segments of the spinal cord that lie in the vertebral canal opposite the lower lumbar vertebrae, ending at the fourth lumbar vertebra. The dorsal and ventral roots of the sacral nerves run in the cauda equina to the sacral foramina, where they join to the form the sacral nerves. The anterior primary rami of these nerves give off the pelvic splanchnic nerves (nervi erigentes), which run to the pelvic plexus and ganglia. The pelvic ganglia are mixed ganglia, containing not only parasympathetic and sympathetic fibres, but also fibres from the enteric nervous system.

PLEXUSES AND GANGLIA OF THE AUTONOMIC NERVOUS SYSTEM

The Sympathetic Chains

Sympathetic chains lie on the ventrolateral surfaces of the bodies of the vertebrae, forming a series of discrete ganglia linked by bundles of nerve fibres. Human terminology indicates three cervical ganglia (superior, middle and inferior), but in the rat the middle ganglion is often missing, and the inferior ganglion is sometimes combined with the anterior thoracic ganglia to form the stellate ganglion. The relatively larger superior cervical ganglion is always present.

The thoracic ganglia include the stellate ganglion, and one per vertebra as far as the 13th thoracic vertebra. Four or five lumbar ganglia, and three to four sacral ganglia complete the chain. The chains approach each other and the midline as they come to an end and meet at the small ganglion impar (impar, Latin, means odd or unequal, perhaps implying that this ganglion is not paired).

In man, not all the grey rami are connected with ganglia. In the cervical region, some of the grey rami connect with the bundles of nerve fibres that run from ganglion to ganglion, but whether this is the case in the rat is not clear from published descriptions.

Although some postganglionic fibres such as the splanchnic leave the ganglia directly, others exit the ganglia in a variety of ways. These include:

1. turning up or down the chain before leaving at some distant point;
2. leaving the ganglia via the grey rami to rejoin the primary rami of the spinal nerves for distribution with those nerves;
3. leaving the chain to join plexuses around blood vessels and
4. leaving the chain to join other plexuses, such as the oesophageal plexus.

The sympathetic chains do not enter the head. All postganglionic sympathetic fibres reaching the head do so via the periarterial sympathetic plexuses, such as those found around the carotid arteries. Most of these fibres are distributed along with the arteries and their branches, but some leave to form specific nerves, for example the deep petrosal nerve, or join cranial nerves.

Those preganglionic fibres that do not synapse in the chain ganglia follow similar routes to reach more peripheral ganglia where they do synapse. One unusual group runs to the adrenal medulla; the endocrine cells of the medulla may be considered to be modified postganglionic neurones.

Plexuses and Ganglia of the Thorax

These plexuses are found around the oesophagus, the roots of the lungs (pulmonary plexuses), and the great vessels as they enter and leave the heart (the cardiac plexus and ganglia). These mixed plexuses are formed by preganglionic fibres of the vagus nerves and largely postganglionic sympathetic fibres from the cervical sympathetic chain. Parasympathetic fibres either synapse with the cells of the ganglia, or run on to the walls of the target organs where they synapse with postganglionic neurones. The distinction made by human anatomists between deep and superficial cardiac plexuses does not seem to have been made in the rat.

The Abdominal Prevertebral Plexuses and Their Ganglia

The bundles of nerve fibres that leave the thoracic sympathetic chains to form the splanchnic nerves cross the diaphragm to reach the prevertebral plexuses of the abdomen. Human terminology defines the greater, lesser and least splanchnic nerves, but these individual nerves are more

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TABLE 21.3.1 Functional Components of Cranial Nerves

Nerve	Rat	Man
	Ganglia of sympathetic chain (Greene, 1935)	Ganglia of sympathetic chain
Great splanchnic	8th, 9th, 10th thoracic and 1st lumbar	5th–9th or 10th thoracic
Lesser splanchnic	3rd lumbar	9th and 10th thoracic
Least splanchnic	3rd or 4th lumbar	12th thoracic

TABLE 21.3.2 Distribution of Efferent Nerve Fibres From the Three Prevertebral, Abdominal, Plexuses and Ganglia

Target Organ	Coeliac Plexus	Superior Mesenteric Plexus	Interrenal Ganglia	Inferior Mesenteric Plexus	Lumbar Sympathetic Chain
Stomach	+ + +	+			
Proximal small intestine	+ + +	+ + +			
Remainder of small intestine		+ + +			
Proximal colon	+	+ + +			
Remainder of colon				+ + +	
Spleen	+	+			
Kidney			+		+ + +
Pancreas	+ + +	+			

Note: + + + = a major supply.
+ = a minor supply.

clearly seen in larger mammals than in the rat. The origins of the splanchnic nerves of the rat are shown in Table 21.3.1; the ganglionic origins of the nerves in man are shown for comparison (Greene, 1935; Furness, 2014).

Coeliac Plexus and Ganglia

This plexus contains two discreet ganglia that lie at the root of the coeliac artery, ventrolateral to the aorta, and consists of preganglionic parasympathetic fibres from the vagus nerves, and postganglionic sympathetic fibres via the splanchnic nerves, which have several ganglionic swellings along their length (Furness, 2014). Efferent fibres from the coeliac plexus follow the branches of the coeliac artery.

Superior (or Anterior) Mesenteric Plexus and Ganglia

The coeliac, superior and inferior mesenteric plexuses can be thought of as one extended plexus with an increased density of fibres around the roots of the related arteries. In the rat, there is a single superior mesenteric ganglion located near the origin of the superior mesenteric artery. Inter-renal ganglia have also been described.

Inferior Mesenteric Plexus and Ganglia

Two ganglia are found in the rat: one anterior to, the other caudal to, the origin of the inferior (or posterior) mesenteric artery. Paired hypogastric nerves connect the plexus with the pelvic plexus and ganglia.

Distribution of efferent nerve fibres from the three prevertebral, abdominal, plexuses and ganglia are shown in Table 21.3.2.

Table 21.2 is intended to provide only an outline of the nerve supply to the target organs listed. For further details, see Chevendra and Weaver (1991). Quinson et al. (2001) reported that the interrenal ganglia also projected to the gut.

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Pelvic Plexus and Ganglia

The main pelvic ganglia are a pair of mixed ganglia with synapses between the terminals of preganglionic fibres and postganglionic neurones of both the parasympathetic and sympathetic systems (Furness, 2014). The ganglia are found at the base of the bladder near the dorsolateral lobes of the prostate in the male, and near the junction between the uterus and cervix in the female. The pelvic plexus and ganglia receive sympathetic fibres from the outflow from spinal levels L1 and L2, and parasympathetic fibres from the sacral parasympathetic outflow L6−S1. The parasympathetic outflow forms the pelvic splanchnic nerves, which run to the plexus that is wrapped around the pelvic organs.

Distribution of Postganglionic Fibres

The postganglionic fibres of the ANS may be distributed by spinal and cranial nerves and also via the periarterial plexuses. The latter are particularly important for the sympathetic system.

THE AFFERENT COMPONENT OF THE AUTONOMIC NERVOUS SYSTEM: THE GENERAL VISCERAL AFFERENT SYSTEM

One of the simplest, but most useful ways of thinking about the CNS, is to imagine it as an integrator that sits between inputs from afferent systems, and outputs via efferent systems. The reflex arc is perhaps the simplest form of neurological activity, with an input via afferent systems linked to a specified output over efferent systems. But even simple reflexes such as the flexor withdrawal or crossed extensor reflex involve a good deal of central integration to proceed smoothly. It is a matter of common knowledge that not all afferent input reaches consciousness. At least in man it is to some extent a matter of choice. For instance, as you read this text you are probably not conscious of the position or state of your feet, but can find out where they are, and whether they are warm or cold by thinking about them. It could be said that one can call information from one's feet to consciousness at will, but if your feet are very cold you soon become aware of that as the afferent input forces itself upon your mind. Although it is a small step to say that we either are, or can become, conscious of a range of sensations by conscious thought, we cannot call to mind our blood pressure or arterial oxygen tension. Despite having no appreciation of such physiological variables we know that such variables are monitored and regulated by the nervous system. This leads to a distinction between what used to be called the 'internal economy' of the body, and those features of the body and the external world that we can consciously appreciate. In general terms, we can say that the internal economy of the body is monitored by the general visceral afferent (GVA) system. Information from the carotid body and carotid sinus, or from stretch receptors in the walls of the bladder and rectum, flows continuously to the CNS and commands appropriate responses. Although some of this afferent input reaches consciousness, for instance we know when our bladders are full and need emptying, as soon as we begin to think about how these aspects of nervous system functioning apply to nonhuman species we are in difficulties. If a cat lies close to the fire on a cold day, we assume that the cat felt cold and moved to the fire. But what the cat actually feels is quite unknown to us, indeed it is quite unknowable. Cats lack the facility of descriptive language, and so it seems unlikely that they can think in words as we do. It is almost certainly nonsense to imagine that on a cold day the cat thinks: 'it's chilly today, I wonder whether the fire in the living room has been lit, ah yes, I'll go and lie by it.' That would be asking too much of even a very intelligent cat! On the other hand, it is certain that the cat remembers there is often a comfortable place in the house and seeks it out on a cold day. The reader will appreciate how difficult it is to talk, write or think about such problems; there is no reason to think that the cat thinks in terms of coldness or comfort. Indeed, we do not know how a cat thinks, or even whether it thinks at all.

Some reflex activities involve both the GSA and GVE systems. Skin temperature is monitored via temperature receptors (GSA); vasodilatation and sweating are controlled by the sympathetic system (GVE). Separating somatic sensory input from visceral sensory input is to some extent a matter of convenience. In both systems a receptor acts as a transducer, and converts a change in the local environment into frequency modulated input to the CNS over afferent nerve fibres. This input is integrated with other inputs (and, at least in man, with information derived from memory) to trigger an innate or learned response that is signalled to effector systems.

The visceral afferent system comprises a system of receptors and afferent nerves that convey information to the CNS. The receptors include those that monitor blood gas tensions (partial pressures), blood pH, blood pressure and distension of hollow viscera. The cell bodies of the nerve fibres that convey the information to the CNS are located in the dorsal root ganglia of the spinal nerves and the sensory ganglia of the cranial nerves, such as the nodose ganglion of the vagus nerve, and the semilunar ganglion of the trigeminal nerve. Afferent nerve fibres from the limbs run in the spinal nerves, and from the gut in the splanchnic nerves, returning to the spinal ganglia via the grey rami communicantes to the anterior primary rami of the spinal nerves and thence to the dorsal roots. The cell bodies are located in the dorsal root ganglia.

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THE ENTERIC NERVOUS SYSTEM

Interest in the enteric nervous system has developed greatly over the past 25 years, though it is interesting to note that as long ago as the early years of the 20th century Langley divided the ANS into sympathetic, parasympathetic and enteric components. The enteric nervous system is now regarded as the brain of the bowel. Detailed reviews of the system have been published by Goyal and Hirano (1996), Hansen (2003a,b,c), Sasselli et al. (2012), Furness et al. (2014) and Avetisyan et al. (2015).

It has long been known that the wall of the gut contains two plexuses of nerve fibres and ganglion cells. The myenteric plexus of Auerbach lies between the circular and longitudinal layers of muscle, and the submucosal plexus of Meissner lies in the submucosa. A deep muscular plexus in the inner part of the circular layer of smooth muscle has also been described. The ganglion cells of these plexuses were long assumed to belong to the parasympathetic system, synapsing with preganglionic fibres from the vagus nerve and pelvic splanchnic nerves before giving rise to postganglionic fibres. In part, this is true, but there are several other types of neurone present in the enteric nervous system. In the rat types I to III have been identified, and up to type VIII in larger mammals. As many as 20 subtypes have been identified by immunohistochemical methods. The large type II cells appear to be afferent neurones, similar to those of the GVA system that are found in the dorsal root ganglia of the spinal nerves: type I neurones appear to be motor neurones.

The system controls every aspect of gut function, including smooth muscle activity, secretion and the movement of substances across the mucosa, as well as the function of associated organs such as the pancreas and the biliary tract. A large number of transmitter substances have been identified in the cells of the system, and many cells produce more than one transmitter. The complexity of the enteric nervous system approaches that of the spinal cord, and appears capable of integrating afferent input and controlling the functions of the gut. It is a semi-independent system that can operate alone, but is usually influenced by input from the CNS via the parasympathetic and sympathetic systems.

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Chapter 22

The Eye

The mammalian eye is a subject of intense research, and ophthalmic or visual physiology is now a well-defined and distinct subject to which textbooks and journals are devoted. In the short account provided here we have been selective; the histology of the retina is not covered in much detail, nor have we described the topographical anatomy of the intra-orbital nerves, or attempted a synopsis of the evolution of the vertebrate eye. For reviews of the evolution of the vertebrate eye and its many variations, we suggest Fernald (2000, 2004) and Gehring (2004). Readers with access to a good library may wish to consult the classic work, the *Vertebrate Eye and Its Adaptive Radiation* (Walls, 1963). Davson's famous book *Physiology of the Eye* (1990) provides an outstanding, but rather dated account of visual physiology. Excellent accounts of the retina are available in textbooks of human histology, but beware the pitfalls of transferring the histological features of man to the rat. Books by Tansley (1965), Gregory (1966) and Pirenne (1967), though more than 50 years old, are still useful, and contain much of interest on structure, function and comparative physiology. There are also many fine up to date textbooks of human and veterinary ophthalmology. Grant's *Toxicology of the Eye* (1993) is a large compendium of the effects of compounds on the eye, but does not deal with structure and function.

DIFFERENCES BETWEEN THE RAT AND HUMAN EYE

Clearly the rat eye is substantially smaller than the human eye (5.5 mm in diameter compared with 24 mm), and by way of introduction we have provided a list of differences between the eyes of man and rat.

1. The lens of the rat eye is close to spherical in shape, and unlike the lens of the human eye, cannot be brought to a shorter focal length by contraction of the ciliary muscle which is poorly developed in the rat, so the rat lacks the capacity of accommodation.
2. The depth of focus (depth of field is a better term) of the eye is defined by the minimal distance from the eye at which objects can be focussed without changes to the focal length of the optical system. In man objects can be brought in from infinity to 2.3 m without loss of focus if the diameter of the pupil is held at 2 mm (Campbell, 1957). A smaller pupil increases the depth of focus by reducing spherical aberration introduced by the periphery of the lens, so the rat has a greater depth of focus than man: from infinity to 7 cm (Powers and Green, 1978). This large depth of focus offsets the lack of accommodation in the rat.
3. The lens of the rat eye is close to spherical, that of man is lenticular (meaning like a lentil or lens) and biconvex.
4. The rat has poor visual acuity compared with man. In the rat the minimal angle subtended at the retina by distinguishable points is about 1 degree of arc, in man it is about 30 seconds of arc (0.0083 degrees of arc).
5. The rat is hypermetropic (long sighted) compared with man. If one were thinking in terms of human ophthalmic optics then the rat would be described as 7 dioptres hypermetropic (Glickstein and Millodot, 1970). The power of a lens expressed in dioptres is the reciprocal of its focal length in metres.
6. As a nocturnal animal, the rat has a higher ratio of rods to cones in its retina than man; 99% of the receptors in the rat retina are rods.
7. The rat lacks a fovea, the small central area of the human retina that is occupied only by cones and where the retina is thinned. This accounts for the high visual acuity of man.
8. Man has good colour vision based on cones that are sensitive to red, green and blue light, but in the rat, only cones sensitive to green and blue/ultraviolet light are found.
9. Rod development depends in part on dihydroxyphenylalanine (DOPA), a compound not produced in albino animals, so as a result albino rats have poorly developed rods.
10. Binocular stereoscopic vision is dependent on the overlap of visual fields. The laterally placed eyes of the rat have less overlap of fields then frontally placed human eyes.

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All this means that the rat sees less well than man. Compared with man, the image produced by the rat eye is blurred with poor colour definition. Of course, the rat is a macrosmatic mammal and relies on its sense of smell to a large extent, and using its whiskers to provide it with information on close objects (see Chapter 10: Nasal Cavity).

DEVELOPMENT OF THE EYE

Development of the eyes begins with the evagination of the right and left optic vesicles, which arise from the ventral aspect of the forebrain. The optic vesicles and the stalks that connect the vesicles to the brain are hollow. As the vesicles grow, they come into contact with the ectoderm of the lateral aspect of the head. Interaction between neuroectoderm and surface ectoderm induces formation of the lens placode from which the lens of the eye will develop. The developing lens sinks below the surface and invaginates the optic vesicle, which assumes the shape of a cup. The cup has an outer layer that will give rise to the pigment epithelium of the retina and an inner layer that gives rise to all the other elements of the retina.

Mesenchyme (mesoderm) condenses around the developing eyeball and forms the choroid layer and the sclera. The collagen of the sclera is produced by fibroblasts, so although the sclera and the cornea are essentially continuous, they are produced by cells of different embryological origins. The choroid provides the bulk of the ciliary body, and is covered by two layers of epithelium derived from the retina that continue over the ciliary body and form the posterior epithelium of the iris. The remainder of the iris and its anterior surface are thought to be formed from mesenchyme. In man the ciliary body contains the smooth muscle cells that allow accommodation; in the rat these are poorly developed, the rat has minimal powers of accommodation. The iris contains the constrictor and dilator muscles of the pupil, also thought to be derived from mesenchyme.

The epithelium of the anterior surface of the cornea is derived from surface ectoderm, and the posterior epithelium (often called endothelium), Descemet's membrane and the cells that produce the collagen fibres of the corneal stroma from neural crest tissue that infiltrates between the developing lens and the anterior surface of the developing cornea. The collagen fibres of the corneal stroma are produced by keratocytes, a cell similar to the fibroblast, but not to be confused with the keratin producing keratinocytes, although both words have the same derivation – the Greek, kerato, meaning horny.

With the exception of the microglial cells and endothelium of the capillaries and other blood vessels, the retina develops from the retinal progenitor cells of the optic vesicle.

Differentiation, migration and maturation of retinal cells are closely controlled by regulatory genes. In the mouse, development of the cones, ganglion cells, horizontal cells and amacrine cells begins at gestational day (GD) 11. Development of the cones and horizontal cells is complete at birth, with ganglion and horizontal cells continuing to develop for a few days after birth. Rods, bipolar cells and Müller cells begin to develop between GD 12 and 16 and continue to do so after birth until the eyes open. The axons of the ganglion cells grow into the stalk of the optic vesicle and form the optic nerve (Bassett and Wallace, 2012). The development of the eye of the rat has not been documented in such detail but it is thought to be similar, beginning on GD 11, with the eyelids forming at about GD 17 and fusing shortly thereafter, with the result that rats are born with their eyes closed, only opening at around day 12 of postnatal life. The period of eye-closure is important for the normal development of the rat eye (Meng et al., 2014).

The eye of the rat is much less well developed at birth than the human eye, and development continues for around 3 weeks after birth. Corneal development continues for a week or so after the eyes have opened, with the anterior epithelium of the cornea increasing in thickness from two to six layers of cells. At birth the lens of the eye is biconvex, and development into the almost spherical lens of the adult rat occurs after birth. The blood vessels that run through the hyaloid canal of the vitreous to the lens disappear between 7 and 22 days of postnatal life; in man they have disappeared by birth. There are two sets of hyaloid vessels in the rat, a central group of three to five arteries running to the posterior pole of the lens, and a peripheral set that runs over the surface of the retina to reach the equator of the lens (Heywood, 1973).

The development of the eye is substantially more complicated than described above and for a detailed account we suggest Walling and Marit (2016), who have provided a short account of prenatal development, and a more detailed account of postnatal development. Publications by Bassett and Wallace (2012), Kuszal et al. (2004), Zieske (2004), Meng et al. (2014), Braekevelt and Hollenberg (1970) and Ju et al. (2012) should also be consulted for further details.

THE GENERAL ANATOMY OF THE RAT EYE

Although the rat eye is similar in general structure to other mammalian eyes, it differs from man in that it is not completely surrounded by bone, so is held in place by the Harderian and intra-orbital lacrimal glands and the extraocular muscles, with the lateral aspect of the eye being relatively unprotected. The rat eye bulges forwards from the face, in human terms the rat is exophthalmic. The bulging of the eyes is increased during chewing by contraction of

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that part of the masseter muscle that runs through the orbit (see Chapter 6: The Musculature of the Rat). The optic nerve runs from the eye to the optic foramen.

Conventional Axes and Planes of Reference

An easy way to remember the axes and planes of reference of the eye is to imagine the planet earth lying on its side. The poles are thus anterior and posterior, and the equator runs vertically around the globe. The optic nerve is not quite in line with the centre of the cornea, it leaves below and medial to the point where the optical axis, running horizontally from the centre of the cornea to the retina, meets the back of the eyeball. An equatorial section divides that the globe into anterior and posterior halves is not much use for histological work, but if the vitreous is removed from the anterior half the posterior surface of the lens and the surrounding ciliary processes can be seen. Planes passing through the axis are described as meridians. For histological and pathological purposes, sections that are taken in either the horizontal or vertical meridian will show approximately the same appearance as the eye is close to symmetrical around the optical axis. Fig. 22.1 shows a diagram of a section taken through the vertical meridian.

The Eyeball

The wall of the eyeball comprises three layers, a tough outer sclera, a vascular middle layer called the uveal tract (more often called the choroid membrane or the choroid), and the retina. The sclera is made up of collagen and some elastic fibres, and is continuous with the cornea at the front of the eye, although the radius of curvature of the cornea is smaller than that of the sclera. Human anatomists use the analogy of a watch glass sitting on a cricket ball to represent the eye.

At the back of the eye, the dura mater that surrounds the optic nerve blends into the sclera, and where the optic nerve penetrates the sclera, bundles of Type IV collagen fibres are arranged as a lattice through which the interstices of the bundles of nerve fibres run. The nuclei of the fibroblasts that produce the collagen and elastic fibres of the sclera can be seen pressed between these bundles of collagen fibres. The uveal tract (or vascular tunic) can be divided into the choroid, which underlies the retina, the ciliary process and the iris, which forms the diaphragm that defines the pupil of the eye. The light-receptive retina occupies all the inner surface of the eye posterior to the lens. The anterior portion runs forwards over the ciliary body and its processes onto the posterior surface of the iris. Here the retina is thin and does not function as a photoreceptor.

The interior of the eyeball can be divided into three parts. The anterior chamber lies between the cornea and the iris and has a volume of around 15 μL. The posterior chamber lies between the posterior surface of the iris, the lens and the suspensory ligaments that link the lens with the ciliary processes. The anterior and posterior chambers contain aqueous humour, which is produced by the ciliary

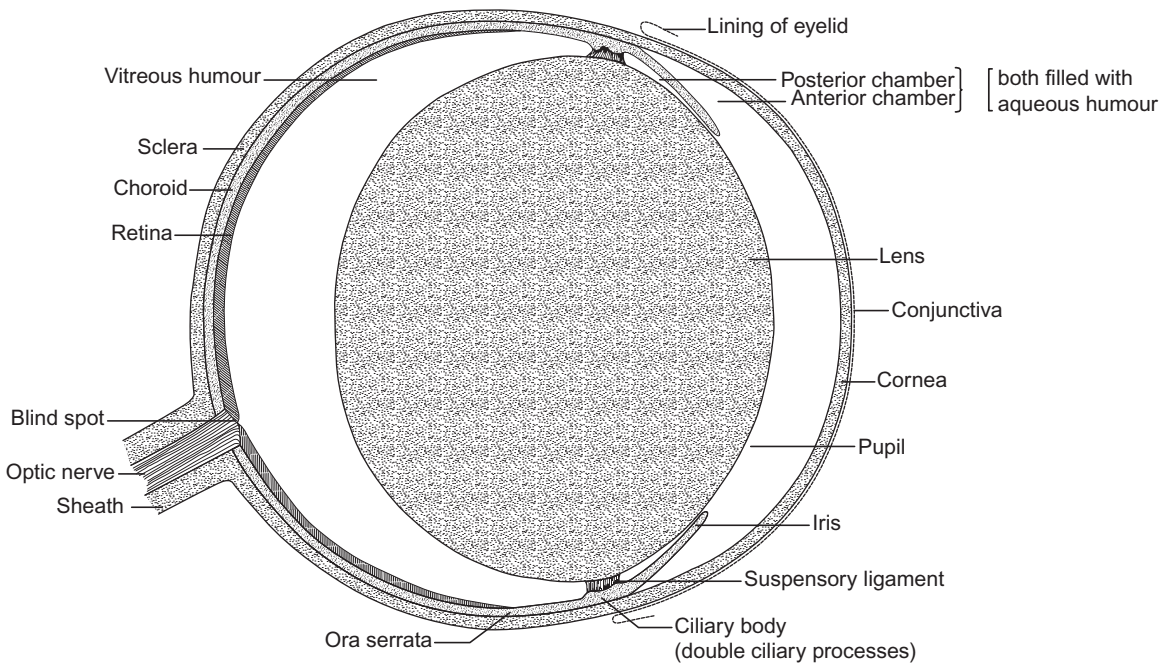


FIGURE 22.1 Eyeball (vertical section).

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processes of the posterior chamber and percolates between the iris and the lens into the anterior chamber where it is absorbed at the irido-corneal angle. The vitreous body (the vitreous) fills the space between the lens, its suspensory ligaments and the retina. The vitreous is a gel with a volume of about 35 μL (Hebel and Stromberg, 1976). Substances found in the aqueous humour can diffuse into the vitreous, but beyond that little is known of its turnover (Davson, 1972). Note that the vitreous does NOT occupy the posterior chamber.

One of the most important things to grasp about the structure of the eye is that the iris and ciliary body are circular structures. Similarly, the suspensory ligaments of the lens form a circle around the periphery of the lens. The canal of Schlemm, see below, runs in a circle around the irido-corneal angle.

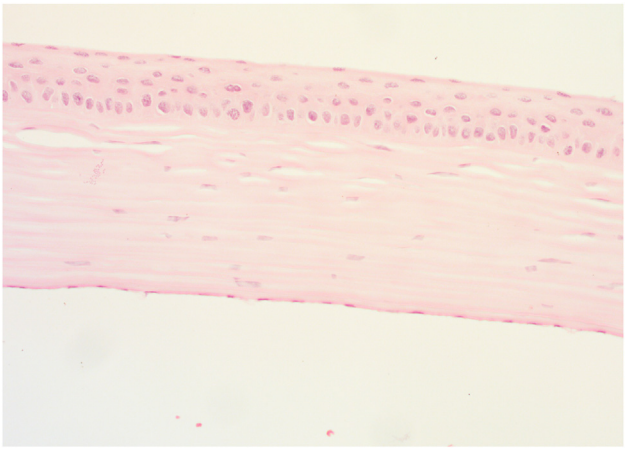


FIGURE 22.2 The cornea.

The Anterior Parts of the Eye

The Conjunctiva

The surface of the cornea is covered by a nonkeratinised, stratified squamous epithelium that is continuous with the conjunctival epithelium covering the sclera (bulbar conjunctiva) and the inner surfaces of the eyelids (palpebral conjunctiva), blending into the skin at the edges of the eyelids. The bulbar and palpebral parts of the conjunctiva meet at the fornices of the conjunctival sac (Fornix is Latin for an arch).

The conjunctival sac sometimes causes confusion. If you shut your eye the sac is a closed space limited by the cornea, sclera and the posterior or inner surfaces of the eyelids. The conjunctiva is more than an epithelium, it includes a subepithelial lamina propria that contains many blood and lymphatic vessels and many nerve fibres. In cases of conjunctivitis, blood vessels dilate and the inner surfaces of the eyelids and the bulbar conjunctivae become reddened. Although no capillaries enter the cornea, severe damage to the cornea may lead to capillaries growing into it from the conjunctiva. Sensory nerve fibres running in the conjunctiva mean the cornea is exquisitely sensitive to touch. Secretions from the lacrimal and Harderian glands flow into the lateral part of the conjunctival sac, and are drained by the two lacrimal ducts at the medial ends of the upper and lower eyelids. These join to form the lacrimal sac from where the fluid drains via the nasolacrimal duct to the nasal cavity.

The Cornea

The rat cornea is about 250 μm in thickness, somewhat thicker than the sclera (Fig. 22.2). The surface epithelium is continuous with that of the bulbar conjunctiva and rests on a basement membrane. In man, but not in the rat, this basement membrane is supported by a well-defined layer of fine collage fibres, Bowman's membrane. Basal cells of the epithelium divide in response to injury and areas of denudation are covered by sliding of epithelial cells. This sliding of epithelial cells is an interesting phenomenon as these cells interdigitate with one another and are connected to one another by desmosomes.

The bulk of the corneal stroma is made up of bundles of collagen fibres produced by fibroblasts and arranged as lamellae or sheets. Nuclei can be seen in the bundles of fibres in adjacent lamellae running at 90 degrees to one another. The most important functional characteristic of the cornea is that it is transparent. Transparency is maintained by the actions of endothelium of the posterior surface of the cornea that controls the amount of fluid that lies between the bundles of fibres; if the cornea becomes oedematous then blurring of vision occurs. These endothelial cells lie on a thickened basement membrane known as Descemet's membrane, which is present in both man and the rat. These cells pump ions from the interstitium of the cornea and water follows. The water and ions are subsequently secreted into the aqueous humour of the anterior chamber.

The Choroid

A number of names and eponyms have been given to the layers that can be defined in the choroid. In essence, there is an outer layer containing large blood vessels, a middle layer containing fenestrated capillaries and an inner membrane. The inner membrane is known as Bruch's membrane, a combination of the basement membrane of the pigment epithelium of the retina and the endothelial cells of the capillaries of the choroid with an intervening layer of fine collagen and elastic fibres. It is frequently described as vitreous or glassy because of its glistening

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appearance in histological sections. Bruch's membrane has been subdivided into five layers that can be defined at electron microscopy (Fawcett, 1994). Many melanocytes are found in the choroid of pigmented animals but not in the albino rat.

The Ciliary Body

The bulk of the ciliary body (Fig. 22.3) is made up from connective tissue containing a wealth of fenestrated capillaries. In histological sections the ciliary body looks like a wedge, but is, in fact, a ridge that runs round the inner surface of the eyeball. Extending from the surface of the ring are about 100 finger-like ciliary processes reaching from the apex of the ring towards the lens. From these 'fingers', fine collagenous filaments run to the capsule of the lens to make up its suspensory ligament. Ciliary processes are less well developed in the rat than they are in man, and not much smooth muscle is found in the ciliary body of the rat, accounting for its poor powers of accommodation.

The epithelium that covers the ciliary body and processes is both interesting and unique. Two layers of cuboidal cells, each layer resting on a basement membrane, are arranged with their apices facing each other across a narrow gap. Aqueous humour is secreted by this double layer of epithelium. This unique arrangement has an embryological basis, the outer layer being derived from the outer wall of the original invaginated cup, and the inner layer from the inner wall of the cup, and the narrow gap between the apical surfaces of the two layers represents the remains of the original optic vesicle. The outer layer is thus no more than a forward extension of the pigment epithelium of the retina and the inner layer a forward extension of the retina itself. However, the 10 layers of the retina have been scaled down to a single layer of cuboidal cells and a basement membrane that probably represents the inner limiting membrane of the retina proper. Note that this basement membrane is in contact with the aqueous humour of the posterior chamber. The apices of the cells are characterised by microvilli and their basal surfaces by marked infolding of the basal cell membrane. In pigmented animals, the cells of the outer layer are laden with melanin granules. Similar granules appear in the cells of the inner layer as it runs forwards from the ciliary processes to the posterior surface of the iris, but again only in pigmented animals.

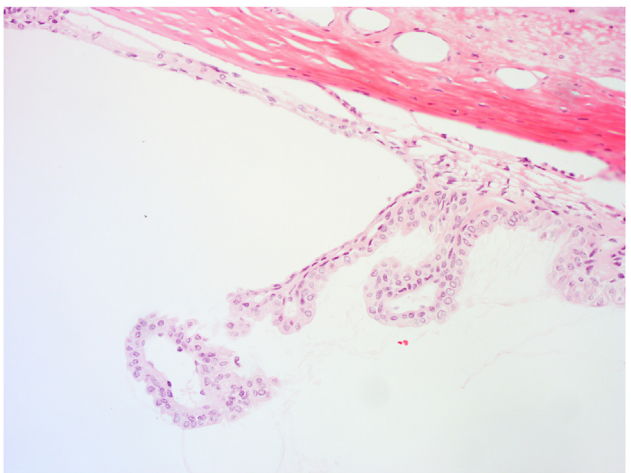


FIGURE 22.3 The ciliary body and the canal of Schlemm.

The Iris

The choroid continues forwards from the ciliary body to form the iris. Like the ciliary body, the posterior surface of the iris is covered by a double layer of epithelial cells that is heavily pigmented in non-albino animals. The constrictor muscle of the iris is arranged in a ring around the inner edge that defines the pupil; the dilator muscles are placed peripherally and arranged radially. The constrictor muscle is under parasympathetic control, and the dilators under sympathetic control, although a good deal of dilation of the pupil results from a reduction of parasympathetic input to the constrictor muscle. The bulk of the iridial stroma comprises a loose vascular connective tissue in which two arterial circles, one near the margin of the pupil, and one at the periphery of the iris, can be seen. The anterior surface of the iris is described by some authors as being covered by endothelium that is continuous with the posterior surface of the cornea, and by others as comprising a surface made up of fibroblasts and connective tissue fibres.

The Irido-Corneal Angle

This angle, which can be seen in histological sections, is a cross section of a narrow groove spanned by trabeculae of connective tissue, and runs round the inner surface of the eye. Aqueous humour percolates between the trabeculae and enters the canal of Schlemm (seen in Fig. 22.3 as several small spaces) that runs around the eyeball at this location. Fluid flows from the canal into the unique aqueous veins of the sclera (we are now beyond the periphery of the cornea) and thence to the veins that drain the sclera (Fig. 22.3).

The Lens

The lens is enclosed by the thickened (11–18 μm) basement membrane of the epithelial cells that cover the anterior surface of the lens: the lens capsule. The lens forms as a vesicle, the cells of the posterior wall of the vesicle lengthen and form long curved ribbons. These cells lose their nuclei and become the lens fibres, becoming arranged as a series of intricately spiralling lamellae

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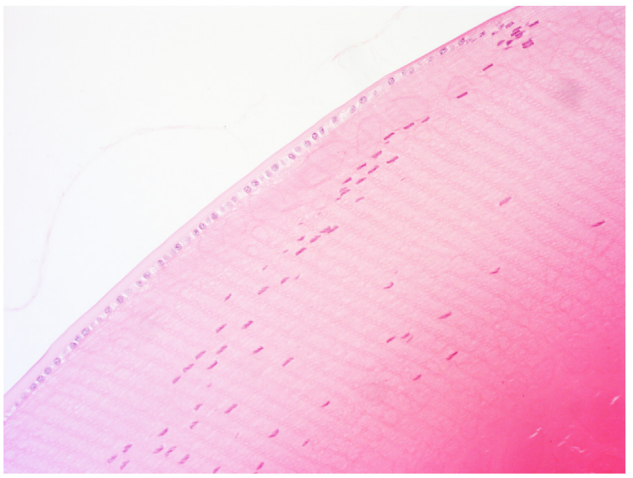


FIGURE 22.4 The lens in the area of the bow.

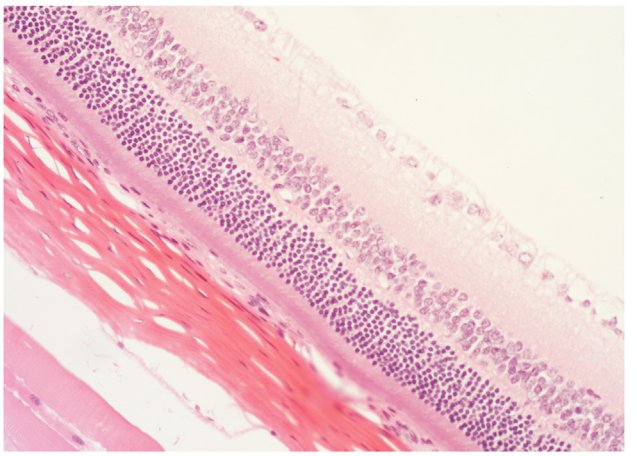


FIGURE 22.5 The retina.

(Kuszal et al., 2004). The lumen of the vesicle is lost, and the adult lens comprises the lens fibres and an anterior epithelium. At the equator or bow of the lens (an upright equator in a plane parallel to the equator of the eye itself), nuclei can be seen turning in from the anterior layer (Fig. 22.4). Sivak and Dovrat (1983) have shown that the optical properties of the rat lens change with age. They noted that the lens of the rat was over corrected for spherical aberration, indeed it showed negative spherical aberration that increased with age. This has been regarded as an adaption for nocturnal living (Walls, 1963).

The Vitreous

The transparent vitreous is a gel made up of a liquid phase comprising mostly hyaluronic acid, and a solid phase made up of fine collagen fibres. Hyalocytes produce both phases, but are difficult to detect in histological sections, although occasional nuclei are found at the periphery of the vitreous. In young rats the remains of embryonic blood vessels can be seen, these having reached the lens via the hyaloid canal during development.

The Retina

The structural complexity of the structures described earlier is as nothing compared with that of the retina (Fig. 22.5). Detailed accounts of the structure of individual types of cells may be found in textbooks of histology and in Bron et al. (1997). The retina comprises the pigment epithelium that develops from the outer wall of the invaginated optic cup, and the many layers of cells that develop from the inner wall (these may become separated and form a 'detached retina'). As the rat retina ages, the thickness and the area of the internal surface of the eyeball covered by retina decreases. It appears that ganglion cells are more affected by age than other cells of the retina, and are reduced in number by 20%–25% (Cavallotti et al., 2001).

Most accounts divide the retina into no less than 10 layers. These are set out, for reference, in Table 22.1. The albino rat has no pigment in the 'pigmented layer' of the retina, but the rods and cones of the albino rat do contain visual pigment.

The time-honoured 10 layers of the retina do scant justice to the complexity of the tissue. Many accounts refer to:

The vertical system of neurons: photoreceptors – bipolar cells – ganglion cells.

The horizontal system of neurons: horizontal cells and amacrine cells that connect across the junctions of the vertical system and sharpen the coding of the retinal image.

The supporting cells: those of Müller than run vertically through the retina and the astrocytes and microglial cells that occur amidst the other cells.

The details of the cytoarchitecture of the retina inspire a feeling of awe. Masland (2001) reported 55 distinct cell types, including 29 types of amacrine cells and 10–15 types of ganglion cells. In 2000, Masland and Raviola asked whether it was necessary to treat so many types of cells as distinct entities and pleaded 'surely nature is not malicious'! Upon detailed consideration they concluded that the distinctions were necessary. In addition to the 55 types of cells, there are at least 6 neurotransmitters, mainly amino acids, acting at conventional chemical synapses and gap junctions serving as electrical synapses. Reading recent accounts of the retina is likely to inspire feelings of elation or despair, depending on the reader's familiarity with the field. It seems likely that when the cytoarchitecture is eventually fully understood and the 'wiring diagram' properly modelled, the nonspecialist will be unable to understand, let alone remember, these details.

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TABLE 22.1 Structure of the Retina

Layer Number	Layer Name	Description
1	Pigmented epithelium	Cuboidal, heavily pigmented cells with processes that extend between the tips of the outer segments of the rods and cones
2	Bacillary layer	Outer segments of the rods and cones containing the stacks of membranous discs that contain the visual pigment
3	Outer limiting membrane	Not a true membrane but formed by the peripheral processes of the cells of Müller that support the rods and cones. These processes are held together by junctional complexes
4	Outer nuclear layer	Nuclei of the rods and cones. The segments of the rods and cones containing the nuclei are connected to the outer segments containing the photoreceptors by modified, intracellular, cilia
5	Outer plexiform layer	Synapses between the photoreceptors (rods and cones) and the bipolar cells and horizontal cells
6	Inner nuclear layer	Nuclei of the bipolar cells
7	Inner plexiform layer	Synapses between the bipolar cells and the ganglion cells and the amacrine cells
8	Ganglion cell layer	Nuclei of the ganglion cells
9	Nerve fibre layer	Unmyelinated axons of the ganglion cells running to the optic disc when they form the nerve fibre bundles of the optic nerve
10	Inner limiting membrane	Basal foot processes of the Müller cells linked by junctional complexes

The Blood–Retinal Barrier

The retina can be regarded as a displaced part of the brain, and like the brain, is defended by a barrier that allows only selected substances to reach its neurones. The barrier comprises the pigment epithelium (unpigmented in the albino rat), the epithelium of the ciliary processes, and the tight junctions between the endothelial cells of the retinal capillaries. Damage to the blood-retinal barrier can be caused by a number of toxic substances and by light. Phototoxic damage promotes the formation of free radicals that damage lipid-rich structures such as cell membranes and the membranous stacks that make up the photoreceptors of the rods and cones. The pigment epithelium is much more than merely a dark coat that prevents reflection of light, playing an important role in 'nibbling away' at the distal ends of the outer segments of rods and cones, and removing 'worn out' photoreceptor membrane. New photoreceptor discs are synthesised continuously by the rods and cones with a rod turning over its complement of photoreceptors every 10 days (Fawcett, 1994). The pigment epithelium also cycles visual pigment cycles to and from the rods and stores the Vitamin A that is an essential component of the pigment.

The Extraocular Muscles

The rat has all the extraocular muscles found in man, plus the retractor bulbis muscle, which lies beneath the eyeball and pulls it back into the orbit. The four rectus muscles (lateral, medial, superior and inferior) arise from just below the optic foramen, and are inserted into the sclera of the eyeball just behind the equator. The lateral (or external) and medial (or internal) muscles turn the eye laterally and medially; the superior rectus turns the eye upwards and a little inwards, the inferior rectus turns the eye downwards, and again, a little inwards. The superior oblique muscle runs a rather unusual course. From the orbital aspect of the frontal bone it forms a tendon that runs through a tiny cartilaginous or bony hook attached to the bone, before swinging back to the eyeball as a second muscle belly that is inserted into the sclera. It turns the eye downwards and a little outwards. The inferior oblique runs from medial to lateral beneath the eyeball and turns the eye upwards, and again, a little outwards. Acting in concert the combination of their actions permits movement of the eyeball in all directions. The splendidly named levator palpebrae superioris muscle that lifts the upper eyelid lies in the roof of the orbit and is inserted into the connective tissue of the upper lid.

The extraocular muscles are supplied by different cranial nerves that enter the orbit through the foramen orbitorotundum (see Chapter 21(3): The Autonomic Nervous System). The nerve supply of the individual muscles is as follows:

Superior oblique by the trochlear nerve, CN IV (trochlea means pulley).

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Lateral rectus by the abducens nerve, CN VI (so named because it supplies the muscle that abducts the eyeball).

Superior and inferior recti, the medial rectus, levator palpebrae superioris and inferior oblique by the oculomotor nerve, CN III.

Autonomic Supply of the Eyeball and Associated Structures

Preganglionic parasympathetic fibres travel from the Edinger–Westphal nuclei of the midbrain to the orbit via CN III, the oculomotor nerve. Here they leave the oculomotor nerve and run to the ciliary ganglion found deep in the orbit. From that ganglion, postganglionic fibres run as the short ciliary nerves to the eyeball to innervate the constrictor muscle of the pupil and the weak ciliary muscle.

Postganglionic sympathetic fibres reach the orbit in the plexus around the ophthalmic artery and join the long ciliary nerves (branches of the ophthalmic nerve (CN V (1)). The long ciliary nerves run to the eyeball and supply the dilator muscle of the pupil and provide sensory fibres to the cornea.

Blood Supply of the Eye and Associated Structures

In man the ophthalmic artery arises within the skull from the internal carotid artery, and travels to the orbit with the optic nerve via the optic foramen. In the orbit it runs close to the optic nerve giving off the long and short posterior ciliary arteries that pierce the sclera and supply the choroid. As the long posterior ciliary arteries run forwards they are joined by the anterior ciliary arteries, which arise from the arteries supplying the extraocular muscles, forming an arterial circle at the outer edge of the iris. The ophthalmic artery also gives off the central artery of the retina, which pierces the sheath of the optic nerve and finds its way deep into the nerve to appear on the surface of the retina in the middle of the optic disc.

In the rat, the ophthalmic artery arises from the palatine branch of the pterygopalatine artery, itself a branch of the internal carotid, taking a circuitous route through the foramen orbitorotundum to the posterior corner of the orbit-temporal fossa. The palatine branch of the pterygopalatine artery also gives rise to the lacrimal artery in the rat, which in man is a branch of the ophthalmic artery. Greene (1935) stated that the lacrimal artery supplies the intra-orbital lacrimal gland; Hebel and Stromberg (1976) stated that both the intra- and extra-orbital lacrimal glands were supplied by branches of the facial artery. In both the rat and man the extraocular muscles are supplied by branches of the ophthalmic artery. The posterior ciliary artery of the rat runs inside the sheath of the optic nerve, dividing into the long posterior ciliary arteries and the central artery of the retina. There are no short posterior ciliary arteries in the rat (Sugiyama et al., 1999). These authors made no mention of anterior ciliary arteries and refer to the posterior ciliary artery as a terminal artery (end artery).

In the rat, the central artery of the retina supplies more of the layers of the retina than in man, where it supplies only the ganglion cells and their nerve fibres, the remaining layers being supplied from the choroidal vessels. In the rat branches of the central artery (five to eight) of the retina radiate like the spokes of wheel, running a straight course over the surface of the retina towards its periphery (Heywood, 1973). Retinal veins run alongside the arteries, appearing darker and wider than the arteries when examined with an ophthalmoscope. The yellow-pink/creamy optic disc seen in man is not apparent on examination of the rat eye. This has been attributed this to the shape of the cup of the head of the optic nerve, described as 'narrow, funnel-shaped, and oblique to the disc surface', which gives the disc a dark appearance (Cohen et al., 2003).

Venous blood from the choroid drains to the several vorticeal veins that penetrate the sclera and join the anastomosing veins behind the eyeball. The vorticeal veins are so named because the many venous channels of the choroid appear to sweep up to these veins like a vortex. The veins of the retina run with the arteries, but leave at the optic nerve to join the ophthalmic veins. Venous blood from all the tissues of the orbit is collected by the superior and inferior ophthalmic veins, joining to form the ophthalmic vein, which drains to the cavernous and petrosal sinuses within the skull. Timm (1979) reported that the veins behind the eyeball of the rat form a plexus, but in the mouse there is a confluent venous sinus. Some drainage from the veins of the orbit to those of the pterygopalatine fossa also occurs.

Because the albino rat has no melanin in the 'pigmented layer' of the retina or its choroid, the blood vessels of the choroid can be seen more clearly on ophthalmoscopy than in pigmented animals. It is important not to confuse melanin (absent in the albino rat) with the visual pigment of the rods and cones of the retina, this is present in both white rats and albino humans, without it neither could see.

Lymphatic Drainage of the Eye

It has often been said that the eye lacks a system of lymphatic drainage, but more recent work has shown that this is not true, and that the drainage system of the aqueous humour might best be regarded as at least an auxiliary part of the lymphatic system (Nakao et al., 2012). Lymphatic drainage of the uveal tract has also been suggested.

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The extraocular structures, muscles and the intra-orbital lacrimal gland and the Harderian gland all appear to have a conventional lymphatic drainage system.

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Chapter 23

The Ear

The ear can be divided into the external auditory meatus (or outer ear), the middle ear and the inner ear. The first is relatively simple, the second is complicated, and the third is very complicated. The ear is of interest to toxicologists because of the effects of drugs and other chemicals on auditory and vestibular function. Ototoxic effects were initially noticed soon after the introduction of the aminoglycoside antibiotic streptomycin for the treatment of tuberculosis. The toxicologist is also interested in Zymbal's gland, a large sebaceous gland associated with the external ear canal (the external auditory meatus) that may develop tumours in response to carcinogens (see Chapter 24: The Skin or the Integument).

Some of the following account is perhaps a little too detailed for the general toxicologist, but can be reserved for reference if a specific point about the ear structure arises in the course of toxicological work. The interested reader is encouraged to consult Mason (2013, 2015), Ekdale (2016), Li et al. (2015) and Wysocki (2008) for accounts of the anatomy of the middle ear (Li et al. and Wysocki deal with the whole temporal bone; Wysocki reviews, critically, other accounts of the subject); Maier and Ruf (2016) for an account of the evolution of the mammalian ear, and Anthwal and Thompson (2016) and Sienknecht (2013) for accounts of development of the ear. Both the accounts of the development of the ear contain details of the genes controlling ear development: we do not explore this fascinating area in this chapter. The physiology of the inner ear is also not explored in any detail: Fettiplace and Hackney (2006) have provided a useful review of the functioning of the hair cells of the cochlea; Pickles' book (2013, fourth revised edition) provides an up to date account of auditory physiology. Hitier et al. (2016) have provided an excellent and well-illustrated account of the anatomy of the rat's vestibular system. Descriptions of the topographical anatomy of the human ear can be found in any textbook of anatomy, that provided in the ninth edition of *Cunningham's Textbook of Anatomy* (Brash, 1951), though now nearly 70 years old, is particularly detailed. The reader is, as always, warned about the dangers of extrapolating details of anatomy from man to the rat. The histology of the ear is dealt with in detail by Fawcett (1994).

Sound waves travel through the air contained in the external auditory meatus and cause vibration of the tympanic membrane (ear drum). Movement of the tympanic membrane is relayed by the chain of ossicles to the inner ear. The third ossicle, the stapes, is set in an oval window in the inner ear. Movement of the footplate of the stapes sets up pressure waves in the fluid of the inner ear, the perilymph (all this will be explained in more detail shortly) causes displacement of the basilar membrane and stimulation of the auditory receptors. The space containing the perilymph is a closed space (almost closed: see below for a note on the aqueduct of the cochlea) with a bony wall, but limited by membrane at the round window that lies between the inner ear and the middle ear. This window, which is very small and partially hidden in the rat, is closed by the second tympanic membrane. The official names for the windows just mentioned are the fenestra ovalis (or fenestra vestibuli) and the fenestra rotundum (or fenestra cochleae). The middle ear is connected to the pharynx by the Eustachian tube, which allows the pressure in the middle ear to be equalised with that of the pharynx. Swallowing opens the tube, which is why boiled sweets are provided on some aircraft. The tube opens when you swallow, and air that has expanded under low pressure in the middle ear (aircraft cabins are pressurised to lower than atmospheric pressure at sea level) flows through the tube relieving the feeling of pain in the ear. The inner ear comprises the cochlea, which contains the structures essential for hearing, and the three semicircular vestibular canals that are important for balance.

DEVELOPMENT OF THE EAR

It has been generally agreed that the malleus and incus develop from the first branchial (pharyngeal) arch and the stapes from the second. The origin of the incus from the first arch has been disputed; Whyte et al. (2009) have argued that, at least in man, part of the incus is derived from the second arch. Work on the comparative anatomy,

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development and evolution of the mammalian ear continues, and the rodent ear has been the subject of much recent research. This work has thrown up a mass of new facts and ideas, as well as clarification of the anatomy of the middle ear. The complexity of processes involved in the development of the ear is remarkable, but beyond the scope of this account (Maier and Ruf, 2016; Ekdale, 2013, 2016; Mason, 2013, 2015).

THE EXTERNAL EAR

The external ear of the rat is leaf-shaped with a folded margin, supported by elastic cartilage and covered by thin skin bearing fine hairs. Ears are moved by extrinsic muscles innervated by branches of the facial nerve (CN VII). The external auditory meatus, the canal that runs inwards to the tympanic membrane or ear drum, is lined by skin and supported in its outer part by cartilage. The inner part of the meatus is 1−2 mm in length, and has a bony wall provided by the ectotympanic bone, which also forms the tympanic bulla. Zymbal's gland, lying partly within, and partly outside the meatus, provides the rat equivalent of human cerumen or wax (see Chapter 24: The Skin or the Integument).

In man, there is no tympanic bulla, instead there is a tympanic plate which forms the floor, most of the anterior, and part of the posterior wall of the bony part of the meatus. The tympanic plate is much expanded as the tympanic bulla in the rat and in other mammals. It is a large structure enclosing the middle ear cavity that resembles a rather flat sugar bowl turned on its side; the lid being the tympanic membrane, and the base (the medial wall of the middle ear) provided by the petrous temporal bone. The petrous temporal bone to which the bulla is rather loosely attached, also forms part of the roof of the middle ear, extending over the inner ear as the tegmen tympani (tegmen = roof).

THE MIDDLE EAR

The middle ear is divided into three regions: the epitympanum (above), which contains most of the malleus and the incus, the empty hypotympanum (below), and between them the mesotympanum.

THE TYMPANIC MEMBRANE OR EAR DRUM

The oval ear drum of the rat provides the lateral wall of the mesotympanum, and measures about 6 mm by 4 mm, the longer diameter being vertical. It is tilted so that the upper edge lies lateral to the lower edge at an angle of about 75 degrees between the drum and the floor of the meatus (Dr. M.J. Mason, 2016, personal communication). The drum is about 5 μm thick, and covered on both surfaces by a simple squamous epithelium (Hebel and Stromberg, 1976). Between the epithelia lies a thin stratum of connective tissue with fine collagen fibres running radially, and at the periphery, circumferentially. Most of the circumference of the drum is attached to a groove in the wall of the tympanic bulla. This groove is incomplete superiorly, and here the drum is attached to the petrous temporal bone. In both man and rat, the drum can be divided into an upper, smaller, pars flaccida and a larger, lower, pars tensa. The rat has a pars flaccida that is proportionally much larger than that found in man.

THE MIDDLE EAR OR TYMPANIC CAVITY

The middle ear contains a auditory ossicles, a chain of three small bones that connects the tympanic membrane with the inner ear. These are the malleus (mallet or hammer), incus (anvil) and stapes. The stapes really does look like a stirrup, its footplate occludes the oval window (fenestra ovale or fenestra vestibuli), one of the two openings between the inner ear and the middle ear. The function of this chain of ossicles is to overcome the mismatch of impedance between the air through which sound waves travel to the ear drum, and the fluid, of the inner ear. The fenestra ovale is much smaller than the tympanic membrane, so that pressure applied to the tympanic membrane is increased proportionally.

The middle ear connects to the nasopharynx via the Eustachian tube, which has a partially bony wall as it leaves the anterior part middle ear, but soon becomes entirely cartilaginous. The medial wall of the middle ear is not flat, but bears a promontory that extends towards the tympanic membrane and is somewhat flatter in the rat than it is in man. The relationships of the structures of the middle ear are shown in Figs. 23.1 and 23.2. In Fig. 23.1, the malleus and incus have been removed to show the details of the medial wall of the middle ear.

Note that there is a second opening, the round window (fenestra rotundum) that lies posterior to the promontory with the oval window above its posterior end. The position of the oval window is shown by the stapes which is shown lying horizontally. The round window is hidden in a niche and much less obvious than shown in the diagram.

The middle ear is lined with a low cuboidal epithelium that is continuous with the epithelium of the Eustachian tube and the nasopharynx. The Eustachian tube is lined by a pseudostratified ciliated columnar epithelium that extends over the medial wall of the middle ear below the promontory, before turning up to the posterior edge of the epitympanum and running up to the anterior edge of the epitympanum (Albiin et al., 1986). Secretory cells,

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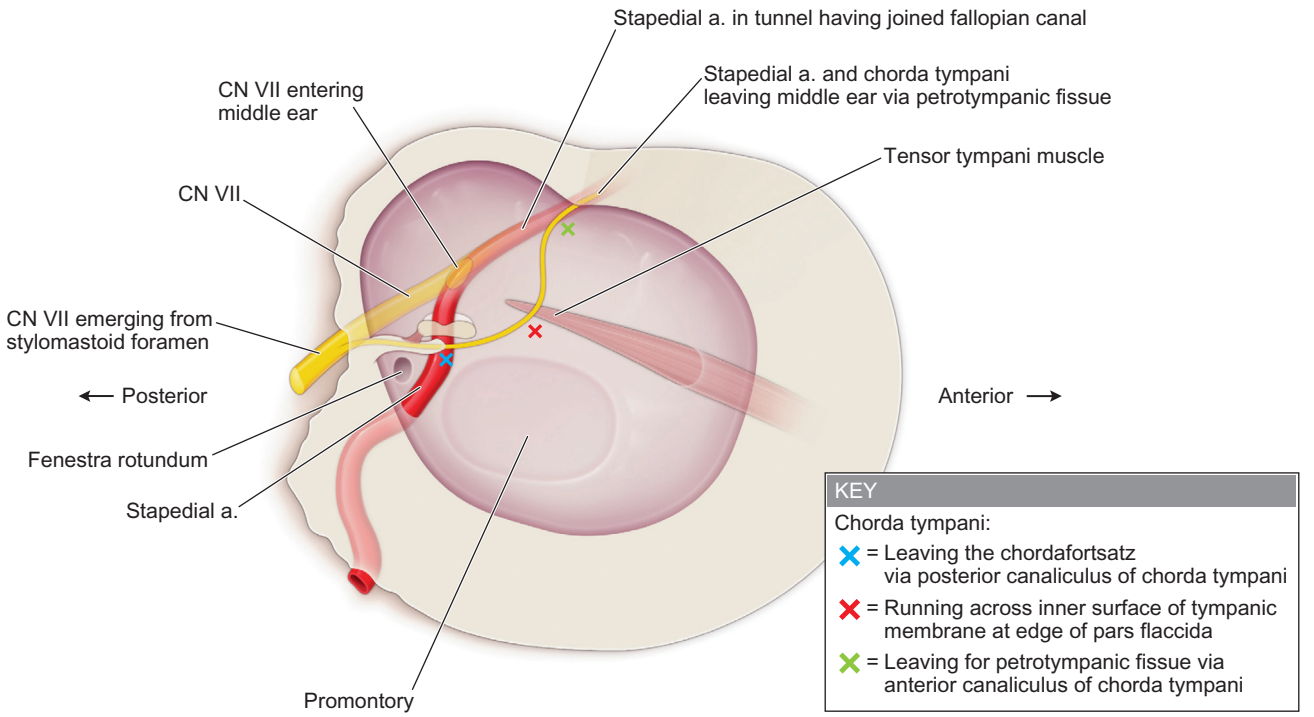


FIGURE 23.1 Medial wall of rat middle ear with ossicles removed.

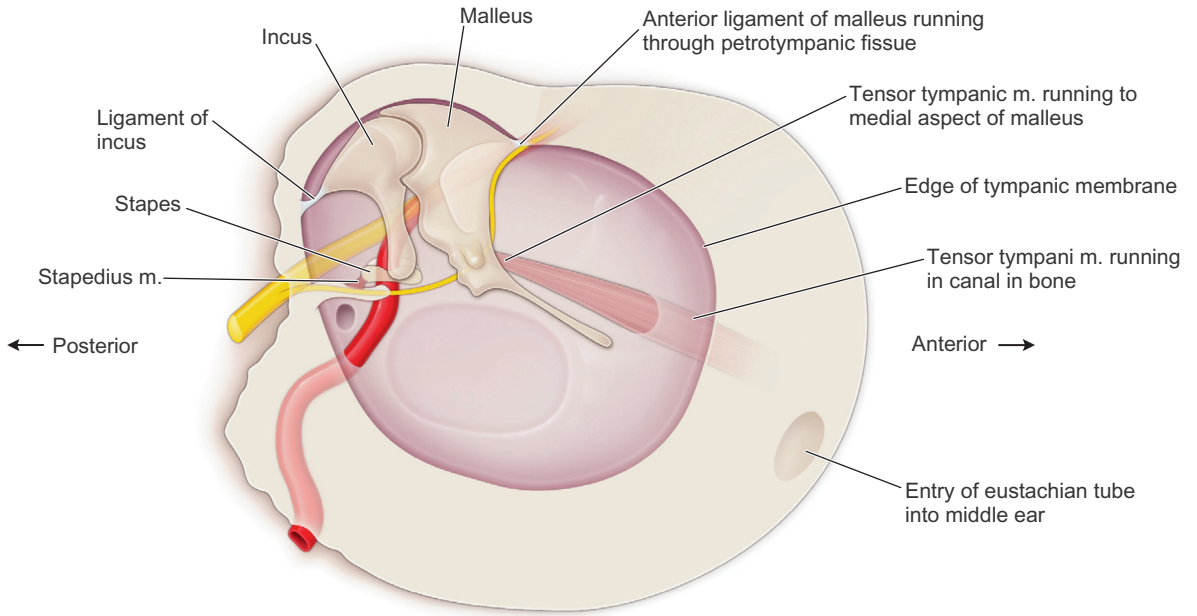


FIGURE 23.2 Medial wall of rat middle ear with ossicles in place.

but not goblet cells, are found among the ciliated cells of these tracts. A thin layer of connective tissue containing blood and lymphatic vessels and nerve fibres underlies the epithelium of the middle ear. What is often not realised is that the ossicles of the middle ear are entirely draped with epithelium.

The anatomy of the middle ear is completed by one artery, two nerves and two muscles.

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The pterygopalatine artery leaves the internal carotid just below the tympanic bulla and enters the middle ear, becoming the stapedial artery as it runs through the arch of the stapes (see Fig. 23.1). In the rat the stapedial artery gives off the middle meningeal artery, which passes through the stapes and enters a tunnel (or canal) in the medial wall of the middle ear, often joining the Fallopian canal. The artery runs on, hidden in its canal, to emerge at the petrotympanic fissure (Glaser's fissure) on the base of the skull, a busy fissure as the chorda tympani and the anterior ligament of the malleus also emerge from it.

The facial nerve enters the inner ear from the middle cranial fossa. In man it enters the inner ear via the internal auditory meatus with the vestibulo-auditory nerve (CN VIII) and a small artery. There is no well-defined internal auditory meatus in the rat, so the nerves enter the petrous temporal bone separately. The facial nerve never really appears in the middle ear, rather it runs across the medial wall in the Fallopian canal. This canal forms a ridge above the fenestra ovale as it passes caudal and downwards towards the posterior wall of the bulla to emerge at the stylomastoid foramen behind the tympanic bulla. Before the facial nerve emerges it gives off the chorda tympani, an important branch that runs back into the middle ear through the caudal chordafortsatz (fortsatz is German for process). This well-defined process arises from the posterior wall of the bulla. The chorda tympani emerges at or near the tip of this process, via the posterior canaliculus of the chorda tympani and runs free across the inner surface of the tympanic membrane, the medial surface of the malleus, and then along the anterior process of the malleus to enter the anterior canaliculus of the chorda tympani and emerge from the lower surface of the skull at the busy petrotympanic fissure. Although the caudal chordafortsatz is well developed in the rat (Mason, 2015), it has not been described in man. Throughout its passage through the middle ear, the chorda tympani follows the edge of the pars flaccida of the tympanic membrane.

The two muscles of the middle ear are the stapedius and the tensor tympani. Stapedius originates from the posterior wall of the bulla, its origin in man lies inside a small conical projection from the wall called the pyramid, but in the rat, it emerges from the upper surface of the caudal chordafortsatz (the point of emergence is called the pyramid in some accounts), and is attached to the stapes by a tendon. The tensor tympani runs from a tunnel in the anteromedial wall of the bulla to the muscular process on the medial surface of the malleus. In man, the tensor tympani arises from within a bony tunnel that lies superior to the Eustachian tube.

The tensor tympani muscle, true to its first branchial arch origin, takes its innervation from the trigeminal nerve (CN V). The stapes and stapedius, as second arch derivatives are innervated by the facial nerve (CN VII). The facial nerve also gives off a small branch, the greater superficial petrosal nerve that runs through the tegmen tympani to emerge inside the skull.

In man a branch of the ninth cranial nerve (glossopharyngeal) enters the middle ear via narrow canaliculus. This is Jacobson's nerve. It ramifies over the promontory and is the origin of the lesser superficial petrosal nerve. This arrangement is not found in the rat (see Chapter 21).

For further details of the muscles and their nerve supply, see Van Den Berge and Wirtz (1989a,b) and Van Den Berg and Van Den Wal (1990).

The Auditory Ossicles

The ossicles are shown in Fig. 23.3. Fig. 23.2 shows the manubrium (from the Latin manus, meaning hand and

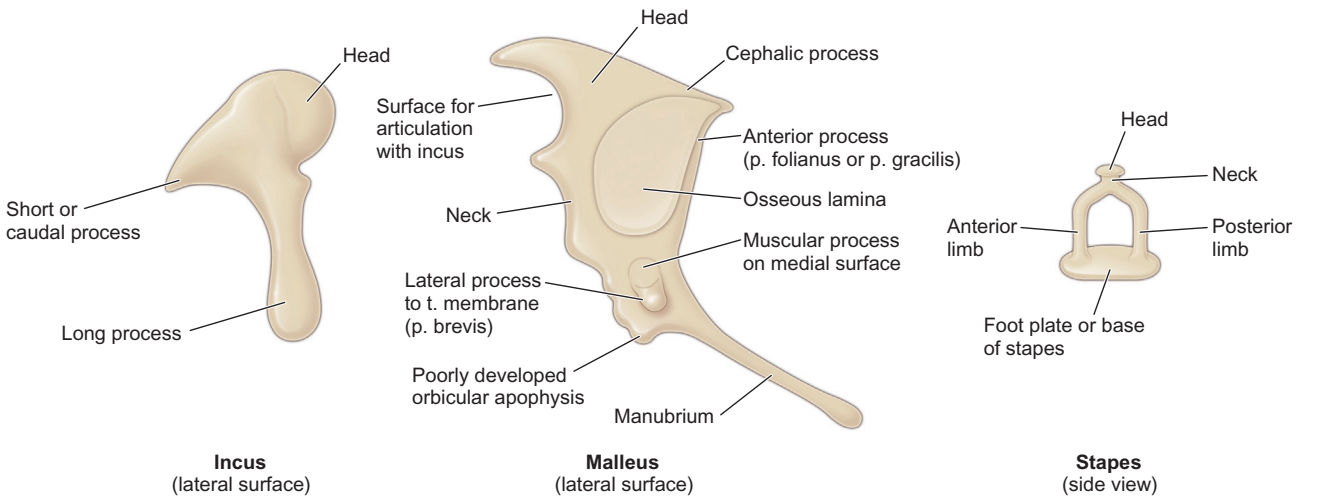


FIGURE 23.3 Rat auditory ossicles.

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manubrium meaning handle) of the malleus running downwards and forwards in the rat; in man, it runs downwards and back.

In man, the malleus and incus are connected by a synovial joint which is a synchondrosis in the rat, an immovable cartilaginous joint. The joint between the incus and the stapes is synovial in the rat.

The anatomical features of these bones have been used in rodent classification, and for a detailed description of the auditory ossicles of rodents Mason (2015) should be consulted.

THE INNER EAR

The inner ear of all mammals consists of a complicated series of spaces that form the bony labyrinth within the petrous temporal bone. These spaces contain a complicated series of membranous canals known as the membranous labyrinth. Some anatomy textbooks insist that the bony labyrinth has no independent existence, but is merely a series of spaces. This is not quite correct, as the spaces of the bony labyrinth are surrounded by unusually dense bone, and in the rat the bony labyrinth, with its walls, can be separated from at least a part of the petrous temporal bone.

The Bony Labyrinth

The bony labyrinth is a complex space of three parts: the conical cochlea, the semicircular canals and the vestibule, which connects the former two parts.

The entire bony labyrinth is lined with periosteum and contains perilymph derived from the capillaries of the periosteum, in the same way as lymph is formed elsewhere in the body. The periosteum is mostly covered by a thin squamous endothelium, but in the osseus spiral lamina and the spiral ligament, the endothelial lining is deficient and bone cells come into contact with perilymph (Chole and Tinling, 1994).

The wall of the bony labyrinth is perforated by three single openings, and several multiple openings that allow auditory and vestibular nerves and accompanying blood vessels enter (or leave) the inner ear. The three single openings are the fenestra rotundum (closed by the secondary tympanic membrane), the fenestra ovale (closed by the footplate of the stapes) and the minute opening of the aqueduct of the cochlea: a short narrow passage way that allows perilymph to come into contact with cerebrospinal fluid. It is thought the perilymph is absorbed into the cerebrospinal fluid. In man the aqueduct runs through the petrous temporal bone to emerge in or near the jugular foramen, but the location in the rat is less well described. On its way to the middle ear, the facial nerve passes over the junction between the cochlea and the vestibular system.

Perhaps the most difficult aspect of the bony labyrinth to grasp is its position in the skull. It lies roughly in the long axis of the petrous temporal bone with the cochlea in front, the vestibule in the middle and the semicircular canals behind. The cochlea points forwards, outwards (laterally) and downwards; its basal turn bulges the medial wall of the inner ear and underlies the promontory.

The Cochlea

The cochlea is a double tube wound around a bony, conical structure called the modiolus. The modiolus is shaped like a wood screw, the threads of which partially divide the two tubes from one another. The division is completed by the basilar membrane that runs from the edges of the 'threads' (correctly called the osseous spiral lamina, and which makes two and a half turns to the spiral ligament which springs from outer wall of the cochlea (Fig. 23.4)).

Understanding the cochlea is easier if we imagine it straightened out, see Fig. 23.5.

One of the two tubes, the scala vestibuli (scala meaning ladder, vestibuli meaning that it is connected with the vestibule) is connected, at the helicotrema (trema means a hole and helico is derived from helix) with the scala tympani (scala as before, tympani because this tube ends at the secondary tympanic membrane) which is blind ended. In vertical sections of the cochlea, the scala vestibule always lies above the scala tympani.

The complexity of the cochlea is increased by the cochlear duct, or scala media, which is a part of the membranous labyrinth. It is inserted into the scala vestibuli and runs its full length before ending blindly (Fig. 23.5). The blind end of the cochlear duct helps to limit the size of the helicotrema. From its distal end, the basilar membrane narrows as it runs from the edge of the osseous spiral lamina to that of the spiral ligament, separating the scala tympani from the cochlea duct (Fig. 23.4). The cochlear duct is separated from the scala vestibuli by the thin Reissner's (vestibular) membrane (see Fig. 23.4).

Membranous Labyrinth

The membranous labyrinth is a real structure that can be removed with difficulty from the bony labyrinth for examination as a distinct anatomical entity. Along with the cochlear duct, the membranous labyrinth is composed of the small saccule and utricle that lie within the vestibule of the osseous labyrinth, and the semicircular canals that arch outwards from the utricle. The majority of the membranous labyrinth lies rather loosely in the bony labyrinth, largely surrounded by perilymph, although in some places it lies against the wall of the bony labyrinth.

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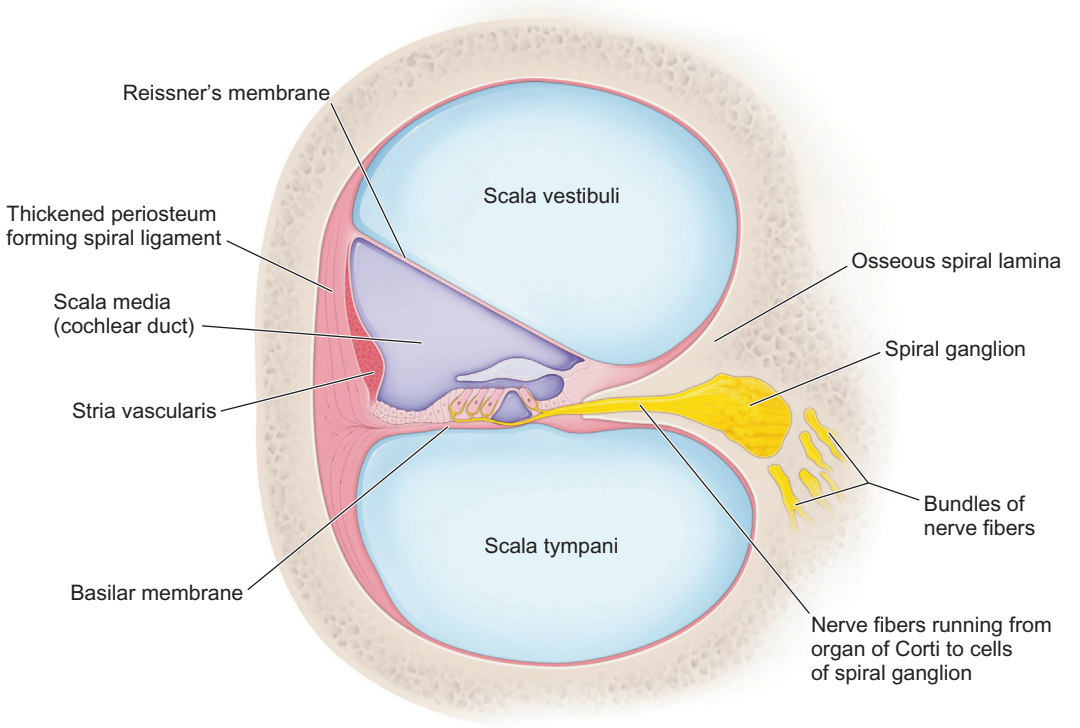


FIGURE 23.4 Section of cochlea showing the scalae.

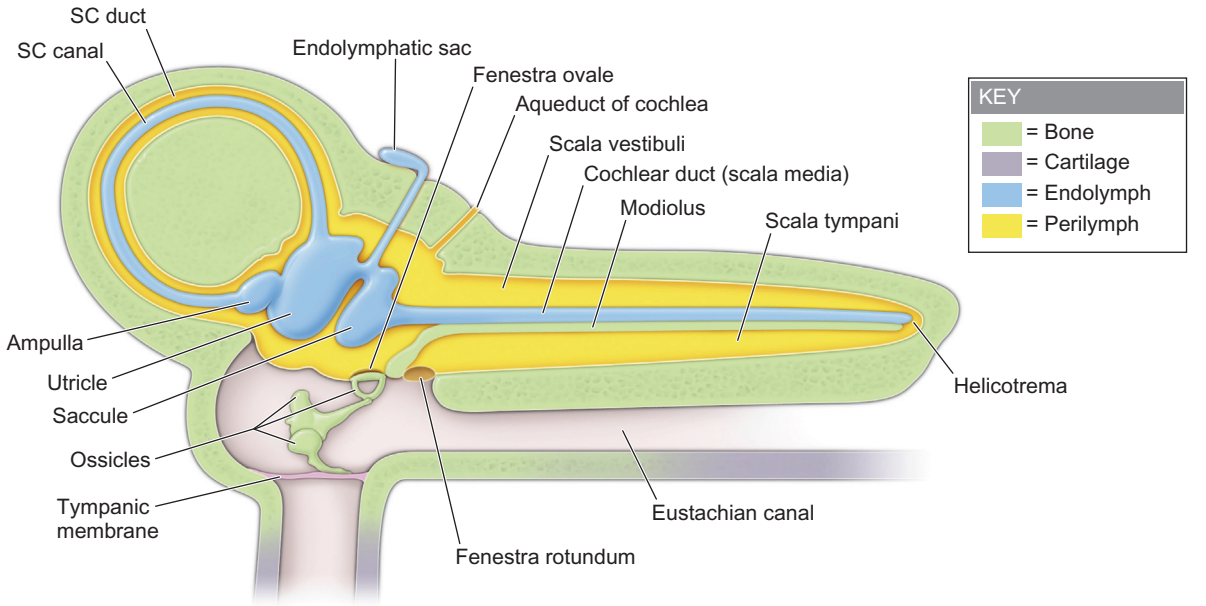


FIGURE 23.5 Generalised schematic diagram of mammalian ear. *SC*, semicircular canal.

The three semicircular canals (anterior, posterior and lateral) are about 6 mm in length. The anterior and posterior canals are about 0.6 mm in diameter, and the lateral 0.2 mm in diameter. All are of course narrower than the semicircular ducts of the bony labyrinth, and are partially surrounded by perilymph (Cummins, 1924). One end of each canal is expanded to form an ampulla (each ampulla is surrounded by an expansion of the bony labyrinth also

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called, rather confusingly, an ampulla), the other end of the anterior canal joins the saccule, but the other ends of the lateral and posterior canals join to form a common canal (the common crus) before joining the saccule. The common crus does not occur in all mammals, but is present in both man and the rat, although there are minor strain differences between the lateral semicircular canals of Wistar and Sprague–Dawley rats (Lindenlaub and Oelschläger, 1999).

Visualising the orientation of the semicircular canals presents a few difficulties. The posterior canals lie along the main axis of the petrous temporal bone, the anterior canals lie at 90 degrees to this axis and the lateral canals lie horizontally. This orthogonal arrangement is important to ensure that similar information from both sides of the head results from acceleration in a specific direction. The arrangement of the canals is seldom perfectly orthogonal, but whether this deviation from geometric perfection is important seems to be unknown (Berlin et al., 2013).

The utricle is about 1.3 mm in length, 0.4 mm in width and 0.7 mm in height. The saccule is a flattened structure about 0.9 mm in length and 0.4 mm in width (data adapted from Hebel and Stromberg, 1976). The utricle and saccule are connected by a narrow duct that runs in the petrous temporal bone, and gives of a branch that connects to the endolymphatic sac located in the subdural space to the side of the cerebellum. It is believed that endolymph is absorbed across the walls of this sac. The endolymph that fills the membranous labyrinth is produced by the stria vascularis, and is very different from the perilymph that surrounds the membranous labyrinth. In composition, endolymph is similar to intracellular fluid with a high potassium concentration. Perilymph, on the other hand, has a composition similar to that of intercellular fluid.

The three ampullae, the utricle and the saccule all contain receptor organs and are innervated via tiny canals in the walls of the bony labyrinth by the vestibular division of the vestibule-auditory nerve (CN VIII).

Receptor Systems of the Inner Ear

It is conventional to divide the receptor systems of the inner ear into those concerned with reporting changes in position of the head (receptor systems of the utricle and saccule), those that monitor the acceleration that occurs at the onset of movement of the head (the receptor systems of the semicircular canals), and those that monitor the frequency and amplitude of sound waves impinging on the outer ear (receptor system of the cochlea). Each is based on the conversion of a mechanical displacement into action potentials that travels to the brain via the vestibular or auditory nerve.

Activity of the mechano-electrical transducers, which relies on the conversion of mechanical displacement into receptor and action potentials, is mediated by the bending of stereocilia. These are modified microvilli with a well-developed central core of microtubules. Each stereocilium has a narrow base, where that bending of the structure occurs. In the semicircular canals, bending is induced by the relative movement of the stereocilia and the overlying gelatinous cupula that is surrounded by endolymph; in the utricle and saccule by displacement of the overlying otolith membrane containing fine granules of calcium carbonate, and in the cochlea by displacement of the basilar membrane, which moves the stereocilia of the hair cells relative to the tectorial membrane. The stereocilia are inserted into an overlying structure that is acellular and comprises glycoproteins and fine collagen fibres.

The receptor systems of the three semicircular canals are the cristae of the canals (one crista per ampulla); those of the utricle and saccule are the maculae of these structures, and that of the cochlea is the organ of Corti. The maculae of the saccule and utricle are arranged so that the otolith membranes of each macula lie at 90 degrees to each other. Each macula responds to both the pull of gravity on its otolith membrane and displacement of the membrane due to movement.

Common to all these receptor systems is that the cells that bear the stereocilia (the hair cells) are held in place by specialised supporting cells. The hair cells are contacted by afferent nerve endings and a variety of different forms of synapse have been described. In the organ of Corti, the outer hair cells (OHCs) are innervated mainly by efferent nerve fibres, and act not as receptors but as modulators of the activity of the inner hair cells. Detailed descriptions of the cells of these receptors may be found by Fawcett (1994).

Histology of the Sound Receptor System of the Cochlea

The fundamental mechanism of hearing involves vibration of the basilar membrane induced by the movement of the footplate of the stapes, and the propagation of pressure waves into the perilymph of the scala vestibuli. A range of theories of how the cochlea works have been proposed, but there is little doubt that sound waves of differing frequencies produce maximum displacement of the basilar membrane at specific locations along its length. Displacement of the basilar membrane leads to propagation of pressure waves in the scala tympani, and the secondary tympanic membrane of the fenestra rotundum vibrates in sympathy with the footplate of the stapes.

The fibres across the width of the basilar membrane increase in length as it winds its way up the cochlea. The

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irresistible similarity between this set of fibres and the strings of a piano or harp was noticed in the 19th century, and it seemed obvious that individual 'strings' would vibrate according to the frequency of the sound wave producing the disturbance of the membrane. This idea, the place theory of frequency detection, has since been modified, but remains a useful basis for discussing the reception of sound energy. Displacement of the basilar membrane clearly stimulates the hair cells of the organ of Corti, which act as transducers in converting mechanical displacement into changes in their membrane potential, and so causing action potentials to be produced in the fibres of the auditory nerves. Although an oversimplification, it could be said that the frequency of the incoming sound determines which hair cells are activated, and the amplitude determines the degree of displacement, which in turn is reflected by the frequency of firing of action potentials of the afferent nerve fibres in synaptic contact with these cells.

The histological structure of the organ of Corti is similar in all mammals and is illustrated in Fig. 23.6.

A number of eponyms have been applied to the cells, and groups of cells of the structure are shown in the figure; detailed accounts can be found in textbooks of histology (Fawcett, 1994). A number of points should be noted. Refer to Figs. 23.4 and 23.6 when reading the following text.

1. The three scalae are separated from each other by the basilar membrane and the vestibular membrane (Reissner's membrane). The basilar membrane is relatively thick and reinforced by the connective tissue 'strings' of the membrane. Blood vessels run in the connective tissue of the basilar membrane. Reissner's membrane comprises little more than two layers of squamous epithelial cells.
2. The osseous spiral lamina and the spiral ligament.
3. The stria vascularis: a unique epithelium in that it is invaded by capillaries.
4. The gelatinous tectorial membrane is often displaced or poorly preserved in histological sections.
5. The triangular inner tunnel defined by the inner and out pillar cells. This tunnel and the outer tunnel (space of Nuel) are filled with perilymph, not endolymph. Perilymph is said to diffuse into these spaces through the basilar membrane.
6. The several rows of OHCs and the single row of IHCs.
7. The cells of the spiral ganglion lying in the spiral tunnel of the modiolus and the nerve fibres running via the osseous spiral lamina to the organ of Corti.

The basilar membrane moves about a point defined by the foot of the inner pillar cell. The stereocilia of the OHCs are in contact with the tectorial membrane, but those of the IHCs are not.

Inner Hair Cells

These are the essential receptor cells of the organ of Corti. Linkage of their stereocilia by lateral links and tip links (fine thread-like fibres running from the top of one

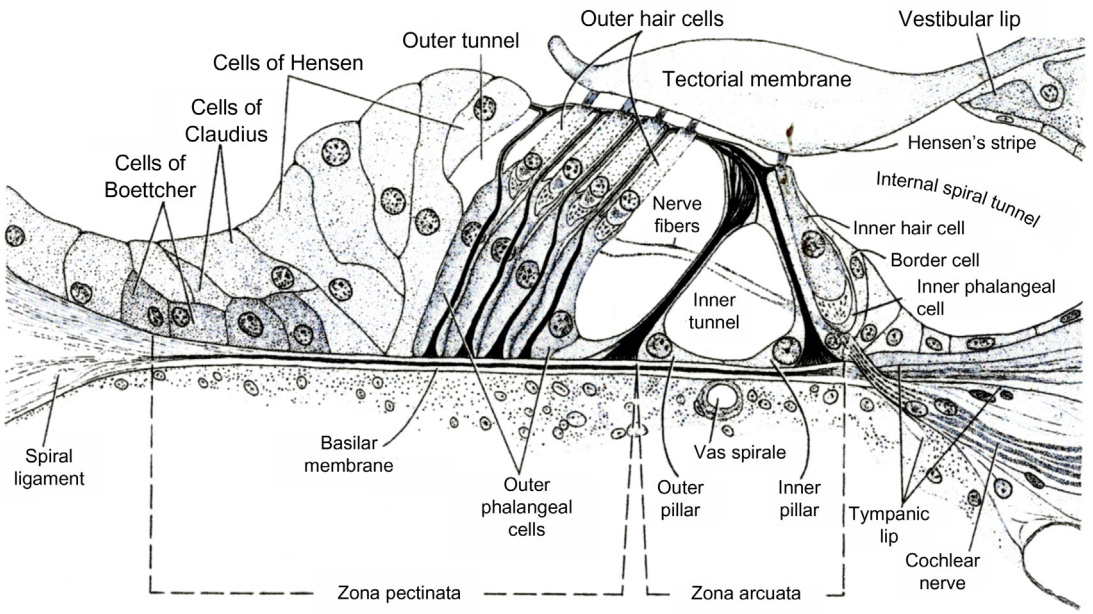


FIGURE 23.6 Radial transection of the organ of Corti, from the upper part of the first coil of human cochlea. *Slightly modified from Fawcett, D.W., 1994. A Textbook of Histology, twelfth ed. Chapman & Hall, New York, London.*

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stereocilium to the lateral aspect of an adjacent, taller, stereocilium) cause the stereocilia to move as a group. Displacement leads to the opening of mechanically gated ion channels and generation of receptor potentials. According to Fettiplace and Hackney (2006) the stereocilia are not embedded in the tectorial membrane and respond to 'basilar membrane velocity rather than displacement'.

Outer Hair Cells

There are three rows of OHC. Each hair cell carries a group of stereocilia arranged in a W formation (this is also the case with the single row of IHC). The major function of the OHCs seems to be to amplify the movement of the basilar membrane, which they appear to do by shortening when stimulated by interaction of their stereocilia with the tectorial membrane. Shortening is dependent on a contractile protein molecule akin to myosin. Amplification of the movement of the basilar membrane leads to stimulation of the IHCs, and a sharpening of the frequency response of the system. This response is further sharpened by efferent nerve fibres running back to the organ of Corti from the auditory nuclei of the brainstem. Activity of these nerve fibres leads to inhibition of IHC adjacent to those which have been stimulated and results in a form of 'surround inhibition' that reduces the response to a specific frequency (Fettiplace and Hackney, 2006; Dallos, 1992).

OTOTOXICITY

The general toxicologist may be wondering why these cells have been discussed in even the modest level of detail provided above. The reason is that a small, but important, number of drugs have been shown to be capable of damaging the activities of hair cells of both the vestibular system and of the cochlea. Although the aminoglycoside streptomycin was the first drug recognised as capable of damaging hearing and causing disturbances of balance, other aminoglycosides, including gentamicin and neomycin have shown the ability to cause apoptosis of hair cells, potentially resulting in permanent damage to hair cells. The vestibular system is particularly sensitive to gentamicin, whereas the cochlea hair cells are particularly sensitive to neomycin.

Sensitivity to the ototoxic effects of aminoglycosides has a genetic component thought to depend on inheritance of a mutation of maternal mitochondrial DNA that affects mitochondrial ribosome function, and renders them particularly susceptible to damage by aminoglycosides (Huth et al., 2011). It has been suggested that if mitochondria evolved from intracellular prokaryotic symbiotes, mitochondrial ribosomal function should be expected to be more susceptible to the effects of aminoglycosides than that of cytosolic ribosomes, after all aminoglycosides work by disrupting ribosomal function in prokaryotes, that is in bacteria.

The antimitotic drug cisplatin produces damage to the cochlea, and the loop diuretics frusemide and ethacrynic acid, salicylates and quinine are other well-known drugs with these side effects. In the case of the loop diuretics, the damage may be to the stria vascularis of the cochlear duct. The interested reader will find further discussion of ototoxicity by Huth et al. (2011), Rybak (1986) and Schnacht et al. (2012).

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Chapter 24

The Skin or the Integument

Toxicologists require a detailed knowledge of the histology of the rat's skin, because the rat is used in a wide range of studies dealing with the response of the skin to damage caused by chemicals, radiation and physical injury. Studies of cosmetics, including hair products, were once undertaken in the rat, but public pressure led to an ethical reappraisal, and these studies are now largely undertaken in in vitro systems. Excellent accounts of the histology of the skin are provided in standard textbooks of histology (Fawcett, 1994; Weiss, 1988; Monteiro-Riviere, 1998). The early development of the field is traced by Cowdry (1932), by Trotter (1932), and in the monograph by Montagna (1962, 1974). The reader is advised that many accounts of the histology of the skin do not specify the species described, and there are substantial interspecies differences in skin structure. Human skin has been studied more than that of any other mammal; the skin of the mouse has been studied in more detail than that of the rat, especially as far as the control of the hair cycle is concerned. The rat differs from man in that no apocrine sweat glands are found in the rat. The rat has eccrine sweat glands that are limited to the palmar surfaces of the front and hind paws, but the rat is well supplied with sebaceous glands; those of the skin are described in this chapter. Others include Zymbal's gland, described in this chapter, and the preputial glands described in Chapter 18, Male Reproductive System. Mecklenburg et al. (2013) have provided an excellent description of rodent skin pathology.

INTRODUCTION TO RAT SKIN

The skin and the lining of the respiratory tract provide the most important interface between the rat and its environment. The entire surface of the body is covered by skin which borders the oral, anal and genital orifices, lines the external ear canal and covers the lateral surface of the eardrum and the outer surfaces of the eyelids.

It should be remembered that the skin is the largest organ of the body. In man, it represents around 6%–7% of body weight. Skin protects the body from invasion by microorganisms and from physical injury; is important in thermoregulation and is a vitally important sensory organ. In addition, it acts as an excretory organ, stores vitamins and is metabolically and immunologically active. In wild animals the skin often provides protective camouflage.

Skin can be divided into hairy skin and glabrous (hairless) skin. With the exceptions of the pads of the fore and hind paws, and the small area of skin lateral to the nostrils, the skin of the rat carries a covering of hair or fur. In man glabrous skin is restricted to the volar surfaces of the hands and feet, the umbilicus, nipple, margins of the lips, the medial surfaces of the labia majora and the whole of the labia minora, the glans penis and the prepuce. Hairless and glabrous are not synonymous; the smooth surface of the head of a bald man is not covered by glabrous skin. Hairs play an important role as mechanical transducers. Specialised hairs, the whiskers or vibrissae are found on the face, mainly to the sides of the nostrils, and are described in some details later in this chapter. Large, rather coarse hairs or bristles are found in all areas of the skin, and these too, are important in sensation. The skin of the tail is not hairless, but is covered by scales with hairs emerging between the scales.

GENERAL HISTOLOGY OF RAT SKIN

Skin comprises the dermis, epidermis and subcutis (hypodermis). The epidermis is a cornified stratified squamous epithelium that is generally just a cell or two thick, much thinner than in man. The dermis is a thick layer of connective tissue that supports the epidermis and is well supplied with blood vessels, lymphatic vessels and nerve fibres. The dermis also lodges the hair follicles with their attendant sebaceous glands and the sweat glands where they occur. The epidermis and dermis meet at a rather flat interface. This interface is more complicated in man, with dermal ridges and papillae that project upwards, and intervening downwards projections of the epidermis. This arrangement is seen in the rat only in the thick glabrous skin of the pads of the paws. In thick skin, the large, primary and dermal ridges are separated from one another by primary grooves, which are themselves divided by secondary grooves into two secondary ridges.

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The subcutis comprises a layer of fatty tissue, the panniculus adiposus, beneath which lies superficial sheet of skeletal muscle known as the panniculus carnosus (see Chapter 6: The Musculature of the Rat). Well-developed hair follicles project deep into the panniculus adiposus. The panniculus adiposus comprises mostly white adipose tissue: the fat cells contain single fat droplets surrounded by membrane. In between the shoulder blades, on the ventral aspect of the neck, around the thoracic inlet and in the inguinal region, brown adipose tissue is found. Here, the fat cells contain multiple fat droplets, each surrounded by membrane. Bundles of connective tissue, carrying blood vessels, lymphatic vessels and nerve fibres run though the subcutis.

The Epidermis

The epidermis is a keratinised stratified squamous epithelium. The nomenclature of the epidermis is based on the anatomy of human skin and some of the layers described in man are not readily apparent in the majority of rat skin. Starting at the deepest layer, the following layers are described stratus basale (or germinativum), stratum spinosum, stratum granulosum, stratum lucidum and nearest the surface the stratum corneum (Fig. 24.1).

The stratum basale contains the stem cells of the skin. After division some of the progeny migrate upwards and as they migrate their appearance changes as they produce and accumulate the building blocks of keratin. By the time they reach the stratum corneum, the cells are no more than bags of keratin and can no longer be described as 'alive'. They have lost their nuclei and other organelles, and their cell membranes have been reinforced or replaced by a layer of keratin. Keratin is the characteristic product of the epidermis, so it is hardly surprising that the cells of the epidermis are described as keratinocytes. The most superficial cells of the stratum corneum are shed from the surface of the skin. It is often assumed that this is a passive process but there is some evidence to suggest that it is a little more than that and that some residual cellular activity is involved. The material that is shed is described as being made up of squames

The Stratum Basale

This is a single layer of cells resting on the basement membrane or basal lamina of the epidermis. Under normal circumstances there is no clear basal layer in the rat epidermis, but when the epidermis becomes hyperplastic the basal cells become apparent. The cells are low columnar in shape with their long axes at right angles to the basement membrane. Occasional mitoses can be found among these cells. The cells are anchored to the basement membrane by hemi-desmosomes that cannot be seen at light microscopy, but which are clearly visible at electron microscopy. The hemi-desmosomes are attached by fine filaments of laminin and fibronectin that cross the lamina lucida of the basal lamina and contact the Type IV collagen fibres of the lamina densa. Perhaps we should clarify: the basement membrane of the light microscopist comprises the basal lamina of the electron microscopist (itself divided into a superficial lamina lucida and a deeper lamina densa on the grounds of their appearance), and the lamina reticularis, also described by electron microscopists, made up of fine collagen or reticulin fibres. For

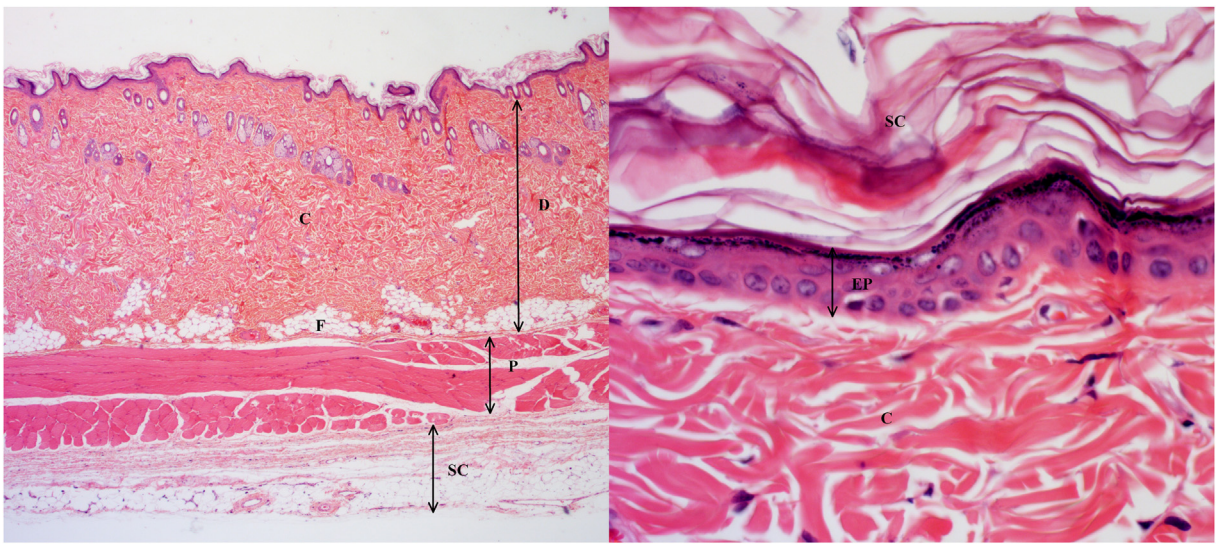


FIGURE 24.1 Dorsal skin at low and high power. D: dermis, as well as sebaceous gland and hair follicles, the dermis contains plentiful coarse collagen fibres (C) and white adipose tissue (F). Beneath the dermis is the subcutis (SC) As can be seen in the high power image the epidermis (EP) is only a few cells thick. Dark staining keratin granules are visible in the surface layer of epithelial cells, above which is the stratum corneum (SC).

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further details see Fawcett (1994). None of these details can be seen at light microscopy, though the basement membrane can be stained for glycoprotein using the periodic acid Schiff (PAS) method or for reticulin fibres using silver impregnation techniques.

Stratum Spinosum

The stratum spinosum is a characteristic of human skin but is not seen in the thin skin of the rat, although it is present in the thick skin of the paw pads. A stratum spinosum is also seen in the keratinised epithelium of the oesophagus and fore-stomach. The cells of the stratum spinosum are linked by desmosomes, there is little space between the cells. Textbooks of histology should be consulted for details of the structure of desmosomes, but we might note that the characteristic cytoplasmic filaments (intermediate filaments or tonofilaments) of epithelia are made of keratin that runs to the desmosomes. The cells of the stratum spinosum are indisputably alive, indeed they are migrating, or being pushed, upwards.

Stratum Granulosum

This stratum is readily visible in most sections of thin rat skin, usually as a single layer of cells and usually more prominent in dorsal surfaces than ventral. It is clearly marked in H&E sections by the deep blue-purple staining of granules within the cells. The stratum granulosum comprises keratinocytes that have matured to the point where they are beginning to produce the large amounts of keratin that will eventually fill the cells. Electron microscopy (EM) and immunohistochemical techniques have demonstrated two types of granule in the cells of this layer:

1. Keratohyalin granules, 1−5 μm in diameter, containing profilaggrin.
2. Lamellar granules (Odland bodies) are 100−500 nm in diameter and visible only at EM. These granules contain lipids and enzymes and are thought to be the source of the lipid-rich intercellular material of the skin.

The keratohyalin granules are the ones seen at light microscopy. The details of the production of keratin are beyond the scope of this book, but we should note that the mature keratin meshwork of the stratum corneum is a combination of keratin tonofilaments and filaggrin. Many different keratins have been identified (Schweizer et al., 2006) and we simply note that the keratin of the stratum corneum is not identical with that of the hair.

Stratum Lucidum

This stratum does not appear in thin skin, but is clearly marked in the thick skin seen on the pads of the paws. The keratinocytes that were actively producing keratohyalin in the stratum granulosum lose their organelles and are difficult to define as individual cells in the stratum lucidum. The cells of this layer contain what was described as eleidin and could be stained by the picro-nigrosin method (Carleton, 1957). Eleidin is best thought of as a stage along the road to keratin.

Stratum Corneum

As we have noted already, the stratum corneum comprises a thin layer of cells that have given up metabolic activity, lost their nuclei and organelles, and been filled with keratin. The most superficial layer is sometimes called the stratum disjunctum. The keratinocytes at this stage in their lives are known as corneocytes. The cells are surrounded by the lipid-rich material we have already mentioned: produced in the stratum granulosum, this provides the cement for the toxicologist's 'bricks and mortar' model of the epidermis.

Epidermal Proliferative Unit

Keratinocytes have been shown to be arranged in stacks in the epidermis. This is particularly clearly seen in the mouse epidermis, but a little less clearly in the rat (Allen and Potten, 1974; Strachan and Ghadially, 2008). The upper part of the epidermal proliferative unit (EPU) consists of a stack of hexagonal corneocytes (4−6 layers in the mouse), beneath which are the keratinising cells and the basal cells. Allen and Potten (1974) estimated that there were 10−11 basal cells per column. Very interestingly, each EPU was found to contain a Langerhans cell (see later) with its cytoplasmic processes extending between the keratinocytes. The corneocytes of the stack were found to be attached to each other by a circumferential band or 'squamosome'. The EPU should not be confused with the epidermal melanin unit (EMU) described by Jimbow et al. in 1976.

The epidermis contains three further cell types.

The Langerhans Cell

Paul Langerhans described this cell type in 1868, 1 year before his better known discovery of the pancreatic islets (Romani et al., 2012). Langerhans cells are dendritic cells with long cytoplasmic processes that ramify between the keratinocytes of the epidermis. Originally regarded as sensory cells (they stain with silver impregnation methods, as do neurones), they are now well established as members of the immune system that collect antigen, become 'activated' and migrate to lymph nodes where they present antigen to lymphocytes. Romani et al. (2012) point out that this view is changing, or at least becoming more complicated. Hosoi et al. (1993) showed that Langerhans cells were influenced by neuropeptides released

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by local nerve terminals. How this relates to their immune function is unknown, though links between neural activity and the inflammatory process are well established (Anderson and Tracey, 2012). Langerhans cells cannot be identified in H&E sections of rat skin and immunohistochemical staining is required to demonstrate them. The electron microscope shows that Langerhans cells contain a unique form of organelle: the Birbeck granule. These little, tennis-racket shaped, granules contain langerin, a protein specific to these cells. Langerhans cells are regarded as being derived not from ectoderm, but from mesodermal mesenchyme. Romani et al. (2012) should be consulted for further details of these interesting cells.

The Melanocyte

Melanocytes are derived from neural crest tissue and migrate to the skin. The ordinary laboratory rat is, of course, an albino mutant, and does not produce melanin due to defect in the gene for tyrosinase. Rather intriguingly, albino rats seem to produce melanin, or some related substance, in their brains and retinae: Kastin et al. (1976), Roffler-Tarlov et al. (2013). In rats that do produce melanin, the melanin is stored in melanosomes within the melanocytes. The melanosomes are transported along the cytoplasmic processes of the melanocytes and transferred by a process describe as cytocrine secretion to the keratinocytes. Each melanocytes supplies a group of keratinocytes: the epidermal melanocyte unit (EMU): Jimbow et al. (1976). Melanocytes without melanin presumably exist in the skin of albino rats, but cannot be identified at light microscopy.

The Merkel Cell

This cell type is discussed below in the section on skin receptors.

HAIR

Hair is a defining characteristic of mammals; no other class of vertebrates produces hair. Partly as a result of human vanity, and partly as a result of interest in diseases affecting hair growth, much interest has developed in the effects of chemicals on the growth of hair. Forensic science has an interest in distinguishing human from animal hair, and in the analysis of hair that carries cellular material to allow DNA fingerprinting.

Most people know that hair is made of keratin. As in the case of the cornified layer of the epidermis, the keratin is produced by cells, the remnants of which can be found in hair. In the case of hair these cells are in the cortex (outer layer) of the hair. The surface of the hair is formed from overlapping scales, the free edges of which point towards the skin. This can be discovered by drawing a human hair between the finger tips. Hairs develop in follicles, epidermal structures that project down from the epidermis into the dermis and, during active hair growth, into the subcutis. Hair growth is cyclical, the lower part of the follicle regenerates and extends during the growth phase (anagen), and degenerates and contracts during the postgrowth phase (catagen). The upper part of the follicle is stable and persists once formed. Sebaceous glands pour their secretions into the upper part of follicles. Larger hairs have a thin strand of smooth muscle, the arrector (not erector) pili that is attached to the connective tissue sheath of the follicle just beneath the sebaceous gland and erects the hair.

Hair Nomenclature

A complex and specialised nomenclature has grown up. This is summarised as follows.

Classification of mammalian hair (based on Noback, 1951):

1. Hairs with specialised follicles containing erectile tissue. Long, stiff hairs with a primary sensory function. Alternative names: feelers, whiskers, sensory hairs, sinus hairs, tactile hairs and vibrissae. Found in all mammals except man. Subclassified as:
   - **a.** Active tactile hairs: under voluntary control
   - **b.** Passive tactile hairs: not under voluntary control
     - **i.** Follicles with a ring sinus.
     - **ii.** Follicles without a ring sinus.
2. Hairs with follicles that lack erectile tissue that are mainly defensive or protective, often with a passive sensory function as a result of nerve supply to follicles:
   - **a.** Coarse
     - **i.** Spines and quills, such as in the hedgehog and porcupine.
     - **ii.** Bristles: protective hairs, primary hairs, overhair and guard hairs. Subdivided by Hebel and Stromberg (1976) with regard to the rat into: Type A, flattened in cross section and Type B, round in cross section and longer than Type A. Type B modified for sensory reception.
     - **iii.** Awns. The awn is the beard of the ripe ear or grain-bearing part of the barley plant. These hairs have a thin root and slender shaft and taper towards the tip, half or two-thirds the length of bristles. The term ‘awn’ should not be confused with the term ‘awl’: see Priestley (1966), though they may describe the same sort of hair.
   - **b.** Fine: underhair, ground-hair, underwool
     - **i.** Wool: long, soft and often curly.
     - **ii.** Fur: thick, fine and relatively short: underhair and wool-hair.
     - **iii.** Vellus: finest and softest of hairs: down hairs, wool, fuzz and lanugo in the foetus.

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Other terms have also been used, a few are as follows.

Monotrich: follicle containing only one hair.

Tylotrich: A term used to describe a monotrich follicle surrounded by the follicles of smaller hairs. This is associated with raised areas of epidermis called Haarscheiben (singular: Haarscheibe) or tactile domes (see English (1977) and later). These raised areas suggest the origin of the name: tylos is the Greek word for a projection or callus. The central follicle produces a Type B bristle hair. Other central follicles, not specialised for sensory activity, may produce awn hairs.

Compound follicles: follicles containing more than one hair. These may be a result of an old hair not being shed and a new hair growing up past it, or to a number of subfollicles opening into a single pilo-sebaceous canal. These are what Hussein (1971) referred to as the 'echte Haarbündel of de Meijere', the true hair bundle of de Meijere.

Awl hairs: straight, shorter than guard hairs and shaped like the blade of an awl.

Auchene hairs: similar to awl hairs but bent at a single constricted region. The name may be derived from the auchen, Greek meaning neck. Some authors refer to these hairs as awn/auchene hairs.

Zigzag hairs: fine hair with a zigzag form

For a more detailed analysis and for details of the configuration of the scales of the hair cuticle and form of the medulla, see Sundberg (1994). Note that more work has been done on the mouse than the rat in this area.

The Development of Hair in the Rat

Rats are born without hair, with the first hairs appearing about 5 days after birth. Hair follicles develop from a small area of epidermis that is induced to form a follicle by a condensation of dermal cells beneath the basement membrane of the stratum basale: an example of ectoderm−mesoderm cooperation. This condensation is the first sign of the dermal papilla, appearing from days 17−20 of gestation (Bulcher, 1951). Epidermal cells of the stratum basale multiply and take on a dark staining appearance before extending into the dermis. The dermal papilla is carried into the dermis ahead of the developing cord of epidermal cells. Note that we are using the term dermal papilla in a special sense, which should not be confused with the dermal papillae of thick skin, which are projections of the dermis into the epidermis. Follicle formation continues until about day 10 of postnatal life.

The lowest part of the epidermal cord expands and becomes wrapped around the dermal papilla. Some authors reserve the term dermal papilla for the group of dermal cells once they are partially surrounded by epidermal cells. Epidermal calls in the expanded lower end of the cord, the bulb of the follicle, divide and pushed upwards into the centre of the solid epidermal cord. This insertion, or invagination, separates an outer layer of cells from an inner cord of cells. The outer layer, part of the original epidermal cord that extended downwards, becomes the outer (or external) root sheath (ORS). The cells of the bulb that are dividing and expanding upwards comprise the matrix of the follicle. Cells in the centre of the matrix begin to develop keratin, and also push up into the central cord of cells, forming a second sheath, the internal root sheath (IRS), around the developing hair. The essential thing to grasp is that the IRS and the hair shaft grow upwards together inside the ORS. The innermost cells of the IRS develop keratin and form scales. Rather elegantly these scales point upwards and interlock with the scales of the hair shaft that point downwards. The IRS and the hair shaft are thus locked together.

The cord of epidermal cells that grows down into the dermis forms two bulges in the upper part of the follicle, becoming a permanent feature of the follicle. The upper bulge forms the sebaceous gland, while the lower, described as 'the Wulst' (German for bead, bulb or collar) is very important, as it contains the stem cells of the follicle (see later). Smooth muscle derived from mesodermal mesenchyme becomes attached to the upper part of the follicle just below the sebaceous gland and forms the arrector pili muscle.

The IRS and hair shaft continue to grow upwards together. The IRS fades out at about the level of the sebaceous gland, where its cells form a thin, keratinous layer lining the follicle, but the hair shaft grows on and emerges from the surface of the skin. The hair follicle has completed its development; from now on it will undergo cyclical degeneration and regeneration.

Histology of the Fully Developed Follicle

Because rat hair follicles pass through cycles of activity, sections of follicles show a range of appearances. This is also true for human follicles although the cycles are longer. A fully developed follicle extends into the subcutis, but a follicle at the end of its degenerative phase extends only into the dermis (Fig. 24.2).

A mature follicle comprises a bulb that partly encloses the dermal papilla, a connective tissue sheath that completely surrounds the follicle and which is connected to the dermal papilla, an ORS of several layers of cells, an IRS also of several layers of cells and, of course, the hair shaft itself. In addition, the sebaceous gland and arrector pili muscle can be seen. Note that smaller hairs lack arrector pili muscles. Learning the details of the various sheaths and layers is a burden to the memory, so here we provide only short notes on these structures.

The connective tissue sheath is well supplied with blood vessels and nerve fibres and is derived from the

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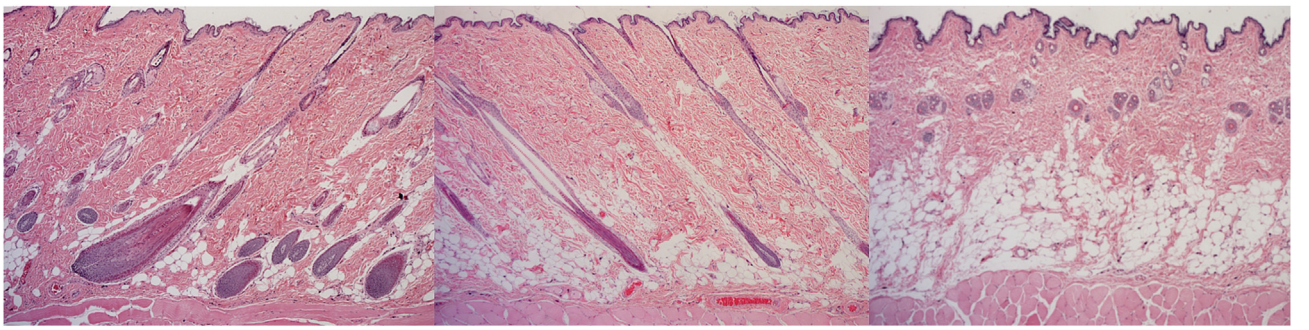


FIGURE 24.2 The skin cycle, from left to right anagen, catagen and telogen.

dermis. Containing both collagen and elastic fibres, it is divided into an outer longitudinal and inner circular layer.

The connective tissue sheath is separated from the ORS by a thickened basement membrane, the vitreous or glassy membrane. This is produced in part by the outermost cells of the ORS (the equivalent of the stratum basale of the epidermis) and in part by fibroblasts of the connective tissue sheath. The vitreous membrane stains bright pink in H&E sections.

The ORS comprises several layers of cells which are equivalent to the stratum basale and stratum spinosum of the epidermis.

The interface between the ORS and the IRS is an unusual epithelium to epithelium interface. The IRS slides on the ORS, they are not anchored one to the other. In most accounts, the IRS is described as having an outer layer of cells (layer of Henle) and several inner layers (comprising the layer of Huxley). Schneider et al. (2009) added a further, outermost layer – the 'companion layer'. In their view, the companion layer is tightly bound to Henle's layer and slides on the ORS. Classical accounts state that the outermost cells of Huxley's layer (the wing cells or Flügenzellen) have cytoplasmic processes that infiltrate between the cells of Henle's layer. The cells of Huxley's layer contain keratohyalin granules, and are said to be the equivalent of those of the stratum lucidum of thick skin. They contain eleidin and their nuclei are often difficult to see.

The IRS develops a cuticle deep in the follicle on its inner surface, where it appears as flat nucleated cells that produce keratin. The nuclei and other organelles of these cells disappear as they become converted into the scales of the cuticle.

The hair shaft has an outer cuticle of overlapping scales (the scales are described as 'imbricated': overlapping like roof tiles), a cortex of cells that lose their nuclei and other organelles as they become filled with keratin as the shaft develops, and a medulla that is not present in small diameter hairs. The medulla contains cells at its base deep in the follicle, but these soon degenerate, leaving gaps that appear to be air-filled. The medulla seldom extends to the tip of the hair and differs in appearance from species to species (Glaister, 1931). The hairs of the albino rat are, of course, not pigmented, but in animals that have pigmented hair melanocytes are found at the upper end of the dermal papilla that transfer melanin in to the cells of the developing hair.

The Hair Cycle

Although, the details of the structure of the hair follicle might be considered a little dull, the hair cycle is a dynamic process and anything but dull! Several authors have pointed out that the hair follicle is a 'mini-organ', and the cyclical processes of degeneration and regeneration are both complex and fascinating. The complexity of the process is increased by the activity of follicles being linked together. In rats, at least in young rats, a wave of follicular activity passes over the body from anterior to posterior and from ventral to dorsal (Durward and Rudall, 1949; Priestley, 1966; Hussein, 1971). The wealth of studies using the techniques of molecular biology that has appeared in recent years has been summarised in a series of stimulating reviews: Sundberg et al. (2005), focuses on mice; Paus et al. (1999), Schneider et al. (2009), Müller-Röver et al. (2001), essential for classification of the stages of follicle activity in the mouse; Plikus and Chuong (2008); and Alonso and Fuchs (2006), an admirable and brief account.

The hair cycle is divided into as follows.

Anagen

This represents the growth phase and lasts about 18 days in the rat but 2–6 years in man. During anagen the hair shaft is growing and is in continuity with the cells of the matrix of the bulb of the follicle. At the end of anagen the follicle is fully developed and reaches down into the subcutis.

Catagen

This is the degeneration phase, lasting about 3 days in the rat but about 2 weeks in man. Cell division in the matrix

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slows and then stops and the cells of the ORS and bulb begin to show apoptotic changes. As the cells of the bulb degenerate the dermal papilla becomes exposed and contracts into a small ball of cells. The hair is now separated from the matrix of the bulb of the follicle and forms an expanded bulb of its own, the club end of the hair.

The lower part of the follicle begins to shorten as the ORS degenerates from its lower end and the follicle retracts upwards. Of course, the IRS and the hair shaft are pushed up as the retraction continues. The mechanism of retraction of the ORS is not well understood, but has been attributed to 'apoptotic force', a splendidly imprecise phrase. However, what is certain is that the dermal papilla is drawn up with the retracting follicle. A thin strand of follicle cell remains attached to the papilla. Interestingly, the papilla itself remains attached to the connective tissue beneath it, also by a strand of tissue.

All this means that the hair shaft continues moves upwards during catagen, following the IRS, which also continues to moves upwards even as the lower part of the follicle degenerates. Thus the hair continues to 'grow' in the sense that the length of the hair above the skin surface increases. Of course, the total length of the hair cannot increase because the lower end of the hair has become separated from the matrix.

Eventually, the follicle has shortened to the extent that the dermal papilla lies just beneath the cells of the bulge in the wall of the ORS, with the result that the dermal papilla is now very close to the stem cells of the follicle. In the rat, the ORS becomes wrinkled in appearance as it retracts. This is said to be associated with thickening of the vitreous membrane. The hair shaft has been pushed up and now lies with its club end, which has a rather furry appearance, at about the level of the entry of the ducts of the sebaceous gland into the upper part of the follicle. The IRS has degenerated and the hair lies more or less free in the short follicle.

Telogen

This is the resting phase, lasting about 10 days in the rat, but about 4 months in man. During this phase nothing apparently happens, the hair remains in the follicle, where it may be pushed out when the next hair grows or it may remain for a while alongside the new hair. In man, the hairs from follicles in telogen are removed by a comb, a cause of mild distress in at least some middle aged males. During telogen, the follicle is short, extending only into the dermis, and comprising only the ORS with a remnant of degenerate IRS and the hair shaft. At the end of Telogen, some signal passes between the cells of the dermal papilla and the cells of the bulge (or Wulst) of the ORS. The latter contains the stem cells of the follicle, which begin to divide and increase in number. The follicle begins to grow downwards, pushing the dermal papilla before it. This downwards drive continues until the follicle has extended deep into the subcutis. The cells of the bulb of the follicle multiply, surrounding the dermal papilla and forming the new matrix, building a new IRS and hair shaft. The IRS and hair shaft grow upwards, forcing apart the cells of the cord of cells that grew downwards during early anagen. The follicle has been regenerated and a new hair shaft is being produced.

It will be clear by now that the upper part of the follicle is a permanent structure but that the lower is not. Much interest has been shown in the stem cells that divide and regenerate the lower part of the follicle. The cells derived from the stem cells are called transit-amplifying cells. These cells replicate for a limited number of times, and then differentiate to form the cells of the matrix of the bulb of the follicle. Taylor et al. (2000) suggested that the follicular stem cells may represent the stem cells of the epidermis, as well as those of the follicle. This links with the observation in human patients, that damaged epidermis can be replaced as long as the hair follicles remain intact. The stem cells of the follicle are also liable to malignant transformation. Jahoda and Reynolds (2001) have pointed out that the cells of the connective tissue sheath have been rather ignored in work on the follicular cycle and stressed their role in the reorganisation of the dermal papilla (Fig. 24.3).

The Sinus Hair

The whiskers or vibrissae form a special group of large modified hairs. It is more accurate to say that their follicles are modified, allowing them to function as transducers, linking contact with objects in the environment with activity of mechano-receptors. The special musculature of the vibrissae is discussed in Chapter 6, The Musculature of the Rat; here we focus on the follicles. Hebel and Stromberg (1976) provided an excellent account of these follicles, based in part on Vincent's paper of 1913. This paper is available via the Biodiversity Heritage Library website, and well worth reading in full.

The rat has 50–60 sinus hairs to each side of the upper lip and nostrils. Further sinus hairs are found below the lower lip, on the chin, above and lateral to the eyes and, interestingly, on the fore-limb proximal to the radiocarpal joint.

During anagen sinus hairs penetrate deep into the subcutis. The follicle comprises the usual layers, but the connective tissue sheath is much modified. It is divided and encloses two blood sinuses. The upper or ring sinus, lies below the level of the sebaceous gland, and below it lies the larger cavernous sinus, which is subdivided into loculi by bands of connective tissue. The bulge or Wulst projects into the ring sinus and has been called the ringwulst.

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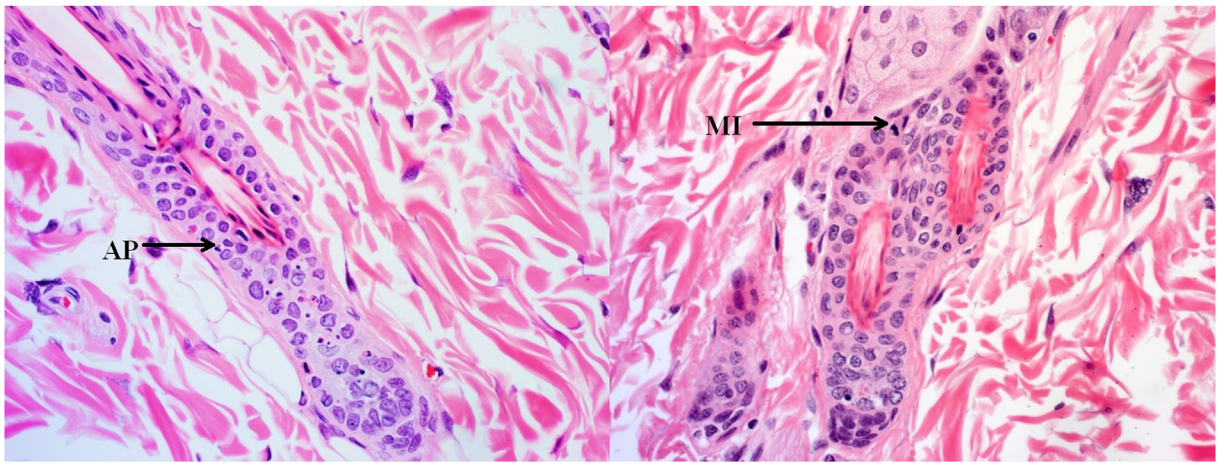


FIGURE 24.3 Catagen and early anagen at high power. Catagen on the left and early anagen can be a little difficult to differentiate. The follicle in early anagen is a little more cellular and you may see the occasional mitosis (MI), but there will not be any of the apoptotic bodies (AP) seen in catagen.

Vincent describes the ringwulst thus: 'It is composed of connective tissue fibres..... which enclose in their meshes great, clear, round, transparent cells with pale nuclei'. Vincent goes on to say that the ringwulst is well supplied with blood vessels and nerve fibres. The follicle itself also has a very rich blood supply. A main artery enters from the side to the lower part of the follicle and sends branches up and down the follicle to supply the sinuses. As in all follicles, blood vessels also enter the dermal papilla.

Above the entry of the sebaceous gland, the connective tissue sheath thickens and forms a structure called the cone. The outer layer of the cone surrounds part of the sebaceous gland and is denser in structure than the inner layer.

The key function of the sinus hairs is the detection of external objects, which depends on deflection of the hair stimulating mechano-receptors in the wall of the follicle. Sinus hair follicles are well supplied with such receptors: Hebel and Stromberg listed:

1. Tactile discs of Merkel that are found in large numbers in the basal layer of the ORS of the upper two-thirds of the ringwulst, which they called the upper enlargement of the epithelial root sheath (the lower enlargement being the bulb of the follicle).
2. Lanceolate (leaf-shaped) nerve terminals reaching the intermediary zone (between the enlargements).
3. Lanceolate nerve endings or similar terminals lying beneath (interior to) the vitreous membrane.
4. Rather simple lamellar bodies (see later) lying beneath the vitreous membrane.
5. The cone is well supplied with unmyelinated fibres with both club-shaped and lanceolate endings.

This list is based on Vincent's paper which should be consulted for further details.

The Sensory Nerve Endings of the Skin

The subject of the innervation of the skin has been treated in detail by Sinclair (1967), whose monograph is wide-ranging and by no means limited to human skin. Cunningham and FitzGerald (1972) have provided a good description of encapsulated nerve endings in the hairy skin of small mammals including the rat.

That the skin has an excellent nerve supply, and is sensitive to a range of stimuli, is general knowledge. The skin of the trunk, tail and limbs is supplied by the cutaneous branches of the spinal nerves. The head is supplied predominantly by the trigeminal nerve (CN V), although other cranial and spinal nerves contribute (see Chapter 21 (1) and (2)). Somewhat surprisingly, the exact mechanisms by which different stimuli are 'recognised' by peripheral receptors are still not entirely clear, despite more than a century of work. It is generally accepted that there are two broad categories of nerve endings. Naked nerve endings, which are characterised by fine unmyelinated fibres, are found throughout the skin, and penetrate between the cells of the epidermis. Nerve endings with more structurally elaborate endings, include the nerve endings that impinge on specialised cells, for example Merkel cells, and those that are characterised by a capsule of some sort, for example Meissner's corpuscle or the Pacinian corpuscle. The taxonomy of sensory nerve terminals can be excessively complicated, and as many as 34 apparently distinguishable nerve endings have been described (Botezat, 1912). It seems likely that many of the differences in structure stressed by early workers were either unimportant, artefacts of preparation, or perhaps represented a range of structural normality. In the early years of research in this field, it was believed that specific receptor structures were associated with specific stimuli

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and sensations: the Krause end bulb for cold and the organ of Ruffini for warmth. Current opinion is that all the encapsulated endings seem to be mechano-receptors, and all the other stimuli seem to be received via naked nerve endings. Another belief to have been abandoned is that encapsulated nerve endings do not occur in hairy skin. There is no doubt that such endings occur in great profusions and variety in glabrous skin, but work by Cunningham and FitzGerald (1972) has demonstrated that such endings also occur in the hairy skin of the rat. The nerve endings they describe could not be detected by methylene blue staining, a widely used method, but were revealed by a histochemical technique that detected acetylcholinesterase (AChE). This raises the question of the function of AChE in sensory, as opposed to cholinergic motor terminals. No answer is yet available but Carr and Proske (1996) and Watanabe and Ide (1986) provide an interesting discussion of this point.

A recent review by Zimmerman et al. (2014) provides the classification of cutaneous mechano-receptors is summarised in Table 24.1.

The Ruffini, Meissner and Pacinian receptors are found mainly in the glabrous skin of the paws. The reader will appreciate that the term 'receptor' is used here in a special sense, and should not be confused with, for example, receptors that respond to acetylcholine or noradrenalin. Hairy skin relies on receptors associated with the hair follicle and the clusters of Merkel cells found in the epidermis of the sensory domes (Haarscheiben) that are associated with guard hairs. These interesting structures have been described by Smith (1970, 1977) and more specifically in rats by English (1977). English reported that in adult rats these small domes of epidermal cells with Merkel cells in their basal layers were of 208 μm average diameter. The Merkel cells have cytoplasmic processes that are anchored to keratinocytes. Expanded sensory nerve terminals impinge on the Merkel cells, which appear to depolarise in response to mechanical distortion. The cell membrane contains the Pizzo 2 channel that allows cations to pass through the membrane. Intriguingly, the Merkel cells also contain dense granules that store neuropeptides. The embryological origin of Merkel cells has been a subject of controversy, but Morrison et al. (2009) have produced evidence to show that these cells are derived from an epithelial cell lineage (from ectoderm) and not, as others have maintained, from neural crest tissue.

Meissner corpuscles are found in the projections of dermis into the epidermis of glabrous skin. These projections are ridges that bear small papillae, not to be confused with the dermal papilla of the hair follicle. The corpuscle has a thin collagenous capsule and contains lamellar cells among which winds the nerve fibre (unmyelinated within the corpuscle) that supplies the corpuscle.

Pacinian corpuscles have a complex lamellated capsule and look rather like small oval onions in cross section. The many layers of the capsule are made up of lamellar cells and collagen fibres. Fine unmyelinated branches of the large myelinated sensory nerve fibre that supplies the corpuscle ramify between the layers of the capsule. Sinclair's account (1967) should be consulted for details. Pacinian corpuscles in man range from 1−4 mm in diameter, up to 1 cm diameter, and more than 100 can occur in the skin of one finger. Large Pacinian corpuscles are found in the mesentery, especially of the cat, and turn up in the connective tissue of the pancreas. Their function in locations other than the skin is far from obvious. Such large structures require a blood supply: see Cauna and Mannan (1958) for a superb account of the structure, blood supply and nerve supply of the Pacinian corpuscle of the human finger.

The organ of Ruffini is an elongated and unlamellated structure with a thin capsule. Sensory nerve endings ramify within the structure. Readers interested in the history

TABLE 24.1 Classification of Cutaneous Mechano-Receptors

Group	Named Structure	Notes
Aβ SA1-LTMR	Merkel cells or discs	Found in hairy skin and in glabrous skin, respond to static input
Aβ SA2-LTMR	Organ of Ruffini	Respond to stretching of skin: found in hairy and in glabrous skin
Aβ RA1-LTMR	Meissner's corpuscle	Found in glabrous skin, respond to low frequency vibration produced by movement of objects across the surface of the skin
Aβ RA2-LTMR	Pacinian corpuscle	Found mainly in glabrous skin, respond to high frequency vibration

Note:
Aβ: nerve fibre type: see Chapter 21, for the classification of nerve fibres.
SA: slow adapting, responding to stimulus through its presence.
RA: rapidly adapting, responding to onset and offset of stimulus.
LTMR: Low threshold mechano-receptor.
Source: Based on Zimmerman, A., Bai, L., Ginty, D.D., 2014. The gentle touch receptors of mammalian skin. Science 346, 950−954.

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of these structures will wish to look at the picture on page 195 of the Schafer's Essentials of Histology (Carleton and Short, 1954). It shows an organ of Ruffini taken by Ruffini from his own finger.

Temperature receptors (thermo-receptors) have been discussed in detail by Spray (1986). It appears that free unmyelinated nerve endings, and not the more complex encapsulated nerve endings described earlier, respond to temperature. Physiologists distinguish between receptors for cold and for warmth, although the structural basis for this distinction is not understood.

GLANDS OF THE SKIN

Athough the rat is well supplied with sebaceous glands, it lacks apocrine sweat glands, and has merocrine (or eccrine) sweat glands only in the glabrous skin of the paws. Zymbal's gland is a large specialised sebaceous gland that surrounds the ear canal. In man the ceruminous glands of the ear canal (modified apocrine sweat glands) produce ear wax, but in the rat these glands do not seem to occur, or if they do they are very small, and it is the secretion of Zymbal's gland that produces the equivalent of the human ear wax.

Specialised sebaceous glands, the Meibomian glands and the glands of Zeis, are present in the eyelid (note Zeis not Zeiss). The glands of Moll (modified apocrine glands found in the eyelid man) are absent in the rat (Hebel and Stromberg, 1976). The preputial and clitoral glands are modified sebaceous glands and are discussed in the Chapter 18, Male Reproductive System, and Chapter 19, Female Reproductive Tract.

Eccrine Sweat Glands of the Glabrous Skin of the Paws

An authoritative account of the structure of these glands has been published by Matsuzawa and Kurosumi (1963). Eccrine sweat glands are tubular glands with a coiled, terminal, secretory portion that lies in the dermis or subcutis. A duct lined by a two layered cuboidal epithelium is straight in the dermis, but spirals as it passes between the cells of the epidermis as a channel unlined by epithelial cells. Contractile myoepithelial cells are wrapped around the secretory tubule and a basement membrane covers both the secretory cells and the myoepithelial cells.

Two types of secretory cells are found in the eccrine sweat glands of man and many other mammals, clear cells and dark cells, so named for their appearance in histological sections. The dark cells contain granules and produce a mucoid secretion. Although both cell types can be found in the young rat, in the adult only one type of cell can be seen. Matsuzawa and Kurosumi (1963) argued that these cells were the equivalent of dark cells of other species and reported apical blebbing of some secretory cells, suggesting an apocrine component in the mechanism of secretion, at least for some cells. Intercellular canals lined with microvilli run between the secretory cells of the glands. The lateral and basal cell membranes are elaborately infolded and interdigitated with microvilli on the apical surfaces of the cells. The sweat glands of the paws are innervated by unmyelinated, adrenergic, autonomic nerve fibres and a well-developed capillary plexus surrounds the glands.

Sebaceous Glands

Sebaceous glands pour their lipid-rich secretions into hair follicles. The glands vary in size: large glands are lobulated. The alveoli of the glands are filled with cells. Near the periphery of the alveoli these are small, generally cuboidal and contain many lipid droplets in their cytoplasm. Occasional mitoses can be found in this layer of cells. The alveoli of the gland are not filled with a liquid secretion, on the contrary they are filled with cells that degenerate as they approach the short duct of the gland. Degenerating cells lose their nuclei and other organelles to become bags of fatty secretion. The cells continue to degenerate, cell membranes are disrupted and a sludge of lipid (triglycerides, squalene and fatty acids) and cellular remnants enter the hair follicle. This type of secretory process whereby the cell and contents are all part of the secretion is known holocrine (Fig. 24.4).

In man, a minority of sebaceous glands open directly to the skin surface, for example the Meibomian glands of the eyelids and the sebaceous glands surrounding the nipple. No sebaceous glands are found in the glabrous skin of the rat's paws.

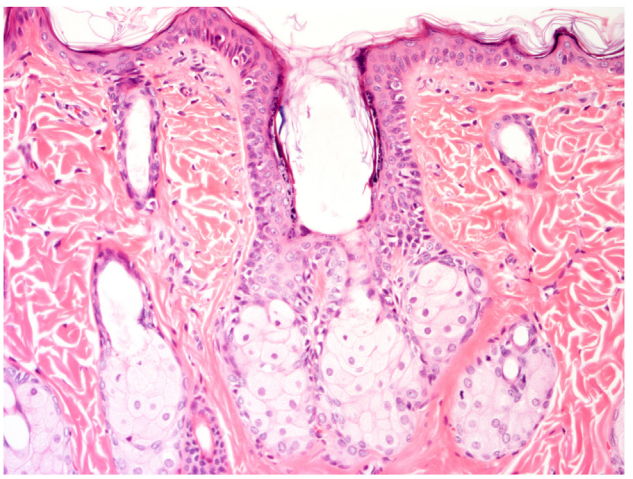


FIGURE 24.4 Sebaceous glands.

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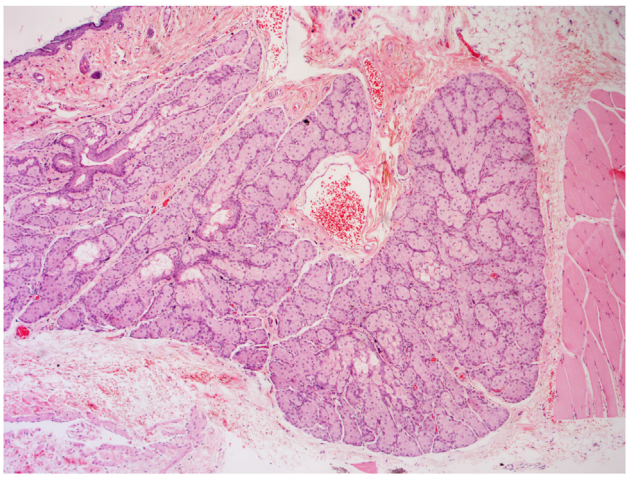


FIGURE 24.5 Zymbal's gland.

Zymbal's Gland

This gland is not found in man. Pliss (1990) has provided an excellent description; the following summary is based upon his work. Three groups of glands can be included as specialised sebaceous glands associated with the external ear canal of the rat, two groups lie beneath the squamous epithelium of the anterior and posterior walls of the canal. The anterior group is the better developed. The third group is found anterior to and below the ear, wrapped around the tympanic bulla (see Chapter 5: The Skull). This group represents what is usually referred to as Zymbal's gland. Pliss described it as 'triangular in shape, pinkish yellow in colour, 3–5 mm in diameter and 7–8 mg in weight'. The gland comprises 3–4 lobules each contributing 40–60 glandular tubules that lead to intra-lobular ducts. These ducts unite to form the main excretory duct that winds between the bone and cartilage of the ear canal and pours secretions onto the floor of the external ear canal. The secretory part of the gland is similar in appearance to that of a sebaceous gland; the main excretory duct is lined with keratinised and stratified squamous epithelium. This gland is of great interest to toxicologists as tumours develop in response to a range of carcinogens (Fig. 24.5).

Blood and Lymphatic Supply of the Skin

Neither blood nor lymphatic vessels penetrate the epidermis. The arterial supply of the skin is largely derived from the cutaneous branches of vessels supplying subjacent skeletal muscle. In man the major exception to this is provided by the blood supply of the scalp, where major arteries such as the superficial temporal artery provide arterial supply. The blood vessels of the skin are arranged as plexuses that run parallel to the skin surface. A superficial plexus lies at the border of the dermis and epidermis, a deeper plexus runs at the border of the dermis and the panniculus adiposus, and a still deeper plexus can be found at the border of the panniculus adiposus and the panniculus carnosus. Connecting vessels between the plexuses run in bands of connective tissue along with bundles of nerve fibres. Venules and small veins run in the opposite direction to the arteries and arterioles. In man many aterio-venous anastomoses are found, characterised by the glomus (Hoyer-Sucquet anastomosis) of the finger tips and nail beds. These anastomoses are important in controlling blood flow to the superficial capillaries, and play a key role on thermoregulation. Dellmann (1998) has provided a good description of the glomus. Subendothelial epithelioid muscle cells control blood flow through these mini-organs that are surrounded by a collagen-rich capsule. The skin is also well supplied by the lymphatic vessels that run with the blood vessels.

CLAWS

The rat has narrow, curved claws on all its digits, except the first digit of the fore-paw, which carries a flattened nail (Hebel and Stromberg, 1976). The terminal phalanx of the digit is conical in shape and partially ensheathed by the claw, which is sharply curved in both its longitudinal and transverse axes and comes to a point at its tip.

If the ventral surface of the free part of the claw is examined it will be found to be hollow or filled with debris. Between the terminal phalanx and the claw bed is a thick, very vascular layer of connective tissue. Hebel and Stromberg (1976) described this as forming a 'longitudinal eminence', which may be responsible for the sharp transverse curvature of the claw.

Like human fingernails, the claw grows from its base. Cells at the base of the claw form the matrix and divide to produce the cells of the claw. These cells produce a hard form of keratin that creates the substance of the claw. Cell division of the matrix forces the developing claw forwards over the curved claw bed (nail bed in man) in such a way that slow sliding is possible. The nail bed itself is a modified epidermis with no stratum corneum.

THE SKIN OF THE TAIL

The scales of the rat tail are formed by a folding of the cornified layer of the epidermis and arranged in rows, forming rings around the tail. A large central hair and two smaller flanking hairs emerge between adjacent scales. In an excellent account of rat thermal biology Gordon (1990) reported that though the tail accounted for only 7% of the body surface area, it was responsible for 20% of total heat loss. The tail has an excellent blood supply, and many arteriovenous anastomoses are present (Staszyk et al., 2003). These are under sympathetic control and

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respond to both central and local changes in temperature. Work by Owens et al. (2002) and Bitar et al. (2014) should be consulted for further details.

HISTOLOGICAL TECHNIQUES

Skin presents some difficulties for the inexperienced histologist. Specimens tend to harden and curl during processing, and penetration of wax into the epidermis may be slow. Hardening is particularly serious if the specimen of skin is left for too long in ethyl alcohol during dehydration prior to clearing. Clearing in xylene should be avoided as it also produces hardening. Carleton (1957) recommended that chloroform should also be avoided as a clearing agent, and older works recommend cedar oil for clearing. Pinning the specimen out on a small piece of cardboard can prevent curling, although stapling skin samples to sheets of acetate is a more durable method. Vacuum embedding speeds penetration by paraffin wax and is recommended. Sections of skin are usually stained with H&E, which is satisfactory for most diagnostic work, but for staining elastic fibres in skin there is no better stain than the Taenzer-Unna orcein method (Drury and Wallington, 1980). Orcein is outstanding for staining the fine elastic fibres of skin, indeed it was developed for this purpose. Counterstaining should be light, as a heavy counterstain for collagen may well obscure the finer elastic fibres. Keratin, eleidin and keratohyalin granules can all be stained by using specific histochemical techniques: see Bancroft and Gamble (2007) or Lillie and Fullmer (1976) for details.

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Chapter 25

Dissection of the Adult Rat

In this chapter, we have outlined a procedure for dissecting a rat so as to discover those aspects of its anatomy that are likely to be of most interest to the toxicologist. Only the main organ systems are described; descriptions of the musculature and skeleton have not been provided (see Chapter 4: Vertebrae, Ribs, Sternum, Pectoral and Pelvic Girdles, and Bones of the Limbs, Chapter 5: The Skull and Chapter 6: The Musculature of the Rat). The procedure set out here can be used for postmortem examinations; a shorter protocol for such work is set out in Chapter 26, Postmortem Examination. Some of what follows may seem elementary and perhaps superfluous if you have already studied mammalian zoology and done some dissection, but for the benefit of those readers who have not, we begin at the beginning. The illustrations in this chapter follow the instructions given in the text.

MAKING A RECORD OF OBSERVATIONS

Making a record of observations is important and often critically important. The extent of the record depends on the purpose of the dissection. If you are new to the anatomy of the rat, try to record what you see and make a few outline drawings to imprint this upon your memory. If you are taking specimens for histological examination you MUST record which specimens have been taken and be able to link the histological sections with the source animal. Imagine seeing something interesting in for example a section of a salivary gland. Off you go to consult an expert who asks you about the sex of the animal. You don't know or don't remember, say so, and look a fool! If a formal (regulatory) postmortem examination is being conducted, detailed records, which may be scrutinised later, MUST be made. These are often made according to a protocol: see Chapter 26, Postmortem Examination, and should include, as a minimum: date, reference number of the animal, sex and weight.

EXAMINING THE ANIMAL AFTER DEATH

It is assumed, and indeed we would suggest, that you will begin by dissecting mature male and female rats.

Before starting to dissect, conduct a thorough external examination of the animal. You will wish to assure yourself that the animal is actually dead. Animals may be killed by exposure to carbon dioxide or by intraperitoneal injection of a barbiturate anaesthetic (see Annex 1). Observe that there is no sign of breathing movements, that no reflex blinking occurs when the cornea is touched and that the foot is not withdrawn when the toes are pinched with forceps. You will soon learn that the appearance of the eyes changes with death. The sparkling bright eye becomes dull and lifeless. On handling, the animal is found to be entirely limp and drapes itself over the hand.

Weigh the animal: record its weight.

Record the sex of the animal.

Lay the body on the dissection board and examine the external features. Note the large scrotal sacs of the mature male rat. In the female, look for the six pairs of nipples running in two lines from the upper thorax to the groin. Note the soft abdomen and the firm thorax, feel the ribs. Examine the animal's mouth and note the large incisor teeth. Turn the animal over and examine its back, recording any abnormalities, including lumps. When we come to the formal postmortem examination this will be very important.

Pin out the animal for dissection. Place the animal on its back and pin out the limbs with strong dissecting pins or awls. The limbs should be extended fully. Push the pins through the front and back paws. If using ties rather than pins, tie them firmly around the wrists and ankles and attach them to the tying points on the dissecting board or table. If you planning to do a bone marrow smear, now is the time to do it (see Chapter 26: Postmortem Examination).

Examine the anus and external genitalia using blunt forceps to examine the penis of the male rat. You can apply gentle pressure at the base of the penis to push it

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out (eversion). The glans of the penis is covered by a prepuce. In the female, look for the clitoris (the female equivalent of the penis) and for the orifice of the vagina, which lies between the clitoris and the anus.

The Abdomen

The abdominal wall comprises thin layers of muscle. Using forceps and scissors make a linear incision in the abdominal wall (Fig. 25.1).

Carry the incision forwards to the lower end of the rib cage. Here you will find the xiphoid process of the sternum. The xiphoid process is made of cartilage and shaped like a little, rounded leaf. Continue the incision laterally along the lower boundary of the ribs. Do not be tempted to make these incisions with a scalpel, as the danger of cutting too deeply and into the organs that lie close to the abdominal wall is real. Now turn back the abdominal wall and pin it out. The flaps of abdominal wall will overlie the skin flaps you have already pinned out. Note that the

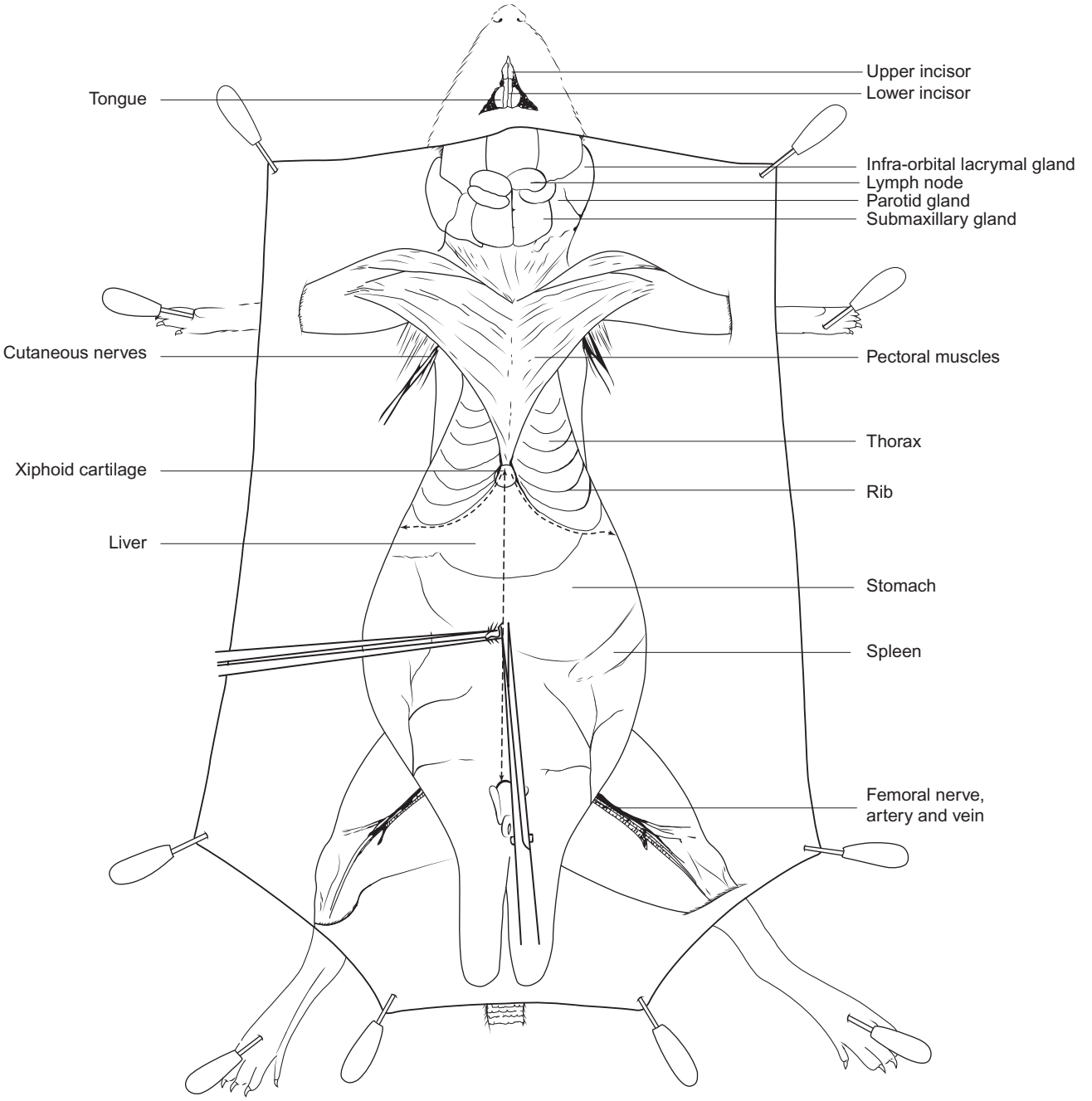


FIGURE 25.1 Male.

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inner surfaces of the abdominal wall are made shiny by the parietal peritoneum (Fig. 25.2).

The gut is suspended from the dorsal abdominal wall by connective tissue called mesentery, which supports the blood vessels and nerves supplying the gut, the gut itself being covered by visceral peritoneum. As the gut develops, grows and rotates, it comes to fill the abdominal cavity and the visceral peritoneum is pushed against the parietal peritoneum, leaving the peritoneal cavity as a small space, which normally contains only a very small volume of peritoneal fluid. This will have escaped when you cut through the parietal peritoneum that lines the ventral abdominal wall. The peritoneal cavity appears to be a closed cavity, one might think of the gut being pushed into peritoneum like a fist into a balloon. It is a genuinely closed space in the male, but in the female Fallopian tubes of the uterus open into the ovarian bursa, which is connected with the peritoneal cavity by a slit (see Chapter 19: Female Reproductive Tract).

You can now examine the contents of the abdomen. Immediately adjacent to the diaphragm, under the lower edge of the rib cage and overlying the stomach, you will see the liver. Unmistakable as a large reddish organ divided into a number of lobes. The stomach is about two centimetres in length, greyish in colour and lies across the abdomen partly covered by the liver. The intestine fills most of the rest of the abdominal cavity. There will be a variable amount of fat in the abdominal cavity, generally increasing with age, and usually more in females. Some strains, such as the CD rat, may become extremely obese in longer term studies, and is not unusual to have rats that weigh well over a kilogram. Using the large forceps, pick up the fat and turn it to the side.

Use the large forceps to lift the stomach and gut and look for the following organs. The spleen, also dark red because it contains a lot of blood, lies to the left side of the stomach. If you lift the intestine gently you will see the delicate, rather lace-like pancreas peeping out between the intestine and the stomach. The pancreas lies in the bend of the first part of the intestine, the duodenum.

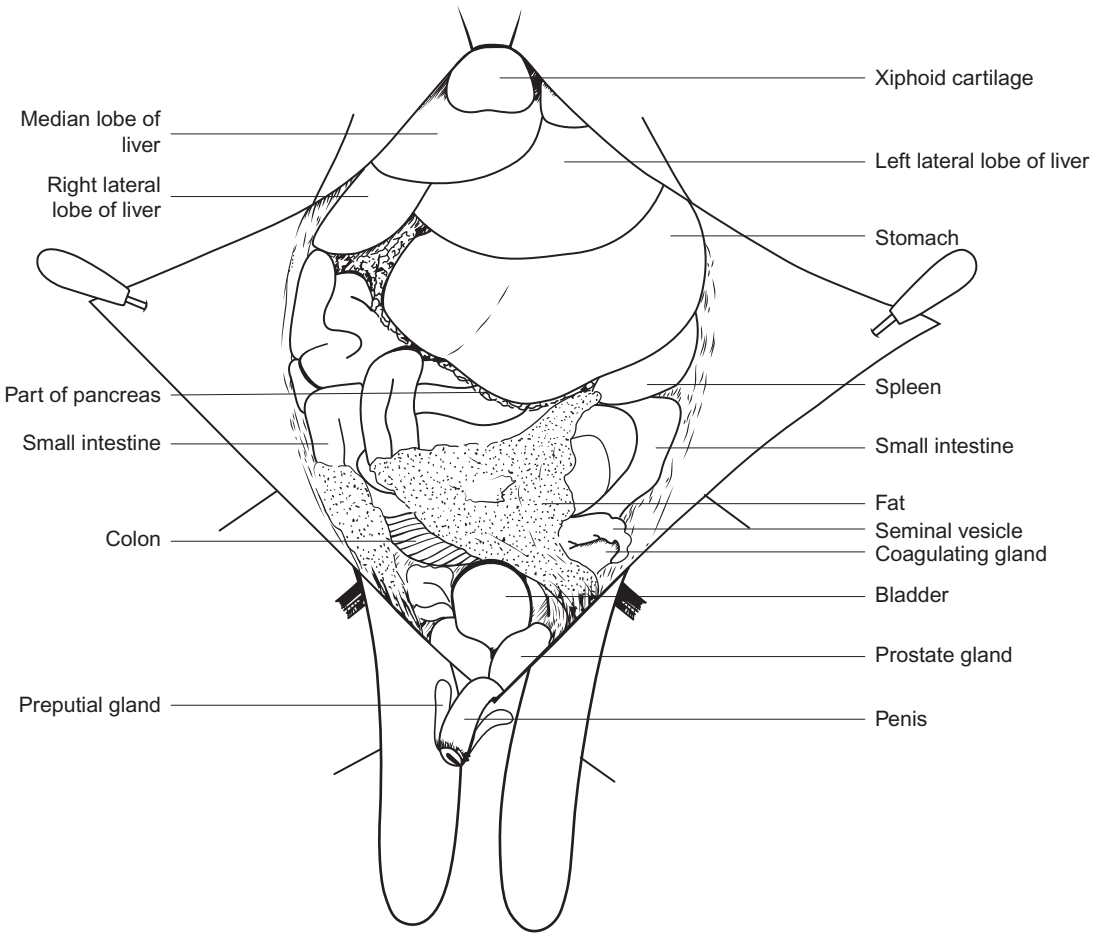


FIGURE 25.2 Male.

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Defining the various parts of the intestine is rather tricky at this stage. Using the forceps look for the caecum, it usually lies low down in the midline or on the left side. The colon originates from the distal part of the caecum and can be recognised it by its striped surface. The stripes are actually ridges on its wall. Lift the lower gut gently and look for the bladder; small, shiny and usually full of urine, lying in the midline. In the male, the nearby seminal vesicles are instantly recognisable by their shape, large and curved, rather like horns with a crinkled margin. You will also see the large preputial glands that are attached to the penis near the glans. A little work with the fine scissors to remove more skin will be needed to display these. In the female, you will not at this stage see the uterus.

Look again at the arrangement of the viscera as you are about to disturb them.

The Stomach and Intestine

Lift the liver to display the full extent of the stomach. The oesophagus passes through the diaphragm (the muscular and tendinous bulkhead that lies between the abdominal and thoracic cavities) just behind the liver. The caudate lobe of the liver is wrapped around the lower end of the oesophagus in the rat. Using your fingers, pick up a coil of intestine and turn it to the animal's left. This will expose the colon and allow you to see the bend of the duodenum, in which the pancreas lies. With a magnifying glass, you will be able to make out fine ducts. One of these, the bile duct, comes down from the liver, although you will probably not be able to recognise it with certainty. The rat is one of the few animals that does not have a gall bladder, so bile passes directly from the liver to the duodenum (that the mouse has a gall bladder is a fact well known to cat lovers, as cats do not eat gall bladders but leave them on the carpet). Following the duodenum down and you will see that it passes behind the colon, a hangover of the complicated rotation the gut undergoes during its development (Figs. 25.3 and 25.4).

We shall now follow Rowett's advice (see Bibliography):

> *Grip the duodenum and colon [with your fingers] and carefully pull them apart, thus exposing the hepatic portal vein.* Note*: BE CAREFUL NOT TO TEAR THE MESENTERIES. Notice the way in which the small and large intestines are twisted over one another. Turn the bulk of the intestines over to undo this twist.*

The entire intestine is now laid before you. You should be able to see the dark red hepatic portal vein through the pancreas. The lace-like mesentery of the intestine spreads out from a rather limited attachment to the dorsal abdominal wall to every part of the small intestine, like a flared and pleated skirt. In addition to nerves and vessels the mesentery contains some fat, and at the base are a series of lymph nodes that lie alongside the portal vein.

Beyond the duodenum lies the jejunum (meaning empty) and the ileum. Some authors deny that the rat has both, speaking only of the ileum. Ileum is derived from the Greek word for severe colic, referring to the intestine. Distally the ileum joins the caecum at the ileo-caecal junction. Emerging from the caecum, the ascending colon curves around the abdominal cavity on its path to the rectum, which usually contains pellets of faeces, and is thus

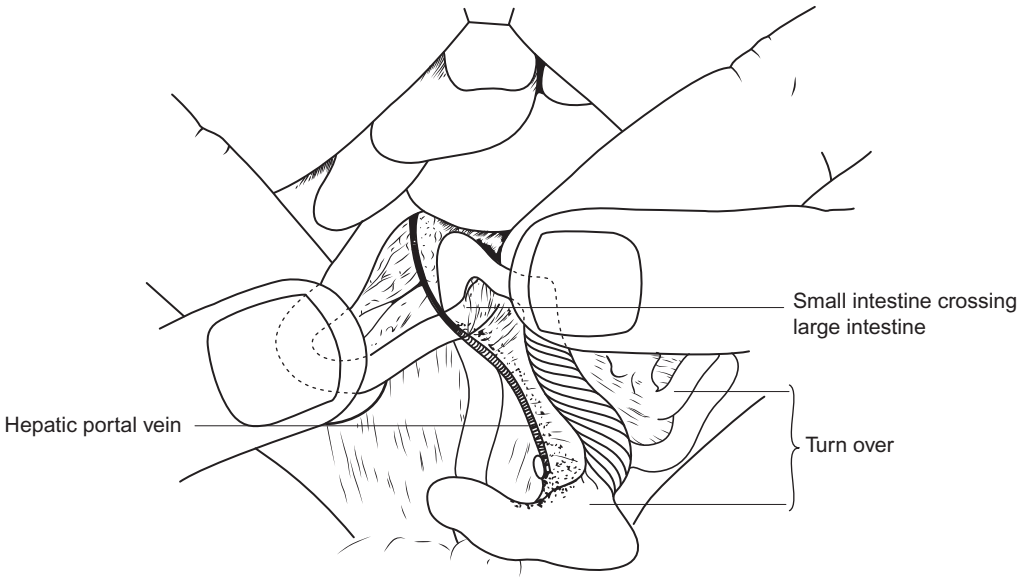


FIGURE 25.3

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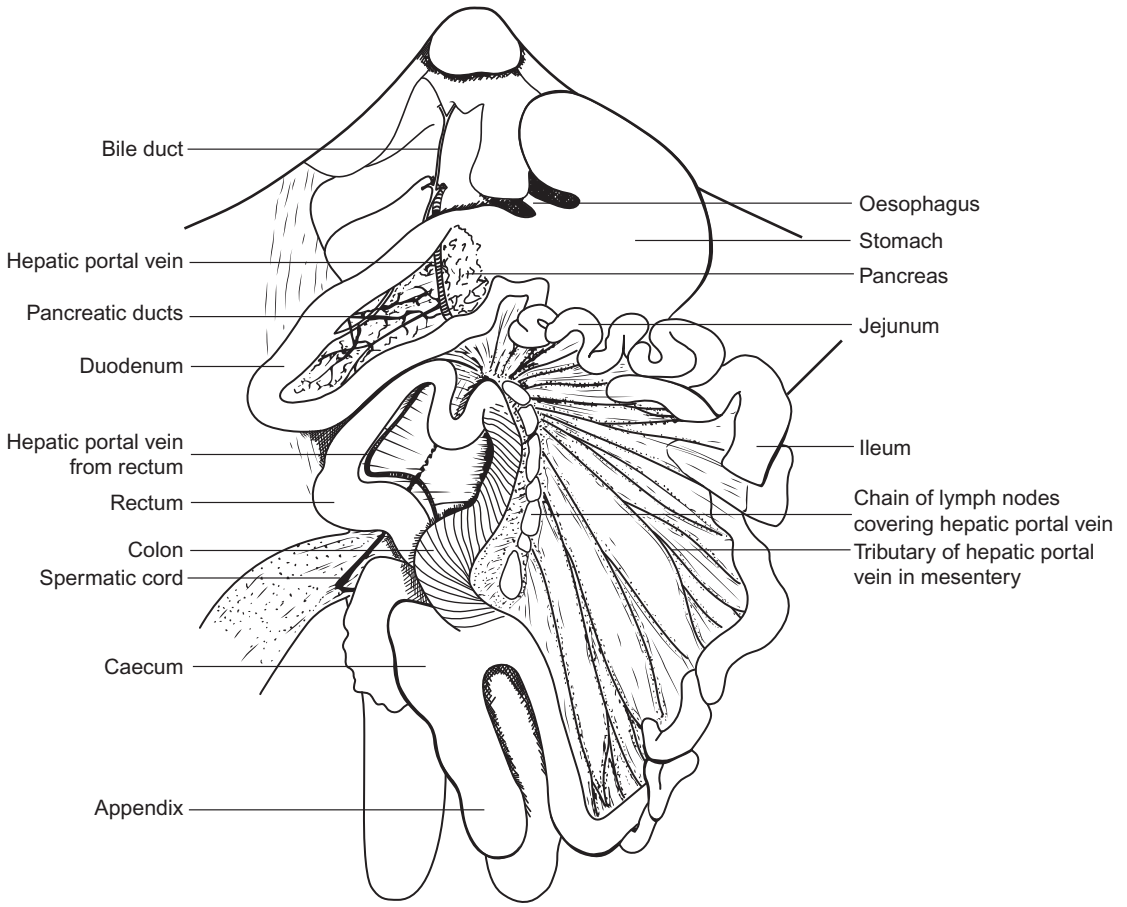


FIGURE 25.4

easy to recognise. The caecum is blind-ended sac; the rat lacks an appendix.

Removal of the Gut

Having examined the gut in some detail, we can now remove it to enable examination of the remainder of the contents of the abdomen. Removal of the stomach and intestines involves cutting nerves and blood vessels, including the hepatic portal vein that carries blood from the intestines to the liver.

The stomach, duodenum, spleen and liver receive their arterial blood supply from branches of the coeliac artery, which arises behind the stomach as a branch of the abdominal aorta. If the stomach is turned to the right of the animal and the tissues behind the stomach are dissected, the origin of the coeliac artery can be seen. Most of the remainder of the intestine is supplied by the anterior mesenteric artery, which branches off the abdominal aorta behind the pancreas. The rectum is supplied by the posterior mesenteric artery, another branch of the aorta that arises from it just before it divides into the iliac arteries in the pelvis.

Lift the liver and find the oesophagus. Pass a length of thin cord (a ligature) around the oesophagus[1] and cut above the tie. Not much fluid will leak from the open lower end of the oesophagus. Lift the stomach using coarse forceps and gently separate the stomach from the liver (they are connected by a very thin mesentery). Continue downwards using the fine scissors to separate the intestine from its mesentery. Cut the rectum as low down in the pelvis as is possible, lift out the stomach and intestine and lay it on a dissecting board. For the majority of regulatory studies, you will be taking samples from the stomach and gut for histological processing. If you are dissecting to produce such material: turn now to

1. If you found passing the ligature around the oesophagus difficult or have not done this before, then this is how to do it. Using fine forceps, dissect away the connective tissue from around the oesophagus. Now pass the points of the forceps behind the oesophagus and seize the end of a suture which you should be holding in one hand (probably your left hand). Draw the suture through and tie it in a double knot around the oesophagus. If you have fine curved artery forceps or a pair of angled forceps as used by dentists as 'packing forceps', then these can be used to draw through the suture.

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Chapter 26, Postmortem Examination. Mop up any further leakage of blood so as to have a good view of the dorsal abdominal wall and at the structures which lie upon it: see Fig. 25.5.

These abdominal structures all lie behind the peritoneum lining the abdominal walls and so are referred to as retro-peritoneal structures. Structures in the pelvis, the bladder for example, also lie outside the peritoneal cavity. In dissecting out these structures you will remove, perhaps without noticing, the remnants of the peritoneum that was left in place when the intestines were removed. The seminal vesicles actually project with their covering of peritoneum into the spaces between the coils of the gut, but the peritoneum is so thin that it cannot easily be seen during dissection.

The Liver

Removal of the intestine leaves the liver attached to the posterior surface of the diaphragm. The liver can be removed at this stage. Separate it from the diaphragm and cut it away from its connection to the vena cava. Take the liver and place it in a Petri dish or on a dissecting board for examination. Note how soft it is, like a jellyfish. Using Fig. 14.1 of Chapter 14, Liver, identify its major lobes, you will need to turn it over to see all the lobes. If you are taking specimens for histological examination refer to Chapter 26, Postmortem Examination.

The Kidney, Ureter and Bladder

The kidneys, shaped appropriately like kidney beans, lie high up on the posterior walls of the abdominal cavity, the right kidney lying somewhat further forwards than the left, partly surrounded by fat. The large renal artery and renal vein pass between each kidney, the aorta and posterior vena cava. The adrenal glands, surrounded by fat, will be found close to the upper pole of each kidney. If you are careful as you dissect out the adrenal glands you will find the blood vessels of the adrenal glands running to and from the renal vessels.

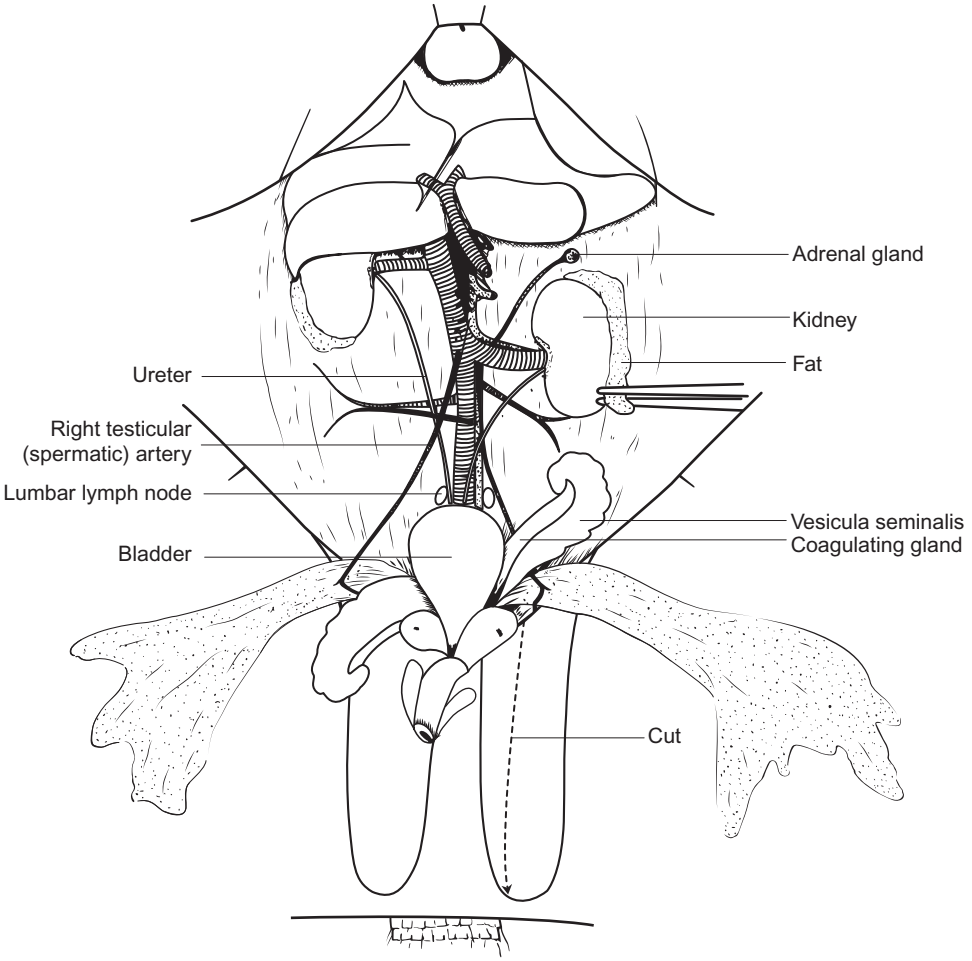


FIGURE 25.5

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Returning to the kidneys, look for the ureters as they leave the hilum of a kidney and run down the posterior abdominal wall to the bladder. They run behind the parietal peritoneum and thus must be looked for through that thin transparent membrane. Once you have found them, remove the transparent tissue lying over them and follow them down to the bladder. Finding the entry point of the ureters low on the bladder's distal surface is tricky, as the anatomy around the lower end of the bladder is complicated. The cavity of the bladder leads into the urethra, which passes above the anterior arch of the pelvis to emerge as the passageway running through the penis to the exterior. Just below the bladder, the ductus deferens and a number of ducts open into the urethra (see Chapter 18: Male Reproductive System).

Blood Vessels of the Dorsal Abdominal Wall

The abdominal aorta and posterior vena cava can be seen running down the midline of the dorsal abdominal wall. The aorta is on the left and the vena cava is on the right. In the male rat, the testicular arteries may arise high in the abdomen, either from the renal artery (usual origin on the left) or from the aorta (usual origin on the right). This is tied up with the embryology and subsequent development of the testes, which involves a descent into the scrotum, taking its blood supply with it. In some animals, for example, the elephant, the testes remain inside the abdominal cavity behind the peritoneum.

The Reproductive Tract

Male Reproductive Tract

In most mammals, including man and rat, the testes lie in the scrotum. Spermatozoa travel from the testis via the epididymis to the ductus deferens, which carries them to the urethra, and thence during copulation, via the penis, to a female rat. The ejaculate contains sperm and the secretions of an imposing array of accessory sexual glands (see Chapter 18: Male Reproductive System). The vas deferens (ductus deferens) passes up in the spermatic cord via the inguinal canal and turns across the wall of the pelvis to reach the urethra just below the bladder. The testicular artery coming down from above joins the spermatic cord just about where the cord enters the pelvis.

Examine the prepuce of the penis, then dissect away the skin of the prepuce and define the large yellowish preputial glands near the tip of the penis. Make an incision (forceps and scissors again) on the ventral surface of the scrotum on the left side of the animal and extend this incision to the edge of the incised abdominal wall (Figs. 25.5 and 25.6). Gentle probing with the blunt forceps will reveal the testes lying in front of the epididymides as you look down from above.

The penis emerges from the pelvis from behind the ventral arch of the pelvis. Note the relationship of the penis to the arch: see Fig. 25.6. Using fine forceps, identify the ventral arch of the pelvis, just above the penis. Clean away the connective tissue until you have defined the arch clearly. Note that the urethra and the lower end of the bladder dive down behind the arch. Carefully insert the tip of one blade of your large scissors between the

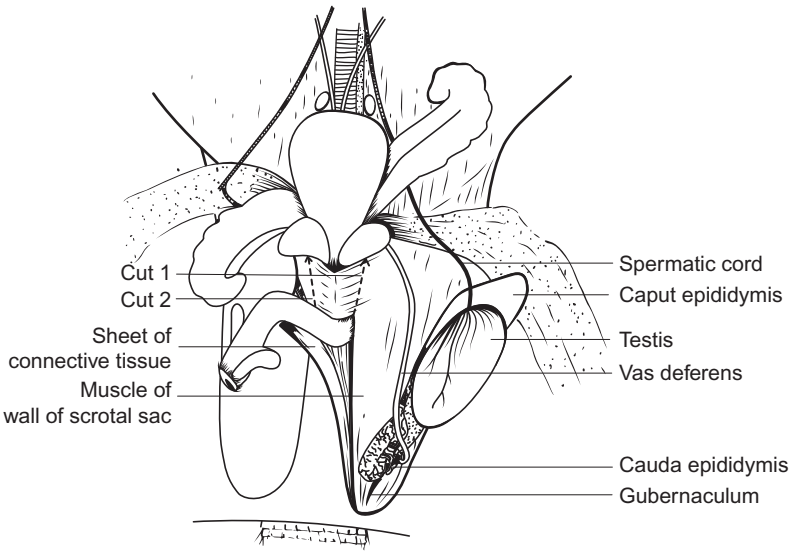


FIGURE 25.6

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surface of the penis and the arch, and work the blade of the scissors up until it emerges above the arch, then cut the arch. Only the arch lies between the blades of the scissors, so you will not cut through any parts of the reproductive tract. Having cut through the arch, remove more of the arch from each side until you have made a wide gap through which you will be able to see the urethra. Your initial cut will have passed through the cartilaginous joint between the right and left sides of the arch so should not have been difficult to make (Fig. 25.6 cuts 1 and 2).

Remove more skin from the scrotum until you have cleared the area as far back as the anus. Turning back to the testis, remove the fat from around the spermatic cord in the scrotum using blunt forceps and define the testes. Note the testicular artery and the accompanying plexus of veins (the pampiniform plexus) running to and from the testes (pampiniform is derived from pampinus: the Latin for the twining shoot of a vine). The veins are wrapped around the artery, and because the blood in the veins is running in the opposite direction to the blood in the artery, cool the arterial blood. The temperature of the testis is a few degrees below that of the body in general.

Clear away more fat from around the testis and define the epididymis, a structure longer than the testis. Explore the lower end of the epididymis (the cauda, meaning tail; the other end is the caput, meaning head) and find the ductus deferens. Clean away the fat from around it and follow it up towards the abdomen. Recalling that the ductus runs with the testicular artery and those twining veins as the spermatic cord, which runs through the abdominal wall in the inguinal region. This is difficult to observe in the rat, as the ductus and the vessels seem to lie apart, but this is a result of your dissection (Fig. 25.7).

The next part of the dissection is the base of the bladder, which is tricky because the structures we shall be looking for are small. An illuminated magnifier is useful for visualising some of the smaller structures.

The seminal vesicles have already been identified so cleaning away any fat and connective tissue lying around

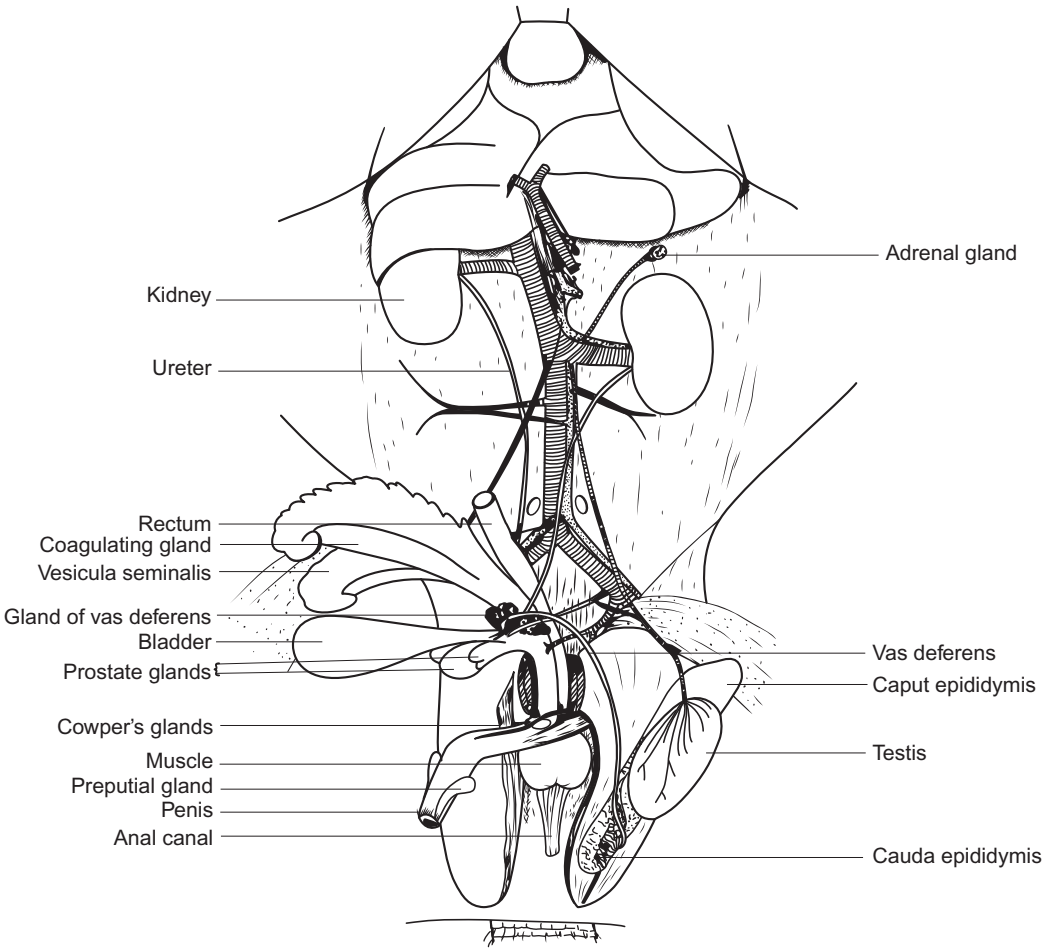


FIGURE 25.7

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them, look for the coagulating gland in the curve of the seminal vesicle. Turn the bladder to one side and dissect around its base, carefully separating tissues and removing connective tissue, looking for the prostate gland. This appears as two lobes lying in front of the urethra close to the lower end of the bladder. Dissect behind the lower end of the bladder and look for the entries of the ductus deferens and the ducts of the seminal vesicles into the urethra. The gland of the ductus deferens is also in the area, but you may not be able to find it. Dissect down the urethra and try to find the small glands of Cowper that lie close to the bend in the penis where it emerges under the anterior arch of the pelvis. These are tiny glands but visible with a magnifier.

By this stage, you should have seen the testis, the epididymis and the ductus deferens, which you have followed to the urethra. You will certainly have seen the seminal vesicles, coagulating glands and the prostate gland, all of which are removed in a single block of tissue when histological specimens are being collected.

Further Examination of the Blood Supply of the Abdomen and Pelvis

Turn the seminal vesicles and bladder down towards you, over the penis and anchor them with a pin pushed into the dissecting board. The entire blood supply of the abdomen and pelvis is now revealed (Fig. 25.8).

Dissect the fat and connective tissue away from the aorta and vena cava, and follow these large vessels down to their bifurcation. Follow the common iliac arteries as they divide into the external and internal iliac arteries. The external leaves the pelvis to supply the leg; the

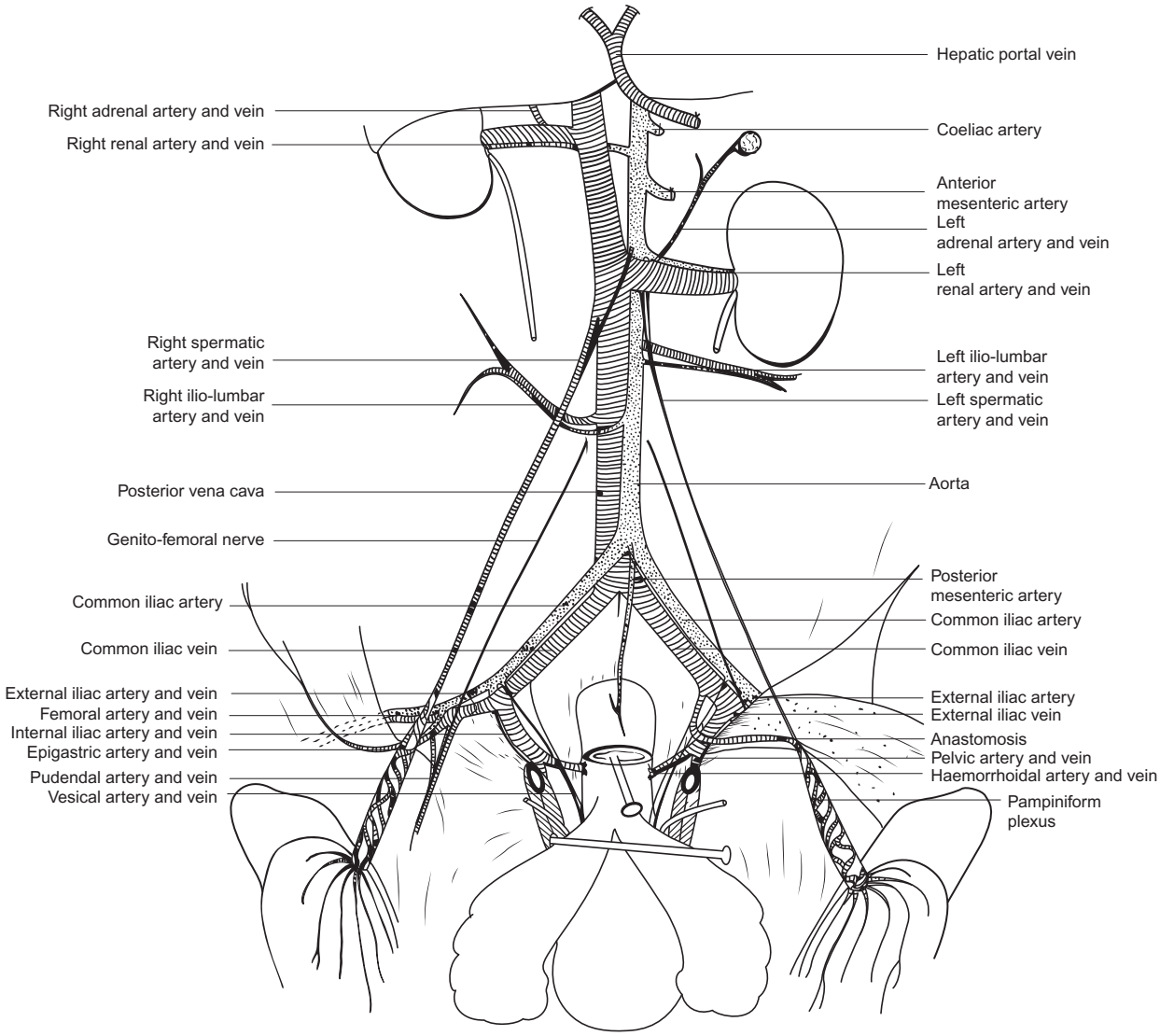


FIGURE 25.8 Male

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internal gives off many small branches that supply the pelvic viscera. These vessels include the vesical artery to the bladder, and the pudendal artery to the external genitalia. The haemorrhoidal artery supplies the lower rectum. Following the veins you may be able to define an anastomosis between the veins of the pampiniform plexus and the common iliac vein.

Female Reproductive Tract

The reproductive tract of the female rat is rather different from that of the human female. The rat has two uterine horns that are inserted into the proximal end of the vagina (see Chapter 19: Female Reproductive Tract). Having removed the intestine you will be able to see the uterine horns running up on the posterior abdominal wall from the vagina behind the bladder. The ovaries lie at about the level of the kidneys and are surrounded by fat. At the upper end, the horns narrow into the Fallopian duct (oviduct if you prefer), which is small, short and coiled in the rat. The ovaries themselves are enclosed in the connective tissue capsule (or bursa) along with the proximal end of the Fallopian tube (Fig. 25.9).

The uterine artery arises from the common iliac artery in the pelvis and the uterine vein drains into the common iliac vein. The ovarian arteries usually arise from the aorta, but the left ovarian artery may arise from the left renal artery. Each ovarian artery supplies the ovary, before sweeping down the inner surface of the uterus. The ovarian vein joins the renal vein on the left, and the vena cava on the right. The ovarian artery and vein form an anastomosis with the uterine artery and vein.

The vagina lies behind the bladder. Draw it and the bladder forwards and repeat the dissection of the pelvic blood vessels outline above for the male.

Cut through the vagina (see below for an alternative) and remove it along with the uteri and ovaries. Lay the reproductive organs out on a dissecting board and examine the upper end of a uterus with a magnifier. Find the ovary and the Fallopian tube at the upper end of the uterus.

Alternatively, examine the external genitalia of the female and repeat the dissection you undertook in the male. Define the clitoris: the female equivalent of the penis. In the females, there are clitoral glands, which are broadly equivalent to the preputial glands in the males, though somewhat smaller. Divide the ventral arch of the pelvis and expose the urethra which runs down behind it. The vagina lies behind the urethra. There are no female equivalents of the prostate gland, seminal vesicles or coagulating glands. Dissect out the vagina (you may take

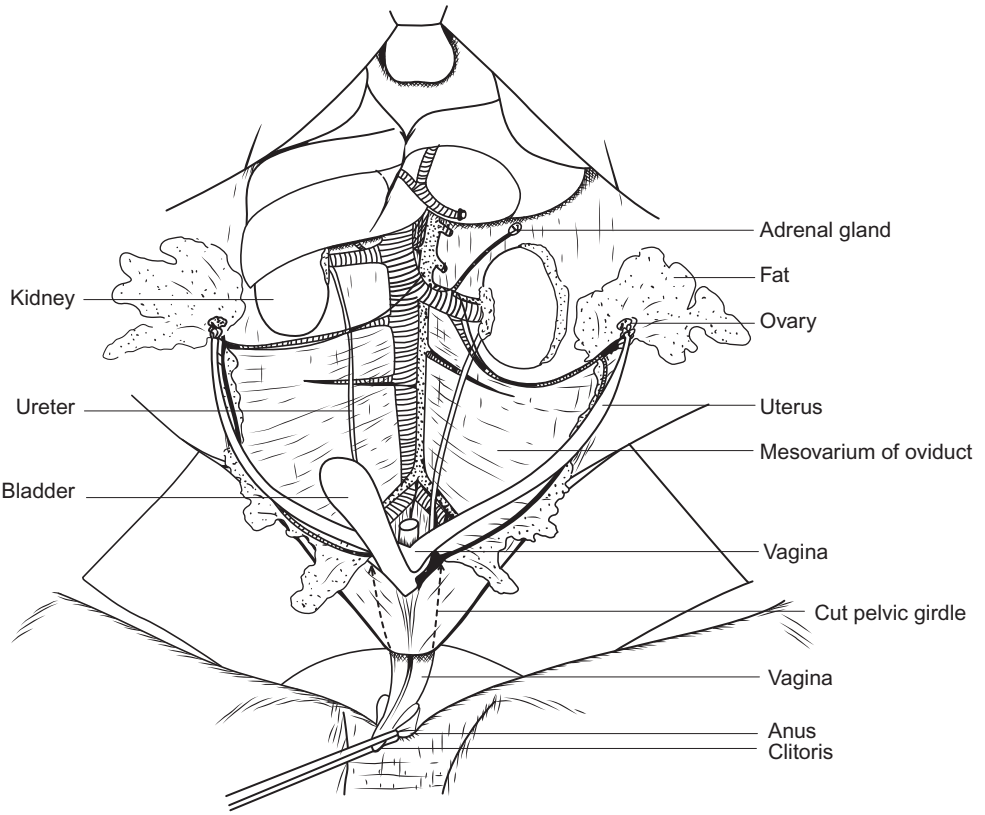


FIGURE 25.9

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the clitoris and bladder attached by connective tissue to its anterior surface, but you will need to cut the ureters to free the bladder) and remove it along with the uteri and ovaries. If you do this you will have the entire female reproductive tract before you.

The vagina and lower parts of the uteri are usually taken as a block for histological examination, as are the Fallopian tube and ovary (see Chapter 26: Postmortem Examination). We suggest you block the entire reproductive tract as one if possible, as this makes staging the reproductive cycle and detecting asynchrony more straightforward. We would also suggest you take longitudinal sections of vagina rather than the transverse sections that seem to be often taken.

This completes the dissection of the abdomen and pelvis. We have not discussed or examined the nerve supply of the viscera, or the nerves that leave the spinal cord and run on the posterior wall of the abdomen. We have also not said anything about the lymphatic system, but you will have come across a chain of lymph nodes running alongside the abdominal aorta. For details of these structures, see the works listed in the Bibliography. Review your dissections and make sure that you can recognise all the structures discussed above and shown in the diagrams.

The Thorax and Neck

The thorax contains the heart, great vessels, lungs and the thymus gland. It is dissected along with the ventral aspect of the neck. Although the contents of the thorax itself present few problems, the neck is a difficult area because so many important structures are packed into a very small space. In postmortem work, this does not matter as the pathologist is not usually seeking to identify the vessels and nerves in detail, but when learning about the anatomy of the rat we must pay some attention to these details.

The thorax is an elastic box that communicates with the neck via the inlet of the thorax. The elasticity is provided by the ribs, which are attached dorsally to the thoracic vertebrae, and ventrally (in regard to the upper seven ribs), direct to the sternum. Ribs 8, 9 and 10 are attached to the sternum via number 7. Ribs 11, 12 and 13 are not attached to the sternum at all, although all are of course attached to the corresponding vertebrae. Unlike man, the rat has 13 thoracic vertebrae and 13 ribs. The first rib bounds the inlet of the thorax by forming an arch that stretches from the vertebral column to the upper end of the sternum. All the great vessels entering and leaving the upper end of the thorax; the oesophagus, the trachea and a number of important nerves have to pass through this narrow inlet. The posterior end of the thorax is closed by the diaphragm. This arched partition of muscle and tendinous connective tissue is attached to the posterior end of the sternum, to the lower ribs, and the vertebral column. It is perforated by the aorta, the posterior vena cava and the oesophagus. Within the thorax are the pleural cavities, closed cavities that envelop the lungs (see Chapter 12: The Lung). The heart lies in the middle of the thorax enveloped by the pericardium. The great vessels, trachea and oesophagus lie in the midline of the thorax in a fat-filled space described in human anatomy as the mediastinum.

Begin by lifting the xiphoid with forceps and examine the undersurface (posterior surface) of the diaphragm. The periphery of the diaphragm is pinkish in colour because of the high muscle content, becoming almost transparent towards the centre. You will see the pink lungs through the semi-transparent diaphragm. Take the fine scissors and pierce the diaphragm on the left side. The lung will immediately draw away from the diaphragm, collapsing because the pleural sac has been punctured. Repeat on the right.

Remove the anterior chest wall by cutting as shown in Fig. 25.10.

You will be cutting through ribs, so use the coarse scissors or bone forceps. The cuts through the ribs join at the lower end of the neck. Be careful not to cut into the tissues of the neck. In cutting towards the anterior end of the chest wall, you will meet the clavicle. This bone connects the anterior end of the sternum with the large bone of the shoulder girdle, the scapula. You will have cut through the first rib, thereby opening up the thoracic inlet. You will not be able to see much in this area just yet and we shall have to nibble away more of the first rib and clavicle in just a moment.

Examining the organs of the thorax you will notice that the heart (easy to find: dark, red and pointed), partially covered by a mass that has a similar appearance to fatty tissue (Fig. 25.11).

This is the thymus, a vitally important part of the immune system. In younger animals it is quite substantial, but may wither away to almost nothing in the older animal and be quite difficult to identify. Dissect out the thymus and associated fat and if you are taking specimens for histology, put the thymus in a jar of fixative (see Chapter 26: Postmortem Examination).

Removing the thymus reveals more fat. Remove this carefully with the fine forceps, or if necessary fine scissors, to reveal the left and right anterior venae cavae. Between the great veins lies the trachea, recognisable from the rings of cartilage in its wall. Before going any further, remove the pectoral muscles. Grip the cut edge of one pectoral muscle and lift it gently, separating it from the underlying tissue. Beneath this is the clavicle, and running in from the arm, the axillary vein. This vein runs behind the clavicle to cross over the first rib to join the external jugular vein, forming the anterior vena cava. If you cut one of these large veins you will need to mop the

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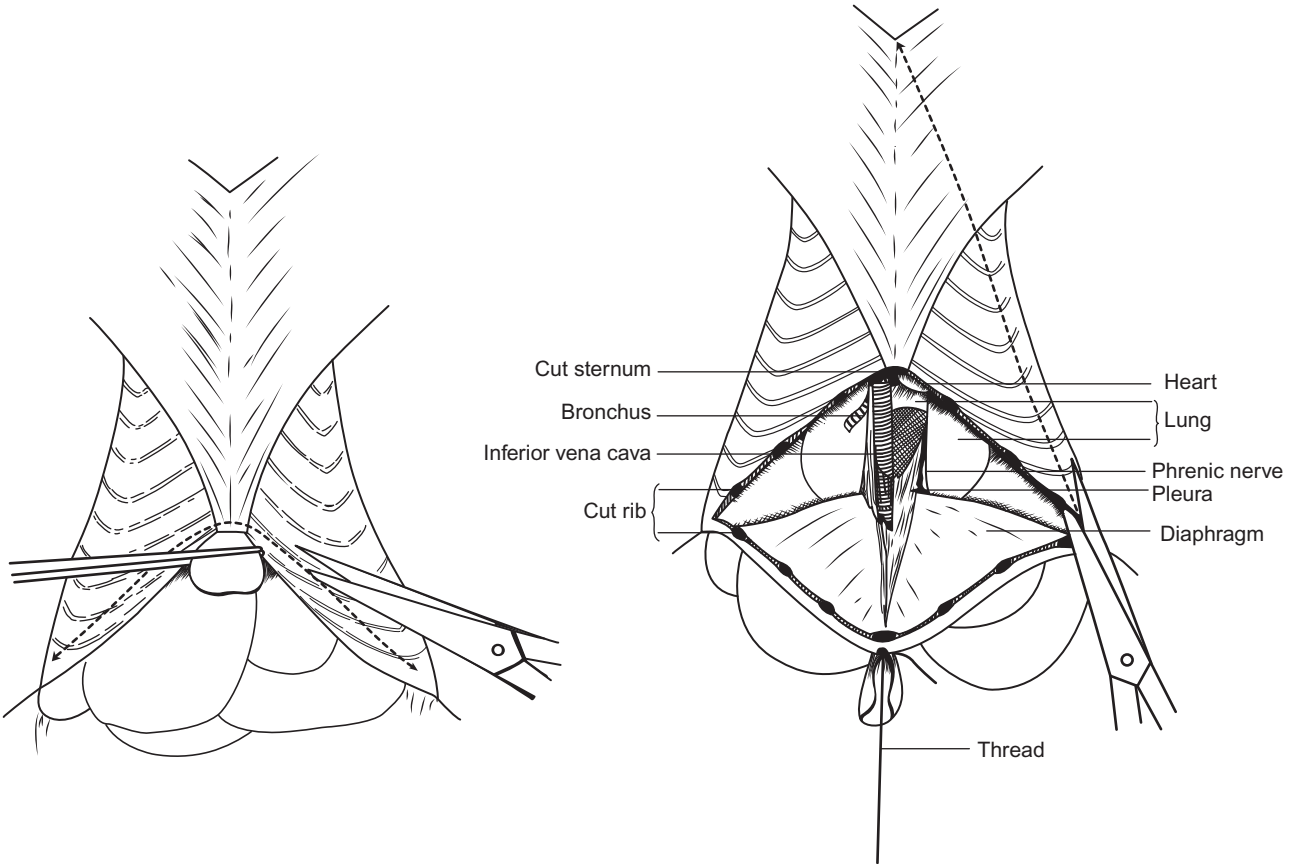


FIGURE 25.10

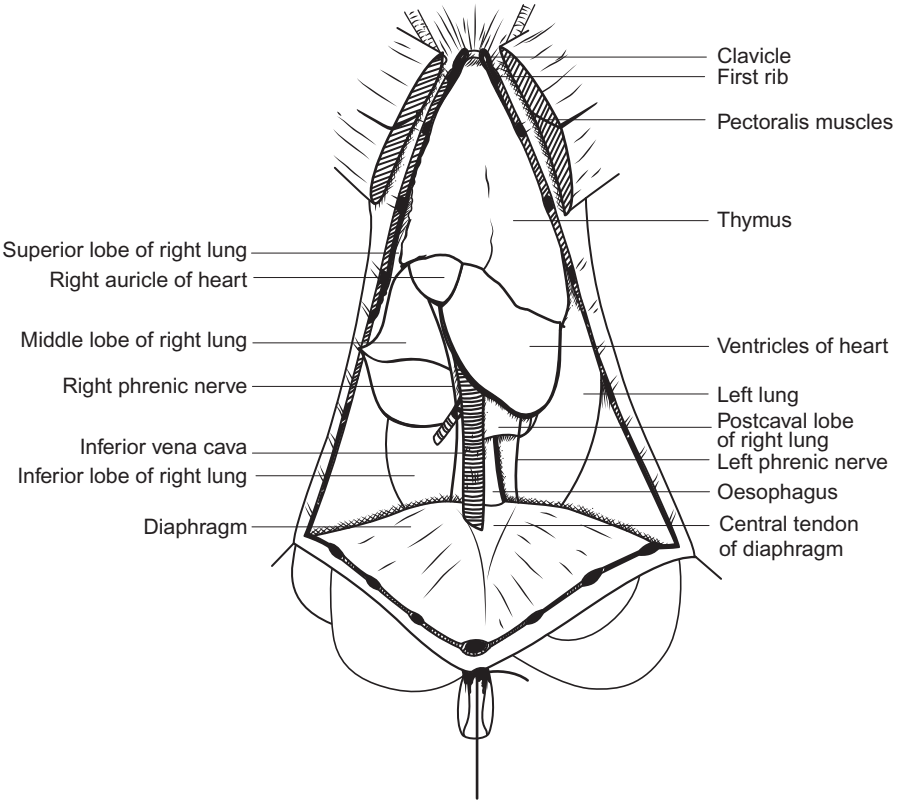


FIGURE 25.11

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area, but try not to, as they are easier to recognise when they are full of blood. If the animal has been bled out after death then, of course, this point does not apply. Repeat on the other side, and nibble away the clavicle and the cut ends of the first rib using the bone forceps. Go easy as the axillary vein is just beneath the bone. What you are doing is to widen the space so that the underlying structures can be dissected. You will notice that the trachea, which you saw in the upper part of the thorax, disappears under a sheet of muscle in the neck. This is the sterno-hyoid muscle, running from sternum to hyoid bone. Pick up the muscle with forceps, make a nick and then extend the incision upwards and remove the muscle. The trachea will now visible running up to the larynx (Fig. 25.12).

Other muscles present on either side include the sternomastoid muscles (the ones that give you a stiff neck if they go into spasm) and the omo-hyoid muscles. Remove them or turn them up out of the way.

For the next part of the dissection, you will need a magnifier unless you have very sharp eyesight. By now, you should have a clear view from the larynx straight down to the heart. Using fine forceps carefully remove as much fat and connective tissue as you can, looking for the vagus and the phrenic nerves.

Observe the external jugular veins as they sweep down the neck, well away from the trachea. Note the large lymph nodes just to the medial side of the vein. These can be removed, although they are not really in the way (Fig. 25.13).

Lying on either side of the trachea are the common carotid arteries, and on their lateral sides the thin vagus nerves that run down the thoracic inlet with the arteries into the thorax. The only structure that you might mistake for the vagus is the sympathetic cord, which links the sympathetic ganglia in the neck, but that is even harder to find than the vagus! Assuming that you have found the vagi, a closer examination of the trachea should reveal two very fine nerves running up on its anterior surface, towards its sides. These are the recurrent laryngeal nerves, branches of the vagus nerves, which are actually running up to the larynx where they supply most of the very small muscles of that structure. On the left, the recurrent laryngeal nerve hooks round the arch of the aorta, actually around the remains of the ductus arteriosus, and runs forwards to the neck. On the right, the recurrent laryngeal nerve merely hooks around the right axillary artery. The phrenic nerve is a branch of the brachial plexus and supplies the diaphragm. It runs back through the thorax to the diaphragm. If you follow the anterior venae cavae and

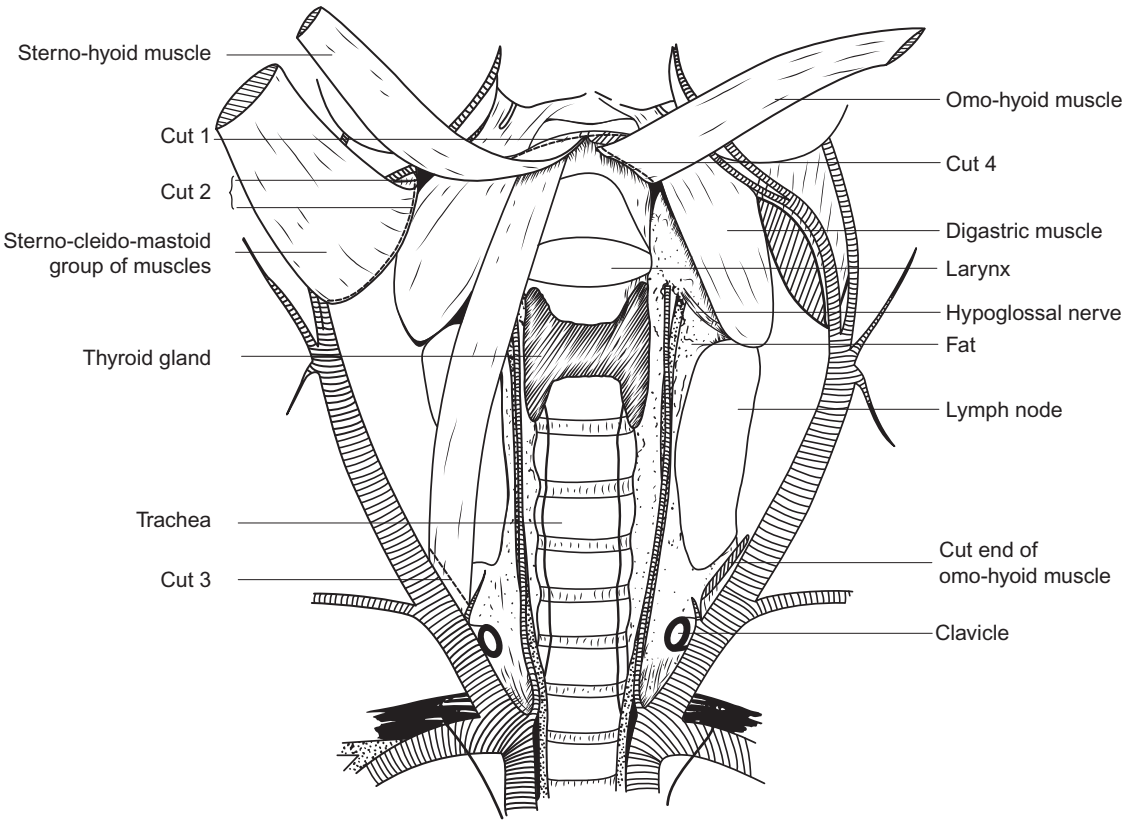


FIGURE 25.12

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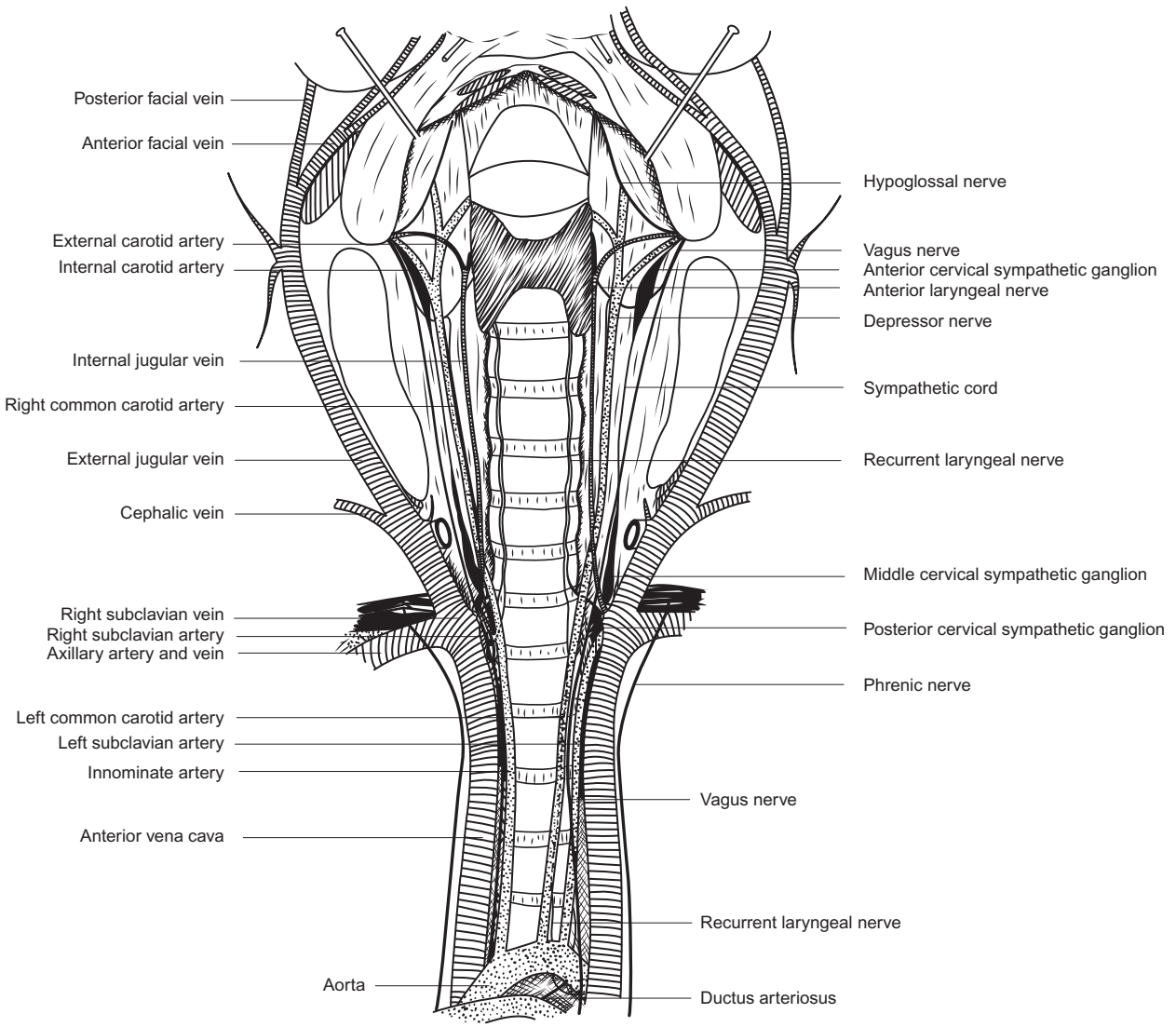


FIGURE 25.13

dissect on their lateral sides the phrenic nerves are not difficult to find.

While looking for the vagus nerves you will have noticed that the common carotid arteries are a lot smaller than the external jugular veins. In this region, the veins of the rat are rather different from the veins in man. In man, the external jugular is small and the internal jugular large; the converse is true in the rat. The rat has two anterior venae cavae, but man has only one. The arteries are however the same as in man, with three large arteries arising from the arch of the aorta. The innominate artery (a particularly pointless name meaning unnamed or nameless) divides into the right axillary artery (running to the axilla or armpit, and thence to the foreleg) and the right common carotid, the left common carotid and the left axillary artery.

Working downwards you will need to dissect carefully to identify these great arteries as they leave the thorax beneath (looking down on the dissection) the veins. Draw the heart to the right to enable the vessels leaving and entering the heart to be seen more clearly. We shall examine them in detail in just a moment.

We may now remove the larynx, trachea, heart and lungs, sometimes known as the thoracic pluck, and continue the dissection away from the rat. Run your fine forceps behind the larynx and cut it as near the lower jaw as you can. Holding the larynx with the forceps and dissecting its dorsal surface, you will find the oesophagus, a pale narrow tube. Cut it behind the larynx as high as you can. Lower down you will find it is stuck to the back of the trachea: leave it there. Cut the great vessels where they enter or leave the thorax and pulling gently, remove the

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heart and lungs in one piece. Cut any structures that prevent you from removing it from the thorax. Cut the posterior vena cava, the aorta and the oesophagus as they pass through the diaphragm. You will soon have the heart and lungs dangling from the trachea and quite free from the rat. Place the specimen you have removed on a dissection board or in a Petri dish. Dissection of the heart and lungs is easier if they have been placed in formalin for 24 hours. In their fresh state, they are rather soft and difficult to handle. Let us assume that you place the specimen in formalin and come back to it 24 hours later.

The Thyroid Gland

Before tackling the heart and lung, it might be sensible to deal with the thyroid gland. The thyroid gland lies across the trachea just below the larynx (see Fig. 25.13). It always stands out clearly in diagrams in books, but is less easy to see in reality. It is a small gland, and the portion lying in front of the trachea linking the two lateral lobes, is really very thin indeed. You certainly will not see the small parathyroid glands embedded in the dorsal surface of the lateral lobes of the thyroid. If you are collecting histological specimens, refer to Chapter 26, Postmortem Examination.

Heart and Lungs

Lay the heart and lung specimen, with its dorsal surface uppermost, on a dissecting board. Examine the oesophagus as it runs in a straight line down the dorsal surface of the specimen. Lift the anterior end of the oesophagus with forceps and using fine scissors, dissect it away from the rest of the specimen. The main difficulty in dissecting this specimen is that it is small and difficult to hold. Pin the specimen down on a dissecting board. A small cork board is very useful for this.

Examination of the dorsal aspect of the trachea shows that the cartilage rings do not make a complete circle (see Chapter 12: The Lung). Dissect down until you find and can clean the bifurcation of the trachea. On the left of the specimen, the aorta arches over the left main bronchus like the handle of an umbrella hooked on a man's wrist (Figs. 25.14 and 25.15).

If you lift the medial edges of the dorsal surfaces of the lungs you will see that while the left has only one lobe, the right lung is divided into several lobes, including the small postcaval lobe that crosses the midline behind the posterior vena cava, preventing you from seeing this vessel.

Dissect the aorta free from the lungs and lift it up above the left main bronchus. Now dissect the trachea, left and right bronchi, and lungs, away from the heart and great vessels. One would wish to leave as much as possible of the great vessels attached to the heart, although you may find this tricky on your first attempt. You now have two specimens, the lungs and the heart.

Lungs

Lay the lungs on a dissecting board, ventral surface uppermost. As laboratory rats neither smoke nor are they exposed to air pollution, their lungs are pale pink. In man, they are often slate grey and in coal miners were often black. Note that on the left, the lung is made up of only one large lobe. Identify the lobes of the right lung by reference to Fig. 12.1 of Chapter 12, The Lung. If you are collecting histological specimens refer to Chapter 26, Postmortem Examination.

Heart

As the rat heart is small, there is much to be said for obtaining a lamb's heart from a butcher and dissecting that. However, if you have time, enthusiasm and a magnifier proceed as follows. When in the body, it is easy to tell one of the surface of the heart from another, but less easy when you have it in your hand. The ventral surface of the heart is the aspect of the heart you examined when the heart was still in the chest. It comprises mainly the right ventricle, with the left ventricle peeping round the left edge. Having identified the ventral surface of the heart, turn the specimen over and look at the dorsal surface. This is much more complicated, so you will have some difficulties in identifying all the vessels. Using Fig. 25.15 as a guide, find the aorta arching over the pulmonary artery. Find the posterior vena cava and follow it to the right atrium. The pulmonary vein (just one in the rat, four in man) runs to the left atrium. Examine the arch of the aorta and confirm that three branches are given off from the arch (see above). If you are taking specimens for histological examination refer to Chapter 26, Postmortem Examination.

Salivary and Lacrimal[2] Glands

The rat has an impressive array of salivary glands, presumably because it eats rather dry food. You will have seen these glands when you made your original incision from the lower jaw to the lower abdomen and reflected the skin.

The arrangement of the salivary glands and the lacrimal glands is shown in Fig. 15.5 of Chapter 15, Exocrine Glands. The rat has essentially the same salivary glands as man. A large parotid gland, a large submaxillary gland (called the submandibular gland in man) and smaller sublingual glands. It is also well equipped with lacrimal glands. An intra-orbital gland lies in the orbit and an

2. Lacrimal can be spelt as lachrymal if you wish.

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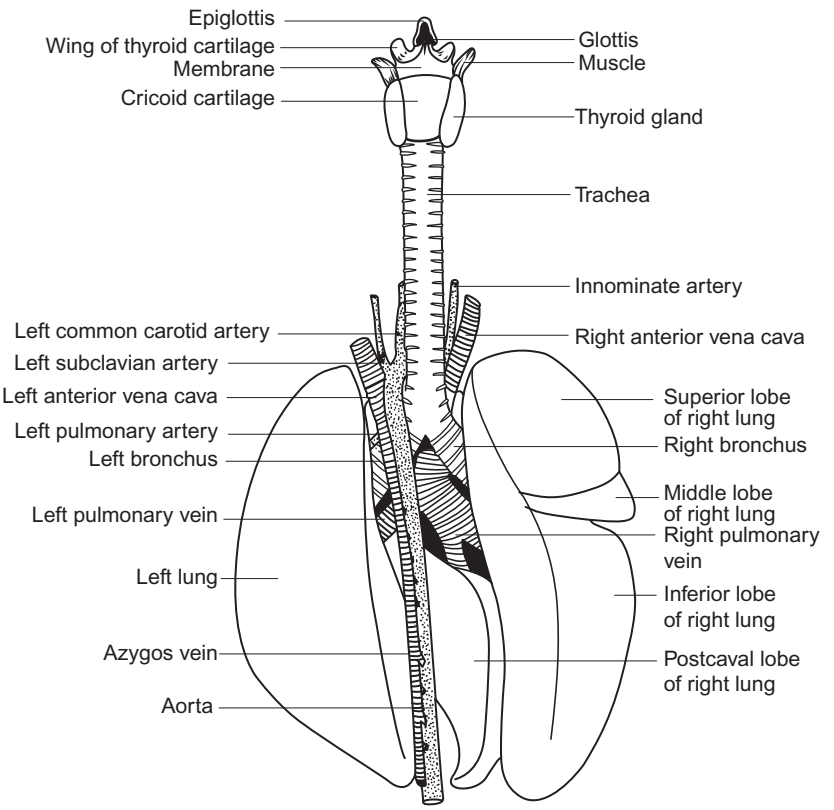


FIGURE 25.14

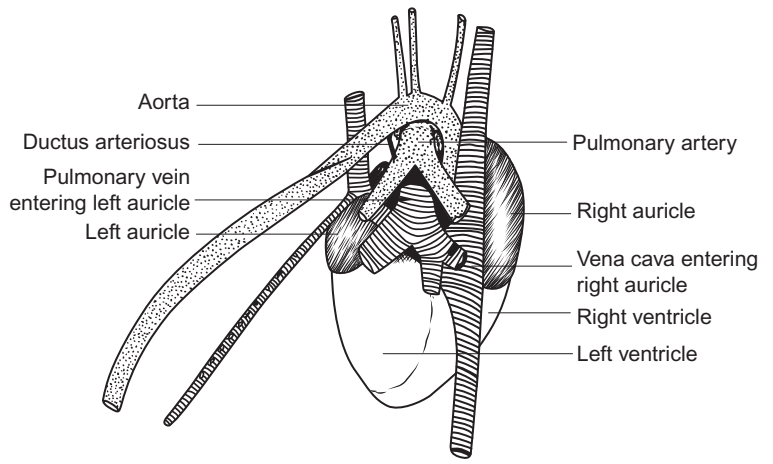


FIGURE 25.15

extra-orbital gland lies on the surface of masseter close to the parotid gland. Man has only an intra-orbital lacrimal gland. The rat also has a Harderian gland, located with the intra-orbital gland. Man does not possess Harderian glands (see Chapter 15: Exocrine Glands).

As you remove connective tissue from around the salivary glands note that there are large lymph nodes in this area that can be easily taken for salivary glands. The parotid and the extra-orbital lacrimal glands look rather alike, but are more finely lobulated than the submaxillary glands. The large masseter muscles that can be seen on either side of the lower jaw allow the rat to gnaw efficiently. Between the masseters lies the mylohyoid muscle that forms the floor of the mouth. If you dissect away the

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mylohyoid muscle you will see the ducts of the submaxillary gland run between the mylohyoid and the tongue. The duct of the parotid gland takes a different route, running over the masseter and opening into the side of the mouth just in front of the edge of the muscle. Clean the connective tissue from the surface of the masseter and look for this duct. If you are taking specimens for histological examination, you can now remove the salivary glands and the extra-orbital lacrimal glands as a single block of tissue (see Chapter 26: Postmortem Examination).

REMOVAL OF THE EYE AND ASSOCIATED GLANDS

The entire eyeball can be removed by gripping the sclera with forceps and pulling the eye forwards in its socket (the orbit). You will notice that the space behind the eyeball is filled with fat. Cut the optic nerve and remove all the contents of the orbit. The intraorbital lacrimal gland and the Harderian gland are wrapped, in part, around the optic nerve. You must remove these with the eyeball itself. For details of histological processing of the eye and associated glands refer to Chapter 26, Postmortem Examination.

REMOVAL OF THE HEAD AND LOWER JAW

The lower jaw articulates with the skull beneath the ear at the temporomandibular (TM) joints. Remove the skin from the head and neck and separate the head from the trunk by cutting through the cervical spine just below the skull with bone forceps. Cut away the muscles attaching the lower jaw to the remainder of the skull and separate it from the skull using bone forceps if necessary to cut through the TM joints. You now have a lower jaw with the tongue intact, and the remainder of the skull.

While you have the lower jaw before you, examine the teeth. The rat has a classic rodent dentition, with large incisors and a gap (diastema) between the incisors and the molars. The diastema is important to the rat. The lips are drawn back into this gap during gnawing thus preventing material getting into the mouth.

Observe the teeth. The rat is monophyodont (man is described a diphyodont because he has two sets of teeth: milk teeth and then permanent teeth). The rat's incisors continue to grow throughout life, and if not worn down by contact with opposing teeth can pierce the skull and kill the animal. The incisors are huge teeth with the open-ended roots that sweep most of the way round the lower jaw. The rat, like man, has three molar teeth in each jaw, but no premolar or canine teeth. Examine the tongue. You will see that it is a long, narrow structure (see Chapter 13: Alimentary Canal or Gastrointestinal Tract). Dissect the tongue from the lower jaw and place it in fixative (see Chapter 26: Postmortem Examination).

Turning to the remainder of the head, place the specimen so that you can examine the roof of the mouth. Note the ridges running across the roof of the mouth and the soft palate that continues back from the hard palate. If you removed the larynx when you took out the trachea you will not be able to confirm that in the rat the epiglottis comes into contact with the soft palate pushing the upper end of the larynx into the nasopharynx.

REMOVAL OF THE BRAIN AND EXAMINATION OF OTHER STRUCTURES OF THE HEAD

We are approaching the end of our dissection. The last part involves removal of the brain. Removal of the brain is easier using a fixed specimen, as the fresh brain has the consistency of a badly set blancmange, and so is easily damaged. Adequate examination of the nasal passages requires the multiple histological sections of the skull, for which decalcification of the skull is a prerequisite. For the decalcified specimen, take the skull on which you have been working (see below) and place it in a jar of formalin for a further 3–4 days, then decalcify according to the methods set out in Chapter 26, Postmortem Examination. We shall use this specimen for examination of the nasal passages and Zymbal's gland (see Chapter 10: Nasal Cavity, Chapter 24: The Skin or the Integument and Chapter 26: Post-mortem Examination).

Perfusion Fixed Specimen

The preferred method for optimal preservation is to perfuse fix the brain (see Annex 2). Although in situ immersion fixation of the brain works well enough for mice and juvenile rats, for an adult rat it is not ideal. After perfusion, we remove the head and place in fixative for around 48–72 hours before we remove the brain. The perfused fixed specimen can also be used for examination of the eye and the intra-orbital glands, as well as for collecting specimens of other tissues for histological examination and for general dissection. Perfusion fixation provides very good fixation of tissues such as kidney, adrenal gland and testis, which are difficult to fix well by other means.

Removal of the Brain

Rowett offered excellent general advice on the removal of the brain:

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'Nervous tissue is very soft and has very little elasticity. Even after "hardening" with formalin it is much softer and more easily cut than other tissues. It is therefore very easily damaged by any slip of the instruments and the removal of the brain requires much care and patience. The skull must be cut away in VERY SMALL PIECES and the pressure on the instruments must be fully under control all the time'.

We shall follow the method outlined by Rowett.

Make a midline incision on the upper surface of the head, from the nose to the upper neck. This incision may be made with a scalpel (one of the few times when the use of scalpel is justified in dissecting a rat!). Use a small blade and do NOT cut into the skull. The skull of the rat is thin and easily damaged. Turn back the skin on both sides using coarse forceps and your fingers. In some specimens, you will be able to see the brain through the thin roof of the skull.

The next step is to make a small hole in the roof of the skull. Rowett's advice on how to do this is particularly helpful and clear:

> *Grip the head of the rat with the thumb and second finger and support the chin with the first finger of the left hand. The free part of the skin helps to give a good grip. [The free part of the skin is the part reflected from the dorsum of the skull when the first incision was made] Hold a scalpel level with the back of the skull, as shown, and pierce the interparietal bone.* NOTE. *It is very important to MAINTAIN THE HORIZONTAL POSITION OF THE SCALPEL. Any tilting will either cause piercing of the brain or failure to perforate the skull. The entry of the scalpel point causes the interparietal bone to crack away along the sutures with the parietals and squamosals (see Fig. 25.16).*

Having opened the skull the bone covering the brain can be nibbled away. In our experience, the easiest way to do this is to use the artery forceps to peel off small pieces of bone, gradually working forwards until the cerebral hemispheres exposed as fully as possible. Other workers remove the roof of the skull by inserting the tip of one blade of a pair of scissor and snipping and twisting out bits of bone. THIS IS A GOOD WAY TO RUIN THE FINE DISSECTING SCISSORS! DO NOT DO THIS WITH YOUR FINE SCISSORS! An old pair of scissors that are useless for anything else might be used, but care is necessary. Do not poke the tip of the blade into the brain. Bone forceps? Unless you have a really small pair these will be too large to use effectively. Orthopaedic surgeons use an instrument called 'bone nibbling forceps'. A small pair of these might be used, but the blades are usually too large as the lower blade has to be thin enough to fit between the skull and the underlying brain. The golden rule is that whichever approach you decide to try, whittle away the skull slowly.

Expose and cut through the dorsal parts of the atlas and axis vertebrae (the anterior two vertebrae of the vertebral column). Cut under the olfactory lobes, severing the olfactory nerves. Working from the side, cut under the posterior part of the brain, again severing cranial nerves. Only the occipital bone, surrounding the undersurface of the posterior part of the brain and importantly and awkwardly (!) the parafollicular lobes of the cerebellum on each side now remains. Cut through the spinal cord as far down it as you can with the point of the scalpel.

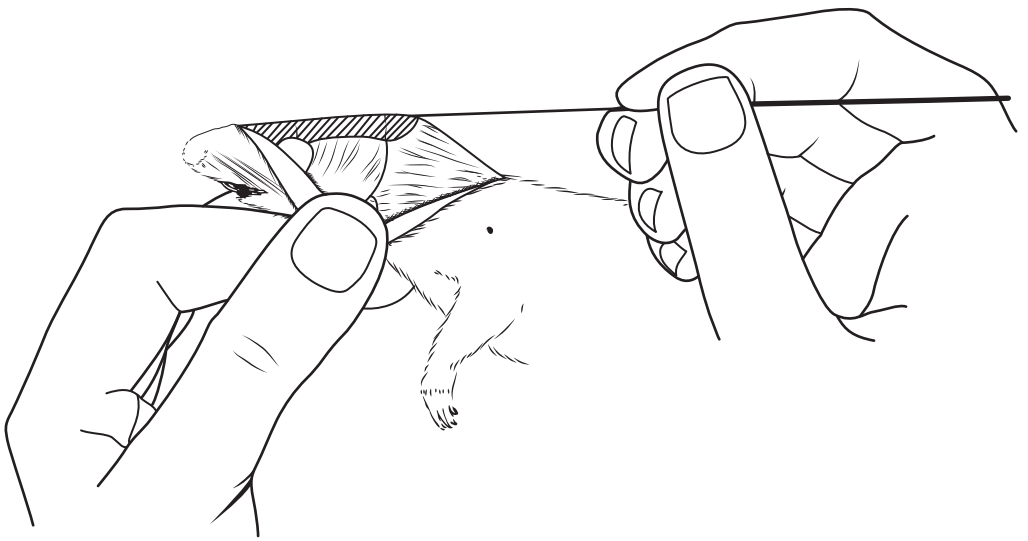


FIGURE 25.16

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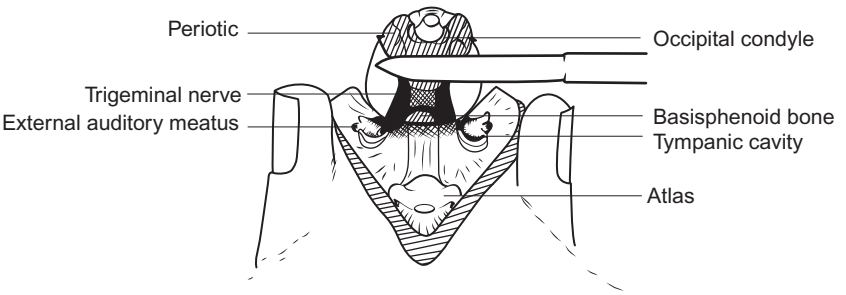


FIGURE 25.17

Now for the step which requires real confidence and some practice.

Rowett again:

> *Change the grip and position of the scalpel. Prise the posterior part of the base of the skull upwards. This breaks the suture between the occipital and basisphenoid bones and also between the tympanic bulla and the periodic (see Fig. 25.17).*

As you lift the occipital bone (still around the undersurface of the posterior part of the brain) you will see the large trigeminal nerves. Cut them as far away from the brain as you can and lift the brain and the remaining attached piece of occipital bone from the rest of the head. As you lift, cut any remaining cranial nerves, but in practice they break very easily.

Lay the brain down on its upper surface on a dissecting board. Cut the occipital bone in the midline using the coarse pair of scissors. Break away the bone on each side using forceps. The bone covering the parafollicular lobes will annoy you as it almost completely covers these little lateral lobes of the cerebellum and removing it without damaging the underlying brain is a real test of patience and skill. Trying to do this in an unfixed specimen is to doom oneself to disappointment.

Remember – do not try to hold the brain with forceps as you will simply cut into it and ruin the specimen. Hold the remaining bits of occipital bone with forceps as you work away at the bone covering the parafollicular lobes, using two pairs of forceps to pull away the last bits of bone is helpful.

The brain should be examined with a hand lens (see Chapter 20: The Brain and Spinal Cord for a description of the external features of the brain).

FURTHER READING

Rowett, H.G.Q., 1952. Dissection Guides. III. The Rat (With Notes on the Mouse), second ed. John Murray, London (reprinted 1965).

ANNEX 1

Dissection: Process and Instruments

Dissection is undertaken for two major purposes:

1. To discover structural relationships for the first time, that is as a journey of exploration or as part of a learning process.
2. As a means of discovering pathological changes in organs and tissues.

For many readers of this book, the latter will be the more important and dissection will be undertaken as a part of the postmortem examination of experimental animals. Before discussing the techniques of postmortem examination (see Chapter 26: Postmortem Examination) it is worth considering dissection itself, as a means of learning and discovery.

All dissection is essentially destructive. The specimen is 'taken apart' to discover its structure. This process involves the removal of tissue that obscures the relationships between structures, fat and connective tissue are also removed. Because of this, it is essential to observe and record appearances as you go. The animal, unlike a car engine or clock, cannot be perfectly reconstructed after it has been taken apart. If, for example, you do not observe and record, at least mentally, the relationships of one abdominal organ to another before you remove them for further examination you will be unable to observe these relationships again. Thus an orderly process is demanded. Dissection should be methodical and initially at least, a slow process.

Relationships and appearances can be recorded verbally by taking notes on paper as you work or by dictating notes. Many pathologists dictate as they work. Drawings can be made, photographs can be taken and films or video recordings can be made. The need to record as you go is often stressed and for good reason. Memory is faulty and a record made after the dissection has been completed will be more subject to error than one made during the dissection. At one time, the making of drawings of

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dissections was a standard requirement in schools and universities. This was tedious for the student and those apparently lacking artistic skills felt at a disadvantage. Teachers stressed that artistic skills were not required, all that was needed was a capacity to observe and record with a pencil what was seen. Measuring, using a pair of dividers and a ruler was recommended and drawings were required to be 'to scale'. It was accepted, and indeed it is true, that one looked more closely, one observed better, when one was required to produce a drawing.

Once a dissection is completed you will have learnt all that you can learn from that dissection. Of course, you could repeat the process and learn more, but in postmortem work this is not possible. Your specimen may be the only specimen that shows certain features and once destroyed by dissection those features, in a physical sense, may be lost forever. The need for care and for the making of records is obvious.

Dissection in More Detail

Dissection requires practice. We have three objectives in view:

1. Dissection undertaken to acquire a working knowledge of the more important aspects of the anatomy of the laboratory rat.
2. Dissection undertaken to acquire specimens for histological study.
3. Dissection undertaken as the postmortem examination of tissues which might show abnormalities.

These three purposes or objectives are not mutually exclusive, but neither are they identical in terms of the approaches needed during dissection. The reader who is familiar with the anatomy of the rat will be mainly concerned with the second and third objective, but for the benefit of those who may not have done much dissection we begin at the beginning and consider firstly the process of dissection.

The Specimen

We assume that the reader will begin by dissecting an adult rat. Both male and female specimens should be dissected. We also assume that the animals will have been killed in accordance with humane principles and Good Laboratory Practice guidelines. Rats may be killed by exposing them to carbon dioxide or by intraperitoneal injection of an overdose of a barbiturate anaesthetic. The latter is an excellent method. An injection of sodium pentobarbitone (100 mg/kg) is effective. The *Universities Federation Animal Welfare Handbook* (*UFAW Handbook*) is the standard reference. Another very useful book is *Animal Anaesthesia* by C.J. Green (see Bibliography). Both books give details of the correct means of ensuring rapid and painless euthanasia of laboratory animals. Euthanasia by neck dislocation is not recommended when anatomical and histological work is planned as damage to some tissues is inevitable. All animals must be treated with respect, there is no case for careless or slap-dash methods when working with experimental animals.

Dissecting Instruments

Many workers seem to show little interest in their instrument, which is a pity because dissection is easier if appropriate, sharp and well-maintained instruments are used. Instruments such as pairs of scissors, forceps and bone scissors (also called bone shears and, for some unfathomable reason, bone forceps) should be bought from a maker of first-class surgical instruments. Nearly all surgical instruments are made of stainless steel and will not rust. The days of nickel- plated instruments have passed. Pairs of scissors need to be cared for, and if used for cutting inappropriate materials the set of the blades will be damaged and the instruments will become useless. Several pairs are necessary. Scalpels are not much used in dissection of small animals, as the free use of a sharp pointed blade can do more harm than good. But scalpels are needed for cutting up tissue so should be available. Scalpels of the Swan Morton type are equipped with disposal blades. Changing the blade of a scalpel is one of the more dangerous tasks in a biological laboratory. A slip may sever a tendon or digital nerves. Experienced workers grip the blade between thumb and index finger of their right hand and pull it away from the handle having flipped up the heel of the blade with their left thumbnail. This method is definitely not recommended. It is much better to lift the heel of the blade with a pair of artery forceps and then to push off the blade away from you. The old rule: 'keep fingers behind the cutting edge' is always worth remembering. Removing a blade which has become stuck in place by an accumulation of blood and debris is more difficult and should not be necessary if instruments are properly looked after. If you do need to do this soaking in hot water with added detergent before attempting removal is advised.

The Basic Kit for Dissection of Small Animals

1. One pair of large, sturdy dissecting scissors. These should have strong pointed blades and handles long enough to provide good leverage. Six-inch handles are very satisfactory, though smaller pairs of scissors are quite adequate. Some workers use such scissors for cutting bone, but it is better to use bone shears.

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The coarse scissors are needed for cutting skin and tendons.

2. One pair of fine pointed dissecting scissors. These should be reserved for fine work and must cut right to their tips. Ophthalmic surgeons use very fine scissors, and these are expensive. Fine scissors with tungsten inserts along the cutting edges of the blades are very effective and long-lasting. Scissors cannot be sharpened or set by anyone except an expert technician, thus scissors need to be kept in good condition by their users. One sure way of ruining fine dissecting scissors is to use them to cut your fingernails! You will use fine scissors more than any other instrument except forceps, so look after them, and obtain the best quality that you can persuade your employer to afford. Slipping a short length of fine bore rubber tubing over the closed blades of the scissors prevents damage to the points when the instruments are not in use.
3. One pair of small bone shears (bone forceps). These are needed for cutting ribs. Only a small pair is needed, the large instruments used in postmortem work on humans and large animals are unwieldy and useless for work on rats. Bone shears are not to be used for cutting wire or pins as the cutting edges of the blades are often not sufficiently hard for such tasks.
4. One large Swan Morton scalpel (Number 4 handle) fitted with a blade with a well-curved, convex, edge – for example, a Number 21 blade. The well-curved edge allows the belly of the blade to be used for cutting rather than the point. Sherrington (see below) stressed that when making an initial incision through the skin the scalpel should help 'comme un archet', like a fiddle-bow, and that the cutting should be done by the belly of the blade and not by the point. It hardly seems necessary to point out that scalpels should not be used for anything other than dissection. Sharpening of pencils, cutting cardboard or rubber tubing, stripping the insulation from wire, etc., requires other instruments.
5. One small Swan Morton scalpel (Number 3 handle) fitted with a small blade with a curved edge (a Number 16 blade, e.g., or the Number 10 if a larger blade is needed) for fine work. Swan Morton makes a large range of scalpels and blades. Although sharp pointed blades (e.g., number 11) are useful, they should be used with discretion as the point can do harm and is easily lost from view while dissecting. Sterile blades designed for use by surgeons, are not necessary for routine dissection.
6. One pair of 'blunt pointed' forceps. These are the coarse forceps of the basic kit. A pair of 5 inches long is satisfactory for work with small animals. Blades should meet well at their tips and should be transversely ridged (serrated) on their inner surfaces to improve the grip on tissues. Toothed forceps, with interlocking sharp pointed teeth set at their tips at 90 degrees to the blades are not necessary, though they do allow a very firm grip to be taken on skin.
7. One pair of fine pointed forceps. Again, a 5-inch pair is satisfactory. The tips should meet precisely and a pin or peg should be present on the inner surface of one blade of the forceps. This pin inserts into a hole in the other blade and prevents the points crossing. Very fine pointed watchmaker's forceps are seldom required. Fine forceps can easily be damaged by inappropriate use. Sir Charles Sherrington, Nobel Prize winner and the most distinguished neurophysiologist of the last century, invented what is known as 'Sherrington's dagger-tipped forceps'. These had fine points, but the blades expanded above the points like the blades of two small daggers. A pin or peg was provided to prevent the points crossing. The great advantage of this instrument was that it provided fine but strong points, much stronger than conventional fine forceps. Very similar forceps are still available as 'splinter forceps' (see www.wms.co.uk › Surgical Instruments › Forceps)
8. One bent seeker or probe. A bent, blunt needle set in a wooden or, better, metal handle. Fine, sharp pointed needles mounted in handles are sometimes suggested: a pin vice provides an excellent way of holding a needle firmly. These are not of much use in routine dissection work.
9. One straight seeker or probe of similar design, but again with a blunt point.
10. Two pairs of fine pointed curved artery forceps that lock when the handles are brought together are useful, but not essential.
11. Large pins or spikes set in wooden handles (steel awls) are needed for pinning out the animal for dissection.
12. A selection of fine-pointed artery forceps is useful. Before using, practice locking and unlocking the handles using one hand.

Additional Equipment

13. A dissecting board is essential. A wooden board is satisfactory, but plastic boards like those used by chefs for cutting vegetables on are better. Metal, glass and granite composition surfaces should be avoided as they rapidly blunt the edges of scalpel blades. If dissecting pins are used, then a wooden board into which they can be driven is essential. A cork covered board makes insertion of the pins easier. If pins are not to be used then clips that can

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be attached to the animal's skin and by cords to tying points of the dissection table should be used. Surgical towel clips are effective, though spring-loaded tie clips are just as good, but increasingly difficult to find.

14. A supply of cotton wool or tissues for mopping up blood and other fluids.
15. Some fine but strong cord is needed for tying off structures while dissecting. Surgical suture silk is ideal. Do not choose a very fine silk suture because it snaps so easily, on the contrary, choose a coarse one: 2-0 or 0 gauges are suitable.
16. A good light is essential.
17. A magnifier that incorporates a light (a circular fluorescent tube around a large diameter lens) is a luxury, but a very desirable one. Older workers find they need both a good light and some magnification to see small objects. Loupes (magnifying spectacles) are very effective. Those made by Keeler (www.keelerusa.com/ophthalmic/index.../surgical-loupes-and-lights.html) are outstanding, but their high-quality optics make them costly. Such instruments offer a good field of view and a long working distance. A reading glass mounted or a long adjustable, spring support is a cheap alternative. A dissecting microscope will not often be needed unless you are interested in for example following the ramifications of the cranial nerves.
18. A balance is needed for weighing animals and organs, a top-loading laboratory balance is ideal.
19. Gloves. It is sensible to wear surgical gloves during dissection. Surgical gloves are powdered with starch and should be rinsed from the hands before dissection begins. Starch granules can contaminate histological sections and can be recognised by their well-known Maltese Cross appearance when viewed under polarised light. A face mask is also a sensible precaution. Appropriate clothing should be worn. Avoiding infection is obviously important. This is vital when a recently killed, unpreserved, animal is being dissected. If the body has been preserved in for example formalin, the risk of infection is reduced very substantially.
20. A workbench or table is an obvious requirement. Some attention should be given to the chair or stool which you to sit on. An adjustable chair with a good back support will prevent strain. Crouching over a dissection leads to backache. Pay attention to ergonomics!

The blowpipe and section lifter usually found in dissection kits are not of great help. The blowpipe was used for inflating organs such as the gut, bladder and lung: it is not required. The section lifter is used for lifting rather thick histological sections from one bowl of processing fluid or staining solution to another. The camel's hair brush, also often provided, is for use when making histological preparations. Scissors with angled blades, curved blades or with one blade ending in a small knob (used by pathologists for opening intestines), are in general not necessary. Angled forceps (packing forceps as used by dentists) are not essential, but are useful when ligating blood vessels. The instruments that you will use more than any others are the fine scissors and the blunt forceps. All instruments should be washed after use in hot water containing an antibacterial solution and then in cold water and dried before being put away safely. Instruments should not be thrown as a heap into a drawer. Putting the instruments away in a canvass roll is helpful. Having a personal set of instruments is a great advantage: look after them.

The Process of Dissection

Dissection involves the removal of fat and other connective tissue to display the relationships of organs and other structures. Dissection should involve as little cutting with scissors or scalpel as possible. The art of good dissection lies in the separation of tissues by means of a probe. When large animals are dissected the probe is often best provided by the fingers, but when small animals such as the rat are dissected, a blunt seeker replaces the exploratory finger. Dissection is a gentle process: tissues are separated without the application of much force. Gentle teasing with forceps and separation of tissue planes by the spreading of blades of scissors that have been inserted into connective tissue are the keys to effective work. Always identify structure before you cut them or remove them. Small blood vessels and some nerves will inevitably be sacrificed, but this should be done knowingly rather than in ignorance. When a structure is being cleaned of connective tissue it helps to pull gently on the structure: put it on stretch. Dissection of a recently killed animal is rather different from dissection of one that has been preserved in formalin. Unfixed tissues are softer and tear more easily, but blood vessels are more obvious. Pouring a little 70% alcohol onto the tissue makes nerves taken on a whitish colour and this can be helpful in their identification.

ANNEX 2

Perfusion Fixation

Fixation by perfusion of a solution of fixative through the vasculature provides the most rapid and effective method of fixing tissues. It is, of course, a more complicated process than fixation by immersion of pieces of tissue in a

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solution of fixative. Perfusion fixation is recommended for the brain. Any fixative can be used but formalin is effective.

Fixation by perfusion is best undertaken in an anaesthetised rat. The animal should be anaesthetised by intraperitoneal injection of sodium pentobarbitone (40 mg/kg). Place the anaesthetised animal on a heated pad. Full anaesthesia is established within 5−10 minutes. The apparatus needed for perfusion fixation is simple: two reservoirs, one of normal (0.9%) saline and one of fixative are required. These should be placed 30 cm above the bench, on a shelf or held by a retort stand. Plastic tubing leading from the reservoirs should be connected via a three-way tap to a cannula. The cannula may be made of plastic or metal, should be about 2 mm in diameter and should have a bevelled point. The saline and fixative should be at 37°C or a little higher to allow for cooling in the tubing. Ensure that there are no air bubbles in the system by running saline and fixative through the tubing before beginning the process of fixation.

When the animal is fully anaesthetised open the abdomen, identify the margin of the rib cage and cut the ribs to allow removal of most of the chest wall. Bleeding is inevitable, but avoid cutting the internal mammary arteries that run close to the sides of the sternum. Turn back the chest wall to expose the heart which will be beating. Grip the apex of the ventricles with artery forceps and identify the left ventricle lying dorsal to the right ventricle. Insert the cannula into the left ventricle and advance the tip into the root of the aorta, where it may be tied or held in place. Snip the right atrium to allow fluid to escape from the venous side of the circulation. Perfuse with saline. The fluid escaping from the right atrium will be bloody at first but will soon clear. The liver will become pale and blood is washed from it. As soon as the escaping fluid is clear switch over to the fixative and run in about 100 mL. The animal will stiffen as the fixative flows through its blood vessels as the muscles of the limbs contract. Death will occur as the fixative is perfused and the heart will cease to beat.

Once the perfusion is complete the head and the roof of the skull can be removed to expose the brain. The head, with the brain still in place should be placed in a beaker of fixative for 24 hours. The brain can then be removed for further study.

Some authorities recommend adding heparin to the saline to prevent the formation of blood clots that may block capillary vessels. Others have added gum acacia to the saline to prevent the formation of oedema of the tissues. Neither seems to be necessary.

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Chapter 26

Post-Mortem Examination

This chapter sets out the procedure used for post-mortem examination of the rat and the collection of tissues for histopathology. There is some overlap between this chapter and that on the dissection of the rat: Chapter 25, Dissection of the Adult Rat. That chapter is intended for those undertaking dissection to learn the anatomy; this chapter is for those working in regulatory toxicology.

PREPARATION AND PRELIMINARIES

The order in which tissues are removed at post-mortem examination varies from worker to worker: the order given here is the one we would suggest you use routinely for the types of tissue lists specified for regulatory studies. These lists also require organ weights to be recorded. Although this is a schedule for regulatory work, we suggest it provides a sound basis for a rigorous harvesting of tissues from animals used in other types of work.

We suggest the following basic equipment list:

- dissecting instruments as required (scalpels, forceps, scissors, etc.);
- cork boards and pins;
- 'Petri' dishes;
- post-mortem pots or tubes, containing fixative where required;
- saline (0.9% sodium chloride in water);
- pot labels;
- cassettes/acetate sheet/glass slides;
- wax blocks and pins;
- CO_2 euthanasia chamber connected to CO_2 cylinder (our standard method of euthanasia is exposure to a rising concentration of carbon dioxide);
- clippers for removing fur;
- balances;
- syringe, cannula and cotton thread; and
- sieve and pot.

Some form of personal protection, we suggest a lab coat, protective gloves and face mask as a minimum.

As indicated earlier, the excision order may be varied from the one set out here to the specific requirements that you may have. Most regulatory studies require organs to be weighed: our normal procedure is for contra-lateral organs to be weighed together, but this is largely a matter of personal preference. Before beginning the autopsy ensure your pots and tubes are filled with the appropriate fixatives and appropriately labelled. To produce the best quality tissue for histology you need to get the tissues into fixative in the shortest possible time, so you need to have everything to hand. We find it useful to place small pieces of tissue, such as adrenal glands and ovaries, in plastic histology cassettes; this makes checking the tissue harvest at the end of the study more straightforward: you would not be searching around in a large pot for tiny pieces of tissue.

Before you start check the in-life history of the animal, if available, and record the presence of any masses or other lesions, this may cause you to reconsider your approach to the autopsy.

Being a commercial operation we capture all our data directly onto a computer system, but if this is not available we suggest you design for yourself a post-mortem form that you can use. It is good practice to capture the following data.

- the date of necropsy;
- the circumstances of death;
- method of euthanasia;
- the time of necropsy; and
- the animal's identification, for example, a number allocated by the experimenter.

During the post-mortem all macroscopic abnormalities should also be recorded on your form.

POST-MORTEM PROCEDURE

The most commonly used method of euthanasia in commercial laboratories is exposure to a rising concentration of carbon dioxide. Once the animal appears dead, check for absence of any reflex before proceeding.

Now that the preliminaries are complete you are ready to begin. Begin by weighing the animal and recording the dead bodyweight. It is important to confirm the death of the animal, which can be done by opening abdominal

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cavity exsanguinating to animal from the caudal (posterior) vena cava or alternatively the spinal cord can be severed. We would suggest severing the spinal cord in juveniles and pups, and animals from which bone marrow smears are being taken. To do this, tilt the chin forwards to create a convexity in the cervical region. Identify a gap between two cervical vertebra and slide a scalpel blade between the two, cutting the cord cleanly.

If you are planning to examine bone marrow smears now is the time to take them, if you delay for more than a minute of two you will find that the smears are uninterpretable. For rats the normal procedure is to extract marrow from the shaft of the femur which is transected with bone forceps. We use a fine paint brush to remove the marrow and 'paint' it onto a slide before fixing in methanol, but other methods work equally well.

Sampling of any external abnormalities, for example, tumours, can be done at any point, but this is probably a convenient time. If these abnormalities have been noted in-life it is good practice to identify them on your post-mortem form, creating a history of the lesion. External abnormalities will also include injection sites (usually the tail in the rat) and dermal application sites (usually dorsal skin). If a large area for application or injection has been used it is probably best to take the entire area for histological study if that is practicable. Skin can be mounted on acetate sheet to prevent curling and twisting as it fixes.

These preliminaries completed, we move to the head. The eyes, with a length of optic nerve still attached, are removed along with the Harderian glands and placed in modified Davidson's fixative.

Davidson's (sometimes called Hartmann's) fixative is a rapid fixative which gives good nuclear detail with minimal formalin pigment. Davidson's (or modified Davidson's) is used for testes and eyes in regulatory studies because penetration and fixation of specimens is rapid. Exposure to the fixative should be limited to 24 hours (tissue may be transferred to neutral buffered formalin or 70% alcohol for storage).

A recipe for Davidson's is as follows:

- 500 mL strong formalin (37%);
- 750 mL alcohol;
- 250 mL glacial acetic acid: 1 part or 250 mL;
- 750 mL tap water; and
- eosin to colour.

The skin over the cranium is incised and reflected and the dorsal cranial bone removed. The meninges and surface of the brain are examined and the brain removed. The pituitary gland is removed by carefully incising the dural membrane that lies dorsal to the gland, but if the pituitary weight is not required it may be more convenient to fix the gland in situ. Also fixed in situ is the Zymbal's glands and the nasal cavity. These structures are sectioned after decalcification.

When decalcifying tissues you can either use a container labelled with the animal identifier, or alternatively you can use a communal container where numerous animal's tissues are decalcified together, in which instance the required tissues should be placed into a labelled cassette, or identified with an identification tag. Either way ensure you container is filled with sufficient acid solution to ensure that all tissues are fully covered. Your containers should clearly labelled with the start date and solution being used.

The tissues are left to decalcify in the acid solution until soft, which will vary depending on the age and size of the animal. You should check you tissue on a regular basis. Some recommend daily checking, but it is probably sufficient to check a smaller rat after 4–5 days and then daily thereafter. We find a large rat (up to 1.2 kg) will take up to 9–10 days to adequately decalcify. For a rodent you should not need to change the acid in the container during the decalcification process. Before tissue processing, you should wash the decalcified tissues in running tap water to remove any acid.

There are several commercially available decalcifying solutions available, all very similar, but as they are universally corrosive care should be taken with handling.

The animal is now turned over and secured to the dissecting board, ventral aspect uppermost. The site of the mammary gland and skin are removed from the inguinal region, and mounted on acetate sheet. These samples should include skin with a nipple, the underlying connective tissue and mammary tissue. If an abnormality is present in the chosen area (very common in longer term studies) then the nearest unaffected (normal) gland, should be removed and taken as a control sample. This should be clearly recorded.

At this stage the preputial/clitoral glands are also removed.

Moving to the neck the salivary glands and the submandibular lymph nodes are removed followed by the lacrimal glands.

The ventral region skin and muscles are reflected to enable access to the abdominal and thoracic cavities. Lateral cuts are made through the rib cage, which is then folded back. This opens the thoracic cavity and exposes the thymus, which is removed, allowing the heart to be dissected free and also removed.

The larynx, thyroid gland (with parathyroid glands attached), trachea and lungs are freed from cervical and mediastinal attachments. The larynx is removed with the thyroid attached and placed into fixative as is. If thyroid weight is required we record this after fixation.

The lungs are removed with a length of trachea and are slowly expanded with fixative by means of a syringe and cannula. The trachea is then ligated with a piece of cotton thread.

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Moving into the abdomen the spleen and the pancreas are removed. The gastrointestinal (GI) tract is now exposed. This is a good opportunity for a first look at the serosa, the serous membrane that lines the peritoneal cavity, although this should be kept under observation throughout sampling. The caecum is taken as a whole, along with approximately 50 mm of jejunum (from the midsection), ileum and colon. The caecum is opened along its greater curvature, washed in saline and fixative, and the mucosa examined. Before placing in the pot we flush all sections of the GI tract with fixative: this substantially improves fixation and quality of histological sections.

The stomach is preserved with the oesophagus and approximately 50 mm of duodenum. The stomach is opened along the greater curvature along with the first 10 mm of the duodenum. The tissue is washed in saline and fixative and the mucosa examined before being pinned to a wax block, mucosa uppermost. This prevents curling and twisting during fixation.

You can take the mesenteric lymph nodes at any convenient time when sampling the GI tract.

The liver is now removed. As the liver is fairly substantial the lobes are incised with a scalpel to aid fixation; the cut surfaces are examined.

The abdominal cavity is now almost empty. A length of thoracic aorta should be taken followed by the adrenals, which will often be hidden by fat, and the kidneys. After the kidneys are removed, each kidney is cut transversely to one side of the hilus and examined, unless the study requires transversely and longitudinally sectioned kidneys. In this case one kidney should be cut longitudinally and the other transversely.

REPRODUCTIVE ORGANS AND URINARY BLADDER

Males

The testes are removed along with the epididymides and separated, we fix both in modified Davidson's fixative. The tunica albuginea may carefully be nicked at one end to aid fixation.

The urinary bladder is next and you may choose to gently inflate this with fixative and ligature. Do not over inflate as this can mask subtle pathology. Try to knot the ligature at the same point every time – ventrally is probably best, as this will allow the histologist to orientate the bladder in the correct plane. Lastly remove the prostate and seminal vesicles (including coagulating glands), which can all be preserved together.

For females we adopt a slightly different procedure, and the urinary bladder is removed first. The pubic symphysis is divided to allow the uterus, cervix, vagina and ovaries (including oviducts) to be removed intact. If you require organ weights for the ovaries you will need to dissect them out at this stage, otherwise leave the ovaries attached by the oviducts to the uterus.

The rectum is now exposed and can be removed with the anus attached.

There are now just a few remaining tissues to sample. The easiest place to take a sample of skeletal muscle is probably the quadriceps muscle; at the same time you can collect a piece of sciatic nerve. This needs to be collected carefully, avoid stretching because this can create histological artefacts. This will leave you with the remains of the hind limb which should be taken so as to include the femur shaft, tibia and fibula.

Finally, remove the vertebrals column including the spinal cord. If you are examining the nasal cavities and Zymbal's glands preserve what is left of the head.

Having followed this plan you should have a pot full of tissue and the remnants of the carcass, which may be preserved, although most will discard the remains at this point, but not before you have performed a thorough check to ensure you have harvested all your essential tissues. Empty the pot through a sieve and have a colleague double check your collection to ensure nothing has been missed, then return the tissues to the pot for a week or so to fix thoroughly. It is always worth keeping a camera or smartphone handy to take photographs of any unusual observations. Keep a record of the images by placing a piece of card with the animal identifier next to the abnormality when you capture your image.

As we stated, this excision order is not intended as anything more than a guide: if you have a specific interest in a particular organ system then always harvest that first. Although much of the above may seem excessive for your requirements, it is worth remembering that any omissions or mistake at this stage of an experiment are not recoverable, and repeating work with live animals is both expensive and open to criticism on ethical grounds.

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Annotated Bibliography

Anatomy

One of the advantages of anatomy as a subject is that the fundamental facts do not change with time though, of course, new interpretations of these facts continue to appear and, occasionally, new facts are discovered.

Textbooks of Comparative Anatomy

Comparative anatomy is a less popular subject today than it was 50 or a 100 years ago. The following list includes some recent works and a number of older texts that are still helpful. The books by Romer and by Young are strongly recommended.

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