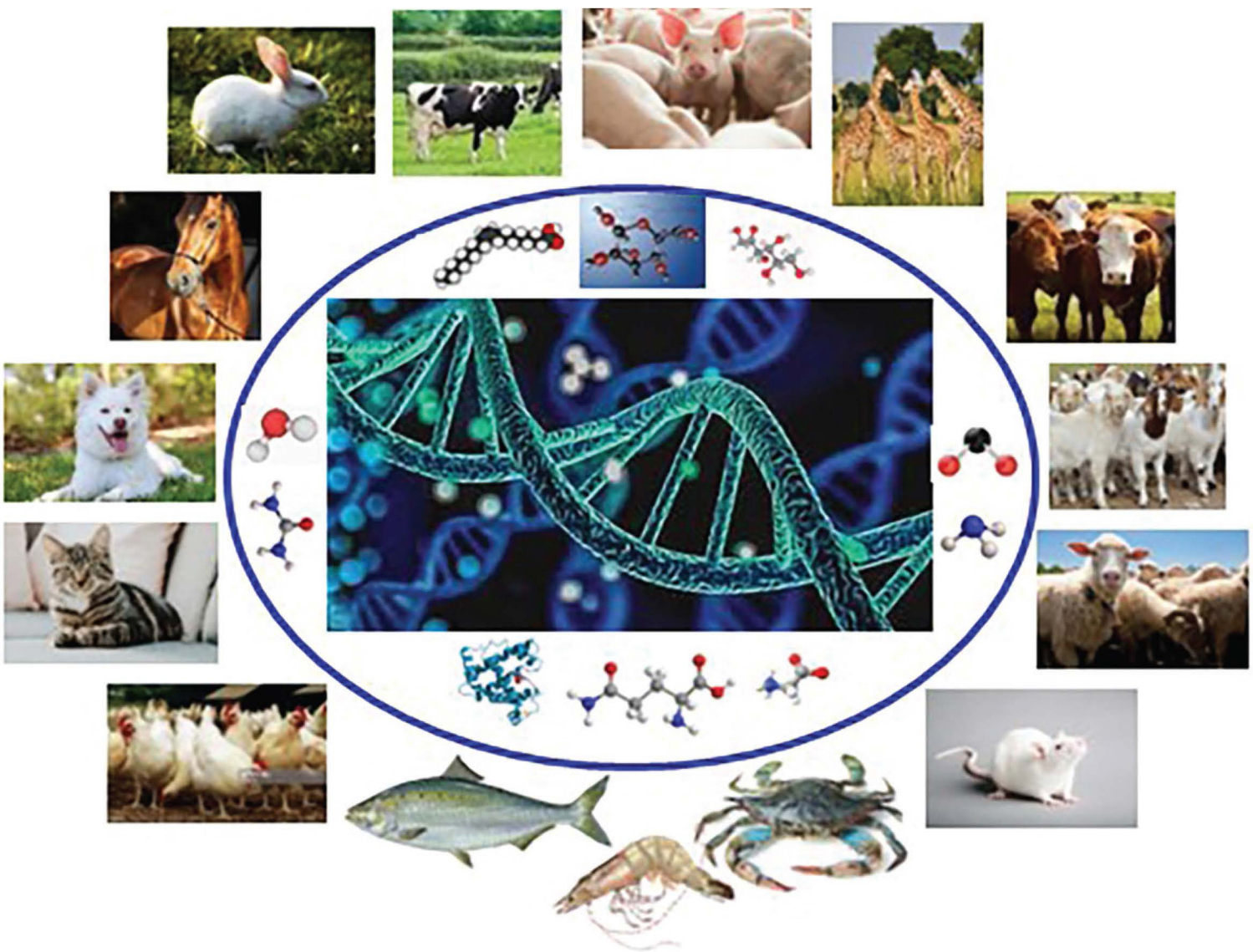
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Principles of Animal Nutrition

Guoyao Wu

SECOND EDITION



Principles of Animal Nutrition

The second edition of *Principles of Animal Nutrition* presents comprehensive coverage of nutrition and metabolism in ruminants, nonruminant mammals (e.g., swine and horses), and poultry, as well as aquatic and companion animals. Substantially revised, expanded, and updated to reflect scientific advances (including the use of artificial intelligence in diet formulations and feeding), this book highlights the foundational aspects of nutrition that are essential to animal growth, development, reproduction, lactation, health, and survival.

The book contains an overview of the physiological and biochemical bases of animal nutrition, followed by a detailed description of the chemical properties of carbohydrates, lipids, protein, and amino acids. The text also covers the digestion, absorption, transport, and metabolism of macronutrients, energy, vitamins, and minerals in animals. To integrate the basic knowledge of nutrition with practical animal feeding, the book continues with a discussion of the nutritional requirements of animals for maintenance, production, and health, as well as the regulation of food intake by animals. Finally, the chapters explain feed additives, including those used to enhance animal growth and survival (e.g., enzymes, amino acids, organic acids, and animal co-products), improve feed efficiency for protein production, replace feed antibiotics, and promote circular bio-economies.

Written by a distinguished professor of animal nutrition, Guoyao Wu, PhD, *Principles of Animal Nutrition, Second Edition* is the authoritative reference for academic researchers, practitioners, and beginners in the fields of animal and human nutrition, biomedicine, and allied health, as well as institutional and government policymakers.



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Preface

Principles of Animal Nutrition explains how animals obtain and utilize nutrients for physiological functions, including maintenance, growth, development, reproduction, and survival. Since the first edition of this book was published in 2017, the author has received constructive feedback from students, instructors, and other readers for expanding the coverage of nutrition and metabolism in ruminant, avian, aquatic, and companion animals. This content has now been incorporated in its second edition. The structure of the first edition of *Principles of Animal Nutrition*, which was well appreciated by its users for the logical flow of the complex knowledge of the subject matter from the basic to the applied, has been retained in the current edition. However, all chapters have been substantially revised, expanded, and updated to reflect significant advances through research over the past eight years. This book presents comprehensive coverage of these scientific developments, while providing a helpful reference for students, researchers, and practitioners in agriculture and biomedicine, and for the public.

Specific additions to this edition include (1) antinutritive and toxic substances in foods; (2) major features of nutrition and metabolism in aquatic animals (fish and crustaceans such as shrimp and crabs); (3) recent advances in amino acid, carbohydrate, and lipid metabolism in ruminants, swine, horses, and poultry; (4) characteristics of nutrition and metabolism in companion animals (e.g., dogs, cats, and horses); (5) roles of nutrients in the immunity of animals; (6) unique aspects of nutrition in connective tissues (bone, skin, and hair); (7) the measurement of energy expenditure in animals using doubly labeled water; (8) mechanisms for calcium and phosphorus transport in ruminants and swine; (8) metabolic disorders and diseases related to nutrient deficiencies or excesses; (9) alternatives to feed antibiotics; (10) the nutrient composition of protein-rich foods and the "ideal protein" concept; (11) uses of animal models for studying human nutrition and diseases; and (12) conventional and computerized methods for formulating animal diets.

Animals are biological transformers of dietary matter and energy into high-quality foods (e.g., meats, eggs, and milk) for human consumption, raw materials (e.g., wool and leather) for clothing and accessories for humans, and nutraceuticals for human use. Through biotechnological techniques, animals are also employed to produce enzymes and proteins to treat a wide array of human diseases. Mammals, birds, fish, and crustaceans possess both common and divergent metabolic pathways for their maintenance and adaptations, but all of them need food to survive, grow, develop, and reproduce. Thus, animal nutrition is a foundational subject of great importance to the modern production of livestock (e.g., cattle, goat, pig, rabbit, and sheep), poultry (e.g., chicken, duck, goose, and turkey), fish (e.g., common carp, bass, salmon, and tilapia), and crustaceans (e.g., shrimp, crab, and lobster), as well as the health and well-being of companion animals (e.g., cat, dog, horse, ferret, gerbil, and parrot). As an interesting, dynamic, and challenging discipline in the biological sciences, animal nutrition spans an immense range from chemistry, biochemistry, anatomy, and physiology to reproductive biology, immunology, pathology, and cell biology. Knowledge of these subjects is necessary for an adequate understanding of the chemistry, intake, digestion, absorption, metabolism, functions, excretion, requirements, and deficiency or excess symptoms of nutrients and related substances in animals.

There is a rich history of research on laboratory, farm, and companion animals. *The Principles of Animal Nutrition, with Special Reference to the Nutrition of Farm Animals*, published by H.P. Armsby in 1902, summarized the knowledge of animal nutrition gained in the 19th century. In the past 65 years, since I.E. Coop wrote *The Principles and Practice of Animal Nutrition* in 1961, the fields of animal nutrition and metabolism have developed rapidly because of great advances in biochemistry, physiology, and analytical techniques. Of particular note, many species differences in the digestion, assimilation, and excretion of nutrients have been illustrated in *Animal Nutrition* (6th ed., 1979) by L.A. Maynard and J.K. Loosli; *Comparative Animal Nutrition and Metabolism* by P.R. Cheeke and E.S. Dierenfeld (2010); and *Principles of Companion Animal Nutrition* (2nd ed., 2010) by J.P. McNamara. Additionally, more applied books include *Basic Animal Nutrition and Feeding* (5th ed.) by W.G. Pond, D.C. Church, K.R. Pond, and P.A. Schoknecht (2004); *Animal Nutrition* by P. McDonald, J.F.D. Greenhalgh, C.A. Morgan, R.A. Edwards, L.A. Sinclair, and R.G. Wilkinson (2021); and *Animal Feeding and Nutrition* by M.H. Jurgens, K. Bregendahl, J. Coverdale, and S.L. Hansen (2012). Although those publications highlighted the feeds and practical feeding of livestock and poultry, a comprehensive and systematic coverage of the biochemical or physiological bases of animal nutrition is warranted to better understand the "black box" of animal systems. With this in mind, and with the support of CRC Press, the second edition of *Principles of Animal Nutrition* has now been published.

Principles of Animal Nutrition, Second Edition, consists of 13 chapters. The book begins with an overview of the chemical, biochemical, and physiological bases of animal nutrition (Chapter 1). This chapter is followed by a detailed description of the chemical properties of carbohydrates (Chapter 2), lipids (Chapter 3), and proteins/amino acids (Chapter 4). The book then advances to the current understanding of the digestion, absorption, transport, and metabolism of carbohydrates (Chapter 5), lipids (Chapter 6), protein/amino acids (Chapter 7), energy (Chapter 8), vitamins (Chapter 9), and minerals (Chapter 10) in animals, as well as interactions among nutrients (Chapters 5-10). To integrate the basic knowledge of nutrition with practical

animal feeding, the text continues with discussion on nutritional requirements of animals for maintenance, production, and health (Chapter 11), as well as the regulation of food intake by animals (Chapter 12). Finally, the text ends with information on feed additives, including those used to enhance animal growth and survival (e.g., enzymes, amino acids, organic acids, and animal co-products), improve feed efficiency for protein production, replace feed antibiotics, and promote circular bio-economies. Although the classical and modern concepts of animal nutrition are emphasized throughout the book, every effort has been made to include the most recent progress in this ever-expanding field (including the use of artificial intelligence in diet formulations and feeding) so that readers in various biological disciplines can integrate biochemistry and physiology with nutrition, health, and disease in mammals, birds, and aquatic species. At the end of each chapter, references are listed to provide readers with both comprehensive reviews of the chosen topics and original experimental data. Reading scientific literature is essential for (a) a thorough understanding of the history of the field, (b) creative thinking and innovations, and (c) the rigorous development of students as future productive scientists. In the Index section, a list of keywords, phrases, and abbreviations are provided to help readers quickly find information presented in all chapters.

This book owes its origin to the lecture notes of four graduate courses (ANSC/NUTR 601 General Animal Nutrition; ANSC/NUTR 603 Experimental Nutrition; ANSC/NUTR 613 Protein Metabolism; and ANSC/NUTR 641 Nutritional Biochemistry taught by the author at Texas A&M University as a faculty member over the past 34 years. This book can be used as a reference or textbook for graduate students and senior undergraduates majoring in aquaculture, animal science, biochemistry, biomedical engineering, biology, human medicine, food science, kinesiology, nutrition, pharmacology, physiology, plant science, toxicology, veterinary medicine, and other related disciplines, and for government policymakers.

Animal agriculture plays an important role in improving human nutrition, growth, and health, as well as economic and social developments worldwide. The global population is 8.2 billion in 2025 and has been projected to be 9.8 billion in 2050. With increases in global population and the human consumption of meats, milk, and eggs per capita, demands for animal-sourced protein and other animal products are expected to increase by 50% between 2025 and 2050 worldwide. With a daunting task to fully understand animal biology, maximally enhance the efficiency of metabolic transformations of feeds into desired animal products, and continuously sustain animal agriculture and global food security, nutritionists face tremendous challenges to produce livestock, poultry, fish, and other seafood for feeding humans and fighting hunger. *Principles of Animal Nutrition, Second Edition* is expected to guide and transform the practice of animal nutrition to greatly facilitate the achievement of this noble goal in the coming years. The fundamental knowledge of nutrition in farm, companion, laboratory, and zoo animals has important implications for improving human growth, development, and health, as well as the prevention of metabolic and infectious diseases.

Guoyao Wu
July 25, 2025
College Station, Texas, USA

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The animal nutrition sciences have been built on the shoulders of many giants and pioneers, as well as thousands of researchers worldwide. The author has enjoyed reading their published works and gained a great deal of knowledge from them. Their seminal contributions to the field have made this book possible and therefore the author must apologize to those whose published works are not cited in the text due to space limitations. Furthermore, special thanks are extended to the author's past and current students for constructive comments on his courses and for helpful discussions to improve the classroom presentations of the subject matter. Finally, the author thanks his family for their patience and support during the writing of the entire manuscript.

The Author

Dr. Guoyao Wu is a University Distinguished Professor, University Faculty Fellow, and Texas A&M AgriLife Research Senior Faculty Fellow at Texas A&M University. He received a BS in animal science from South China Agricultural University at Guangzhou, China (1978–1982); an MS in animal nutrition from China Agricultural University at Beijing, China (1982–1984); and an MSc (1984–1986) and PhD (1986–1989) in animal biochemistry from the University of Alberta at Edmonton, Canada. Dr. Wu completed his postdoctoral training in diabetes, nutrition, and biochemistry at McGill University Faculty of Medicine in Montreal, Canada (1989–1991) and Memorial University of Newfoundland Faculty of Medicine at St. John's, Canada (1991). He joined the Texas A&M University faculty in 1991. Dr. Wu conducted a human obesity study during his sabbatical at the University of Maryland School of Medicine in Baltimore, USA (2005).

Dr. Wu has taught graduate (experimental nutrition, general animal nutrition, protein metabolism, and nutritional biochemistry) and undergraduate (problems in animal science, nutrition, and biochemistry) courses at Texas A&M University over the past 34 years. He has given numerous lectures at other institutions in the United States, Canada, Mexico, Brazil, Europe, and Asia. His research focuses on the biochemistry, nutrition, and physiology of amino acids (AAs) and related nutrients in animals at genetic, molecular, cellular, and whole-body levels. His research interests include: (1) functions of AAs in gene expression (including epigenetics) and cell signaling; (2) mechanisms that regulate intracellular synthesis and catabolism of proteins and AAs; (3) hormonal and nutritional regulation of homeostasis of metabolic fuels; (4) biology and pathobiology of nitric oxide and polyamines; (5) key roles of AAs in preventing metabolic diseases (including diabetes, obesity, and intrauterine growth restriction) and associated cardiovascular complications; (6) essential roles of AAs in survival, growth, and development of embryos, fetuses, and neonates; (7) dietary requirements of AAs and proteins in the life cycle; and (8) animal models (e.g., pigs, rats, and sheep) for studying human metabolic diseases.

Dr. Wu has published 786 papers in peer-reviewed journals, including *American Journal of Physiology, Biochemical Journal, Journal of Animal Science, Journal of Nutrition,* and *Proceedings of National Academy of Science USA*. In addition, he has published two text/reference books, *Principles of Animal Nutrition* and *Amino Acids: Biochemistry and Nutrition*, as well as 99 chapters in various books. Dr. Wu's work has been extensively cited more than 105,000 times in Google Scholar, with an H-index of 153. Four of his papers have each been cited more than 3,500 times. Thus, he has been recognized as a Most Cited Author and a Most Influential Scientific Mind in the Web of Science and among the 10 most cited scientists in the field of agricultural sciences worldwide. Dr. Wu was ranked No. 2 worldwide in animal and veterinary sciences by Research.com (2024).

Dr. Wu has received numerous prestigious awards during his professional career, including the American Heart Association Established Investigator Award, FASS–AFIA New Frontiers in Animal Nutrition Research Award from the Federation of Animal Science Societies and American Feed Industry Association, the Morrison Award from American Society of Animal Science, and the Top Agri-Food Pioneer Award from The World Food Prize Foundation.

Dr. Wu is a member and an elected fellow of the American Association for the Advancement of Science and a member of American Heart Association, American Society of Animal Science, and Sigma Xi Society. He has served on editorial advisory boards for *Biochemical Journal* (1993–2005), *Journal of Animal Science and Biotechnology* (2010–Present), *Journal of Nutrition* (1997–2003), *Journal of Nutritional Biochemistry* (2006–Present), *Journal of Poultry Science* (2019–Present), and *Critical Reviews in Food Science and Nutrition* (2024–Present). In addition, Dr. Wu has served as an editor and a consulting editor for *Amino Acids* (2008–Present), as well as an editor for *Advances in Experimental Medicine and Biology* (2018–2024), *Animal Agriculture: Challenges, Innovations, and Sustainability* (Elsevier 2020), and *The Encyclopedia of Animal Nutrition, 2nd edition* (CABI 2020–2023).



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1 Chemical, Biochemical, and Physiological Bases of Animal Nutrition

The word "animal" is derived from the Latin word *animalis*, meaning "having breath," "having soul," and "living being." All animals are multicellular, eukaryotic, and motile organisms of the kingdom *Animalia* (Dallas and Jewell 2014). With the adequate intake of nutrients, they survive, grow, develop, and reproduce as important parts of the ecosystem. The lifespans (years) of animals vary among species (e.g., cats, 12–18; cattle, 20–25; chickens, 5–10; dogs, 10–16; goats, 8–12; horses, 25–30; rabbits, 6–15; rats, 2.5–3; sheep, 10–15; and swine, 15–20), and are affected by their breeds, sex, and living environment (e.g., nutrition and disease; Austad and Fischer 2016; Fox et al. 2002; Popov and Vashurina 2020). The current taxonomic system for animals is based on domain (eukarya), kingdom (animalia), phylum, class, order, family, genus, and species. Examples are given for animals commonly used in nutrition research (Table 1.1).

TABLE 1.1
Classification of Animals Commonly Used in Nutrition Research

Animal	Phylum	Class	Order	Family	Genus	Species
			Carnivores			
Cat[a]	Chordata	Mammalia	Carnivora	Felidae	*Felis*	*F. catus*
Dog[a]	Chordata	Mammalia	Carnivora	Canidae	*Canis*	*C. familiaris*
Ferret[a]	Chordata	Mammalia	Carnivora	Mustelidae	*Mustela*	*M. furo*
Lion[a]	Chordata	Mammalia	Carnivora	Felidae	*Panthera*	*P. leo*
Mink (Am)[a]	Chordata	Mammalia	Carnivora	Mustelidae	*Neogale*	*N. vison*
Mink (Eur)[a]	Chordata	Mammalia	Carnivora	Mustelidae	*Mustela*	*M. lutreola*
Tiger[a]	Chordata	Mammalia	Carnivora	Felidae	*Panthera*	*P. tigris*
Dolphin[b]	Chordata	Mammalia	Cetacea	Delphinidae	*Delphinus*	*D. delphis*
Whale (blue)[b]	Chordata	Mammalia	Cetacea	Balaenopteridae	*Balaenoptera*	*B. Musculus*
Whale (white)[b]	Chordata	Mammalia	Cetacea	Monodontidae	*Delphinapterus*	*D. leucas*
Atlantic salmon[c]	Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Salmo*	*S. salar*
Pacific salmon[c]	Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	Seven species[f]
HS bass[c]	Chordata	Actinopterygii	Moroniformes	Moronidae	*Morone*	*M. saxatilis*
LM bass[c]	Chordata	Actinopterygii	Perciformes	Centrarchidae	*Micropterus*	*M. salmoides*
Rainbow trout[c]	Chordata	Actinopterygii	Salmoniformes	Salmonidae	*Oncorhynchus*	*O. mykiss*
Red drum[c]	Chordata	Actinopterygii	Acanthuriformes	Sciaenidae	*Sciaenops*	*S. ocellatus*
Shark[c]	Chordata	Chondrichthyes	Lamniformes	Lamnidae	*Isurus*	*I. oxyrinchus*
BT shrimp[d]	Arthropoda	Malacostraca	Decapoda	Penaeidae	*Penaeus*	*P. monodon*
Mantis shrimp[d]	Arthropoda	Malacostraca	Stomatopoda	Squillidae	*Squilla*	*S. mantis*
			Herbivores (ruminants)			
Antelope (NA)[a]	Chordata	Mammalia	Artiodactyla	Bovidae	*Antilocapra*	*A. americana*
Buffalo[a]	Chordata	Mammalia	Artiodactyla	Bovidae	*Bubalus*	*B. bubalis*
Cattle[a]	Chordata	Mammalia	Artiodactyla	Bovidae	*Bos*	*B. taurus*
Deer (red)[a]	Chordata	Mammalia	Artiodactyla	Cervidae	*Cervus*	*C. elaphus*
Giraffe[a]	Chordata	Mammalia	Artiodactyla	Giraffidae	*Giraffa*	*G. camelopardalis*
Goat[a]	Chordata	Mammalia	Artiodactyla	Bovidae	*Capra*	*C. hircus*
Sheep[a]	Chordata	Mammalia	Artiodactyla	Bovidae	*Ovis*	*O. aries*
			Herbivores (nonruminants)			
Camel (Arab)[a]	Chordata	Mammalia	Artiodactyla	Camelidae	*Camellus*	*C. dromedarius*
Elephant (Afr)[a]	Chordata	Mammalia	Proboscidea	Elephantidae	*Loxodonta*	*L. africana*
Horse[a]	Chordata	Mammalia	Perissodactyla	Equidae	*Equus*	*E. ferus*
Kangaroo (red)[a]	Chordata	Mammalia	Diprotodontia	Macropodidae	*Osphranter*	*O. rufus*
Rabbit (EN)[a]	Chordata	Mammalia	Lagomorpha	Leporidae	*Oryctolagus*	*O. cuniculus*

(*Continued*)

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TABLE 1.1 (*Continued*)
Classification of Animals Commonly Used in Nutrition Research

Animal	Phylum	Class	Order	Family	Genus	Species
Rhinos[a]	Chordata	Mammalia	Perissodactyla	Rhinocerotidae	*Rhinoceros*	*R. unicornis*
Zebra[a]	Chordata	Mammalia	Perissodactyla	Equidae	*Equus*	*E. zebra*
Grass carp[c]	Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Ctenopharyngodon*	*C. idella*
Silver carp[c]	Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Hypophthalmichthys*	*H. molitrix*
			Omnivores			
Brown bear[a]	Chordata	Mammalia	Carnivora	Ursidae	*Ursus*	*U. arctos*
Chimpanzee[a]	Chordata	Mammalia	Primates	Hominidae	*Pan*	*P. troglodytes*
Giant pandas[a]	Chordata	Mammalia	Carnivora	Ursidae	*Ailuropoda*	*A. melanoleuca*
Grey squirrel[a]	Chordata	Mammalia	Rodentia	Sciuridae	*Sciurus*	*S. carolinensis*
Human[a]	Chordata	Mammalia	Primates	Hominidae	*Homo*	*Sapiens*
Mouse[a]	Chordata	Mammalia	Rodentia	Muridae	*Mus*	*M. musculus*
Rat[a]	Chordata	Mammalia	Rodentia	Muridae	*Rattus*	*R. norvegicus*
Swine (pig)[a]	Chordata	Mammalia	Artiodactyla	Suidae	*Sus*	*S. domesticus*
Chicken[e]	Chordata	Aves	Galliformes	Phasianidae	*Gallus*	*G. gallus*
Duck[e]	Chordata	Aves	Anseriformes	Anatidae	*Anas*	*A. platyrhynchos*
Goose (grey)[e]	Chordata	Aves	Anseriformes	Anatidae	*Anser*	*A. anser*
Japanese quail[e]	Chordata	Aves	Galliformes	Phasianidae	*Coturnix*	*C. japonica*
Turkey[e]	Chordata	Aves	Galliformes	Phasianidae	*Meleagris*	*M. gallopavo*
Channel catfish[c]	Chordata	Actinopterygii	Siluriformes	Ictaluridae	*Ictalurus*	*I. punctatus*
Nile tilapia[c]	Chordata	Actinopterygii	Cichliformes	Cichlidae	*Oreochromis*	*O. niloticus*
Goldfish[c]	Chordata	Actinopterygii	Cypriniformes	Cyprinidae	*Carassius*	*C. auratus*
Blue crab[d]	Arthropoda	Malacostraca	Decapoda	Portunidae	*Callinectes*	**C. s***apidus*
Indian prawn[d]	Arthropoda	Malacostraca	Decapoda	Penaeidae	*Fenneropenaeus*	*F. indicus*
IW shrimp[d]	Arthropoda	Malacostraca	Decapoda	Penaeidae	*Penaeus*	*P. indicus*
PW shrimp[d]	Arthropoda	Malacostraca	Decapoda	Penaeidae	*Litopenaeus*	*L. vannamei*

All animals belong in the same domain (eukarya) and the same kingdom (animalia).

[a] Terrestrial mammals.

[b] Marine mammals.

[c] Fish.

[d] Aquatic crustaceans.

[e] Terrestrial birds.

[f] Five species of Pacific salmon occur in North American waters: chinook (*Oncorhynchus tschawytscha*), coho (*O. kisutch*), chum (*O. keta*), pink (*O. gorbuscha*), and sockeye (*O. nerka*). The other two species of Pacific salmon, Masu (*O. masou*) and amago (*O. masou macrostomus*), occur only in Asia.

Note: Afr, African; Am, American; Arab, Arabian; BT, black tiger; HS, hybrid striped; NA, North American; EN, European rabbits and New Zealand White rabbits; Eur, European; LM, largemouth

Animals have various mature body weights (BWs) and are classified broadly into different groups according to their anatomical and dietary characteristics (Bondi 1987; Cheeke and Dierenfeld 2010; Dyce et al. 1996; Li et al. 2021a,b). Based on the presence or absence of a backbone, animals are divided into vertebrates and invertebrates. Vertebrates have a backbone (e.g., amphibians, birds, fish, mammals, and reptiles). A subclass of vertebrates is gnathostomata or gnathostomes (from Greek: *gnathos* "jaw" + *stoma* "mouth") with a jaw. The latter comprise about 60,000 animal species and account for 99% of all living vertebrates, including mammals (e.g., humans, cattle, swine), birds, reptiles, amphibians, cartilaginous fish (e.g., elasmobranch fish), and bony fish (e.g., teleost fish). Invertebrates have no backbone and include (a) insects, spiders, and worms; (b) crustaceans (e.g., crabs, crayfish, lobsters, penaeid prawns, and shrimp); and (c) mollusks (e.g., clams, mussels, oysters, and scallops, as well as corals, octopuses, snails, and squids). Crustaceans and mollusks are sometimes collectively referred to as shellfish.

Based on the presence or absence of mammary glands, animals are known as mammals if they have mammary glands (e.g., cats, cattle, dogs, goats, humans, mice, rats, pigs, and sheep) or nonmammals if they do not have mammary glands (e.g., birds, fish, reptiles, and shrimp). Based on the digestive process, animals are called (a) ruminants (e.g., antelopes, cattle, cervids, deer, goats, New World camelids, and sheep) if they have a four-compartment stomach (rumen, reticulum, omasum, and abomasum) or (b) nonruminants (e.g., cats, chickens, crabs, dogs, fish, humans,

pigs, and shrimp) if they have a single-compartment stomach or no stomach in the case of some species of fish. Ruminants primarily digest fibrous plant material through a process of degradation, fermentation (a chemical process by which molecules such as glucose and amino acids are broken down anaerobically), and regurgitation. In contrast, nonruminants generally do not ferment plant material in the stomach but rely more on digestion by animal-sourced enzymes in the small intestine. Hindgut fermenters (e.g., horses, rabbits, and elephants) are a subset of nonruminants that ferment plant material in the cecum and colon, which allows them to digest fibrous plants more effectively than other nonruminants. Based on the primary food animals naturally prefer to eat, they are classified as: (a) carnivores (meat eaters) whose diets consist mainly of nonplant materials such as meat, fish, and insects (e.g., cats, dogs, ferrets, hybrid stripe bass, largemouth bass, minks, and tigers); (b) herbivores (plant eaters) whose diets are composed primarily of plant materials (e.g., cattle, deer, goats, grass carp, horses, rabbits, and sheep); and (c) omnivores (meat and plant eaters) whose diets include both plant and animal materials (e.g., humans, pigs, poultry, rats, mice, and reef fish).

Wilson (1992) estimated that there were 1,032,000 animal species in nature, including 18,800 species of fish and lower chordates, 9,000 species of birds, 6,300 species of reptiles, 4,200 species of amphibians, and 4,000 species of mammals. Despite the vast diversity among vertebrates and invertebrates, animals exhibit greater similarities in physiology, metabolism, and nutrition than differences (Baker 2005; Beitz 1993; Wu 2022a). To date, our understanding of animal nutrition is based primarily on practical feeding and scientific studies of a limited number of terrestrial vertebrate species (e.g., cats, cattle, chickens, dogs, ducks, ferrets, goats, guinea pigs, horses, humans, mice, minks, pigs, rabbits, rats, sheep, and turkeys) and other aquatic and terrestrial species (e.g., fish, crustaceans, insects, and *caenorhabditis elegans*; Cheeke and Dierenfeld 2010; McDonald et al. 2021; NRC 2002). In recent years, tremendous progress in the nutrition of wild animals has been made (Barboza et al. 2009; Meredith 2009). Knowledge learned from research on farm and laboratory animals may be applicable to other species.

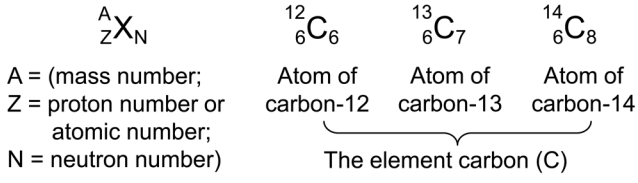
Human civilization has a rich history of studies investigating the nutrient requirements of livestock, poultry, fish, and crustaceans during their life cycle under various physiological and pathological conditions (Baker 2005; Bergen 2007; Li et al. 2021a,b). This is because these agriculturally important animals are the major source of high-quality proteins, lipids, vitamins, and minerals for consumption by humans and their companion animals to sustain optimal growth, development, reproduction, and health (Davis et al. 2002; Reynolds et al. 2015; Wu et al. 2014a). Animal production accounts for 50–75% and 25–40% of the total agricultural output in industrialized and developing nations, respectively (Wu et al. 2014b). Thus, research on farm animals has great scientific, social, and economic significance. In addition, rats and mice have long been used as animal models to discover dietary requirements for nutrients and metabolic diseases caused by their deficiencies (Wu 2020b). Furthermore, the elucidation of metabolic pathways has been facilitated by the study of inherited diseases (e.g., by knocking down or out genes) in humans and companion animals (Brosnan et al. 2015; Vernon 2015). Extensive knowledge exists in the literature regarding the physiological and biochemical basis of nutrition in farm and laboratory animals (Asher and Sassone-Corsi 2015; Dellschaft et al. 2015; Huang et al. 2018; Rezaei et al. 2013), as well as humans (Bennett et al. 2015; Meredith 2009). The integration of these large databases provides a strong foundation for this book.

FUNDAMENTAL CONCEPTS IN ANIMAL NUTRITION

Chemical Basis of Animal Nutrition

Chemistry is the study of properties and reactions of materials. Their tremendous varieties in nature result from the combinations of approximately 100 substances called elements. Each element is a unique kind of atom, such as carbon (C), hydrogen (H), nitrogen (N), or oxygen (O)] (Miśkowiec 2023a,b). C, H, and N were discovered by Antoine Lavoisier (a French chemist) in 1772, Henry Cavendish (a British scientist) in 1766, and Daniel Rutheford (a British scientist) in 1772, respectively. In addition, oxygen was independently discovered by Carl-Wilhelm Scheele (a German-Swedish chemist) in 1771, Joseph Priestley (a British scientist) in 1774, and Antoine Lavoisier (a French chemist) in 1778.

An atom consists of protons (positively charged particles) and neutrons (uncharged particles) in the nucleus, as well as electrons (negatively charged particles) that move in circular orbits around the nucleus. The element carbon includes 3 different isotopes (i.e., $^{12}C_6$, $^{13}C_7$, and $^{14}C_8$) that have the same number (6) of protons (also known as the atomic number) but different numbers of neutrons (6, 7, or 8). The differing number of neutrons results in different atomic masses (12, 13, or 14) and chemical properties. Dalton (Da) is the unit of an atomic or molecular mass, with 1 Da being equal to one twelfth of the mass of the carbon-12 atom. Some of the elements commonly studied in animal nutrition are listed in Table 1.2.



Two or more elements or atoms (e.g., H and O) are joined together via specific bonding to form molecules (e.g., H_2O). The sum of the atomic weights of all atoms in a molecule is called molecular weight (or molecular mass). The chemical properties of matter depend on both the composition and

TABLE 1.2
Elements Commonly Studied in Animal Nutrition and Biomedicine

Element	Symbol	Atomic Number	Atomic Weight[a]	Element	Symbol	Atomic Number	Atomic Weight[a]	Element	Symbol	Atomic Number	Atomic Weight[a]
Aluminum	Al	13	26.982	Hydrogen	H	1	1.008	Phosphorus	P	15	30.974
Arsenic	As	33	74.922	Iodine	I	53	126.90	Potassium	K	19	39.098
Boron	B	5	10.811	Iron	Fe	26	55.845	Selenium	Se	34	78.971
Bromine	Br	35	79.904	Lead	Pb	82	207.20	Silicon	Si	14	28.086
Cadmium	Cd	48	112.41	Lithium	Li	3	6.941	Silver	Ag	47	107.87
Calcium	Ca	20	40.078	Magnesium	Mg	12	24.305	Sodium	Na	11	22.990
Carbon	C	6	12.011	Manganese	Mn	25	54.938	Sulfur	S	16	32.065
Chlorine	Cl	17	35.453	Mercury	Hg	80	200.59	Thallium	Tl	81	204.38
Chromium	Cr	24	51.996	Molybdenum	Mo	42	95.95	Tin	Sn	50	118.71
Cobalt	Co	27	58.933	Nickel	Ni	28	58.693	Tungsten	W	74	183.84
Copper	Cu	29	63.546	Nitrogen	N	7	14.007	Vanadium	V	23	50.942
Fluorine	F	9	18.998	Oxygen	O	8	15.999	Zinc	Zn	30	65.380

[a] Also known as the atomic mass of an element. The atomic mass unit or the molecular weight unit is dalton (Da).

the arrangements of its constituent atoms. The amount of a substance, expressed as mol, is calculated as the mass of the material (g) divided by its molecular weight. In a solution, the molar concentration (M) of a substance is calculated as mol divided by the volume (L) of the solution. Note that mol is not M. Let us consider, as an example of calculations, dissolving 90 g of glucose (with a molecular weight of 180 Da) in 1 L of H_2O.

$$\text{The Avogadro constant: 1 mole (mol) of a substance} = 6.022 \times 10^{23} \text{ atoms, molecules, or ions}$$

$$\text{Mole (mol)} = \text{g of substance/its molecular weight.}$$
$$[\text{e.g., 90 g glucose/180 Da} = 0.50 \text{ mol}]$$

$$\text{Molar concentration (M)} = \text{mol of substance/volume (L).}$$
$$[\text{e.g., 0.5 mol glucose/1 L solution} = 0.50 \text{ M}]$$

Both foods and animals are composed of organic and inorganic matter. Organic substances may be defined as any carbon-containing compounds (e.g., protein, lipids, carbohydrates, vitamins, and urea) other than carbonate (CO_3^{2-}), bicarbonate (HCO_3^-), cyanide (CN^-), cyanate (NCO^-), carbide (made of carbon and a metal), and gaseous oxides [e.g., CO_2 and carbon monoxide (CO)] (Jesperson et al. 2012). Inorganic substances may be defined as any carbon-free compounds (e.g., H_2O, minerals, NH_3, and H_2S) with the aforementioned exceptions of carbonate, bicarbonate, cyanide, cyanate, carbide, and gaseous oxides. These chemical definitions are helpful for the study and practice of animal nutrition. As indicated by Silverberg (2021), the boundaries between some "organic" and "inorganic" compounds may be blurred.

Chemistry provides foundational knowledge and methodologies for studies of animal nutrition, with an understanding of topics such as such as pH, anion gaps, intestinal transport, tissue-specific metabolism, excretion, growth, development, and health (Baker 2005; Bauman et al. 2011; Las et al. 2007; Liao et al. 2010; Webb et al. 1993). Nutritionists must understand (a) the chemical composition, structures, and properties of foods and animals; (b) chemical reactions of ingested foods in the gastrointestinal tract (GIT) and their resultant products during metabolic transformations in animals; and (c) chemical mechanisms for the excretion of physiological metabolites, the detoxification of food-derived toxins, and the adaptation to environmental and pathological changes. Thus, organic, inorganic, and biological chemistries are essential to advance the understanding of nutritional sciences. For convenience, the phosphate group (PO_3^{2-}) and inorganic phosphate (HPO_4^{2-}) are abbreviated as P and Pi, respectively.

Definition of Nutrients, Foodstuffs, and Diets

A nutrient is defined as a compound or substance needed to support the maintenance, growth, development, lactation, reproduction, and health of animals. A list of known nutrients required by animals is given in Table 1.3. Food is an edible material that contains nutrients and is generally called "food" when fed to humans and companion animals. Food for farm animals (e.g., livestock, poultry, fish, and crustaceans) is commonly known as "feed." Based on their composition and use, feeds are classified as (a) dry forages (e.g., dried pasture, leaves, stems, green chop, and hay) and roughages (any plant feeds with high fiber content, including forages, straws, and hulls; containing >18% crude fiber); (b) green pasture, range plants, and freshly fed green forages (e.g., forage feeds); (c) silages [e.g., ensiled corn, alfalfa (also known as lucerne), and grass]; (d) energy feeds (e.g., corn, wheat, barley, and rice; containing <18% crude fiber); and (e) protein feeds (e.g., soybean meal, meat and bone meal,

TABLE 1.3
Nutrients Required by Animals

Water and Macro Organic Nutrients	Vitamins, Quasi-Vitamins, and Minerals
Water	Vitamins
Glucose and fructose	Water-soluble vitamins
Protein or amino acids[a]	Thiamin (vitamin B_1)
Proteinogenic amino acids	Riboflavin (vitamin B_2)
Alanine	Niacin (nicotinic acid and nicotinamide; vitamin B_3)
Arginine	Pantothenic acid (vitamin B_5)
Asparagine	Pyrodoxine, pyridoxal and pyridoxamine (vitamin B_6)
Aspartate	Cobalamin (vitamin B_{12})
Cysteine	Biotin
Glutamate	Folate (pteroylglutamic acid)
Glutamine	Ascorbic acid (vitamin C)
Glycine	Choline
Histidine	Myo-inositol
Isoleucine	Lipid-soluble vitamins
Leucine	Vitamin A
Lysine	Vitamin D
Methionine	Vitamin E
Phenylalanine	Vitamin K
Proline	Macrominerals
Serine	Sodium
Threonine	Potassium
Tryptophan	Chlorine
Tyrosine	Calcium
Valine	Phosphorus
Nonproteinogenic amino acid, Taurine (all carnivores and some omnivores)	Magnesium
Fatty acids	Sulfur
Linoleic acid (ω6, 18:2) and arachidonic acid[b]	Microminerals[c]
α-Linolenic acid (ω3, 18:3), EPA[b], and DHA[b]	Possibly essential ultra-trace elements[d]
Fibers of plant source (for healthy intestine)	

Source: Pond et al. (2005) and Rezaei et al. (2013).

[a] Animals may not be able to sufficiently synthesize all amino acids to meet the needs for maximal growth and production performance or for optimal health and well-being (Hou et al. 2016; Wu 2022a).

[b] Small amounts in diets may be necessary for maximal growth and production performance or for optimal health and well-being. These two fatty acids are nutritionally essential for cats.

[c] Including boron (B), bromine (Br), chromium (Cr), cobalt (Co), copper (Cu), fluorine (F), iodine (I), iron (Fe), manganese (Mn), selenium (Se), molybdenum (Mo), nickel (Ni), silicon (Si), tin (Sn), vanadium (V), and zinc (Zn).

[d] Possibly essential ultra-trace elements in animal nutrition include arsenic (As), barium (Ba), cadmium (Cd), rubidium (Rb), and strontium (Sr).

Note: DHA, 5,8,11,14,17,20-docosahexaenoic acid; EPA, 5,8,11,14,17-eicosapentaenoic acid.

blood meal, poultry byproduct meal, fish meal, and milk replacer). Synthetic amino acids, mineral supplements, vitamin supplements, and other additives (e.g., antibiotics, coloring materials, flavors, phytochemicals, hormones, and medicines) may be included with feeds to prepare complete diets for animals.

Foodstuff is any material used for food. An ingredient of food is a component or constituent that comprises the food. Thus, foodstuffs and food ingredients are often interchangeable terms. Feed ingredients for farm animals may be grains, milling byproducts, animal- and plant-derived products, vitamin and mineral premixes, fats, oils, and other nutritional sources. Animal-derived products include blood meal, feather meal, fishmeal, meat and bone meal, and spray-dried poultry plasma. Products of plant origin comprise alfalfa hay, corn grain, cottonseed meal, dried distillers grains with solubles (DDGS), and soybean meal. Concentrate foodstuffs are foods rich in energy, protein, or both.

A diet is a mixture of foodstuffs that supplies nutrients to an animal. The ration is the daily allowance or amount of food provided to an animal. A good ration should be (a) balanced in the proportions and amounts of all nutrients to meet the needs of animals, (b) palatable and uniformly mixed, (c) beneficial for the health and productivity of animals, (d) safe for animal feeding and products (e.g., meat, milk, and eggs), (e) stable during storage, and (f) low cost. A meal is the food consumed by an animal on regular occasions (e.g., morning and evening). The sources of foodstuffs vary among animal species. For example, pigs eat corn- and soybean meal-based diets, whereas grazing ruminants consume pasture. By contrast, wild fish may ingest phytoplankton, zooplankton, microalgae, macroalgae, aquatic plants, insects, and crustaceans, as well as small fish, birds, and mammals (Wilson and Castro 2011).

Definition of Nutrition

According to the *Merriam-Webster Dictionary* (updated May 17, 2025), nutrition is "the act or process of nourishing or being nourished; specifically, the sum of the processes by which an animal takes in and utilizes food substances." Nutrition is also defined as the science that interprets the interaction of nutrients and other substances in food for the maintenance, growth, development, reproduction, and health of animals. Thus, nutrition includes (a) food intake; (b) the digestion, absorption, assimilation, biosynthesis, and catabolism of nutrients; and (c) the excretion of metabolites by animals. This definition implies that nutritional science is a cluster of scientific disciplines related to biology, which include biochemistry, food chemistry, immunology, molecular biology, pediatrics, pharmacology, physiology, public health, reproductive biology, and toxicology. Thus, animal nutrition includes essentially every biological science that can be applied to the study of nutrient utilization and nutritional problems in livestock, poultry, fish, crustaceans, and other species. Animal nutrition, like medicine, is

a field for both scientists and practitioners, and can be considered a unique discipline because of its specific objective: improving the health, survival, growth, development, and productivity of animals by understanding the metabolism and roles of dietary nutrients in their bodies.

Composition of Foodstuffs

All animals depend on food for survival, growth, and reproduction. Thus, it is important to understand its basic components, which are dry matter (DM) and water (H_2O; Figure 1.1). DM consists of organic and inorganic substances. Organic substances include carbohydrates, lipids, proteins, small peptides, amino acids, nucleic acids, other nitrogenous substances (e.g., urea and polyamines), organic acids, and vitamins. Inorganic substances are comprised of minerals that cannot be burned in a furnace (e.g., 400°C) and of other molecules such as ammonia, nitrite, and nitrate. Ash includes macro- and micro-minerals. Some of these minerals are either nutritionally essential [e.g., calcium (Ca), phosphorus (P), sodium (Na), and zinc Zn)] or harmful [e.g., arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb)] to animals (Pond et al. 2005). The composition of nutrients in some foodstuffs is given in Table 1.4, with protein being usually the most expensive one. Comparison of nutrient content among foodstuffs or diets should be made on a DM basis (Li et al. 2011, 2021c).

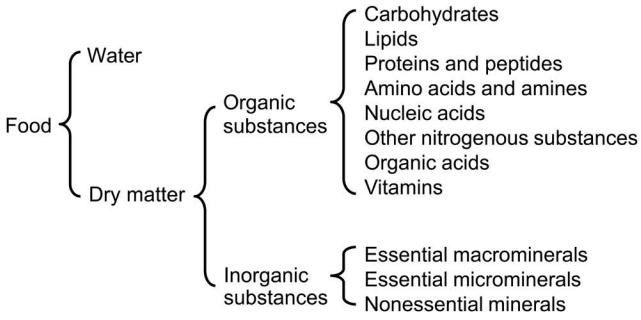


FIGURE 1.1 Composition of foods from plants and animals. Foods consist of plant- and animal-sourced materials. Both plants and animals contain organic and inorganic substances. However, the types and content of proteins, amino acids, carbohydrates, and lipids differ markedly between plants and animals, whereas nucleotides and minerals are the same in foods derived from both types of organisms. Foods from animal sources contain vitamins A, C, D, E, and K, B-series vitamins, and cholecalciferol (the precursor of vitamin D_3). The chemical structures of vitamins C and E, and B-series vitamins in animals are the same as those in plants.

TABLE 1.4
Composition of Nutrients in Foodstuffs and Pig Carcasses[a]

Foodstuff or Pig Carcass	Water	Crude Protein	Crude Fat	Minerals	Carbohydrates
		Composition of nutrients in foodstuffs (%, as-fed basis)			
Blood meal	6.74 ± 0.03	89.3 ± 0.14	1.43 ± 0.06	2.48 ± 0.07	0.05 ± 0.00
Corn grain	10.9 ± 0.05	9.20 ± 0.09	3.80 ± 0.12	1.40 ± 0.03	74.7 ± 1.3
Fish meal	7.95 ± 0.04	63.3 ± 0.33	9.70 ± 0.51	16.2 ± 1.2	2.85 ± 0.19
Meat and bone meal	4.53 ± 0.04	51.8 ± 0.28	9.24 ± 0.63	31.9 ± 1.5	2.53 ± 0.16
Soybean meal (44% CP)	11.0 ± 0.03	43.7 ± 0.12	1.24 ± 0.05	6.36 ± 0.11	37.7 ± 0.94
Sorghum meal	10.9 ± 0.03	9.98 ± 0.10	3.47 ± 0.18	1.65 ± 0.13	74.0 ± 1.6
		Composition of nutrients in pig carcasses (% of wet weight)			
1-day-old pigs (1.5 kg)[b]	82.1 ± 0.07	12.7 ± 0.39	1.38 ± 0.07	3.16 ± 0.07	0.66 ± 0.04
14-day-old pigs (4.2 kg)[b]	69.6 ± 0.82	14.9 ± 0.57	12.0 ± 0.88	3.07 ± 0.06	0.43 ± 0.03
60-day-old pigs (20 kg)[c]	68.2 ± 0.70	14.7 ± 0.51	13.7 ± 0.76	2.98 ± 0.05	0.40 ± 0.02
120-day-old pigs (60 kg)[c]	66.1 ± 0.75	14.4 ± 0.64	16.3 ± 0.82	2.85 ± 0.06	0.35 ± 0.03
180-day-old pigs (110 kg)[c]	49.7 ± 0.52	12.3 ± 0.79	35.0 ± 2.04	2.71 ± 0.08	0.29 ± 0.02
240-day-old gilts (113 kg)[d]	49.4 ± 0.46	12.1 ± 0.82	35.6 ± 1.71	2.60 ± 0.10	0.30 ± 0.02

[a] Values are means ± SEM, n = 6. The composition of nutrients in foodstuffs and pigs was determined as described by Wu et al. (1999). Data on crude protein (CP) content in foodstuffs were taken from Li et al. (2011). The pigs were the offspring of Yorkshire × Landrace dams and Duroc × Hampshire sires and weaned at 21 days of age to a milk replacer (Kim and Wu 2004). After a 10-day period of feeding, pigs were fed corn- and soybean-based diets to meet National Research Council nutrient requirements (NRC 2012). Gastrointestinal contents of the pigs were excluded from chemical analyses of the bodies, which were performed as described by Li et al. (2011).

[b] Whole bodies of the piglets nursed by sows.

[c] Barrows (castrated male pigs).

[d] Gilts (primigravid swine) were fed as described by Li et al. (2010). Briefly, gilts had free access to a corn- and soybean meal-based diet (containing 14% CP, on an as-fed basis) until 8 weeks before breeding at 8 months of age. During the 8-week period before breeding, gilts were fed 2.7 kg/day of the same diet.

Composition of Animals

Like food, an animal also comprises H_2O, nitrogenous compounds (e.g., protein, peptides, amino acids, and their N-containing metabolites), lipids (including fatty acids), carbohydrates (e.g., glycogen, glucose, and fructose), water- and lipid-soluble vitamins, and macro- and micro-minerals (McDonald et al. 2021). In the animal, H_2O and solutes are present in both extracellular and intracellular fluids. The extracellular fluid includes plasma (the noncell component of blood), interstitial fluid (a fluid between cells within tissues outside of capillaries), and transcellular fluid. The latter is present in the peritoneal, synovial, pericardial, and intraocular spaces and within the cerebrospinal tissue that surrounds the brain and spinal cord. Let us use humans as an example to illustrate H_2O distribution in the body. A lean person has ~60% of BW as H_2O, with ~2/3 and 1/3 of the body H_2O inside and outside cells, respectively (Guyton and Hall 2021). Namely, intracellular and extracellular H_2O account for approximately 40% and 20% of BW, respectively. H_2O in plasma, interstitial fluid, and transcellular fluid amounts to about 3%, 15%, and 2% of BW, respectively. In contrast to lean individuals, obese people may have only ~40% of BW as H_2O. In clinical nutrition, the dual-energy X-ray absorptiometry (DEXA, a special imaging modality) allows for the separation of the body into bone mineral, fat, and lean body mass (Shepherd et al. 2017). Lean body mass includes skeletal muscle, internal soft organs (e.g., the heart, lungs, liver, pancreas, kidneys, and GIT), skin, and blood vessels, as well as the non-mineral component of bone and the nonfat component of white adipose tissue (WAT). The GIT harbors diverse species of microbes.

The amounts of vitamins in the body are very small but vital to metabolism and life. The composition and chemistry of proteins, lipids, and carbohydrates differ remarkably between foodstuffs and animals. Animals contain vitamins A, the B series, C, D, E, and K, as well as cholecalciferol (the precursor of vitamin D_3; Bondi 1987). The structures of vitamins C, the B series, and E, as well as nucleotides and minerals in animals are the same as those in plants. The chemical composition of pigs, expressed on a fresh carcass basis, is given in Table 1.4. The content of amino acids in the body protein is generally similar among animal species (Wu and Li 2022). The percentages of fats in animals increase with increased age, but the opposite is true for body H_2O content (Hu et al. 2015). This is because fats are hydrophobic and therefore their deposition reduces the H_2O-holding capacity of the body. Of note, the percentage of proteins in animals increases moderately between birth and a young age but decreases thereafter (Pond et al. 2005). Knowledge about changes in the composition of animals with age helps nutritionists design balanced diets to maximize protein gain and minimize fat accretion in the body.

Proximate or Weende Analysis of Foodstuffs and Animals

The traditional method used to determine nutrient composition in foodstuffs and animals is known as proximate or Weende analysis, which was devised in 1865 by Henneberg and Stohmann at the Weende Experiment Station in Germany. This scheme is outlined in Figure 1.2.

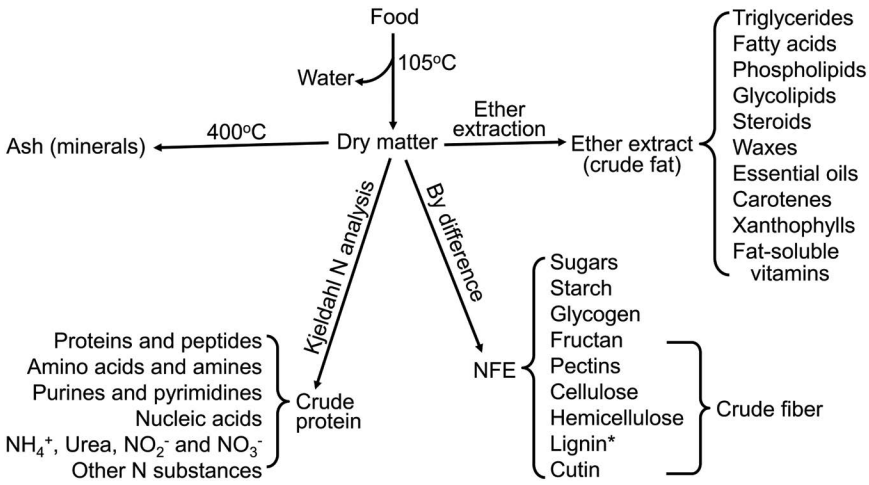


FIGURE 1.2 Proximate analysis of nutrients in foodstuffs and animal tissues. This method provides useful information about the composition of water and dry matter [including CP, crude fats (diethyl ether extracts), crude fiber, and other carbohydrates] in foodstuffs and animal tissues. Cutin is a plant-cell-wall material consisting of water-insoluble polyester polymers of long-chain hydroxyl acids (e.g., C_{16}- and C_{18}-carbon chains) and their derivatives. CP% = 6.25 × N%. Nitrogen-free extract (NFE) consists primarily of carbohydrates; it is a calculated value and is not actually extracted from food. *Lignin is a class of cross-linked complex phenolic polymers that form important structural materials in plant cell walls.

The proximate methods readily provide information on (a) crude protein (CP), which is determined by the Kjeldahl analysis of total N (CP% = 6.25 × N%); (b) carbohydrates, which include crude fiber and N-free extract (NFE; e.g., starch and sugars); (c) crude fat, which is determined by ether extraction; (d) NFE, which is a misnomer in that no extract is involved, is measured as a difference between the original sample weight and the sum of the weights of H_2O, crude fat, CP, crude fiber, and ash, rather than by direct analysis; and (e) volatile compounds [e.g., short-chain fatty acids (SCFAs; acetate, propionate, butyrate, isobutyrate, isovalerate, 2-methylbutyrate, and valerate) and essential oils (e.g., menthol and camphor)], which may be lost when DM is determined at 100–105°C for 12–24 h. In some foods, the value of CP is close to that of true protein. However, the factor of 6.25 cannot be used for the calculation of true protein content in many foods, such as corn grains (5.46), peanuts (5.59), pistachio nuts (5.66), potatoes (3.27), soybeans (6.22), sweet potatoes (4.08), wheat flour (5.69), and white rice (5.49; Hou et al. 2019).

In proximate analysis, CP includes protein and other nitrogenous substances, such as peptides, amino acids, amines, polyamines, carnitine, creatine, purine and pyrimidine nucleotides, nucleic acids, ammonia, urea, nitrite, and nitrate (Wu 2022a). Crude fat consists of triglycerides or triacylglycerols (TAGs), fatty acids and their lipid-soluble metabolites (e.g., prostaglandins), phospholipids, steroids, waxes, aldehydes, carotenes, lipid-soluble vitamins, xanthophylls, and polyphenols (e.g., gossypol). Crude fiber comprises cellulose, hemicellulose (partial), lignin (partial), and cutin. NFE is calculated as the sum of sugars, starches, glycogen, fructans, pectins, hemicellulose (partial), and lignin (partial).

Modified Methods for Analysis of Foodstuffs and Animals

The proximate analysis procedure has been criticized as being imprecise for quantifying specific nutrients (Bach Knudsen 1997; Pond et al. 2005). Procedures for the determination of plant fiber were developed by Peter Van Soest and coworkers at the United States Department of Agriculture (USDA) laboratories in the 1960s. This scheme divides the nutrients in plants into cell walls and cellular contents through the extraction of the test material with a boiling neutral detergent solution [3% sodium lauryl sulfate, 1.86% disodium ethylenediaminetetraacetate (EDTA), and 0.68% sodium tetraborate; pH = 7.0] (Van Soest and Wine 1967). The soluble fraction comprises cell contents (including mono-, di-, and oligo-saccharides; starch; fructans, gums, mucilages, and β-glucans; and lipids, proteins, amino acids, and other nitrogenous substances) plus pectin, whereas the residue or insoluble fraction is cell-wall materials and is called neutral detergent fiber (NDF; Table 1.5). NDF consists mainly of cellulose, hemicellulose, and lignin, and is the most common measurement of plant fibers in the diets of ruminants and horses (Van Soest 1967). NDF is fractionated further by extraction with an acid detergent solution [20 g cetyltrimethylammonium bromide in 1 L of 0.5 M sulfuric acid (H_2SO_4)], which solubilizes hemicellulose and a small amount of fiber-bound protein. Thus, the acid-soluble polysaccharide is hemicellulose, and the other acid-soluble fraction is the fiber-bound protein. The acid detergent fiber (ADF) is the residue (insoluble fraction) obtained after refluxing with 0.5 M sulfuric acid and cetyltrimethylammonium bromide. ADF represents primarily cellulose but also lignin, cutin (which is a waxy substance in the cuticle of plants and consists of highly polymerized esters of fatty acids), insoluble minerals (mainly silica), and acid- and heat-damaged proteins. The acid-soluble polysaccharide is hemicellulose. Therefore, NDF is composed of ADF and hemicellulose. The precise composition of complex carbohydrates can be analyzed by liquid-chromatography-mass spectrometry. The ADF is the least digestible fiber portion of forage and other roughage. The lignification (i.e., the polymerization of lignin macromolecules) of cell-wall carbohydrates affects their total tract digestibility in ruminant and nonruminant animals.

$$\text{Acid detergent fiber (ADF)} = \text{Cellulose} + \text{Lignin} + \text{Cutin} + \text{Insoluble minerals} + \text{Acid- and heat-damaged proteins}$$

$$\text{Neutral detergent fiber (NDF)} = \text{Acid detergent fiber (ADF)} + \text{Hemicellulose}$$

Due to advances in analytical chemistry, amino acids in food and animal proteins are now routinely determined by high-performance liquid chromatography (HPLC) after acidic, alkaline, and enzymatic hydrolysis (Dai et al. 2014; Hou et al. 2015). In addition, HPLC, gas chromatography, mass spectrometry, and enzymatic methods are used for the determination of proteins, nitrogenous metabolites, vitamins, lipids (including fatty acids and derivatives), simple and complex carbohydrates, and other organic substances. Furthermore, atomic absorption spectrophotometry is widely available for the analysis of individual minerals. Thus, adequate laboratory equipment is essential in modern nutrition research.

Feed Processing and Particle Size

Foods for farm, companion, and zoo animals are manufactured by employing a combination of processing technologies, which include physical grinding with hammer and/or roller mills along with hydrothermal treatment (e.g., granulation, expansion, or extrusion). Due to the higher digestibility of dietary carbohydrates, fats, and energy, pelleting is known to improve weight gain, feed intake, and feed efficiency in swine and broilers (chickens raised for meat production) regardless of the grain source, when compared with mash diets (Amerah et al. 2007).

TABLE 1.5
Classification of Forage Fractions Using the Van Soest Method

Fraction	Component	Nutritional Availability	
		Ruminant	Nonruminant
Soluble in neutral detergent solution			
Cellular Content			
Carbohydrates	Mono-, di- and oligo-saccharides, and starch	Complete	Complete
	Fructans, gums, mucilages, and β-glucans	Complete	Medium[b]
Non-carbohydrates	Proteins and amino acids, other nitrogenous	High	High
	substances, lipids (including chlorophyll),	High	High
	vitamins, carotenes, and other solubles	High	High
		High	High
Cell-wall content	Pectin	High	Medium[b]
Cell-wall materials (insoluble in neutral detergent solution)—NDF[a]			
Soluble in acid detergent solution	Hemicelluloses	Partial	Low[b]
	Fiber-bound protein (a small amount)	Partial	Low[b]
Insoluble in acid detergent solution (ADF)	Cellulose	Partial	Low[b]
	Lignin	Partial	Indigestible
	Cutin (waxy polymers of esters of LCFAs)	Partial	Low[b]
	Acid- and heat-damaged proteins	Indigestible	Indigestible
	Insoluble minerals (mainly silica)	Not available	Not available

Source: Van Soest (1967) and Van Soest et al. (1991).

[a] Neutral detergent fiber (NDF) is insoluble in a neutral detergent solution and includes hemicellulose, cellulose, and lignin. NDF is indigestible in nonruminants, while hemicellulose and cellulose are partially digestible in ruminants. NDF is further fractioned using an acid detergent solution into acid detergent fiber (ADF) and hemicellulose; i.e., NDF = ADF + Hemicellulose.

[b] These substances are not digested by animal-sourced enzymes and, in nonruminants, are subject to medium- to low-levels of digestion by intestinal bacteria (primarily in the large intestine).

Note: LCFAs, long-chain fatty acids (e.g., C_{16} and C_{18}).

Appropriate heating can increase the digestibilities of (a) native proteins by unfolding their polypeptide chains and making them more susceptible to digestive enzymes, and (b) starches by disrupting their complex structure. Thus, in addition to nutrient content, food-processing variables (e.g., temperature, pressure, duration, moisture, and mill/sieve sizes) can affect the size, shape, and taste of mash or pelleted diets, thereby influencing food consumption, utilization, and efficiency.

Regarding effects on the digestive tract, grain particle size is more critical for mash diets than pelleted or crumble diets. Compared with large coarse particles, a smaller particle size of food generally confers higher digestibility due to its greater area of surface contact with endogenous and exogenous (supplement and food-borne) digestive enzymes in the GIT, resulting in a greater efficiency for growth and production. However, reducing the size of feed particles, particularly from 600 to 400 μm, requires an increase in the use of electrical energy for milling, leading to a decrease in milling production rates. In addition, the presence of large amounts of fine particles in feeds (e.g., a particle size below 400 μm) for pigs can increase the incidences of gastric ulceration or gastric ulcers in the nonglandular portion of the stomach, despite a short-term increase in animal growth and feed efficiency (Wondra et al. 1995). Gastric ulcers, which occur due to pathogenic and/or dietary factors, are a common and serious problem in swine health and welfare. Another disadvantage of fine grinding is the presence of dusty feed, as well as the clogging of feeders and feed storage bins. Considering both swine productivity and the efficiency of the feed mill, food particle sizes of 650–700 and 600–900 μm are recommended for corn- and soybean meal-based swine and poultry diets, respectively (Amerah et al. 2007).

Because feed is ground in the avian gizzard, the size of the diet particles that enter the small intestine has much less of an effect on the structure and digestive function of the GIT in birds than in monogastric mammals. Interestingly, chickens prefer larger particles of feed, and its coarser grinding to a more uniform particle size can improve the performance of broilers maintained on mash diets, possibly because relatively large feed particle size can stimulate gizzard development (Amerah et al. 2007).

This, in turn, can increase the grinding activity of the gizzard, gut motility, and the digestion of dietary nutrients. There is evidence that growing broilers prefer particles of 1.18–3.36 mm, depending on age (El Boushy and van der Poel 2000).

As for swine and poultry diets, extrusion is also commonly used in the production of aquafeeds for fish and crustaceans. This is because such a processing method confers good stability and desirable flavor of the feed in H_2O, improves feed (e.g., starch and energy) utilization and growth performance in aquatic animals, and removes many anti-nutritional factors (Li et al. 2021a). The size of feed is a critical factor affecting its consumption by fish and crustaceans.

Biochemical Basis of Animal Nutrition

Biochemistry is the science of the chemistry of living organisms, including microbes, animals and plants (Devlin 2011). Thus, this dynamic and diverse field is also a foundation of nutrition. Biochemists study the synthesis, degradation, and roles of protein, amino acids, carbohydrates, lipids, vitamins, and nucleic acids, as well as the function and metabolism of minerals at molecular, cellular, organ, and systemic levels. Biochemists have made significant progress in our understanding of how enzymes catalyze reactions and how molecules interact with each other. These professionals also play a key role in elucidating the mechanisms of diseases in animals (including humans), such as (a) inborn metabolic disorders (e.g., ammonia toxicity due to the lack of urea cycle enzymes, impaired intestinal absorption of amino acids, and sickle-cell anemia); (b) chronic diseases (e.g., diabetes, cancer, and cardiovascular diseases); (c) acquired immune deficiency syndrome; and (d) nutritional diseases due to a deficiency or excess of nutrients (e.g., amino acids, fatty acids, minerals, or vitamins; Wu 2022b).

There is a significant overlap between nutrition and biochemistry. These two disciplines are interdependent and closely related. Biochemistry is the basis for studying the utilization of nutrients by animals (including humans), whereas nutritional studies can provide observations that often lead to exciting biochemical studies. For example, the discovery of the Krebs cycle in 1937 was based on the nutritional question of how the chemical energy in ingested food or dietary protein, carbohydrates, and fats was converted into biological energy in animals. Also, the discovery of the hepatic urea cycle was prompted by the nutritional observation that urinary excretion of urea was elevated when dietary intake of protein was increased in humans. Nutritional and biochemical studies are often indistinguishable because both are intended to understand how organic and inorganic molecules interact to support animal metabolism. In essence, biochemistry helps us understand how animals act as transformers of DM and energy into molecules that are required for all physiological functions.

Physiological Basis of Animal Nutrition

The word *physiology* originated from the Greek *phusiología*, meaning "natural philosophy." It is the study of how living organisms perform their vital functions through the integration of activities among individual cells, tissues, organs, and systems. Physiologists study physical and chemical processes in the body, including (a) sensations, motility, and function of the GIT; (b) blood and lymph circulation; (c) the interorgan transport of inorganic and organic molecules; (d) respiration and exhalation; (e) central and peripheral nervous systems; (f) the excitation and contraction of skeletal muscle and smooth muscle; (g) male and female fertility; (h) hormone secretion and action; and (i) the excretion of metabolites. Thus, knowledge of physiology helps us understand how ingested food is digested, absorbed, and assimilated in animals to sustain homeostasis (maintenance of relatively constant conditions in the internal environment) and support physical activity, growth, development, reproduction, and health.

Physiological research is based, in part, on nutritional observations. For example, the discovery of the synthesis of nitric oxide (NO; a major vasodilator and regulator of hemodynamics) from L-arginine in 1988 was based on nutritionists' observation of a marked increase in urinary excretion of nitrate from lipopolysaccharide-challenged rats. In addition, the discovery of the vitamin K-dependent mechanism for blood coagulation was based on nutritional findings that (a) a hemorrhagic disease occurred in cattle after they had consumed spoiled sweet clover containing dicumarol (an inhibitor of 2,3-epoxide reductase) or in rats fed a lipid-deficient diet, which impairs intestinal absorption of vitamin K, and (b) bleeding in rats could be treated with spoiled fishmeal containing menaquinone (vitamin K_2). The interaction between nutrition and physiology has given rise to a research area known as nutritional physiology, which is the study of how nutrients influence physiological processes in the body and vice versa.

INTEGRATION OF SYSTEMS PHYSIOLOGY IN NUTRIENT UTILIZATION

STRUCTURE OF ANIMAL CELLS

Definitions of Cell, Tissue, Organ, and System

The word *cell* comes from the Latin "cella," meaning "a small chamber." The cell is the basic unit of animals, plants, and microbes. This chapter focuses on cells in animals. Each type of cell [e.g., enterocyte, hepatocyte, myocyte, and red blood cell (RBC, also known as erythrocyte)] performs one or more functions. Many different but similarly specialized cells are held together by intercellular supporting structures to form a tissue [e.g., connective tissue, WAT, and brown adipose tissue (BAT)]. A group of tissues are joined in structural units to form an organ (e.g., the small intestine, liver, kidney, and skeletal muscle) that serves specific functions. A number of different organs work together

to form a system (e.g., the digestive, musculoskeletal, or reproductive system) that carries out a common function, including (a) the breakdown of food to provide nutrients via the GIT, (b) milk production, (c) immune responses, (d) muscle movement through the coordination of the musculoskeletal system, and (e) the production of eggs and offspring through the female reproductive system. Systems physiology is a foundation for studying animal nutrition (Lu 2014; Wu 2022b).

Composition and Function of the Animal Cell

Most animal cells contain a plasma membrane, cytoplasm, mitochondria, and nucleus (Figure 1.3). The cytoplasm is separated from the nucleus by the nuclear membrane, and the plasma membrane (also known as the cell membrane) separates the intracellular components from the extracellular environment (Guyton and Hall 2021). The different substances that make up the cell are collectively known as protoplasm and include H_2O, proteins, lipids, carbohydrates, minerals, and vitamins. Mitochondria are usually reddish-brown in color because of the presence of cytochromes (red and green), flavins (yellow), and iron (Fe)-sulfur (S) clusters (brown).

Nearly all biochemical reactions for nutrient synthesis and degradation take place inside the cell (Brosnan 2005). Thus, it is important to understand the composition and function of cells. The transport of nutrients into the cell is the first step in the utilization of nutrients by animals. Although different cell types in the body differ in many aspects (e.g., shape, morphology, protein expression, metabolites, anatomical location, and functions), all of them have common basic characteristics. Except for mammalian RBCs that have lost all organelles during their maturation process, animal cells are divided internally by membranes into compartments (including nuclei, ribosomes, mitochondria, lysosomes, and other organelles for specific functions). Intracellular compartmentalization is an important feature of animal metabolism (Watford 1991).

The cell membrane is a structure made of a phospholipid bilayer with embedded proteins and cholesterol (Devlin 2011). The fluid nature of the cell membrane permits its

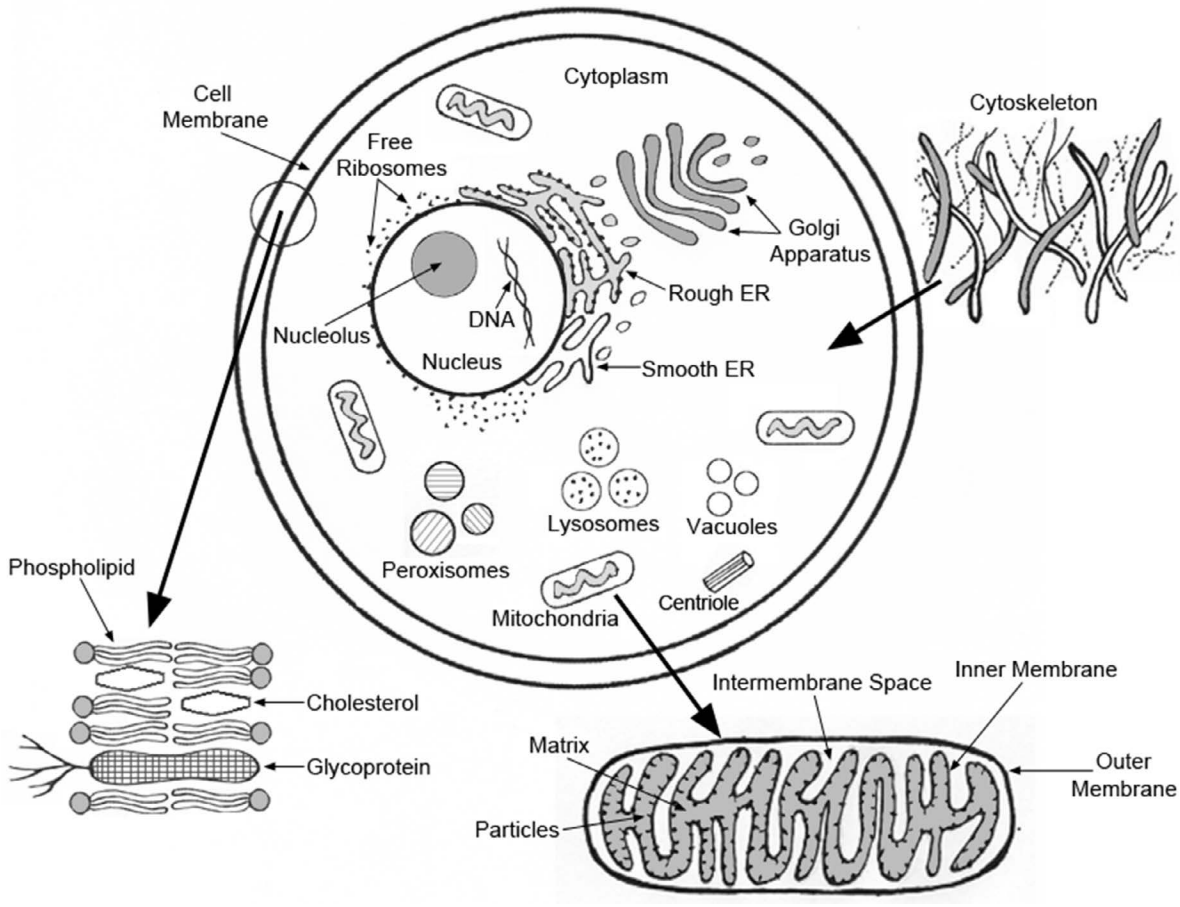


FIGURE 1.3 Structure of an animal cell. The animal cell (except for the red blood cell in mammals) contains the following components: plasma membrane, cytoplasm, and organelles, including the mitochondria, nucleus, endoplasmic reticulum (ER, smooth and rough), centrioles, ribosomes, Golgi apparatus, lysosomes, peroxisomes, vacuoles, and cytoskeletal elements. Note that the mammalian red blood cell does not contain mitochondria or other organelles. The avian red blood cell possesses a nucleus and a limited number of mitochondria. The organization of phospholipids, cholesterol, and glycoproteins forms the lipid-bilayer cell membrane. Mitochondrial components are also presented, with stalked particles (ATP synthetase complexes) containing the F_0 and F_1 domains of ATP synthase.

integral proteins to move laterally along the plane of the bilayers. The selective permeability of the cell membrane allows the cell to control and maintain its internal environment. The cytoplasm (a thick jelly-like solution) consists of cytosol (fluid fraction), organelles, the cytoskeleton, and various other particles. Organelles are subcellular entities enclosed by membranes that are similar to the plasma membrane in structure. These organelles include the mitochondria, rough endoplasmic reticulum, centrioles, smooth endoplasmic reticulum, ribosomes, Golgi apparatus, lysosomes, and peroxisomes (Cooper and Hausman 2016). The nucleus is the largest organelle in the nucleated cell. Of note, mature mammalian RBCs contain no DNA, nucleus, mitochondria, or other organelles (Barminko et al. 2016). By contrast, avian RBCs possess a nucleus and a limited number of mitochondria (Campbell 1995), whereas RBCs of fish [e.g., rainbow trout (Ferguson and Boutilier 1989) and zebrafish (Laale 1977)] contain both nuclei and metabolically active mitochondria. Note that microsomes are not cellular organelles, but rather are formed artificially from fragments of the endoplasmic reticulum when cells are disrupted during homogenization. A scheme of cell fractionation through centrifugation is outlined in Figure 1.4. The biological functions of the cell fractions and organelles are summarized in Table 1.6.

As the powerhouse of the cell, the mitochondrion consists of a simple outer membrane (a lipid-bilayer structure), a more complex inner membrane (also a lipid-bilayer structure), the intermembrane space (the space between these two membranes), and the matrix (the space encircled by the inner mitochondrial membrane; Cooper and Hausman 2016). The inner mitochondrial membrane is invaginated into folds, called cristae, to augment its surface area. The outer mitochondrial membrane is composed of 30–40% lipid and 60–70% protein, including a high concentration of the integral protein called porin. The porin is also called the voltage-dependent anion channel (VDAC) with its conformational states being influenced by the transmembrane voltage (Devlin 2011). Each porin protein is composed of one transmembrane α-helix and 13 transmembrane β-strands, and has a molecular cut-off size of ~5 kDa for most nonelectrolytes (Lemasters and Holmuhamedov 2006). The porin

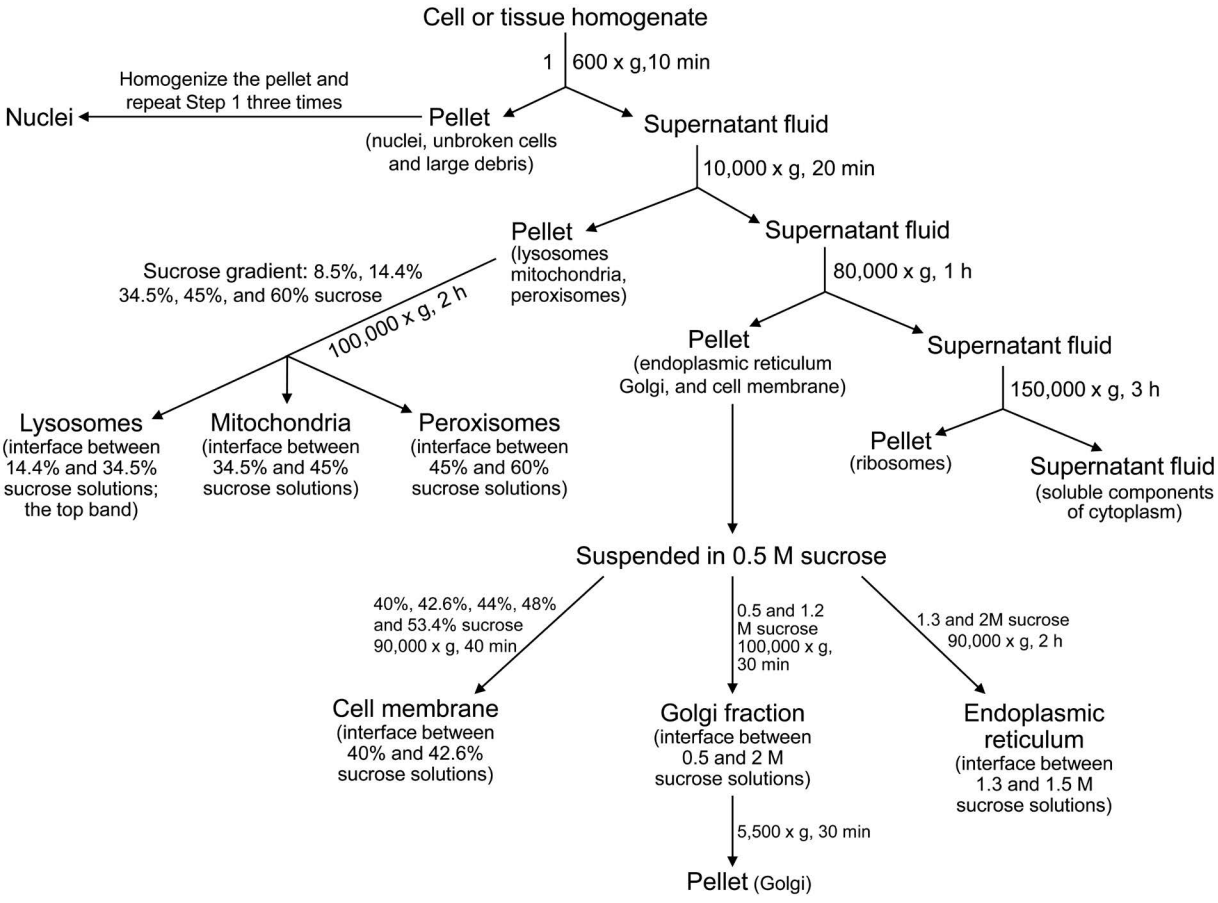


FIGURE 1.4 A scheme for sucrose-based cell or tissue fractionation. Cells or tissues are homogenized in 250 mM sucrose, 5 mM imidazole-HCl (pH 7.4), 1 mM EDTA-Na_2, and protease inhibitors. The homogenate is centrifuged at 600 ×*g* to separate nuclei (pellet) from other cell components (supernatant). High-speed centrifugation of the latter at 10,000 ×g yields a pellet (mitochondria, lysosomes, and peroxisomes) and the supernatant (cytosolic fraction, cell membranes, and endoplasmic reticulum). These two fractions are subject to ultrahigh-speed centrifugation on sucrose gradients, yielding cytoplasm, peroxisomes, mitochondria, lysosomes, Golgi, and endoplasmic reticulum. (Adapted from Cooper and Hausman 2016; Croze and Morré 1984; De Duve 1971.)

TABLE 1.6
Major Functions of the Plasma Membrane, Cytoplasm, and Organelles of the Cell

Cellular Organelle or Fraction	Marker	Major Functions
Plasma membrane	Na^+/K^+ ATPase and 5'-nucleotidase	Separation of the interior of the cell from the outside environment; transport of molecules into and out of cells; intercellular adhesion and communication; receptor for hormones, certain AAs, and other molecules; cell signaling; endocytosis; exocytosis; passive osmosis and diffusion
Cytosol	Lactate dehydrogenase	Control of cell volume, shape, and pH (pH 7.0); suspension of organelles; storage of cellular components; intracellular transport of substances; enzymes for glycolysis, fatty acid and glucose synthesis, and pentose cycle; some enzymes for AA metabolism
Nucleus[a]	DNA	Site of DNA synthesis and storage; site of chromosomes; site of DNA-directed RNA synthesis (in nucleolus); control of protein synthesis and cell growth
Mitochondrion	Cytochrome oxidase	Cellular respiration; ATP production through oxidative phosphorylation; contribution to thermogenesis; enzymes for the Krebs cycle, as well as the oxidation of fatty acids, much glucose, and some AAs to CO_2 and H_2O; regulation of apoptosis, stress response, and cell growth
Rough endoplasmic reticulum	Ribosomal RNA	Site for synthesis of secretory and membrane proteins in cells; protein sorting, processing (e.g., folding and glycosylation), and trafficking
Smooth endoplasmic reticulum	Cytidylyl transferase and glucose 6-phosphatase	Protein sorting, processing and transport; oxidation of xenobiotics via cytochrome P450; synthesis of many lipids (including cholesterol and steroids) and glucose
Ribosomes	High content of RNA	Site for synthesis of endogenous cellular proteins
Golgi apparatus	Galactosyl transferase	Modification (e.g., glycosylation), sorting, and packaging of proteins for secretion; sulfation reactions; transport of lipids; creation of lysosomes
Lysosome	Acid hydrolases	Site of many hydrolases (optimum pH = 4–5); protein degradation via proteases and autophagy
Peroxisome	Catalase and uric acid oxidase	Production and degradation of hydrogen peroxide; initiation of degradation of very long-chain fatty acids through β-oxidation; contains D-amino acid oxidases
Cytoskeleton	Relevant proteins	Microtubules, microfilaments, and intermediate filaments
Centrosome[b]	Ninein (a protein)	Interact with chromosomes to build mitotic spindles in mitosis; facilitate cell division

Source: Cooper and Hausman (2016) and Devlin (2011).
[a] Containing nuclear membrane, nucleolus and chromatin.
[b] Consisting of two centrioles (self-replicating organelles made up of nine bundles of microtubules) surrounded by an amorphous mass of protein.

permits the passage of peptides, nucleotides, metabolites, ions, and nucleic acids. The outer mitochondrial membrane has high permeability for anions (e.g., phosphate and chloride), substrates (e.g., ADP, succinate, malate, and creatine), or products (e.g., ATP, GTP, and phosphocreatine) of mitochondrial energy metabolism, while aiding in the import of RNA and DNA from the cytosol into the mitochondria (Schlattner et al. 2006; Weber-Lotfi et al. 2015).

The inner mitochondrial membrane contains 80% protein and 20% lipid, including unsaturated fatty acids and cardiolipin (diphosphatidylglycerol; Palmieri and Monné 2016). This membrane limits the types of substrates and intermediates that can diffuse from the cytosol into the mitochondrial matrix and vice versa. Specific transporters are responsible for the movement of select substances (e.g., pyruvate ↔ OH^-, phosphate ↔ L-malate, L-malate ↔ citrate, L-malate ↔ α-ketoglutarate, ADP ↔ ATP, glutamate ↔ aspartate, glycerol-3-P ↔ dihydroxyacetone-P, and phosphate and H^+) across the inner mitochondrial membrane from either side (Devlin 2011). The mitochondrial electrochemical gradient generated by proton pumping provides energy for the transport of these molecules. The mitochondrial matrix contains enzymes for the β-oxidation of fatty acids, the whole Krebs cycle, some reactions of amino acid metabolism and antioxidative responses, part of the urea cycle, and the components for mitochondrial protein synthesis. The mitochondria are also essential for cells to synthesize steroid hormones that affect many aspects of the endocrinology and metabolism of animals (Stocco 2000).

Proteins are imported from the cytosol into the inner mitochondrial membrane, mitochondrial intermembrane space, or the mitochondrial matrix through transmembrane pore-forming proteins (Lenkiewicz et al. 2022). Specifically, proteins that are destinated for mitochondria are first synthesized as largely unfolded precursor proteins (containing N-terminal cleavable signal sequences of 15–50 amino acid residues) on ribosomes in the cytosol. Subsequently, these proteins enter the mitochondrial intermembrane space

through the translocase of the outer membrane (a multisubunit complex that serves as the protein receptor and a protein entry gate) and, thereafter, are routed to their final destinations encoded within their amino acid sequences. The translocase of the inner membrane (TIM)-22 (TIM22) and TIM23 insert their respective protein substrates into the inner mitochondrial membrane. The TIM23 substrates are recognized by the presequence translocase-associated motor (PAM) for entry into the mitochondrial matrix, where they undergo the proteolytic removal of their signal sequences to possess biological functions. Note that the oxidase assembly complex inserts the mitochondrially synthesized proteins into the inner mitochondrial membrane.

Transport of Substances Across the Biological Membrane

Substances cross the cell membrane and the membranes of organelles through simple diffusion (passive diffusion), facilitated diffusion (passive transport), or active transport (Figure 1.5). The mode of transport of nutrients depends on their chemical properties and concentrations across the membrane (Cooper and Hausman 2016). Rates of nutrient transport increase with the increase in their extracellular concentrations for influx or in their intracellular concentrations for efflux. Both passive transport and active transport processes require transmembrane proteins.

Simple Diffusion

Simple diffusion refers to the movement of molecules into and out of the cell or its organelles along high-to-low concentration gradients (Friedman 2008). Many small uncharged molecules (e.g., O_2, CO_2, CO, NO, N_2, H_2S, glycerol, and ethanol) can diffuse freely through the cell membrane via simple diffusion (Cooper and Hausman 2016). Another example of simple diffusion is osmosis, which refers to the movement of H_2O across a semipermeable membrane (e.g., the cell membrane) from the solution with a lower osmolarity (osmol/L solution) or osmolality (osmol/L H_2O) toward the solution with a higher osmolarity or osmolality (Reece 1993). This tends to equalize solute concentrations on the two sides of the membrane. Excess flow of H_2O from the extracellular space into cells (e.g., RBCs) can lyse the cells, whereas excess exit of H_2O out of the cells can shrink the

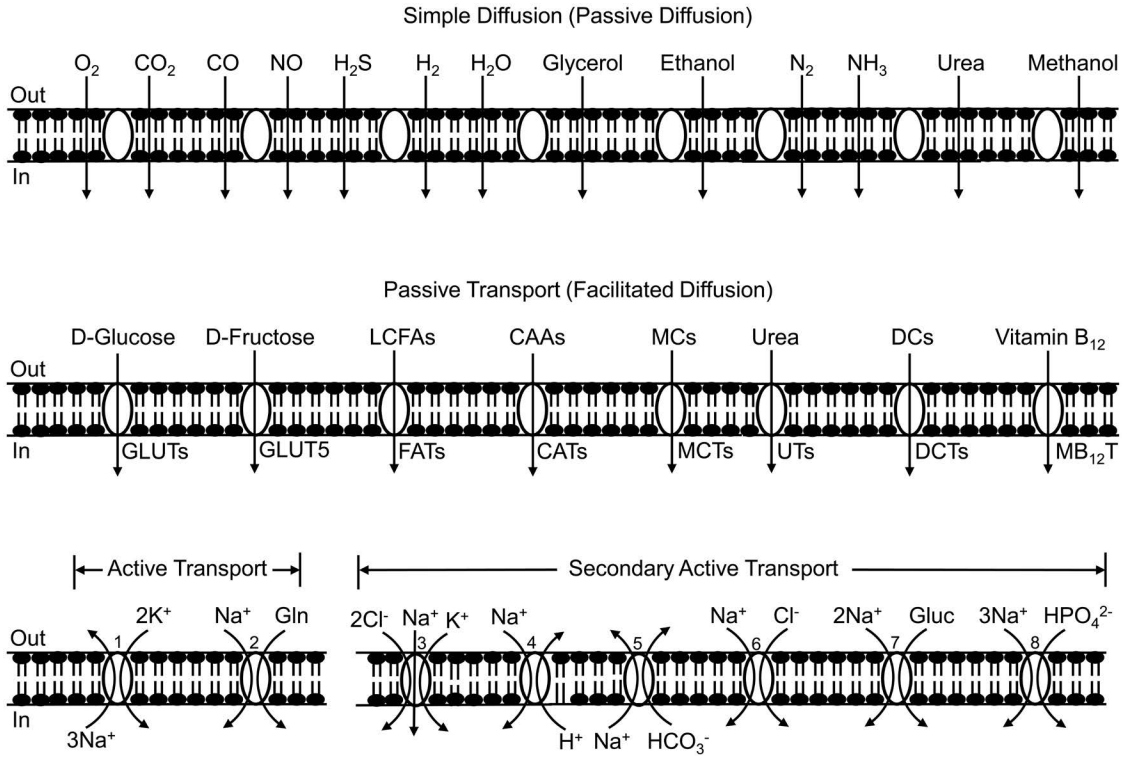


FIGURE 1.5 Transport of substances across biological membranes. Gases, nutrients, and their metabolites are transported through the plasma membrane or the membranes of organelles via simple diffusion or carrier-mediated transporters. Note that the rate of glycerol transport by animal cells is very low via simple diffusion but is relatively high via the passive transport of facilitated carriers (e.g., aquaporins 3, 7, and 9). CAAs, cationic amino acids; CATs, cationic amino acid transporters; DCs, dicarboxylates; DCTs, dicarboxylate transporters; FATs, fatty acid transporters; GLUTs, glucose (Gluc) transporters; LCFAs, long-chain fatty acids; $BM_{12}T$, membrane-bound vitamin B_{12} transporter; MCs, monocarboxylates; MCTs, monocarboxylate transporters; UTs, urea transporters. Active transport or secondary active transport is indicated by a number: 1, Na^+-K^+-pump; 2, glutamine (Gln) transporters; 3, Na^+-K^+-Cl^- cotransporter; 4, Na^+/H^+ exchanger; 5, Na^+-bicarbonate cotransporter; 6, Na^+-Cl^- cotransporter; 7, SGLT1 (sodium-glucose linked transporter 1); and 8, Na^+-phosphate cotransporter.

cells. While the cell membrane itself is actually impermeable to H_2O, it is considered freely permeable to H_2O because there are many aquaporins (channels that allow H_2O to pass through) present and open at all times.

$$\text{Osmolarity} = \text{Osmol/L solution}\left(\text{e.g., 110 mmol } Na^+/\text{L plasma}\right)$$
$$\text{Osmolality} = \text{Osmol/L } H_2O\left(\text{e.g., 110 mmol } Na^+/\text{L } H_2O\right)$$

Osmoregulation is essential to life. Osmolarity values of physiological fluids in animals are as follows (mOsmol/L): the plasma of terrestrial mammals and birds, ~300; the blood of freshwater fish, >250; the extracellular space of freshwater crustaceans, 200–400 (with values of 50 and 600 in some mollusk species and the Chinese mitten crab, respectively); the blood of teleost fish, 260–380 [with the values of 260 and 360–380 in the blood of carps (freshwater fish) and the European sea bass (marine fish), respectively] (Lee et al. 2022).

Carrier-Mediated Transport

Large polar molecules (e.g., vitamin B_{12}, glucose, and amino acids) and ions (e.g., H^+, Na^+ and Cl^-) cannot cross a lipid-bilayer membrane (e.g., the plasma membrane and the inner mitochondrial membrane) by simple diffusion, and instead they cross the cell membrane through specific transmembrane proteins called transporters, channel proteins, or protein pores (Friedman 2008). Based on the requirement of ATP or electrochemical potential, carrier-mediated transport is classified as facilitated diffusion, active transport, or secondary (coupled) active transport (Cooper and Hausman 2016).

Facilitated Diffusion

Facilitated diffusion refers to the energy-independent, transporter (carrier protein)-mediated movement of substances across the membrane in the direction of their concentration or electrochemical gradients. Examples of facilitated diffusion are the intestinal absorption of long-chain fatty acids (LCFAs), basic amino acids (e.g., arginine and lysine), small peptides (di- and tri-peptides), monocarboxylates (e.g., pyruvate and lactate), and dicarboxylates (e.g., α-ketoglutarate and succinate) from the lumen of the small intestine into the enterocytes. The transport of these substances requires fatty acid transporters, cationic amino acid transporters, the H^+-driven peptide transporter 1 (PepT1), monocarboxylate transporters, and dicarboxylate transporters, respectively (Beitz 1993; Friedman 2008). Other examples of facilitated diffusion are the aquaporin-mediated rapid movement of H_2O and glycerol across some cell membranes, and glucose transport by glucose transporter-4 in mammalian skeletal muscle. Aquaporin 7 transports glycerol out of adipocytes, and aquaporin 9 takes up glycerol from the extracellular space into hepatocytes (Lebeck 2014). Of note, protein channels for ion transport (also known as ion channels) provide the passive transport of large numbers of ions down their electrochemical gradient when the channels are open.

Active Transport

Active transport refers to the energy-dependent, carrier protein-mediated movement of substances through the biological membrane against their concentration or electrochemical gradients. An example of active transport is the uptake of glutamine from the plasma (e.g., 0.5–1 mM) into skeletal muscle (e.g., 10–20 mM) by transporter N (Xue et al. 2010). The energy required for active transport is almost exclusively provided by ATP hydrolysis. The most familiar example is Na^+-K^+-ATPase, which is found in many cells throughout the body and helps to maintain osmotic equilibrium and membrane potential in cells by moving 3 Na^+ out of the cell and 2 K^+ into the cell (both against their gradients and thus requiring ATP).

Secondary (coupled) Active Transport

Secondary active transport refers to a form of active transport where a substance crossing the biological membrane is coupled with the movement of an ion (typically Na^+ or H^+) down its electrochemical potential (Friedman 2008). Secondary active transport does not directly require energy (ATP, GTP, or UTP) but is coupled with the hydrolysis of ATP by Na^+/K^+-ATPase to provide energy for pumping the influxed Na^+ out of the cell (active transport). Secondary active transport requires a carrier protein and is commonly referred to as ion-coupled transport. Based on the direction of movement of coupled solutes, the transporters for secondary active transport are known as symporters (cotransporters) or antiporters (exchangers or counter-transporters) when solutes move in the same or opposite direction, respectively. An example of secondary active transport is the entry of glucose from the lumen of the small intestine into the enterocyte through sodium-glucose linked transporter-1 (SGLT1; a symporter) on the apical membrane (brush-border membrane). SGLT1 utilizes the co-movement of Na^+ down its electrochemical gradient to drive the complete uptake of glucose from the intestinal lumen (Boron 2004). The Na^+/K^+-ATPase in the basolateral membrane of the enterocyte pumps Na^+ from the cell. In contrast, the Na^+/H^+ exchanger, which plays a major role in regulating intracellular pH and Na^+ homeostasis, is an antiporter.

STRUCTURE OF PROKARYOTES

The GIT of animals contains prokaryotes, including bacteria and archaea. These microbes contain a cell (plasma) membrane, an enveloping cell wall, and the cytoplasm but lack interior membranes and organelles (i.e., nuclei, lysosomes, and mitochondria). The cytoplasm possesses DNA, RNA, proteins, and enzymes that participate in cell metabolism and growth. The relatively porous cell walls of Gram-positive and negative bacteria consist of about 90–95% and 5–10% peptidoglycans, respectively, to provide structural rigidity and protection from the environment. These macromolecules have polysaccharide

backbones consisting of alternating *N*-acetylmuramic acid and *N*-acetylglucosamine residues. Gram-positive bacteria have a relatively thick peptidoglycan cell wall that retains the primary stain (crystal violet) during the decolorization step and appear purple or blue under a microscope. Using the same staining procedure, Gram-negative bacteria have a low ability to retain the primary stain because of their thinner peptidoglycan layer and appear pink or red. In contrast to bacteria, the cell wall of archaea contains surface-layer proteins called S-layers (with the "S" standing for "surface") and does not possess peptidoglycans. Over 25% of all prokaryotes are fermentative organisms, using >40 substrates in complex metabolic pathways and releasing >50 end products (Hackmann 2024). In prokaryotes, various electron transport systems exist in the cell membrane to oxidize NADH to NAD^+, along with the presence of proton pumps (as many as three pumps) to export protons and generate an electrochemical proton gradient for ATP synthesis by ATP synthase.

OVERVIEW OF THE ANIMAL SYSTEM

The anatomical structures of animals are diverse and are generally comprised of nine systems (nervous, digestive, circulatory, musculoskeletal, respiratory, urinary, reproductive, endocrine, and immune systems) and five sense organs (Dyce et al. 1996). Utilization of dietary nutrients by animals involves the cooperation of all the organs in the body. Examples are how (a) the nervous system controls the food intake and behavior of animals; (b) the digestive system is required for the digestion and absorption of enteral nutrients in diets; (c) the circulatory system is needed for transport of absorbed nutrients from the stomach and intestine into the general circulation; (d) the respiratory system is responsible for the supply of O_2 and the expiration of CO_2; (e) the endocrine system regulates nutrient metabolism under physiological and pathological conditions; (f) the immune system protects the animal from infection and ensures a healthy state; (g) the urinary system excretes metabolites from the body; (h) the musculoskeletal system provides the structure, support, and movement (e.g., walking, chewing, swallowing, and breathing) of the organism, with skeletal muscle being the major component of growth; and (i) the reproductive system ensures the continuous propagation of the animal species (Davis et al. 2002; Field et al. 2002; Scanes 2009). The excretion of metabolites takes place via the kidneys, digestive tract, lungs, and skin in all vertebrates and also occurs in the gills (plate-like filaments covered by an array of lamellae enclosing a capillary blood network) of fish and crustaceans. Thus, it is important for nutritionists to understand the complexity and interactions of all the anatomical systems in animals. Figure 1.6 provides an overview of the utilization of food by animals through digestion, absorption, transport via lymphatic vessels and blood circulation, metabolism, and excretion.

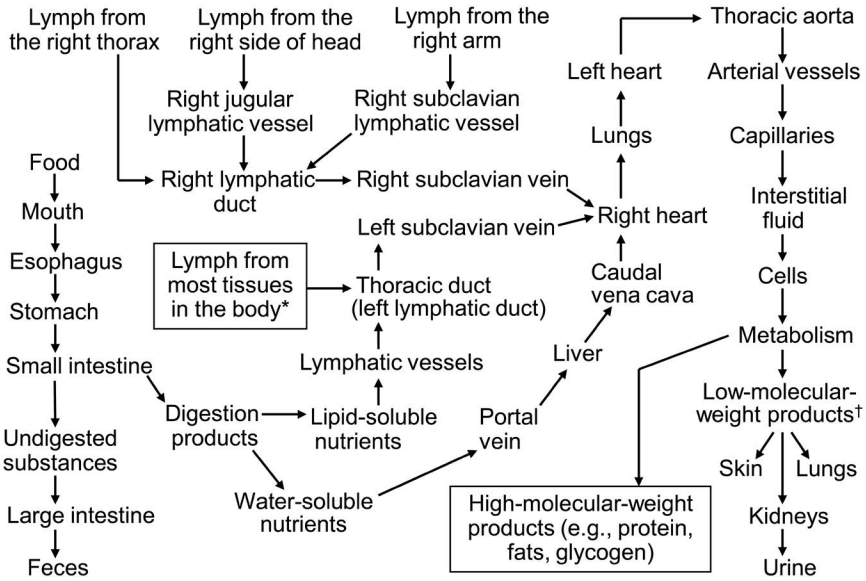


FIGURE 1.6 An overview of food utilization by animals. Ingested food is digested in the stomach and the small intestine to form water-soluble and lipid-soluble products. The water-soluble digestion products enter the portal vein and then the liver. The substances that escape hepatic catabolism are transported to the right atrium of the heart. In contrast, lipid-soluble digestion products enter lymphatic vessels, the left subclavian vein, and then the right atrium of the heart. The lymphatic system also provides a route for both fluid and large-molecular-weight substances (e.g., proteins, lipoproteins, and large particulate matter) in the interstitial space to return to the blood. Through the microcirculation, nutrients are transported to cells for use. Metabolic products are either deposited in the body or excreted through the skin, lungs, and kidneys. Undigested food is fermented in the large intestine and its metabolites are excreted in the feces. *Including parts of the chest region, the lower part of the body, as well as the left side of the head and the left arm. †Including H_2O, CO_2, NH_3, NH_4^+, urea, nitrate, and sulfate.

THE NERVOUS SYSTEM

The nervous system includes central and peripheral nervous systems (Figure 1.7) and is the commander of the body that controls its voluntary and involuntary actions (He and Wu 2020). The neuron (also known as the nerve cell) is the basic unit of the nervous system. The complex neural network includes neurons, neurotransmitters, and glial cells (nonneuronal cells). The word "glial" means "glue" in Greek. The glial cells support and nourish neurons, produce myelin, and participate in signal transmission in the nervous system (Brodal 2004).

The Neuron

The neuron has all the components of the animal cell and additional specialized parts: a cell body, dendrites (thin, branching structures from the cell body), and an axon (also known as a nerve fiber). The types of neurons include (a) sensory neurons in the sense organs; (b) motor neurons, which are efferent neurons that originate in the spinal cord and form synapses with skeletal muscle to control muscle contraction; and (c) interneurons that connect neurons within the central nervous system (Sporns et al. 2004).

As an electrically excitable cell, the neuron maintains an electrochemical potential across the plasma membrane through ion pumps to control the flux of ions (e.g., Na^+, K^+, Cl^-, and Ca^{2+}). The electrochemical pulse travels along the neuron's axon to the synapse (a junction between two neurons) and then onto the synapse of another nerve cell or the receptor site of a muscle or gland cell (Kaeser and Regehr 2014). Upon stimulation by the electrical signal, the transmitting neuron releases chemical neurotransmitters into the synaptic cleft (the extracellular space between two neurons). These neurotransmitters (e.g., acetylcholine) bind to their receptors (e.g., acetylcholine receptor, a ligand-gated Na^+ channel) in the post-synaptic membrane of the recipient cell and transmit an action potential to the next cell (Figure 1.8). The neurotransmitters may then be inactivated by uptake and destruction, depending on the neurotransmitter and tissue (Brodal 2004). Thus, neurons process and transmit information through electrical and chemical signals through synapses or to the receptor site of other target cells (e.g., muscle and gland cells). Neurons are connected to each other, forming complex and well-integrated neural networks.

Neurotransmitters

Neurotransmitters, either excitatory or inhibitory, are the signals released by neurons at the synapses (Table 1.7). These molecules are released from the neuron on the presynaptic side of the synapse into the synaptic cleft and then bind to specific receptors in the cell membrane of the neuron on the post-synaptic side of the

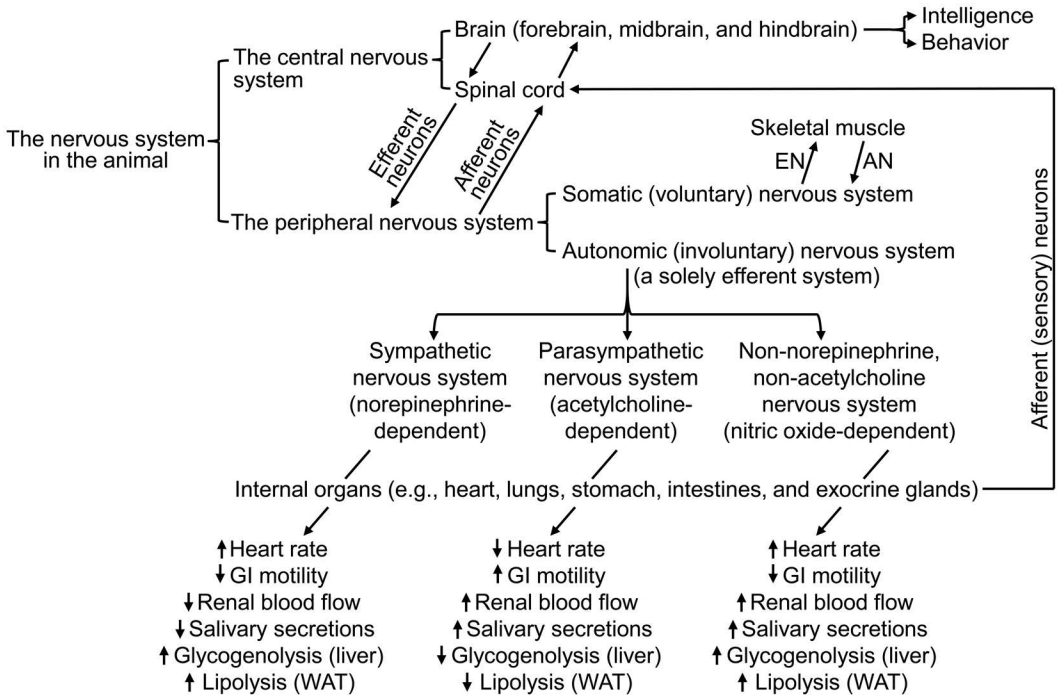


FIGURE 1.7 The nervous system in animals. The nervous system comprises the central nervous system (the brain and spinal cord) and the peripheral nervous system. The brain processes and interprets sensory information transmitted from the spinal cord. Afferent neurons conduct impulses from the peripheral nervous system toward the spinal cord, whereas efferent neurons carry impulses from the spinal cord to the peripheral nervous system. AN, afferent neurons; EN, efferent neurons; GI, gastrointestinal; WAT, white adipose tissue. ↑, increase; ↓, decrease.

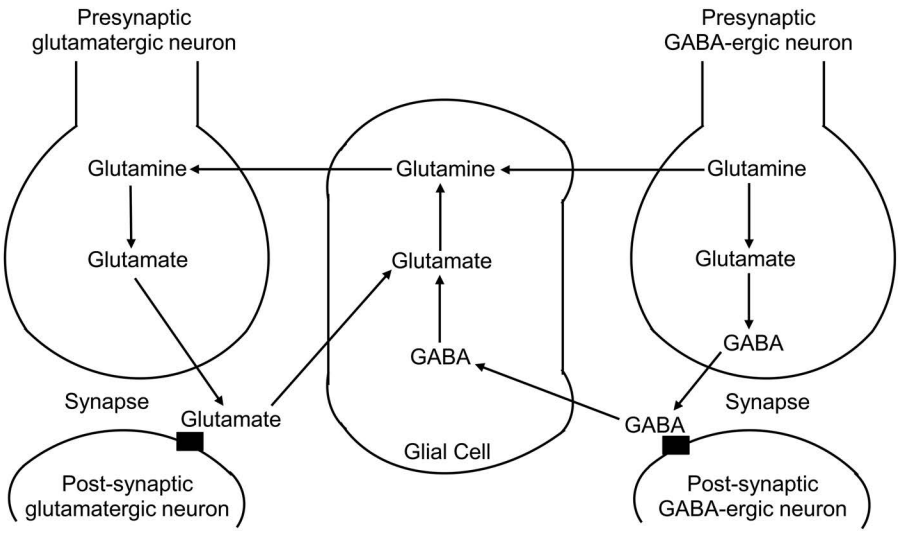


FIGURE 1.8 Communications between neurons through neurotransmitters in animals. A synapse (a small gap between two cells) allows for the first neuron (the presynaptic neuron) to communicate with the second neuron (the post-synaptic neuron) through a neurotransmitter [e.g., L-glutamate or γ-aminobutyrate (GABA)]. The presynaptic neuron releases a neurotransmitter into the synapse, and then the neurotransmitter acts on the post-synaptic neuron through a specialized receptor, followed by the uptake of the neurotransmitter into the glial cell.

TABLE 1.7
Major Neurotransmitters in the Central Nervous System (CNS) of Animals

Neurotransmitter	Physiological Roles	Site of Production within the CNS
Acetylcholine	A neurotransmitter in the brain; allows impulses to be fired at the synaptic cleft; vital for cognition and perception; modulates arousal, attention, memory, and motivation	Cholinergic neurons in the striatum
γ-Aminobutyrate (GABA)	The primary inhibitory neurotransmitter in the mature brain but primarily excitatory in the developing brain; a major inhibitory neurotransmitter in the spinal cord (50% sharing with glycine)	GABAergic neurons[a]
D-Aspartate	An excitatory neurotransmitter in the brain; binds to NMDA receptors; evokes L-glutamate release; promotes LTP	Neurons and glial cells; spinal cord
L-Aspartate	A major excitatory neurotransmitter in the CNS; an agonist for NMDA receptors; enhance synaptic plasticity and the neural network, as well as learning and memory	Neurons and glial cells; spinal cord
Carbon monoxide (CO)	A neurotransmitter in the brain; modulates synaptic plasticity and neuronal activity; promotes LTP	Heme oxygenase is localized in many regions of the brain[b]
Dopamines	A neurotransmitter in the brain; critical for motor control, mood, addiction, and response to stress; modulates learning, rewards, emotion, and executive function	Dopaminergic neurons in midbrain and forebrain[c]
Epinephrine	A neurotransmitter in the brain; modulates metabolism, attention, focus, panic, and excitement; enhances memory formation	Medulla oblongata of the brainstem
L-Glutamate	The primary excitatory neurotransmitter in the brain; an agonist for NMDA receptors; enhances synaptic plasticity and the neural network, as well as learning and memory	Neurons and glial cells; spinal cord
Glycine	A major inhibitory neurotransmitter in the spinal cord and lower brainstem; a co-agonist with L-glutamate at NMDA receptors	Neurons and glial cells; spinal cord
Histamine	A neurotransmitter in the brain and spinal cord; plays an essential role in the control of circadian rhythms; modulates motivational behavior; promotes wakefulness	Histaminergic neurons[d]
Hydrogen sulfide (H_2S)	A neurotransmitter in the brain; modulates synaptic plasticity and neuronal activity; facilitates hippocampal LTP	Astrocytes, microglia and neurons[e]

(*Continued*)

TABLE 1.7 (*Continued*)
Major Neurotransmitters in the Central Nervous System (CNS) of Animals

Neurotransmitter	Physiological Roles	Site of Production within the CNS
Nitric oxide (NO)	A neurotransmitter in the brain; modulates synaptic plasticity and neuronal activity; inhibits NMDA receptor activity	Various regions[f]
Norepinephrine	A neurotransmitter in the brain; modulates emotion, sleep, attention, focus, learning, and response to ANS	Mainly neurons of locus coeruleus; also the lateral tegmental field
Nonopioid peptides	Neurotransmitters in the brain and spinal cord; modulates analgesia, hypothermia, locomotion, and dopamine pathways	Histaminergic neurons[d]
Opioid peptides	Neurotransmitters in the brain and spinal cord; modulates pain perception, analgesia, euphoria, and the actions of other neurotransmitters in the CNS	Histaminergic neurons[d]
Serotonin	A neurotransmitter in the brain; modulates sleep-wakefulness cycles, the processing of afferent inputs, mood, and behavior	Serotonergic neurons mainly in the brainstem raphe complex[g]

Source: He and Wu (2020) and Wu (2024).

[a] These GABAergic neurons are located in the hippocampus, thalamus, basal ganglia, hypothalamus, and brainstem.

[b] Including the hypothalamus and olfactory bulb.

[c] Dopaminergic neurons extend from the midbrain (e.g., the substantia nigra pars compacta, the ventral tegmental area, the arcuate nucleus, and the retrorubral area) through the forebrain (e.g., the olfactory bulb).

[d] Histaminergic neurons are confined to the tuberomamillary nucleus of the posterior hypothalamus.

[e] H_2S is produced by cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (3MST); CBS is highly expressed in the astrocytes and microglial cells of the hippocampus and cerebellum; CSE is mainly expressed in the cardiovascular system but is also present in microglial cells, spinal cord, and cerebellar granule neurons; and 3MST is expressed in the neurons and astrocytes of the brain.

[f] Neuronal nitric oxide synthase (nNOS) is expressed in the neurons and pineal gland of the brain and in the spinal cord; in the juvenile and mature hippocampus and neocortex, nNOS is primarily expressed in subpopulations of GABAergic interneurons.

[g] A midline complex closely associated with the reticular formation within the midbrain, pons, and medulla.

Note: ANS, autonomic nervous system; LTP, long-term potentiation; NMDA, N-methyl-D-aspartate

synapse (Brodal 2004). Some amino acids are neurotransmitters, such as L-glutamate, L-aspartate, glycine, D-serine, and γ-aminobutyric acid (GABA). Most of the other neurotransmitters are either amino acid metabolites or peptides formed from amino acids, which include (a) gasotransmitters [NO, CO, and hydrogen sulfide (H_2S]); (b) monoamines [dopamine, norepinephrine (noradrenaline), epinephrine (adrenaline), histamine, agmatine, melatonin, and serotonin]; (c) peptides [substance P, cocaine, opioid peptides, *N*-acetylaspartylglutamate, gastrin, cholecystokinin (CCK), oxytocin, vasopressin, peptide YY, and neuropeptide Y]; and (d) acetylcholine (Kaeser and Regehr 2014; Wu 2024).

The Central Nervous System

The central nervous system consists of the brain (situated in the head) and the spinal cord (located inside the spinal column made up of bones called vertebrae). The brain (the commander of all neural networks) has three parts (forebrain, midbrain, and hindbrain; Figure 1.9) that are encased within the skull and protected by the cranium (Brodal 2004). The brain processes and interprets sensory information transmitted from the spinal cord. The latter is a tube-like structure containing a bundle of nerves and cerebrospinal fluid, which protects and nourishes the cord. The spinal cord connects with peripheral tissues via neurons. Afferent neurons conduct impulses from the peripheral nervous system toward the spinal cord, whereas efferent neurons carry impulses from the spinal cord to the peripheral nervous system (Sporns et al. 2004). Both the brain and spinal cord are protected by three layers of a connective tissue called the meninges. The central nervous system is responsible for processing information received from the whole body. The forebrain plays a central role in cognitive (e.g., attention, memory, reasoning, planning, and problem solving), sensory, and endocrine functions, and in the regulation of sleep wake states, behavioral responses to emotions (e.g., stress and fear), and hormonal secretions. The midbrain controls motor movement and audio/visual processing. The hindbrain manages coordination, balance, and autonomic functions such as breathing, heart rate, and sleep.

The Peripheral Nervous System

The peripheral nervous system, which consists of all nervous structures outside the brain and the spinal cord, is divided into the somatic nervous system and autonomic nervous system. The somatic nervous system (also known as the voluntary nervous system) innervates the skeletal muscle. The autonomic nervous system (also known as the involuntary nervous system) innervates internal organs (e.g., heart, lungs, stomach, intestines, and exocrine glands) and acts largely unconsciously (Brodal 2004). Of note, the

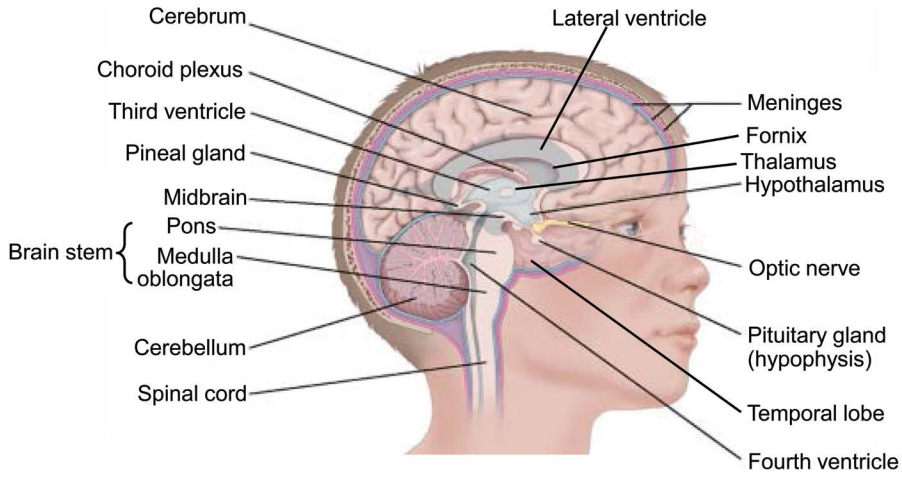


FIGURE 1.9 The central nervous system in mammals. It comprises the brain and the spinal cord. The brain has three parts (forebrain, midbrain, and the hindbrain). The forebrain contains the cerebrum (including the temporal lobe), thalamus, hypothalamus, hippocampus, amygdala, the pineal gland, and the pituitary gland. The midbrain consists of the colliculi, the tegmentum, and the cerebral peduncles and lies on top of the brainstem. The hindbrain comprises the medulla oblongata, pons, and cerebellum, with the first two parts being collectively called the brainstem. The pituitary gland, which contains a front lobe (anterior part) and a back lobe (posterior part), is located below the hypothalamus. The pineal gland is behind the third ventricle. The spinal cord is a tube of tissues that lies caudally to the brain and is protected by the vertebrae. [Reproduced, with modifications and additions to labeling, from a photo that is freely available in the public domain (St. Jude 2024).]

autonomic nervous system requires a sequential two-neuron efferent pathway in that the preganglionic neuron synapses onto a postganglionic neuron before innervating the target organ. Overall, the peripheral nervous system provides a means of communicating signals from the external environment and internal tissues to the central nervous system, and from the central nervous system to the proper effector organs in the body (e.g., skeletal muscle, smooth muscle, the small intestine, and glands).

Based on the type of neurotransmitter, the autonomic nervous system can be divided into: (a) the norepinephrine-dependent sympathetic nervous system ("fight or flight"), (b) the acetylcholine-dependent parasympathetic nervous system ("rest and digest"), and (c) NO-dependent, non-norepinephrine and non-acetylcholine nervous system (McCorry 2007). A tissue (e.g., the small intestine, heart, renal tubules, or the vasculature) can be innervated by both sympathetic and parasympathetic nervous systems, which often have opposite and complementary effects. The sympathetic nervous system increases heart rates and the contractility of cardiac myocytes by releasing norepinephrine, relaxes the bladder wall for urine storage, reduces renal blood flow (and glomerular filtration rate), inhibits salivary gland secretion, decreases the motility of the GIT, and promotes hepatic glycogenolysis and adipose tissue lipolysis (McCorry 2007). In contrast, the parasympathetic nervous system decreases the heart rate, constricts the bladder wall for urine emptying, enhances renal blood flow (and glomerular filtration rate), stimulates salivary gland secretion, increases the motility of the GIT, and inhibits hepatic glycogenolysis and adipose tissue lipolysis (Brodal 2004).

THE CIRCULATORY SYSTEM: BLOOD CIRCULATION IN MAMMALS AND BIRDS

Blood

Blood is a tissue contained within the circulatory system and accounts for 6–8% of BW in mammals and birds, depending on species, age, and physiological state (Fox et al. 2002). The blood consists of RBCs, white blood cells (also known as leukocytes), platelets, and intercellular material (plasma). The two primary cell types in the blood, red and white cells, can be separated based on differences in their cellular density. The percentages of packed cells occupied by RBCs and white blood cells in a spun blood sample are defined as hematocrit and leucocrit, respectively. Unlike other tissues, the intercellular material in the blood is a fluid called plasma. Serum, which is utilized for laboratory analyses, is the defibrinated plasma formed after fibrin and cellular components are removed from plasma. Both the cells and the plasma move within the vascular system. Some leukocytes may migrate through blood vessel walls to sites of injury or inflammation. This leukocyte recruitment is central to the immune response. Monocytes exit blood circulation and develop into macrophages in the interstitial space. When blood vessels in the kidneys are injured, RBCs leak to the lumen of the renal tubule and appear in urine.

Blood, plasma, and serum are often used for the analysis of physiological compounds (e.g., amino acids, urea, lipids, glucose, and hormones). In the laboratory, plasma is obtained when a sample of fresh blood is treated with an anticoagulant (e.g., heparin or EDTA) to prevent clotting, followed by centrifugation to remove cells (i.e., the pellet).

By contrast, serum is collected after a sample of fresh blood (without anticoagulant treatment) is permitted to clot and then centrifuged. One ml of blood contains 0.70–0.75 ml plasma or 0.58–0.62 ml serum, depending on species, age, and physiological state. In the 20-kg pig that has 1.4 L blood, 1 ml blood contains 0.735 ml plasma or 0.616 ml serum.

Hemoglobin, the major protein in RBCs, is a heterotetrameric protein ($\alpha_2\beta_2$) consisting of two α-subunits and two β-subunits, with an α-subunit and a β-subunit containing 141 and 146 amino acid residues, respectively, and each of the four subunits containing a porphyrin ring of the Fe^{2+}-heme center for O_2 binding. This protein is structurally and functionally similar among vertebrates. Hemoglobin can reversibly bind O_2, CO_2, NO, nitrite (NO_2^-), and sulfide (S^{2-}) [including H_2S]. Through binding the thiol group of the cysteine residue in the β-chain of hemoglobin to form *S*-nitrosothiol, NO from endogenous and exogenous sources is transported in the blood for release at sites of reduced tissue oxygenation (Premont et al. 2020). Here, the *S*-nitrosothiol dissociates into NO and thiol again when the hemoglobin releases O_2 from its heme site. Of note, NO also binds to the heme Fe^{2+} in the hemoglobin for oxidation to nitrite. In addition, the heme Fe^{2+} in the hemoglobin can irreversibly bind CO, cyanide (CN^-), and sulfur monoxide (SO) without changing the oxidation state of the iron but reducing O_2-binding and resulting in toxicity. Importantly, the binding affinity of hemoglobin for CO is 250 times greater than that for O_2, and thus CO strongly competes with O_2 at the heme binding site and is a toxic gas at elevated concentrations. Oxyhemoglobin (oxygenated hemoglobin) is formed when O_2 binds to the heme component of the hemoglobin in RBCs, and deoxygenated hemoglobin (deoxyhemoglobin) is the form of hemoglobin without the bound O_2. When hemoglobin Fe^{2+} is oxidized to the ferric state (Fe^{3+}), the resultant protein (methemoglobin) cannot bind O_2. Under normal conditions, RBCs contain a cytochrome b5-based enzymatic system to rapidly reduce Fe^{3+} in the methemoglobin to the Fe^{2+} state.

Normally, the pH of blood is 7.4 ($[H^+] = 40$ nM), ranging from 7.35 to 7.45 (Guyton and Hall 2021). For comparison, the cytosol of mammalian and avian cells has a normal pH of 7.0 ($[H^+] = 100$ nM). Thus, there is a natural H^+ gradient across animal cells. CO_2/HCO_3^- is the most important buffer system in the blood, and the concentration of CO_2 or HCO_3^- can be affected by respiratory or metabolic alterations. Blood pH can be calculated according to the Henderson/Hasselbalch equation. A decrease in blood pH to a value below 7.35 is acidosis and occurs due to either an increase in blood H^+ concentration or a decrease in blood HCO_3^- concentration. Severe acidosis occurs when blood pH drops below 7.2 as in uncontrolled type-1 diabetes mellitus. A blood pH below 6.8 is incompatible with life because of damage to proteins (including hemoglobin and myoglobin) and tissues (including the brain, heart, liver and kidneys) in the body. In contrast, an increase in blood pH to a value above 7.45 is alkalosis and occurs because of either a decrease in blood H^+ concentration or an increase in blood HCO_3^- concentration. When blood pH is 7.5, the Ca^{2+} ion starts to precipitate, affecting blood flow and cell metabolism. Severe alkalosis occurs when blood pH increases to 7.65, leading to severe electrolyte derangements and cardiac dysfunction. Acidosis and alkalosis can be either respiratory or metabolic in origin. In general, alkalosis is less life-threatening than acidosis.

$$pH = -\log[H^+];\ [H^+] = 10^{-7}\ \text{M (i.e., 100 nM)},$$
$$pH = 7;\ [H^+] = 10^{-7.4}\ \text{M (i.e., 40 nM)},\ pH = 7.4.$$

Henderson/Hasselbalch equation:

$$pH = pK_a + \log\left([HCO_3^-]/[CO_2]\right) = 6.10 + \log(25\ \text{mM}/1.25\ \text{mM}) = 7.40$$

The Heart and Blood Vessels

Mammals and birds have a closed dual-circuit circulation system in that deoxygenated blood is pumped out of the right heart into the lungs, undergoes gas exchange in the lungs for oxygenation, returns as oxygenated blood to the left heart, and is then pumped out of the heart to the aorta for flow to organs and tissues in the body. In these animals, the circulatory system consists of the heart that has four chambers (upper left and right atria plus lower left and right ventricles), as well as an extensive system of arterial and venous vessels for the circulation of blood (Guyton and Hall 2021). For comparison, amphibians and most reptiles have a three-chamber heart consisting of two atria and one ventricle (Dyce et al. 1996). Except for the pulmonary artery and the umbilical artery (see below), arteries supply oxygenated blood to a tissue, whereas veins carry low-O_2 blood (referred to as deoxygenated blood herein) away from the tissue. Blood flow in the major veins of the lower extremities depends, in part, on the contractions of leg skeletal muscle. There are valves in most veins to prevent backflow of venous blood and ensure its return to the heart. NO is the major vasodilator of blood vessels for the regulation of blood flow. Thus, restoring NO synthesis by endothelial cells improves the endothelium-dependent relaxation of the aorta in diabetic animals (Kelly et al. 2024).

Direction of Blood Flow in Postnatal Animals

Understanding blood circulation is important for understanding the utilization of food nutrients, designing physiologically sound experiments, and interpreting experimental data. A general scheme of the circulation in postnatal mammals and birds is shown in Figure 1.10. In these species, the venous deoxygenated blood from the body enters the right atrium and then the right ventricle of the heart, and subsequently is pumped through the pulmonary artery into the lungs for gas exchange. Thereafter, the oxygenated blood flows through the pulmonary vein to the left atrium and then to the left ventricle of the heart, and ultimately is pumped through the thoracic aorta and its branches to the rest of the body via small arteries and then arterioles

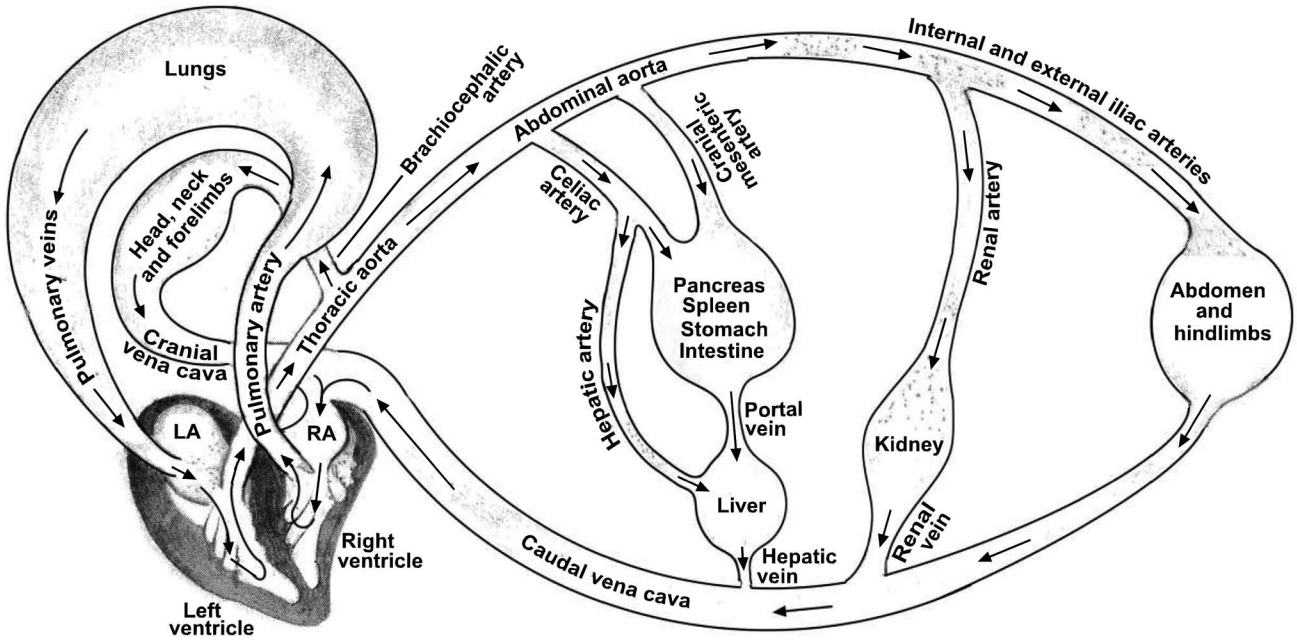


FIGURE 1.10 Blood circulation in postnatal mammals and birds. This is a closed dual-circuit circulation system in which blood is always contained inside blood vessels. The arteries supply O_2-rich blood to tissues, and the veins drain low-O_2 blood from the tissues. Most veins contain valves to prevent the retrograde flow of venous blood and ensure its return to the heart. The hepatic portal vein drains blood from the stomach, small intestine, pancreas, spleen, and large intestine (collectively known as the portal-drained viscera) to the liver. The caudal (inferior) vena cava carries venous blood from the abdomen, internal organs, and hindlimbs to the right atrium of the heart. The cranial (superior) vena cava carries the venous blood from the head, neck, and forelimbs to the right atrium of the heart. The venous blood enters the right atrium and then the right ventricle of the heart, and through the pulmonary artery, flows to the lungs for gas exchange. The O_2-rich arterial blood flows through the pulmonary vein to the left atrium and then the left ventricle of the heart, and ultimately is pumped into the thoracic aorta for flow to the various parts of the body. (Adapted from Dyce et al. 1996.)

(Dyce et al. 1996). In fish, the venous deoxygenated blood from the body enters the atrium via the sinus venosus (a thin-walled chamber), flows into the ventricle, and is then pumped through the bulbus arteriosus into the gills for gas exchange, after which the oxygenated blood flows to the rest of the body (Guyton and Hall 2021). In amphibians and most reptiles, the left atrium and the right atrium receive oxygenated blood and deoxygenated blood from the lungs and the body, respectively; both types of blood are mixed in the ventricle and pumped through pulmonary and skin circulation into the lungs and skin, respectively, for gas exchange (Farmer 1997). In all species studied, the hepatic portal circulation is an important exception to the usual arrangement of the systemic circulation, in that the liver receives much more blood from the portal vein than from the hepatic artery. In mammals, the hepatic artery and portal vein supply 25–30% and 70–75% of blood to the liver, respectively (Dyce et al. 1996). Venous blood drained from the stomach, spleen, small intestine, pancreas, and large intestine enters the liver via the portal vein before entering the general circulation.

Direction of Blood Flow in Fetuses

Although postnatal animals receive enteral nutrients from the small intestine, growing mammalian fetuses are nourished by their mothers through the umbilical vein (Figure 1.11). Similarly, nutrients required for avian embryonic growth are derived from the yolk sac of the fertilized egg via blood vessels. Understanding fetal circulation is essential to understanding fetal nutrition and metabolism. Fetal circulation differs from adult circulation in several respects (Dyce et al. 1996). First, the maternal uterine artery supplies oxygenated blood to the uterus, and this blood enters the placenta through its microvasculature. Subsequently, the umbilical vein carries oxygenated blood from the placenta to the fetal liver and heart. Second, much of the blood from the caudal end of the fetal aorta is transported to the placenta by two umbilical arteries. Metabolic wastes in the fetal umbilical arteries enter the maternal circulation sequentially through the placenta and uterine vein. Furthermore, blood from the fetal umbilical arteries undergoes gas and nutrient transfer through the placental capillaries and then returns to the fetal heart through the umbilical vein. Third, because the fetal lungs are nonfunctional, a relatively small amount of the total blood volume is present in the pulmonary circulation at any given time. There are two bypasses, or shortcuts, between the right side and the left side of the fetal heart, and between the pulmonary artery and the aorta, permitting blood to bypass the lungs in fetal circulation. These bypasses are (a) the foramen ovale that connects the right atrium and the left atrium of the fetal heart and (b) the ductus arteriosus that

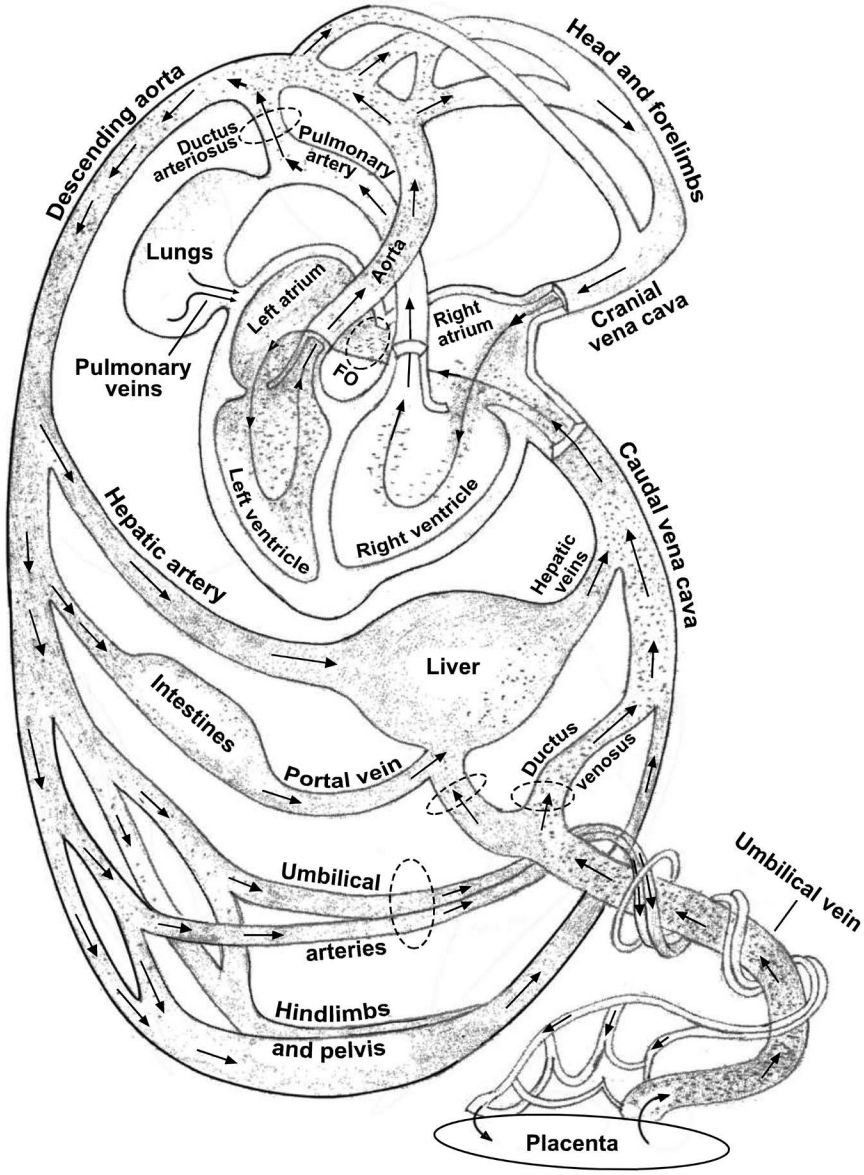


FIGURE 1.11 Blood circulation in fetal-placental tissues. The umbilical vein supplies oxygenated blood from the placenta to the fetus. More than two-thirds of the umbilical venous blood enters the fetal liver through the fetal portal vein, while the remainder bypasses the liver and flows through the ductus venosus to the caudal vena cava for delivery to the fetal right atrium. Because fetal lungs are nonfunctional, two bypasses (i.e., shortcuts) carry blood to the fetus: (a) the foramen ovale (FO) that connects the right atrium and the left atrium of the fetal heart; and (b) the ductus arteriosus that connects the pulmonary artery and the aorta of the fetus. Fetal low-O_2 blood returns to the placenta via two umbilical arteries by way of the internal iliac arteries. (Adapted from Dyce et al. 1996.)

connects the pulmonary artery and the aorta of the fetus (Dyce et al. 1996). At birth, placental blood flow ceases and lung respiration begins. Both the foramen ovale and ductus arteriosis close shortly after birth when the newborn begins to breathe, and the lungs are perfused.

Microcirculation

Within the microcirculation, blood flows from arterioles (with an internal diameter between 10–20 μm) to capillaries and then to venules (very small veins with an internal diameter of 7–50 μm). The capillaries, with an internal diameter of 4–9 μm, are the site for the transport of nutrients from arterial blood to tissues, and the venules are the site for the removal of metabolites from the tissues to the general circulation (Figure 1.12). The blood capillaries are extremely thin, tubular structures with a single layer of semi-permeable endothelial cells (Granger et al. 1988). Each capillary is surrounded by a basement membrane. Between two adjacent endothelial cells, there is a thin intercellular junction, which allows fluid and small solutes to leave the circulation and enter the interstitial space of the perfused tissue. The intercellular junction varies in size, with its diameter ranging

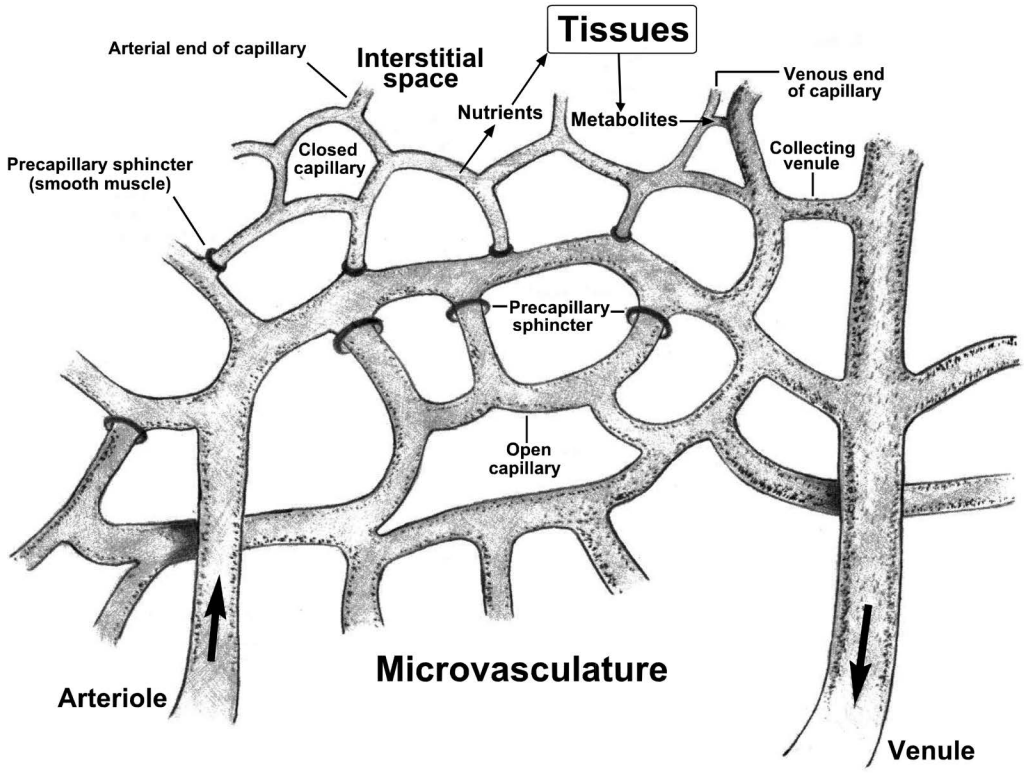


FIGURE 1.12 Blood circulation in microvasculature. Blood flows through the arterioles and then the metarterioles (terminal branches of the arteriole) to the capillaries. Nutrients and gases in the lumen of the capillaries undergo continuous exchange with those in the interstitial space of a tissue. At a point where a capillary originates from a metarteriole, a smooth muscle ring (the precapillary sphincter) encircles the capillary to modulate blood flow. Metabolites in the interstitial fluid may also be taken up into lymphatic vessels. (Adapted from Dyce et al. 1996.)

from <1 nm in the brain and spinal cord to 15 nm in the kidney glomerulus and to ~180 nm in hepatic sinusoids (blood capillaries in the liver; Sarin 2010).

At the capillaries, continual exchanges of fluid and low-molecular-weight substances (<10 kDa) between the arterial plasma and the interstitial fluid occur by either transcellular diffusion and transport or intercellular diffusion (Guyton and Hall 2021). This exchange occurs in a state of near equilibrium, whereby the fluid filtered out of the capillary almost exactly equals the fluid returned to the capillary by reabsorption; the slight disequilibrium that occurs accounts for fluid that is returned to circulation via the lymphatics. The forces that govern filtration and reabsorption in the capillary are Starling forces, which refer to the balance of hydrostatic and oncotic pressures that drive fluid movement between blood vessels and surrounding tissues. Hydrostatic pressure pushes fluid out of the capillaries, while oncotic pressure, created by plasma proteins, pulls fluid back in, thereby regulating fluid balance within tissues (Guyton and Hall 2021). The capillaries are permeable to most molecules (e.g., O_2, CO_2, amino acids, glucose, fatty acids, TAG, minerals, vitamins, ammonia, and urea) in the plasma except for large proteins. Compared with H_2O (100% permeable), the permeability of the following molecules is as follows: NaCl (58.5 Da), 96%; urea (60 Da), 80%; glucose (180 Da), 60%; sucrose (342 Da), 40%; inulin (5 kDa), 20%; myoglobin (17.6 kDa), 3%; hemoglobin (68 kDa), 1%; and albumin (69 kDa), 0.1% (Guyton and Hall 2021). After passing through the capillaries, blood enters the venule and then a large vein. The driving forces for the venous return are (a) the pressure gradient provided by the heart and (b) the contraction of skeletal muscles. Thus, the major function of microcirculation is to transport nutrients and O_2 to tissues and to remove cellular metabolites (including CO_2) from the tissues.

THE CIRCULATORY SYSTEM: BLOOD CIRCULATION IN FISH

Fish have a closed single-circuit circulation system to transport gases (including O_2, CO_2, NO, and NH_3), nutrients, and their metabolites throughout their bodies. These animals have a two-chamber heart that is composed of one atrium and one ventricle (Figure 1.13), as well as four other components: sinus venosus, atrioventricular segment, conus arteriosus, and bulbus arteriosus. The latter two parts are collectively called the outflow tract. The proportion of conus arteriosus to bulbus arteriosus varies among species. The conus arteriosus constitutes the major component of the outflow tract in rays, sturgeons, and bichirs but the bulbus arteriosus is more predominant in advanced teleosts

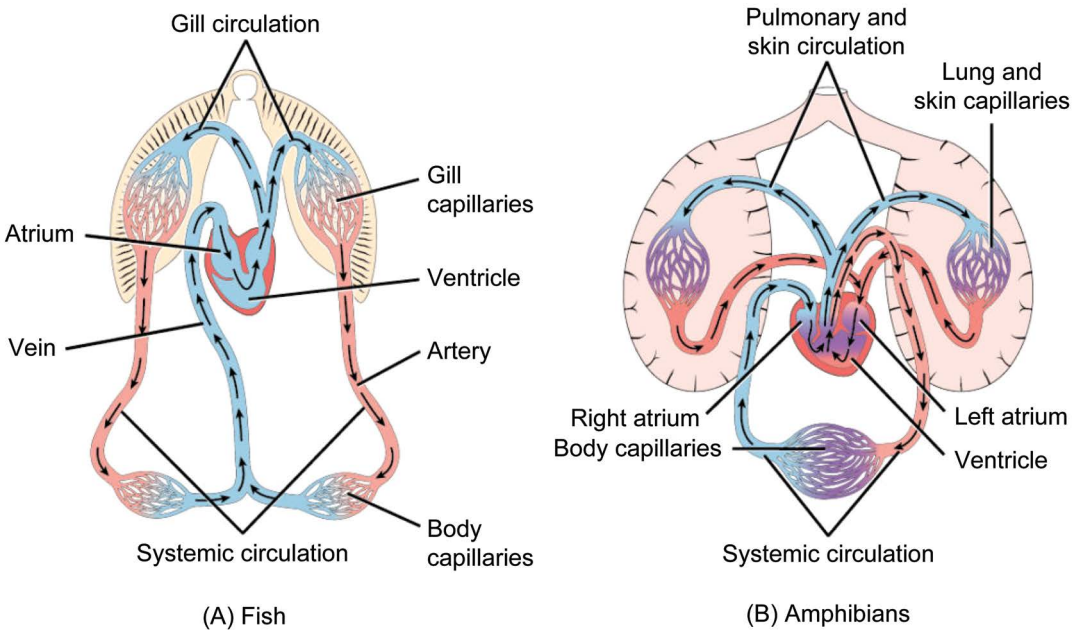


FIGURE 1.13 Blood circulation in water-breathing fish (A) and in amphibians (B). Fish have a two-chambered heart within a closed single-circuit circulation system, where the ducts of Cuvier (also known as common cardinal veins that are not depicted in the figure) are formed from the merging of the anterior and posterior cardinal veins. The gills receive the deoxygenated blood from the ventricle (ultimately the body) and serve as the sites of gas exchange in fish. For comparison, there are a four-chambered heart (two atria and two ventricles) but no ducts of Cuvier in adult mammals and birds. In contrast, amphibians have a three-chambered heart (two atria and one ventricle) within a closed two-circuit circulation system, where (a) deoxygenated blood from the body enters the right atrium and then the lungs and skin for gas exchange; and (b) oxygenated blood from the lungs and skin enters the left atrium and is subsequently pumped by the ventricle into systemic circulation. [Adapted from photos freely available in the public domain (OpenStax 2022).]

such as the European eel and yellowfin tuna. Interestingly, the cyclostomata (hagfish and lampreys) do not have the outflow tract. Expressed as the percent (%) of BW, most fish generally have less blood than mammals and birds. The total blood volume is 3–4%, 5–8%, and 18% of BW in most teleost fish, most elasmobranchs, and hagfish, respectively (Eliason and Stecyk 2021). In fish, the concentrations of proteins, ions (mainly Na^+ and Cl^-), and metabolites in the plasma, as well as its osmolarity are tightly regulated to meet environmental challenges but differ substantially among their diverse species.

As in mammals and birds, the blood of most fish contains RBCs, white blood cells (leukocytes), and plasma. Like terrestrial vertebrates, most fish have hemoglobin within their RBCs in the form of a heterotetramer. Fish and mammalian hemoglobins have high sequence homology but differ in amino acid composition. The heme moiety of hemoglobins is identical in every fish species studied to date, but the globins of hemoglobin differ among species and isoforms. Most fish have several isoforms of hemoglobin. There are four types of hemoglobin in the RBCs of rainbow trout: type IV, which exhibits a reduction in O_2-carrying capacity at low pH or elevated CO_2 concentration (the Root effect), and types I–III, which do not show the Root effect. In some species of fish, such as the cyclostomes (lampreys and hagfish), the hemoglobin, which is more closely related to cytoglobin than to the gnathostome hemoglobin (Hoffmann et al. 2010), reversibly dissociates to monomers in response to O_2 binding. In lampreys, the oxygenated hemoglobin is monomeric, undergoing associations with dimers and tetramers when deoxygenating (Qiu et al. 2000). In the hagfish, the oxygenated hemoglobin exists as three monomers, whereas the oxygenated hemoglobin forms heterodimers and heterotetramers (Muller et al. 2003). Interestingly, Antarctic icefish do not have RBCs or hemoglobin, and their blood volume is 12.7% of BW (Acierno et al. 1995); in these animals, O_2 simply diffuses from the frigid seawater into the plasma of the circulating blood via their enlarged gills and smooth skin.

Water-breathing fish have a closed single-circuit circulation system in that deoxygenated blood is pumped out of the heart into the gills, undergoes gas exchange in the gill lamellae for oxygenation, and then returns as oxygenated blood to the heart. Specifically, the ventricle of the heart pumps the deoxygenated blood via ventricular contraction to the outflow tract, from which the deoxygenated blood flows to the ventral aorta and several pairs of afferent branchial arteries, which enter the gills, where exchanges of gases and ions take place. The oxygenated blood flows to the dorsal aorta and other arteries, and then to arterioles and capillaries. Exchanges of nutrients, gases, and metabolites occur between the capillaries and the interstitial fluid of tissues. Efferent branchial arteries supply blood to the head, the hepatic artery to the liver, the gastric artery to the

stomach, the superior mesenteric artery (arising from the abdominal aorta) to the intestines and the spleen, the renal artery to the kidneys, and the caudal artery (continuing from the dorsal aorta) to the tail. The deoxygenated blood returns to the atrium of the heart via various venules and veins. The large veins include (a) the hepatic portal vein, which collects venous blood from the GIT, spleen, pancreas, swim-bladder (when present), and gonads into the liver; the blood exits the liver through the hepatic vein into the sinus venosus; and (b) ducts of Cuvier (named after the French scientist G. Cuvier; also known as common cardinal veins). The latter are formed from the merging of the anterior and posterior cardinal veins to collect venous blood from the head plus the neck and the kidneys plus the tail, respectively, into the sinus venosus. Note that the anterior cardinal vein is formed by the confluence of the external jugular vein (which drains the gills and the head) and the smaller internal jugular vein [which drains the roof of the oral cavity and pharynx (throat)]. The deoxygenated blood flows through the sinus venosus into the atrium of the heart.

In air-breathing fish (e.g., giant mudskipper, climbing perch, and African sharptooth catfish), the modified circulatory system allows for the flow of blood to and from the air-breathing organ (e.g., the lung in lungfish). These animals have either accessory respiratory organs or modified gill structures as an adaptation to hypoxia or other extreme conditions in the aquatic environment (Zaccone et al. 2018). Air-breathing fish are considered to have a double-circuit circulation system in which deoxygenated blood travels from the right side of the heart to both the gills and the lung for oxygenation, and the oxygenated blood leaves (a) the gills to supply nutrients and O_2 for tissues of the body and (b) the lung to return to the heart.

In the heart of fish, the flow of blood from the sinus venosus to the atrium, from the atrium to the ventricle, and from the ventricle to the outflow tract is controlled by sino-atrial valves, atrioventricular valves, and conal valves, respectively. These valves ensure the unidirectional flow of the blood from the heart to the gills, then to tissues and organs of the body, and back to the heart. Overall, the blood circulation system in fish is more similar to that in mammals and birds than to that in amphibians (Figure 1.13) and crustaceans.

THE CIRCULATORY SYSTEM: HEMOLYMPH CIRCULATION IN CRUSTACEANS

The circulation in crustaceans consists of the heart, the hemolymph vascular system [large arteries, small (emanating) arteries, arterioles, and capillaries], hemocoel, hemolymph, the lacunae system [lacunae (small irregular spaces between tissues) and sinuses (large irregular intertissue spaces)], and ostia. The anatomical structures and locations of their hearts vary among crustacean species, such as a two-chamber heart (consisting of an atrium and a ventricle) in mollusks (where the heart is situated in the anterior part of the visceral cavity) and a single-chamber heart (i.e., a single ventricle suspended within the pericardial sinus) in shrimp (where the heart is situated in the center of the head; Guadagnoli et al. 2007) and crabs (where the heart is situated in the center of the body; McGaw and Reiber 2002). The capillaries have open endings, which allow all the hemolymph (equivalent to blood in vertebrates) to enter the interstitial cavity (called the hemocoel) of tissues. This kind of circulation in crustaceans differs from that in vertebrates where (a) blood in arterioles and capillaries continues to flow into venules and veins and (b) nutrients and gases in the arteriole capillaries are exchanged with those in the interstitial fluid of tissues. In addition, most crustaceans (including crabs, crayfish, lobsters, prawns, and shrimp) have no veins, and their lacunar system (consisting of lacunae and sinuses) takes over the function of veins to return the blood and the interstitial fluid (or lymph) back to the heart. The vascular system is not structurally connected with the lacunar system, and there is no clear distinction between the blood and the lymph (or interstitial fluid) when they are mixed within the hemocoel. Thus, in crustaceans, the circulatory fluid is commonly referred to as hemolymph rather than blood. The hemolymph contains hemocyanin (a copper-containing protein that helps to carry O_2 and gives this fluid a pale blue color), but no hemoglobin.

A diagram of the circulatory system in crustaceans is illustrated in Figure 1.14. When the heart [with five pairs of ostia (open-ended pores protected by valves)] contracts, oxygenated hemolymph is pumped out of the ventricle into large arteries. In *Penaeus vannamei*, the large arteries are the paired anterior lateral arteries, the paired hepatic arteries, the unpaired ventral vessel (descending artery), and the unpaired posterior aorta system (Göpel and Wirkner 2020). In crabs, five arteries (the anterior aorta, the paired anterolateral arteries, and the paired hepatic arteries) are arranged anteriorly, whereas the small posterior aorta is connected to the heart posteriorly, and the largest artery (the sternal artery) exits the heart ventrally to nourish the limbs and mouthparts (McGaw and Reiber 2002). The oxygenated hemolymph continues to flow from the largest arteries into the small arteries and then into arterioles and capillaries. The latter are open to the hemocoel, where the oxygenated hemolymph directly bathes the internal organs and tissues (including the liver, GIT, kidneys, striated muscles, smooth muscles, WAT, and legs). Deoxygenated hemolymph from nonleg tissues enters the hemolymph lacunar system, which consists of the trough-like lacuna, the perineural lacuna, the ventrolateral lacunae, afferent gill lacunae (where gas exchanges take place), and the terminal branches of the afferent gill lacunae. The oxygenated hemolymph enters efferent gill lacunae and then branchio-pericardial sinuses. The latter carry the oxygenated hemolymph to the pericardial sinus, from which the oxygenated hemolymph enters the ostia and then the auricle of the heart when the heart relaxes. Meanwhile, deoxygenated hemolymph from the legs returns, through their lacunae, to the ventrolateral lacunae, and after gas exchanges in the afferent gill lacunae, the oxygenated hemolymph enters the heart, as described

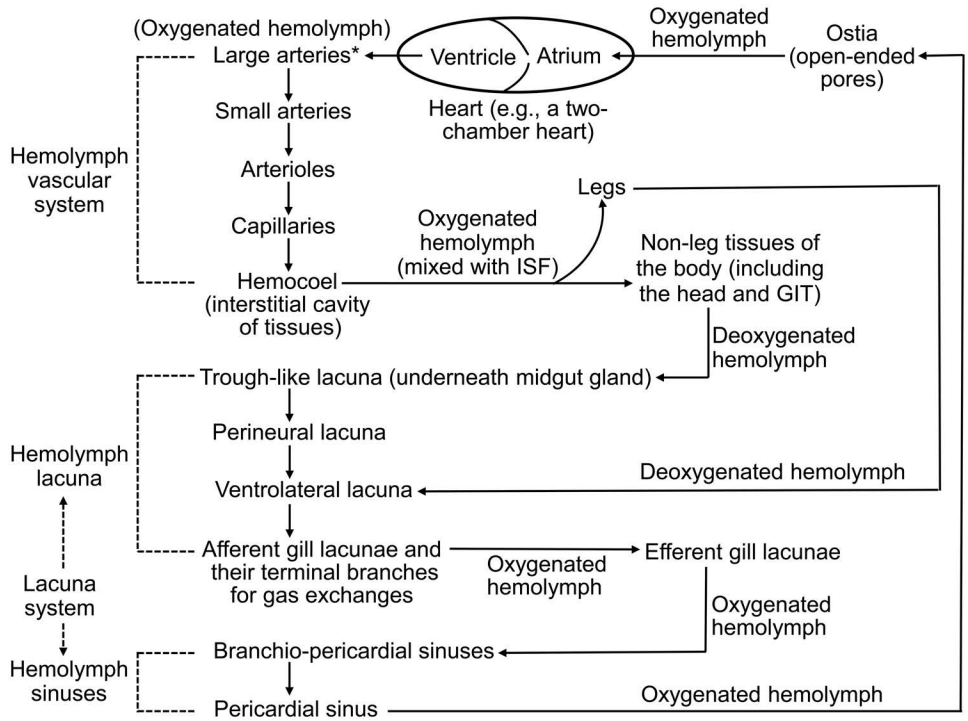


FIGURE 1.14 Hemolymph circulation in crustaceans. When the heart contracts, oxygenated hemolymph is pumped out of the ventricle into large arteries. The hemolymph continues to flow into the small arteries and then arterioles and capillaries. The latter are open to the hemocoel, where the hemolymph directly bathes the internal organs and tissues. Deoxygenated hemolymph from legs enters ventrolateral lacunae and then afferent gill lacunae, where the gas exchanges take place. The oxygenated hemolymph enters the efferent gill lacunae and then the branchio-pericardial sinuses. The latter carry the oxygenated hemolymph to the pericardial sinus, from which the oxygenated hemolymph enters the ostia and then the auricle of the heart when the heart relaxes. Meanwhile, deoxygenated hemolymph from nonleg tissues enters sequentially the trough-like lacuna, the perineural lacuna, and then the ventrolateral lacunae, followed by the same processes as described above.

above. Crustaceans effectively coordinate the opening and closing of multiple ostial and aortic valves.

It follows from the foregoing that circulation in crustaceans depends on hemolymph vascular and lacunar systems, which play important roles in the supplies of nutrients and hormones, the exchanges of gases and metabolites, and the provision of immunocytes. The hemolymph within the vascular and lacunar systems is constantly circulated by the systolic pumping of the heart for outflow and by the diastolic suction of the heart for entry into the heart. In addition, the movement of hemolymph is facilitated by muscular contractility. Like other animals, crustaceans maintain the homeostasis of their hemolymph through complex mechanisms, which involve the transport of H_2O and ions across the gills and in the hemolymph vascular and lacunar systems (Péqueux 1995).

BLOOD-BRAIN BARRIER IN ANIMALS

A unique feature of the brain's vasculature is the blood-brain barrier. It refers to structures in the endothelium of cerebral capillaries and the choroid plexus epithelium that prevent the exchange of certain substances (e.g., saturated LCFAs) between the microvasculature and brain. Clearly, the microvascular endothelial cells of the brain form a tight barrier that regulates the movement of substances between the bloodstream and the brain for protection. There is evidence that a small amount of polyunsaturated fatty acids [e.g., linoleic acid ($C_{18:2}$ ω6) and linolenic acid ($C_{18:3}$ ω3)] can cross the blood-brain barrier from the blood into the brain (Moore et al. 1990). Understanding this blood-brain barrier is essential to understanding why LCFAs serve as an important energy source for peripheral, extraintestinal organs (e.g., the skeletal muscle, heart, and liver), but not for the brain.

THE LYMPHATIC SYSTEM

In mammals, birds, and fish, the lymphatic system provides a route for both fluid and large-molecular-weight substances (e.g., proteins, lipoproteins, and large particulate matter) in the interstitial space to return to the blood circulation. These large-molecular-weight substances cannot be removed by absorption directly into the blood capillaries. The return of proteins from the interstitial space of tissues into the general circulation helps (a) preserve oncotic pressure (also known as colloid osmotic pressure) and (b) maintain blood osmolarity, and therefore is essential to life. About 50% of plasma proteins are filtered by blood capillaries into the interstitial space each day, and these extracellular proteins

must be returned to the blood through lymphatic vessels (Gashev and Zawieja 2010). Although ~90% of the fluid filtered from the blood capillaries into the interstitial space is reabsorbed into the venous ends of the blood capillaries, 10% of the blood-derived interstitial fluid initially enters the lymphatic capillaries as a component of lymph (Guyton and Hall 2021). The lymphatic capillaries merge to form larger lymphatic vessels, which are similar to veins in structure but have an intrinsic pumping ability to propel lymph. Two major lymphatic vessels, the thoracic duct (also known as the left lymphatic duct; the largest lymphatic vessel in the body) and the right lymphatic duct (also known as the right thoracic duct), merge with blood vessels at the level of the left and right subclavian veins, respectively, to return the contents of lymph into the blood (Figure 1.6). Thus, lymph vessels collect tissue fluid or lymph and transport it to large veins. There are no separate lymphatics in crustaceans.

The lymphatic system in mammals, birds, and fish consists of (a) lymphatic vessels, including lymphatic capillaries (10–60 μm in internal diameter) permeable to proteins, other large molecules, and microorganisms; (b) larger lymphatic collecting ducts (~100–200 μm in internal diameter) resembling a vein in structure; and (c) a variety of widely scattered aggregations of lymphoid tissues (e.g., lymph nodes and tonsils; Scallan et al. 2010). A lymph node is an oval or kidney-shaped organ that is distributed widely throughout the body and linked by lymphatic vessels. Lymph nodes are the major sites of B-lymphocytes, T-lymphocytes, and other immune cells, filtering foreign particles, damaged cells, and abnormal cells from lymph fluid and also helping the body fight infection. Like blood capillaries, the lymphatic capillaries are comprised of a single layer of endothelial cells. However, unlike the blood capillaries, the lymphatic capillaries either have no basement membrane or contain only a discontinuous basement membrane and thus are not associated with pericytes. Although there is an intercellular cleft (junction or pore) between two adjacent endothelial cells in the lymphatic capillaries, the diameter of this junction is relatively large (~2 μm), allowing fluid and large solutes in the interstitial space to easily enter the lymphatic vessels (Scallan et al. 2010).

Larger lymphatic collecting ducts are lined with a layer of endothelial cells and have endothelial valves (consisting of two endothelial cell leaflets inside the vessel lumen; Dyce et al. 1996). The lymphatic ducts also have a basement membrane, a thin layer of smooth muscle cells, pericytes, and adventitia (binding the lymph vessels to the surrounding tissue). The endothelial valves prevent retrograde lymph flow. Phasic contractions of lymphatic smooth muscle (modulated by NO) propel a unidirectional flow of lymph along the intervalvular segment of lymphatic vessels (Gashev and Zawieja 2010). In contrast to the lymphatic capillaries, the lymphatic collecting ducts have a very low permeability to plasma proteins (e.g., <3% for albumin; Scallan et al. 2010). The lymphatic vascular tree eventually converges with major veins at the junction of the neck and thorax. Nearly all the lymph from the lower part of the body, as well as lymph from the left side of the head, the left arm, and parts of the chest region, enters the thoracic duct and then empties into the large vein at the juncture of the left internal jugular vein and the left subclavian vein. Lymph from the right side of the neck and head, the right arm, and parts of the chest region enters the right lymph duct and then empties into the large vein at the juncture of the right internal jugular vein and the right subclavian vein (Guyton and Hall 2021). Overall, the flow of lymph is as follows: lymph (containing proteins, nutrients, and metabolites from interstitial fluid) → lymph capillaries → collecting lymphatic vessels → lymph trunks → lymph ducts → subclavian veins (Figure 1.6). Lymphatics also play an essential role in transporting, eventually into the right atrium of the heart, dietary lipids and lipid-soluble substances absorbed from the small intestine.

The lymphatic system in mammals, birds, and fish functions to (a) drain interstitial fluid and large molecules from tissues back into the blood circulation; (b) transport LCFAs, cholesterol, and lipid-soluble vitamins absorbed from the small intestine into the blood circulation; (c) maintain whole-body fluid homeostasis; and (d) conduct the trafficking of immune cells into the lymphatic vessels. The total flow of lymph into the lymphatics in a healthy adult human is 2–4 L/day (Guyton and Hall 2021). Thus, the lymphatic capillaries take up excess extracellular fluid in the body tissues (mainly fluid and protein filtered out of the blood capillaries), and the lymphatic vessels return this fluid to the heart. Impairment of the formation and circulation of lymph results in edema, i.e., the accumulation of interstitial fluid in abnormally large amounts (Gashev and Zawieja 2010). With aging, the contractility of lymphatic vessels and the transport of lymph are decreased in animals.

THE DIGESTIVE SYSTEM

Overview of Nutrient Digestion and Absorption in Animals

The digestive system is responsible for the prehension and digestion of food, as well as the absorption of digestion products into circulation. Anatomic locations of digestive organs within the abdomen of terrestrial animals are shown in Figure 1.15. Dietary protein, complex carbohydrates, TAGs, and other nutrients have no or little nutritive value unless they are digested in the GIT and the products of digestion are absorbed into the systemic circulation. Digestion is defined as the enzymatic hydrolysis of chemical bonds in proteins, lipids, and carbohydrates into smaller molecules. In this process, food is broken down by digestive enzymes in the lumen or the brush border (luminal) membrane of the GIT [i.e., the stomach (simple or multi-chambered) and the gut (small and large intestines)]. The enzymatic hydrolysis of proteins, lipids, and carbohydrates yields small peptides plus free amino acids, monoglycerides plus free fatty acids, and monosaccharides, respectively. Digestibility is

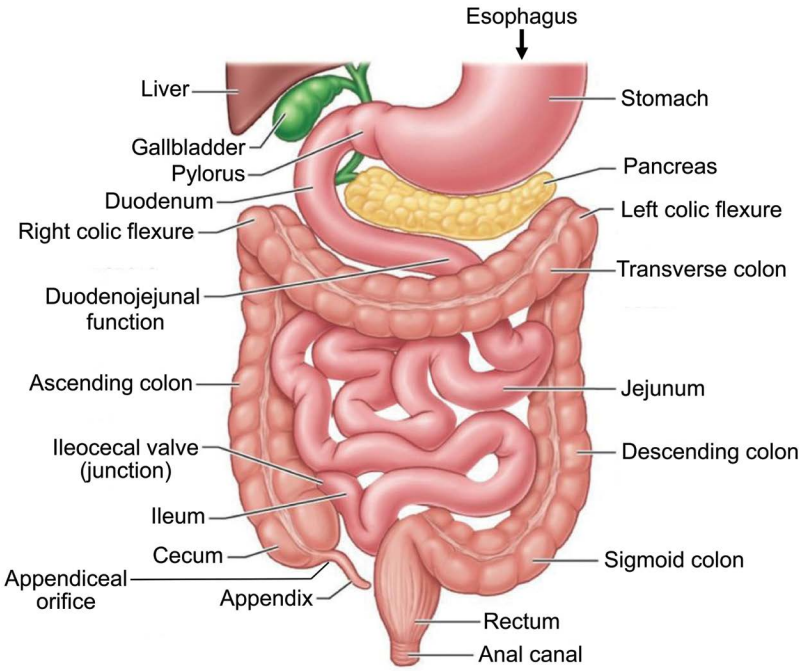


FIGURE 1.15 Anatomic location of organs in the digestive system of animals. Not all animals have a gallbladder or an appendix. [Reproduced from a photo freely available in the public domain (Belleza 2024).]

generally expressed as the percentage of a dietary nutrient that is broken down into small molecules for absorption by the GIT. Digestibility values can be either true or apparent, depending on measurement methods.

Absorption refers to the movement of substances (e.g., nutrients) across the mucosal cells of the GIT into the interstitial fluid of its lamina propria, from which they enter the capillaries (into the blood) and lymphatic vessels called lacteals (into the lymph) in vertebrates (Guyton and Hall 2021) or exit to hemolymph in crustaceans (McGaw and Curtis 2013). During this process, the products of digestion are absorbed across the epithelial cells of the digestive organs into the systemic circulation via the hepatic portal vein and intestinal lymphatic vessels. Digestion and absorption are regulated by intestinal structure, neural control, and gastrointestinal hormones (e.g., gastrin, secretin, CCK, somatostatin, and glucagon-like peptide-1). These factors affect gastric emptying, the distention and motility of the GIT, and intestinal integrity, as well as salivary, gastric, pancreatic, and intestinal secretions such as digestive enzymes and ions (Brodal 2004; Bondi 1987; Goff 2018). Note that not all absorbed substances are fully available for use by animals due to chemical modifications and imbalances or antagonisms among dietary factors. The term "bioavailability" refers to the proportion of a dietary nutrient that is absorbed and then used by animals for physiological function.

Digestive Systems in Vertebrates

As shown for terrestrial mammals and birds (Figure 1.16) and fish (Figure 1.17), vertebrates have evolved to possess diverse digestive systems for the digestion of different types of foods. The number and structures of teeth differ among carnivores, omnivores, and herbivores (Figure 1.18). In addition, some vertebrates (e.g., pigs, poultry, and most fish) have a single stomach with a pH between 1.5 and 2.5 in adults, some (e.g., ruminants) have multi-chambered stomachs, and some species of fish have no stomach. Mammals, birds, and most teleost fish have a spiral small intestine, but the intestine of shrimp is straight without turns. Thus, the digestive tract differs (a) between ruminants (e.g., cattle and sheep) with four stomachs and nonruminants (e.g., swine and poultry) with one stomach, and (b) even among monogastric animals. The digestive system of vertebrates generally includes the mouth [teeth, tongue, and salivary glands (except for teleost fish)], pharynx, esophagus, stomach, small and large intestines, and accessory digestive organs (the pancreas, liver, and gallbladder; Dyce et al. 1996). The mouth is situated in the head to serve as the body orifice through which animals ingest food and plays an important role in the physical breakdown of the food or prey before its entry into the GIT. As a hollow tube, the pharynx starts behind the nose, extends to the neck, and ends at the top of the trachea and esophagus. The esophagus (a muscular tube) runs through the central portion of the neck and behind the trachea to carry food and liquids from the throat into the stomach, which is located between the esophagus and the duodenum. In vertebrates, the stomach, small intestine, large intestine, pancreas, liver, and gallbladder are situated within the visceral cavity. The ileocecal valve separates the small intestine from the

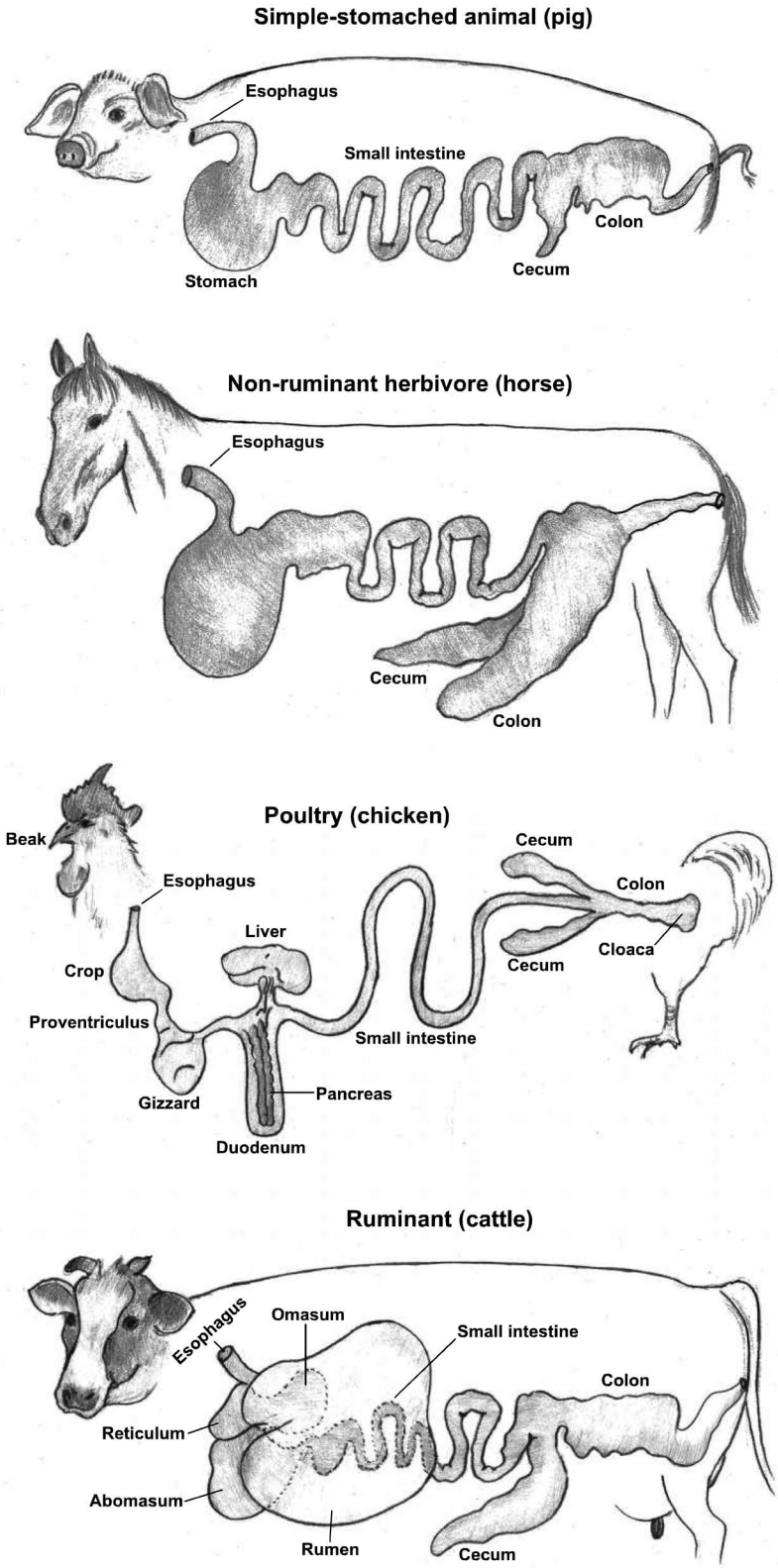


FIGURE 1.16 Comparative aspects of the digestive systems in mammals and birds. Nonruminants (e.g., pigs, horses, and chickens) have a single stomach, the small intestine (duodenum, jejunum, and ileum), and the large intestine (colon and cecum). The digestive system of birds differs markedly from that of nonruminant mammals in that birds have the crop (a temporary storage pouch), the proventriculus (also known as the "true stomach"), and the gizzard (ventriculus; also known as the mechanical stomach). In ruminants, the stomach consists of four compartments: rumen, reticulum, omasum, and abomasum, with the first three parts being called the forestomach. The rumen contains large amounts of bacteria to ferment dietary fibers, other carbohydrates, and proteins. The abomasum in ruminants acts like the stomach in nonruminants. (Adapted from Bondi 1987.)

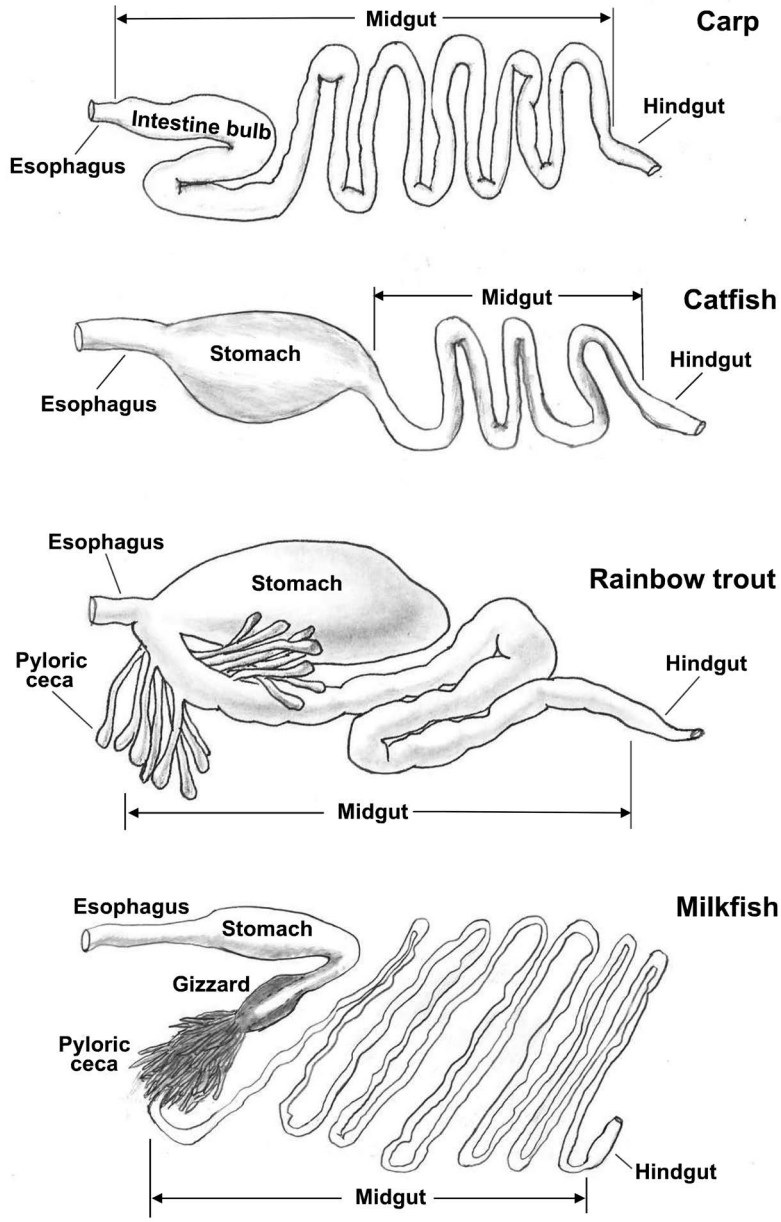


FIGURE 1.17 Comparative aspects of digestive systems in fish. Depending on the species, fish have very different digestive tract morphologies. Some fish have a stomach, but some do not. In contrast to mammals and birds, the gut of fish is divided into three segments (anterior, mid-, and posterior intestines) rather than the small and large intestines.

large intestine. The entire digestive tract, including the mouth, stomach, and intestines, harbors various kinds of microbes with high metabolic activities (Table 1.8). In addition, the liver and pancreas are required for the efficient digestion of nutrients, such as fats, proteins, and carbohydrates (Figure 1.19). Due to the presence of large populations of microbes in the rumen, dietary proteins and carbohydrates are extensively fermented, and dietary unsaturated fatty acids are hydrogenated there before entering the abomasum in ruminants. In all vertebrates, the microbial degradation of proteins, amino acids, lipids, complex carbohydrates, and sugars occurs in the large intestine. Not all degraded protein, fat, and complex carbohydrates are fermented because some of their carbons are diverted into the formation of new microbes. Overall, the digestive apparatus is qualitatively similar among vertebrates that have evolved complex digestive systems to adapt to their foods for survival, growth, development, and reproduction.

Digestive Systems in Invertebrates

Some invertebrates (e.g., flatworms, coral, and jellyfish) have the simplest digestive tract consisting of a hollow, tubular gastrovascular cavity with only one opening (i.e., the "mouth" also serves as an "anus"). Some (e.g., earthworms) have an alimentary canal composed of the mouth

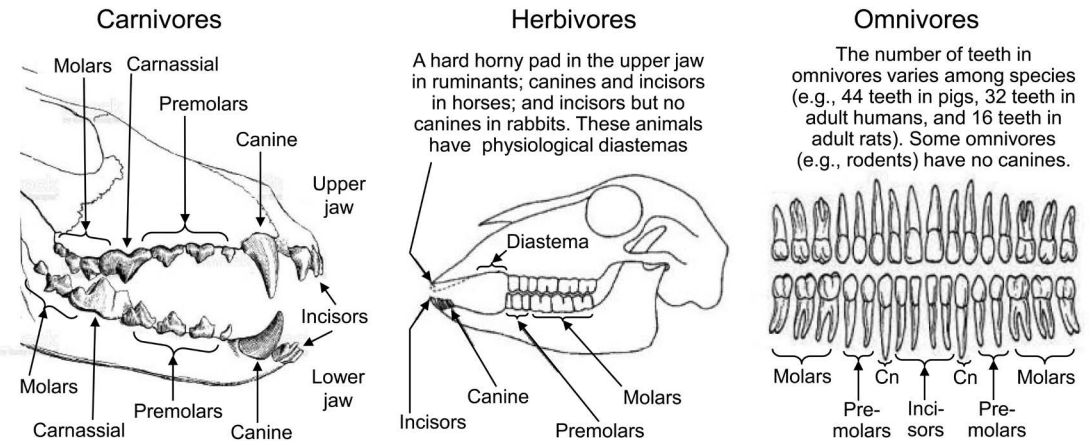


FIGURE 1.18 Structures of teeth in vertebrates. The types of teeth are incisors (also known as front teeth), canines (also called dog teeth; the relatively long, pointed teeth), molars (also known as back teeth), and premolars (transitional teeth located between the canine and molar teeth). For adaptation to their foods, carnivores, herbivores, and omnivores have very different structures of teeth. Specifically, carnivores (e.g., dogs and cats) generally have long, sharp incisors (that help catch and tear their prey) and narrow, sharply serrated molars (much like the blade of a knife). Herbivores (e.g., cattle and sheep) have broad and flat molars with rough surfaces, as well as sharp incisors to grind tough plant tissues. Omnivores (e.g., humans and pigs) possess premolars and broad, flat molars, as well as wide, narrow incisors for grinding and chewing foods of plant and animal origins Cn, canine.

TABLE 1.8
Major Bacterial Species in the Gastrointestinal Tract (GIT) of Animals

Phylum	Species	Primary Origin	Location in the GIT
Firmicutes	*Lactobacilli*	Human, baboon, swine, rat, mouse, chicken, ruminant	Stomach, rumen, SI, LI
	Streptococci	Human, baboon, swine, rat, mouse, ruminant	Mouth, stomach, rumen, SI, LI
	Clostridia	Human, baboon, swine, rat, mouse, chicken, ruminant	Stomach, rumen, SI, LI
	Veillonella	Human, swine, rat, chicken	Mouth, stomach, SI, LI
	Peptostreptococcus	Human, swine, rat, ruminant	Stomach, rumen, LI
	Staphylococci	Human, baboon, swine, rat, mouse	Stomach, SI, LI
	Eubacterium	Human, mouse, ruminant	LI, Rumen
	Bacillus	Human, swine, rat, mouse	LI
	Peptococcus	Human	LI
	Ruminococcus	Human, swine, ruminant	SI, LI, Rumen
	Acidaminococcus	Human	LI
	Butyrivibrio	Human, ruminant	Rumen, LI
	Megasphaera	Human, swine, ruminant	Mouth, rumen, LI
	Faecalibacterium	Swine	LI
	Selenomonas	Ruminant	Rumen
	Lachnospira	Ruminant	Rumen
	Anaerovibrio	Ruminant	Rumen
Bacteroidetes	*Bacteroides*	Human, baboon, swine, rat, mouse, chicken	Mouth, stomach, SI, LI
	Prevotella	Human, swine, ruminant	Mouth, rumen, LI
Proteobacteria	*Actinobacillus*	Human, baboon, swine, rat, mouse	Mouth, stomach, SI, LI
	Escherichia	Human, baboon, swine, rat, mouse, chicken, ruminant	Stomach, SI, LI
	Succinivibrio	Human, ruminant	LI, Rumen
	Salmonella	Chicken	LI
	Ruminobacter	Ruminant	Rumen
	Succinimonas	Ruminant	Rumen
	Wolinella	Ruminant	Rumen
Actinobacteria	*Bifidobacteria*	Human, swine, rat	Stomach, SI, LI
	Propionibacterium	Human, mouse	LI
Fusobacteria	*Fusobacterium*	Human, swine, mouse	Mouth, LI
Fibrobacteres	*Fibrobacter*	Ruminant	Rumen

Source: Booijink (2009), Dai et al. (2015), Robinson et al. (2010), Russell and Rychlik (2001), and Savage (1977).
Note: SI, the small intestine; LI, the large intestine.

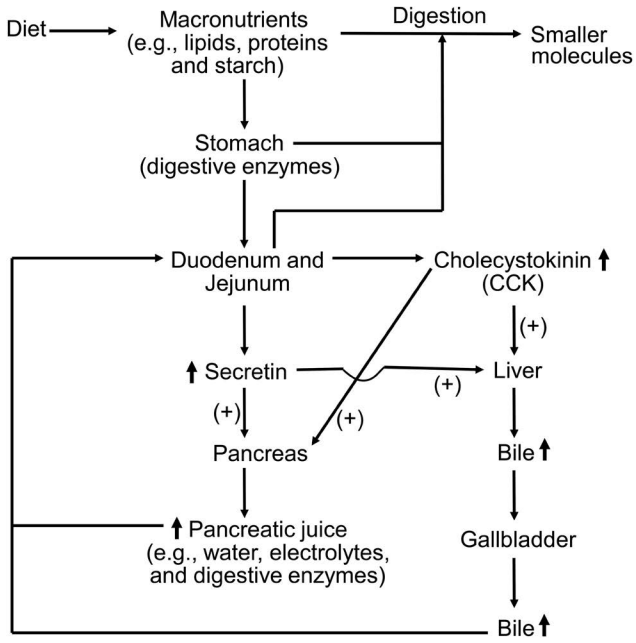


FIGURE 1.19 Coordination among the stomach, small intestine, liver, and pancreas for efficient digestion of nutrients, such as fats, proteins, and carbohydrates. The organs of the digestive tract release enzymes to hydrolyze dietary fats, proteins and carbohydrates, as well as hormones to regulate nutrient digestion, absorption, and metabolism in the body. Ghrelin released from the stomach and the small intestine induces appetite and feeding, whereas motilin from the small intestine stimulates the movement of food through the gastrointestinal tract.

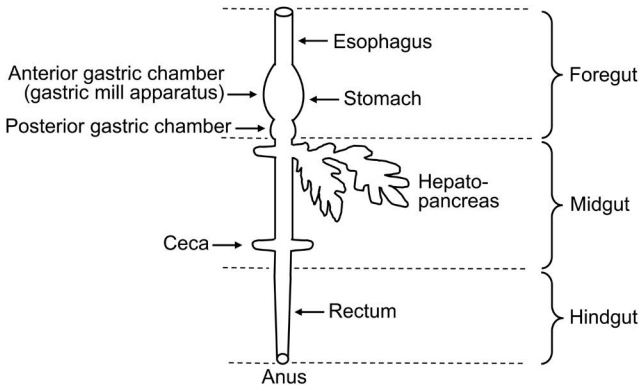


FIGURE 1.20 The digestive tract of crustaceans. It consists of the foregut (a tubular esophagus and a stomach), midgut (a simple tube, the hepatopancreas, and ceca), and hindgut (rectum and anus). The stomach is divided into the anterior cardiac and the posterior pyloric portions. Cutting and grinding of ingested foods, as well as their initial digestion (by digestive enzymes released from the hepatopancreas) occur in the anterior chamber of the stomach (the gastric mill apparatus). The food particles enter the anterior midgut and then the tubules of the joining hepatopancreas. The tubules of the hepatopancreas are the major sites for the extracellular digestion of foods and absorption of digestion products or simple nutrients into the hemolymph. Undigested foods and unabsorbed nutrients enter the rectum and exit the gut through the anus. (Reproduced from Li et al. 2021b, with permission from Springer.)

(the first opening), esophagus, crop, gizzard, intestine, and anus (the second opening); some (e.g., shrimp) have a hollow simple digestive tract containing a large part of stomach in the head and the digestive gland (hepatopancreas) as a component of the midgut. In addition, the gut of crustaceans (e.g., crabs and shrimp) is essentially an internal tube (Figure 1.20). Thus, invertebrates have very different digestive systems than vertebrates.

Retention Time of Food in the Digestive Tract of Animals

The diverse structures of the digestive systems among animal species provide the physiologic basis for the consumption and use of different foods available to them in the ecosystem (Liao et al. 2010; McDonald et al. 2021). This explains why the retention time of food in the digestive tract varies greatly among animals. The mean retention time (h) of food in the digestive tract of animals is as follows: water shrew, 2; mink, 4; fish, 5–7; chicken, 5–9; cat, 13; rabbit, 15; dog, 23; rat, 28; horse, 29; elephant, 33; pig, 43; goat, 43; man, 46; sheep, 47; and cattle, 60 (Blaxter 1989); small copepods (crustaceans), 0.5; larger lobsters, 150 (McGaw and Curtis 2013); and penaeid shrimp, 2–4 (Beseres et al. 2006). Transit times through the digestive tract can be influenced by the type and size of the meal; the physiological state, physical activity, and gastrointestinal health of animals; and the environmental temperature and other factors (e.g., salinity and O_2 tension for aquatic animals).

Digestive Systems in Noncarnivorous Mammals and Birds

The Mouth in Omnivorous Mammals

In omnivorous mammals, the teeth, tongue, and salivary glands are parts of the mouth, as noted previously. The number of teeth varies among animals (Table 1.9). The tongue is a muscular organ with a mucosal membrane and functions to facilitate the movement of food during mastication and to assist swallowing. Much of the tongue's surface is covered by taste buds housed in numerous lingual papillae. Taste receptor cells are located on the taste buds of the tongue and connect with nerves that detect and transmit taste signals to the brain, giving rise to five distinct tastes (sweet, sour, bitter, salty, and umami). Interestingly, there are epigenetic effects on taste responses to dietary nutrients in mammals, including dogs and cats (Kuo 1967).

The major salivary glands to secrete saliva in omnivorous mammals are the parotid, submandibular, and sublingual glands. This physiological fluid contains α-amylase (to break down complex carbohydrates into simpler sugars), lingual lipase (to hydrolyze TAGs), proteases (to hydrolyze protein), and lysozymes (to kill bacteria;

TABLE 1.9
Numbers of Teeth and Major Salivary Glands in Adult Animals[a]

	Teeth					Major Salivary Glands	
Animal Species	Total	Incisors	Canines	Premolars	Molars	Pairs	Names
				Carnivores			
Cat[b]	30	12	4	10	4	5	Parotid, mandibular, sublingual, zygomatic, and molar
Dog[c]	42	12	4	16	10	4	Parotid, mandibular, sublingual, and zygomatic
Mink	34	12	4	12	6 (2/4)[d]	---	---
Tiger	30	12	4	10	4	---	---
Wolf	42	12	4	16	10	---	---
				Herbivores			
Cattle	32	8 (0/8)[e]	0	12	12	3	Parotid, submandibular, and sublingual
Deer	32	6 (0/6)[e]	2 (0/2)[f]	12	12	3	Parotid, submandibular, and sublingual
Goat	32	8 (0/8)[e]	0	12	12	3	Parotid, submandibular, and sublingual
Sheep	32	8 (0/8)[e]	0	12	12	3	Parotid, submandibular, and sublingual
				Omnivores			
Elephant	26	2	0	12	12	---	---
Panda	42	12	4	16	10	---	---
Horse[g]	36	12	0	12	12	3	Parotid, sublingual, and mandibular
Human	32	8	4	8	12[h]	3	Parotid, submandibular, and sublingual
Mouse	18	4	0	2	12	3	Parotid, submandibular, and sublingual
Pig	44	12	4	16	12	3	Parotid, submandibular, and sublingual
Rabbit	28	6	0	10	12	4	Parotid, submandibular, sublingual, and zygomatic
Rat	18	4	0	2	12	3	Parotid, submandibular, and sublingual

[a] Unless indicated otherwise, the upper and lower jaws have the same number of teeth. The value for the type of teeth refers to the total number of each type of tooth in adult animals. An animal has hundreds of minor salivary glands within the mouth.
[b] There are 15 teeth on its upper jaw and 15 teeth on its lower jaw.
[c] There are 20 teeth on its upper jaw and 22 teeth on its lower jaw.
[d] There are 2 molars on its upper jaw and 4 molars on its lower jaw.
[e] There are no incisors on the upper jaw. These incisors are on the lower jaw.
[f] There are no canines on its upper jaw. These canines are on the lower jaw.
[g] Some breeds of horses may have an additional 4 wolf teeth and 4 canine teeth. A foal has only 24 teeth (12 incisors and 12 premolars).
[h] Including 4 wisdom teeth.

Nelson et al. 1977). The parotid salivary glands are located in front of the ears, the submandibular salivary glands below the jaw, and the sublingual salivary glands under the tongue. Many other minor salivary glands are present inside the cheeks, as well as throughout the mouth and throat, to secrete saliva. In omnivorous mammals, salivary glands produce a large amount of saliva (e.g., 1 L/kg DM intake/day in the pig). Within the mouth, saliva mixes with food, lubricates the passage of the food to the stomach during swallowing, moistens and protects the oral mucous membrane, maintains tooth integrity, aids in nutrient digestion, and facilitates heat dissipation through the tongue (evaporative cooling called "panting"). Saliva also has roles in buffering pH, clearing debris, and antimicrobial activity (via lysozymes). Thus, oral health is crucial for food intake, particularly during senior adulthood.

The Mouth in Omnivorous Birds

Birds have no teeth, lips, or cheeks but possess a beak, a tongue, a hard palate, and salivary glands within their mouth. The beak is connected to the skull and composed of bone covered by a thin layer of skin and an outer layer of keratin protein. As a tissue, the beak tip contains blood vessels and nerve endings. The hard palate in the roof of the mouth has five transverse rows of backward-pointing, hard, conical papillae and presents a long, narrow median slit to communicate with the nasal cavity. The tongue (a muscular organ) is covered with a thick epithelium and has a few flask-shaped taste buds. The sense of taste appears to be poorly developed in birds. The avian salivary glands, which are small and diffuse, include maxillary (in the roof of the mouth), palatine (on either side of the nasal opening in the roof of the mouth), spheno-pterygoid glands (in the

roof of the pharynx on each side of the common opening for the auditory tubes), anterior submandibular glands (in the area formed by the union of the two halves of the mandible), posterior submandibular glands (arranged in three groups), lingual glands (in the tongue), crico-arytenoid glands (around the opening of the larynx), and some small glands (Hill 1971). Numerous ducts of the salivary glands pierce the hard palate to release their secretions into the mouth cavity. In birds, the food bolus rapidly transits through the mouth and pharynx into the esophagus.

The Mouth in Ruminant Herbivores

There are teeth, a tongue, and salivary glands within the mouth of ruminants. Interestingly, cattle, deer, goats, and sheep have the same number (n = 32) of teeth. Unlike most mammals, ruminants have a tough dental pad in place of incisor teeth on their upper jaw. The cattle use their long and dexterous tongues to wrap around plant parts and pull the food into the mouth, whereas sheep and goats use their lips and teeth as the primary forage-gathering tools. In addition, the dental pad and teeth allow for chewing and tearing apart feeds. The mechanical cutting and crushing of the ingested feed aid swallowing and digestion. A ruminant has three pairs of major salivary glands (parotid, submandibular, and sublingual) and hundreds of minor salivary glands. Together, these glands produce a large amount of saliva (e.g., 6–16 and 50–80 L/day in the sheep and cow, respectively). In addition to the same functions as described previously for nonruminants, saliva has additional roles in ruminant nutrition: recirculating N, minerals, and vitamins, buffering the ruminal contents, and providing nutrients for ruminal microbes.

The Stomach in Omnivorous Nonruminant Mammals

The stomach of nonruminant mammals (e.g., pigs and humans) consists of four functionally and structurally different regions: cardia, fundus, body, and pyloric antrum. Each of these regions contains glands (called gastric glands). Not all gastric glands have the same composition, but they do commonly share five major cell types in different proportions: parietal, chief, neuroendocrine, mucous, and stem cells. Parietal cells (also known as oxyntic cells) are specialized epithelial cells that form and secrete HCl, and in certain species (e.g., birds, cats, and crustaceans), also the intrinsic factor (for the absorption and digestion of dietary vitamin B_{12}). Chief cells (also known as zymogenic cells), which localize at the base of gastric glands, synthesize and secrete pepsinogen and pro-rennin as inactive zymogens. Neuroendocrine cells synthesize and secrete many peptides (e.g., gastrin, ghrelin, and gastrin-releasing peptide) and amines (e.g., histamine and serotonin), thereby regulating gastric acid secretion, motility, and digestion, as well as appetite and food intake. Mucous cells synthesize and secrete a layer of alkaline mucus that coats the stomach surface, which protects the stomach from attack by gastric digestive enzymes and gastric acid while serving as a mucosal barrier against pathogens entering the blood. Finally, stem cells continuously generate new cells within the stomach to replace old, damaged, and sloughed ones.

Relaxation of the stomach stimulates the appetite. The weight of the stomach represents 0.5–0.8%, 1.0–1.3%, and ~0.6% of the BW in suckling, growing, and adult pigs, respectively (Lærke and Hedemann 2012). In the adult pig, the capacity of the stomach (5 L) represents ~10% of the capacity of the total digestive tract (Lærke and Hedemann 2012). Ingested food enters the stomach through the junction between the esophagus and the stomach and leaves the stomach through the pyloric sphincter (the junction between the stomach and the duodenum). Gastric contractions propel the ingested food and chyme (partially digested food combined with gastric juices) into the duodenum. The stomach secretes digestive enzymes and gastric acid to facilitate food digestion. The pH of the luminal fluid of the stomach is ~2.0 in adults, 2–3 in young mammals (e.g., 2.5 in 7-day-old pigs), and 7.0 in the fetus (Pond et al. 2005; Yen 2000). The gastric acidity helps kill ingested pathogens and reduce susceptibility to outbreaks of gastrointestinal disease. Of note, the rabbit has an enlarged stomach to store large meals because they are crepuscular (i.e., eating primarily at dawn and dusk; McDonald et al. 2021). Thus, the stomach functions primarily in both nutrient digestion and health protection.

The Stomach in Birds (Nonruminants)

The stomach of birds differs markedly from that of nonruminant mammals (Hill 1971). In birds (e.g., chickens, ducks, geese, and turkeys), ingested food passes through the esophagus into the crop. The latter is a temporary storage pouch before digestion starts in the proventriculus (also known as the "true stomach"). The main functions of the proventriculus are twofold: (a) producing gastric juice from Cl^- and H^+, and (b) propelling the gastric juice and food bolus into the gizzard. Specifically, within the proventriculus, food is mixed with hydrochloric acid (HCl) and digestive enzymes, as in mammals, to initiate the hydrolysis of proteins and fats. Subsequently, the digesta enters the gizzard (ventriculus; also known as the mechanical stomach) for grinding, mixing, and mashing, which helps to facilitate further digestion in the small intestine. The gizzard may be an important site for controlling the incidence of *Salmonella* and alleviate gastric dysfunction.

The Stomach in Nonruminant Herbivores

The stomach of most nonruminant herbivores (e.g., horses and rabbits) is anatomically similar to that of omnivorous nonruminant mammals. However, there are some exceptions. For example, the terrestrial kangaroo (a foregut fermenter; a strict nonruminant herbivore) has a complex stomach consisting of two chambers: the sacciform chamber (shaped like a large sack; the forestomach) and the tubiform chamber (shaped like a tube). Food may stay in the sacciform chamber for up to 16 h. Like the rumen of ruminants, the sacciform chamber contains bacteria, fungi, and protozoa for the fermentation of the ingested food. Interestingly,

during initial food ingestion, kangaroos may spit up bits of undigested food in the forestomach to be chewed and then swallowed again, much like a cow. This process is known as merycism, which differs from the rumination of ruminants in that, unlike rumination, merycism occurs infrequently and is not essential for food digestion. As the food ferments, its microbial products enter the second stomach chamber (functioning like the stomach of omnivorous mammals) of the kangaroo, where acids and enzymes secreted by gastric epithelial cells aid in the further digestion of food and microbial proteins (e.g., the lysis of bacterial and protozoal cell membranes, as well as the degradation of proteins).

The Stomach in Ruminant Herbivores

Compartments of the Stomach

Ruminants include cattle, sheep, goats, oxen, musk ox, llamas, alpacas, guanacos, deer, bison, antelopes, camels, and giraffes. In these animals, the stomach consists of 4 compartments: rumen, reticulum, omasum, and abomasum (true stomach; Figure 1.21), with each having different functions (Cheeke and Dierenfeld 2010). The first two compartments (collectively known as the reticulo-rumen) are incompletely separated but distinct structurally and functionally (Vieira et al. 2008). The rumen, reticulum, and omasum are also called the forestomach. Ruminants are capable of regurgitation (the act of bringing swallowed food up again to the mouth), remastication (the act of masticating or chewing food repeatedly), and re-swallowing. A unique characteristic of ruminants is rumination, which refers to the process whereby digesta is regurgitated from the forestomach into the esophagus, remasticated by the animal, and reswallowed into the forestomach for further digestion.

The rumen is the first compartment of the forestomach and is divided into cranial, dorsal, ventral, caudodorsal blind, and caudoventral blind sacs by muscular pillars. The wall of the rumen has small and extensive finger-like projections called papillae, which increase the rumen surface area and function to absorb some nutrients and fermentation products from the rumen into the blood (Cheeke and Dierenfeld 2010). Under normal feeding conditions, the rumen exhibits several characteristics: a mean temperature of 39°C (38–40°C); pH 5.5–6.8 (depending on the type of foodstuffs); a mean redox potential of –350 mV (a strong reducing environment with no or little O_2); and the presence of CO_2 and methane (CH_4) as the major gases. Although the rumen is a highly anaerobic environment, O_2 enters the rumen through ingested food and H_2O, saliva, and the blood. Interestingly, studies of cattle, sheep, and goats have shown that the concentration of dissolved O_2 in ruminal fluid before feeding is negligible (0.3–1.63 μM), falls rapidly to be nondetectable within 30 min post-feeding due to its use as an electron sink by facultative anaerobes and protozoa, remains absent until 3 h after feeding, and thereafter returns to the pre-feeding level by ~9 h post-feeding (Scott et al. 1983). The extreme anaerobic environment in the rumen is responsible for its unique features of digestion. Ruminal pH greatly affects microbial fermentation and growth (Hungate 1966). When large amounts of monosaccharides or starches (highly fermentable carbohydrates) are added to forage diets, ruminal fiber digestion is impaired (Wickersham et al. 2009). In the rumen, reductions in the digestibility of organic matter due to a drop in its pH are not equal for all nutrients, in that the degradation of fiber

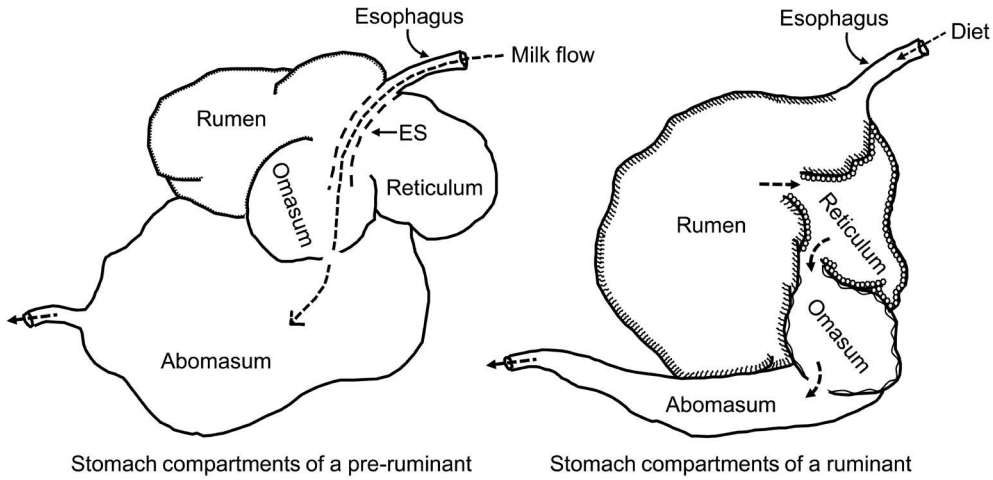


FIGURE 1.21 The stomachs of preruminants and ruminants. These animals have a rumen, a reticulum, an omasum, and an abomasum as a four-compartment stomach. In preruminants, ingested liquid milk enters the abomasum through the esophageal groove (ES). In both preruminants and ruminants, solid food enters, sequentially, the esophagus, rumen, reticulum, omasum, and abomasum for digestion. The rumen has cranial and dorsal sacs for solids, and the ventral sac for liquid. In preruminants, the walls of the rumen, reticulum, and omasum are underdeveloped. By contrast, in well-developed ruminants, the rumen wall has small and extensive finger-like projections called papillae to increase the rumen surface area, the reticulum has folds in the mucosa that appear like a honeycomb, and the omasum has many longitudinal folds called laminae. Digesta flows out of the abomasum (which is similar to the stomach in nonruminants) into the duodenum.

and protein is severely depressed but the catabolism of monosaccharides and starches remains very active.

The reticulum is the second and most cranial compartment of the forestomach. Of note, in camelids (camels, llamas, alpacas, vicunas), the reticulum has gland-like cells and the omasum has a tubular structure that is almost indistinct (Tharwat et al. 2012); therefore, these animals are occasionally incorrectly said to have a three-compartment stomach. In all ruminants, the abomasum is the true stomach, similar to that in nonruminants.

Rumen Development

The development of the rumen as a fermentation chamber for forages and grains is fundamentally important for ruminant nutrition (Hungate 1966). At birth, the rumen is undeveloped and has a limited number of microbes. The rumen and abomasum represent 25% and 60%, respectively, of the total stomach mass in the newborn and increase with age. Rumen development depends on the consumption of fiber-rich diets and the establishment of microbes, including anaerobic bacteria, fungi (primarily multicellular eukaryotes), and protozoa (unicellular eukaryotes). The microbes that colonize the rumen are obtained from the housing environment, dams, other animals with which the neonates come into contact, and food (e.g., milk or replacer milk). The development of the rumen wall and the papillae is also primarily stimulated by butyrate and, to a lesser extent, propionate.

Preruminants are animals with a rumen that is anatomically or functionally immature (Figure 1.21). These animals are similar to single-stomached animals in digestive physiology and are almost solely dependent on their own digestive enzymes. Preruminants have an esophageal groove, which is a muscular fold extending downwards from the opening of the esophagus to the omasum on the wall of the reticulum. Because of neural stimulation, the act of natural suckling or voluntary consumption of a liquid diet induces reflex closure of the esophageal groove into a tubular structure, directing milk into the abomasum for efficient utilization of nutrients and animal growth. Thus, prior to the closure of the esophageal groove and weaning in ruminant animals, the abomasum is the primary recipient of nutrients. This developmental phase is called the preruminant period. Incomplete closure of the groove, primarily due to the lack of suckling reflex (as in force-feeding) results in the initial entry of milk or any liquid food into the rumen or reticulum, which causes indigestion in neonates. Dry food, such as grain mixtures or forage, does not pass through the esophageal groove and therefore flows from the esophagus into the reticulo-rumen to further stimulate its development. The rumen and abomasum represent 65% and 20%, respectively, of the total stomach mass at 3 months of age and 80% and 8%, respectively, of the total stomach mass in adults (Steele et al. 2016). By the time of weaning (e.g., 3–8 months of age, depending on species and farms), the rumen is mature enough to digest fibrous foods. The rumen (including the papillae) continues to develop after weaning. The fluid of a well-developed rumen contains large amounts of anaerobic bacteria (10^{10} to 10^{11}/ml), protozoa (10^5 to 10^6/ml), and fungi (10^3 to 10^4/ml); dietary organic nutrients are extensively fermented, and each papilla can effectively absorb ruminal fermentation end-products (e.g., SCFAs). After absorption into the ruminal epithelial cells, these metabolites enter the bloodstream for utilization.

Digestive Function of the Rumen and the Abomasum in Ruminants

Food passes first from the esophagus into the rumen (cranial and dorsal sacs for solids and the ventral sac for liquids) and then into the reticulum (McDonald et al. 2021). Within the reticulo-rumen, the following reactions take place. First, proteins are hydrolyzed by proteases and peptidases to form small peptides, amino acids, ammonia, other nitrogenous metabolites, carbon skeletons, and SCFAs. Second, small peptides, amino acids, and ammonia are used primarily by bacteria to synthesize new amino acids and proteins, whereas the bacteria are engulfed and digested by protozoa (Firkins et al. 2007). Third, dietary fibers and soluble carbohydrates are fermented to pyruvate, lactate, and large amounts of SCFAs. Fourth, all vitamins are synthesized from the appropriate precursors by microbes. Fifth, unsaturated LCFAs undergo biohydrogenation to form saturated LCFAs and conjugated fatty acids, whereas the oxidation of fatty acids to CO_2 and H_2O is limited (Bauman et al. 2011). The food and its fermented products are mixed freely within the reticulo-rumen before entering the omasum. Finally, the digesta moves into the abomasum and the small intestine for further digestion, which proceeds mostly as in nonruminants. There are essentially continuous abomasal and small-intestinal flows of digesta in cattle, sheep, goats, and related animals. Thus, ruminants can convert low-quality roughages into high-quality protein (e.g., milk and meat) for human consumption.

The rumen contains high urease activity to hydrolyze urea into ammonia and CO_2. Some of the ammonia is utilized locally for the synthesis of amino acids and proteins by microbes; the remaining ammonia enters the blood through the ruminal epithelium and the portal vein and then reaches the liver for urea synthesis (Wu 2022a). Through circulation, the urea becomes part of the saliva and re-enters the rumen for utilization by microbes (Wickersham et al. 2009). This pathway of urea metabolism within the digestive system is called urea recycling. Its role is to maximize the efficiency of utilization of dietary nonprotein N (NPN) and protein N for amino acid synthesis in ruminants.

Fermentation of ingested nutrients (e.g., carbohydrates, lipids, and protein) by bacteria in the rumen generates large amounts of electrons. The latter are used to reduce (a) fermentation intermediates to mainly SCFAs and, to a much lesser extent, succinate, lactate, and ethanol; and (b) H^+ and CO_2 (products and potent inhibitors of fermentation) to H_2 and formate (HCOOH), respectively (Leahy et al. 2022). Through a series of enzyme-catalyzed reactions, H_2 and CO_2 are converted into methane in the rumen.

There are positive relationships among food intake, the turnover rates of ruminal fluid, particles and bacteria, and microbial fermentation efficiency (Hungate 1966). First, the number of ruminal microbes depends on their ability to reproduce at a rate equal to or greater than their losses from the rumen. Second, the survival of ruminal microbes is enhanced by their attachment to food particles. Third, the efficiency of ruminal microbes to digest food is increased when the transit of food particles through the rumen is prolonged. Ruminal digestion of organic matter is reduced when the total number of ruminal microbes decreases. The efficiency of microbial fermentation is very important for the ruminant because the host requires both SCFAs and microbial protein for survival, growth, and reproduction (Stevens and Hume 1998). SCFAs provide 72% (steers) to 78% (sheep) of the total metabolizable energy required by ruminants (Bergman 1990).

The Small Intestine

The intestine of mammals and birds is divided into the small intestine (the portion of the digestive tract between the pylorus and the ileocecal valve) and the large intestine (the portion of the digestive tract between the ileocecal valve and the anus), but the intestine is not separated into small and large intestines in fish. The small intestine of mammals and birds is divided into three parts: the duodenum (the portion of the digestive tract between the pylorus and the jejunum; e.g., 10 cm in length in young pigs), jejunum (beginning at the duodenojejunal junction), and ileum. The jejunum was named for its empty appearance, whereas the ileum may appear darker than the jejunum. The jejunum and ileum constitute 40% and 60%, respectively, of the small intestine length below the duodenum (Madara 1991). Inside the lumen of the small intestine, there is an aqueous diffusion layer (known as the unstirred H_2O layer) that is adjacent to the intestinal mucosal membrane. This unstirred H_2O layer can affect the efficiency of nutrient absorption and drug delivery. Digesta stays in the small intestine of growing steers and pigs for 2.5–3 h. Mechanisms for the digestion and absorption of nutrients in the small intestine are similar between ruminants and nonruminants (Beitz 1993; Bergen and Wu 2009; Goff 2018; Liao et al. 2010).

The small intestine is a highly differentiated and complex organ (Figure 1.22). The wall of the small intestine consists of 4 main layers: the mucosa, the submucosa, the muscularis externa, and the serosa. The epithelium of the small intestine rests on the underlying lamina propria, which is a connective tissue layer containing blood and lymphatic capillaries, as well as mononuclear cells (e.g., lymphocytes, mast cells, plasma cells, and macrophages), polymorphonuclear leukocytes, and nerve fibers (Madara 1991). The blood and lymphatic capillaries carry the absorbed water- and lipid-soluble nutrients to the portal vein and lymphatic vessels, respectively. The lamina propria is covered with an amorphous sheet called the basal lamina on which the epithelium directly rests. The lamina propria is supported by the underlying muscularis mucosa (two thin layers of smooth muscle together with varying amounts of elastic tissue). The epithelium, the lamina propria, and the muscularis mucosa are collectively called the mucosa (Barrett 2014). The mucosa is supported by the submucosa, which is a connective tissue layer containing the plexuses of larger blood vessels. The submucosa is underlain by the muscularis externa (two fairly substantial layers of smooth muscle; inner circular and outer longitudinal for contractions), which propels intestinal luminal contents downward from the duodenum into the cecum. The fourth and outermost layer of the wall of the intestine is the serosa, which is a layer of mesothelial cells.

The small intestinal epithelium consists of two compartments: the villus and the crypt of Lieberkühn. The crypt-villus junction is defined as the point at which the distance between opposing epithelial layers suddenly begins to widen (Madara 1991). The crypt is divided into the Paneth cell zone, stem cell zone, proliferation compartment, and maturation zone. The undifferentiated stem cell of the crypt is the progenitor cell from which intestinal epithelial cells originate. During migration from the crypt to the villus tip via the intestinal crypt-villus axis, stem cells differentiate into one of at least four mature cell types: the villus columnar absorptive cell (enterocyte); the mucin-producing and mucin-secreting goblet cell; the enteroendocrine cell secreting a variety of substances; and the Paneth cell that likely plays a role in the secretion of molecules for protection against microflora (Yen 2000). In the intestinal epithelium, there exist extracellular proteins (occludins, claudins, zonula occludens, and E-cadherens) between adjacent enterocytes to form a tight junction (Goff 2018; Liang et al. 2019). As the epithelial cells proliferate and move up the villus, the activity of digestive enzymes on their brush-border membrane and their capacity to absorb nutrients increase. The small-intestinal epithelium also contains intraepithelial lymphocytes and Peyer's patches as part of the immune system in the body (Li et al. 1990).

Enterocytes constitute >85% of the mucosal epithelial cell population in the small intestine (Klein and McKenzie 1983). A thin layer of mucus (consisting mainly of threonine-rich glycoproteins, known as the glycocalyx) overlies the apical membranes of the enterocytes. The half-life of mucosal epithelial cells is 3–5 days. For example, in 14- to 18-day-old neonatal pigs, the life span of enterocytes in the proximal and distal regions of the small intestine is 4.7 and 10.2 days, respectively (Fan et al. 2001). Each enterocyte has an apical membrane (brush border) composed of microvilli and a basolateral membrane with fairly smooth contours. These two membranes of the enterocyte are chemically, biochemically and physically distinct. The polar organization of enterocytes allows them to receive nutrients from both the arterial blood across its basolateral membrane and the intestinal lumen across its brush border membrane. This has important practical implications for choosing the route of feeding (e.g., enteral vs. parenteral) for nutrient delivery to animals (Burrin et al. 2000). The brush border membrane of the enterocyte is the specific site for: (a) intrinsic

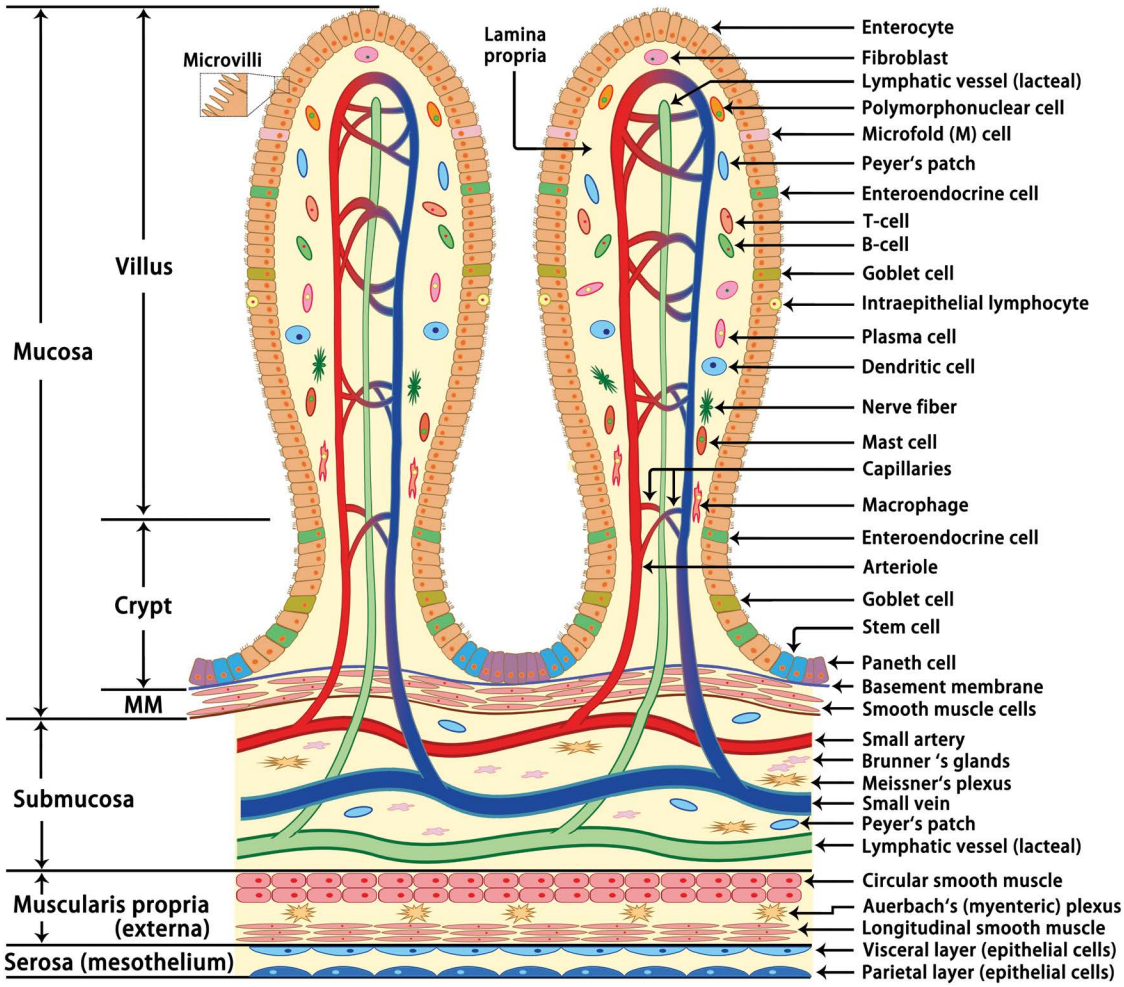


FIGURE 1.22 Structure of the wall of the small intestine. The wall of the small intestine consists of the epithelium in the crypt and villus regions, as well as the connective tissue of the lamina propria, smooth muscle cells, the vasculature, nerves, and basolateral epithelial cells. The lamina propria contains blood capillaries, lymph capillaries, and various types of cells (including lymphocytes and macrophages). Stem cells in the crypt differentiate into four major types of cells: enterocytes, goblet cells, enteroendocrine cells, and Paneth cells. MM, muscularis mucosa.

intestinal digestive enzymes such as disaccharidases (lactase-phlorizin hydrolase and sucrase-isomaltase) and peptidases; (b) transport proteins responsible for the uptake of amino acids, electrolytes, fatty acids, monosaccharides, and vitamins; and (c) binding sites for dietary lectins (Madara 1991). There is also evidence that, like the basolateral surface of the enterocytes, the brush border membrane of the enterocyte has cell-surface receptors for epidermal growth factor (Avissar et al. 2000), insulin-like growth factor-I (Moran et al. 1996), and vitamin D (Nemere et al. 2012). The basolateral membrane does not appear to contain sugar or peptide hydrolases. Only selected substances cross the enterocyte membrane, and an abnormality of intestinal absorption results in diseases such as diarrhea, celiac sprue (an autoimmune disease), food allergies, and drug-induced damage (Li et al. 1990; Mowat 1987). Cell-to-cell interactions via the E cadherin protein (a cell adhesion molecule) are an important feature of polarized enterocytes, and this interaction is maintained during the growth and migration of cells along the crypt-villus axis. Results of pig studies have shown that the activity patterns of digestive enzymes along the crypt-villus axis of the small intestine are generally similar between its proximal and distal regions (Fan et al. 2001).

The small intestine has many nutritional and physiological functions. First, it is responsible for the terminal digestion and absorption of dietary nutrients (Yin et al. 2002) and is therefore essential to the health, growth, development, reproduction, and survival of animals (Barrett 2014). The rates of nutrient digestion and absorption are enhanced by the contractions of the small intestine, which allow for the mixing of luminal contents in a radial direction (from the center of the lumen toward the epithelium), bring the digesta close to the epithelium, and reduce the thickness of the unstirred H_2O layer. When small-intestinal contractions are absent, luminal contents move through the small intestine via laminar flow, just like that occurring in a pipe. Second, in mammalian neonates, the small intestine can

absorb immunoglobulins from colostrum before *gut closure* occurs (e.g., 24 h after birth in calves and goat kids; 24–36 h after birth in piglets and lambs; and 24–28 h after birth in foals, cats, and dogs). This is crucial for the immunity of the newborn. Third, the gut is the barrier separating the internal milieu of the organism from the external environment and therefore is critical for the exclusion of food-borne pathogens and preventing the translocation of luminal microorganisms into circulation. Fourth, nutrient sensing occurs in the small intestine to regulate food intake, nutrient absorption, and hormone secretion from the enteroendocrine cells (Duca et al. 2021). Finally, as the largest lymphoid organ in the body, the small intestine participates in immune surveillance of the intestinal epithelial layer and regulation of the mucosal response to foreign antigens (Mowat 1987). This is of critical immunological importance because (a) the normal intestinal tract encounters enormous dietary antigenic loads, (b) ingested dietary antigens need to be processed and recognized without inducing harmful hypersensitivity reactions, and (c) the integrity of intestinal mucosa must be protected against the invasion of exogenous pathogenic microbes.

The Large Intestine

The large intestine, also called the hindgut or large bowel, is the last part of the digestive system in vertebrates. The large intestine is defined as the combination of the cecum (a pouch-like structure connecting to the ileum), colon, rectum, and anal canal (Barret 2014). In animals [e.g., primates (including humans), opossums, wombats, and rabbits] with an appendix that may function as a mammalian bursal equivalent in immunity (Weinstein et al. 1994), the appendiceal orifice lies at the blind end of the cecum. Many species (e.g., cattle, sheep, goats, pigs, chickens, and horses) do not have an appendix. Like the small intestine, the lumen of the large intestine also has an unstirred H_2O layer. The relative capacities of large intestines differ markedly among animal species. The hindgut harbors large amounts of microorganisms (e.g., bacteria, protozoa, and fungi) that also ferment carbohydrates, proteins, and amino acids to form SCFAs, H_2S, ammonia, indoles, skatole, and other amino acid metabolites just like ruminal bacteria (Dai et al. 2022; Mu et al. 2022; Yang et al. 2014). In all animals, the number of bacteria in the large intestine is several magnitudes greater than that in the small intestine (Table 1.8). Bacterial counts of 10^7 to 10^{12}/g feces and substantial amounts of protozoa (10^3 to 10^8 per g feces) have been reported in the hindgut of mammals, birds, and reptiles. Colonocytes have a high capacity to absorb SCFAs, electrolytes, and H_2O, but these cells take up only a limited amount of amino acids and nitrogenous metabolites from the lumen of the large intestine (Bergen and Wu 2009). In contrast to enterocytes, colonocytes use butyrate as their major energy source and, to a lesser extent, glutamine, acetate, and propionate as metabolic fuels (Barrett 2014; Wu 2022a). Because dietary fiber stimulates intestinal motility and generates SCFAs in the hindgut, the consumption of adequate amounts of these plant-sourced substances is beneficial for optimal intestinal health, longevity, and productivity in animals, particularly lactating dams and breeding stock.

Some nonruminant herbivores, such as horses (Coverdale et al. 2004) and rabbits (Brewer 2006), have a particularly high capacity to digest dietary fiber and plant-sourced protein in the large intestine, which allows them to graze on pasture and consume roughages. They are classified as "hindgut fermenters" and are subdivided into cecal fermenters and colonic fermenters. Cecal fermenters are hindgut fermenters whose cecum is the primary site of fermentation and include the rabbit, guinea pig, ostrich, goose, and duck (Cheeke and Dierenfeld 2010; Clemens et al. 1975). These animals are generally small as adults (e.g., 3–6 kg) with the exceptions of the koala (~10 kg) and capybara (~50 kg; Cheeke and Dierenfeld 2010). Of note, the rabbit directly ingests soft fecal pellets (cecotrophs) from its rectum because of a neurologic licking response (Davies and Davies 2003). Colonic fermenters use the proximal colon as the primary site of fermentation and are usually large as adults. Examples of colonic fermenters are the horse, zebra, donkey, elephant, rhinoceros, monkey, lemur, and beaver. Because of their relatively high capacity to utilize plant cell-wall materials and dietary fiber, hindgut fermenters (particularly rabbits, geese, and ducks) contribute greatly to sustainable animal agriculture, just like ruminants.

The Pancreas

The pancreas, which localizes behind the stomach and next to the duodenum, is an organ with both endocrine and exocrine roles. The islets of Langerhans, which contain α-cells and β-cells, are present in the endocrine pancreas. The exocrine pancreas consists of acinar cells and ductal cells, which account for approximately 80% and 20% of its mass, respectively. The ductal cells are epithelial cells lining the pancreatic duct, which mixes and delivers the secretions of acinar and ductal cells to the lumen of the duodenum. The pancreas is present in all vertebrates, but its shape is very different depending on animal species, with a soft and diffuse organization in rodents as opposed to a firm and pear-shaped structure in pigs (Dyce et al. 1996).

As an endocrine organ, the pancreas synthesizes and releases insulin and glucagon to regulate blood glucose levels. Specifically, glucagon (secreted by pancreatic α-cells) increases glucose concentrations in blood, but insulin (secreted by pancreatic β-cells) has an opposite effect. The autoimmune destruction of insulin-producing ß-cells by activated macrophages results in insulin-dependent (type-I) diabetes mellitus in animals. Interestingly, animals or humans that are genetically prone to type-I diabetes mellitus may not have an onset of this disease, depending on their diets and other environmental factors. In contrast, insulin-independent (type-II) diabetes mellitus occurs in obese animals with insulin resistance as it does in obese humans, although their pancreatic β-cells are capable of synthesizing and secreting insulin. However, some lean and

older individuals may be affected by insulin resistance and type II diabetes mellitus due to dyslipidemia.

As an exocrine organ, the pancreas is a major source of digestive enzymes and bicarbonate in the small intestine (Guyton and Hall 2021). Specifically, pancreatic ductal cells form and secrete a large amount of sodium bicarbonate and, to a lesser extent, sodium carbonate to neutralize acid (derived manly from the stomach) and increase the alkalinity in the lumen of the small intestine. The concentration of $NaHCO_3$ in the pancreatic juice is as high as 140 mM. In addition, pancreatic acinar cells synthesize and secrete (a) proteases, co-lipase, and phospholipase A_2 as pro-enzymes that are activated in the small-intestinal lumen; and (b) α-amylase, lipases, cholesteryl ester hydrolase, ribonuclease (RNAase), and deoxy ribonuclease (RNAase) in active forms. These enzymes hydrolyze dietary protein, starch, glycogen, lipids, and nucleic acids. Furthermore, pancreatic acinar and ductal cells may synthesize and secrete nonenzyme proteins that facilitate the digestion of micro-nutrients (e.g., vitamin B_{12}). Because of these important digestive and metabolic functions, rapid weight loss often occurs in animals with pancreatic diseases. Likewise, young mammals exhibit growth depression during the first week postweaning when the secretion of pancreatic lipase, α-amylase, and proteases is substantially reduced (Yen 2000).

The Liver

The liver is a central organ in the digestion, metabolism, transport, and storage of nutrients, as well as detoxification and immunity. Based on gross anatomy, the liver has four lobes of unequal size and shape: left, right, caudate, and quadrate (Dyce et al. 1996). In the porcine liver, the right lobe is subdivided into the main lobe and a caudate process. The gallbladder, which is present in most vertebrates as a small pouch sitting under the liver, stores bile (a mixture of H_2O, bile salts, organic cations, cholesterol, phospholipids, amino acids, glutathione, and bilirubin) produced by the liver and then releases the bile into the common bile duct (which empties into the duodenum) in response to feeding [e.g., 46 and 38 ml/kg BW/day in 45- and 60-kg pigs, respectively (Yen 2000); ~750 ml/day in a 70-kg adult human (Boyer 2013)]. The hepatocytes release the following substances into the canaliculus: (a) organic cations, mostly by MRP1 [ATP-binding cassette (ABC) B1 (ABCB1); originally called P-glycoprotein]; (b) glutathione (GSH), oxidized GSH (GSSG), and GSH conjugates by the multidrug resistance protein 2 [MRP2 (ABCC2)]; (c) phosphatidylcholine by MRP3 (ABCC3); (d) cholesterol and plant sterols by ABCG5 and ABCG8; and (e) bile salts (i.e., glycocholate and taurocholate) by bile salt export pump (ABCB11; active transport; Boyer 2013). These transporters are localized in the apical canalicular domain of hepatocytes.

The animals with a gallbladder include bears, cats, cattle, channel catfish, chickens, dogs, geese, goats, guinea pigs, hawks, humans, mice, monkeys, owls, pigs, rabbits, sheep, and zebrafish (Oldham-Ott and Gilloteaux 1997). On the contrary, some species of mammals (i.e., horses, deer, rats, seals, and laminoids), birds (e.g., pigeons, parrots, and doves), fish (e.g., lampreys), and all invertebrates lack a gallbladder (Oldham-Ott and Gilloteaux 1997). In the species without a gallbladder, bile flows directly from the liver into the lumen of the duodenum through the bile duct.

As a highly perfused organ, the liver receives 20–25% of cardiac output (Dyce et al. 1996). Nutrients that are absorbed into the portal vein and those that are carried via the hepatic artery enter the liver for extraction by hepatocytes and other types of cells. Nutrients that bypass the liver without uptake and metabolites that are released from the liver both enter the inferior vena cava for utilization (including metabolism) in extrahepatic tissues or for excretion through the kidneys. Of note, the porous endothelium of the liver allows hepatocytes to extract both small and large molecules from the blood and to release large molecules (e.g., lipoproteins and cholesterol) into the blood (Guyton and Hall 2021). However, RBCs do not cross the hepatic sinusoidal endothelium. Kupffer cells (macrophages) in the liver play an important role in fighting pathogens and in the local immune response.

At the histological level, each lobe of the liver comprises many lobules, with the center of each lobule being the hepatic central vein. At the periphery of the lobules are portal triads (portal tracts), which contain five different structures: the hepatic artery, hepatic portal vein, common bile duct, lymphatic vessels, and branches of the vagus nerve (Argenzio 1993). Hepatic lobules are comprised of the parenchymal cells (the functional parts) of the liver (i.e., hepatocytes; 80% of the liver volume or 60% of hepatic cells) and nonparenchymal cells (6.5% of the liver volume but 40% of hepatic cells; Guyton and Hall 2021). Each hepatic lobule is hexagonal in shape and consists of plates of hepatocytes, branches of the portal vein and the hepatic artery, sinusoids (very small blood vessels), hepatic central vein, hepatic vein, bile ducts, and associated nonparenchymal cells (e.g., hepatic stellate cells and Kupffer cells). As noted previously, the portal vein and hepatic artery supply blood to the liver. These two sources of blood mix in the hepatic sinusoids, where nutrients and gases are exchanged with hepatocytes and associated cells through the interstitial space. Thereafter, the blood flows sequentially into the venule end of the hepatic sinusoids, the hepatic central vein, and the hepatic vein, and then leaves the liver through the inferior vena cava (also known as caudal vena cava) into the right atrium of the heart (Dyce et al. 1996).

Based on O_2 supply and nutrient metabolism, the functional unit of the liver is the hepatic acinus, which is oriented around the afferent vascular system (Rappaport 1958). The acinus is divided into three zones. Zone I (periportal hepatocytes) is nearest to the venules of the portal vein and the arterioles of the hepatic artery and is the most oxygenated. Zone III (perivenous hepatocytes), which is farthest from the microvasculature of the entering blood vessels, is around the hepatic central vein and poorly oxygenated (Figure 1.23). Zone II (mid-zone) is the transition zone located between zones I and III. Periportal, mid-zone,

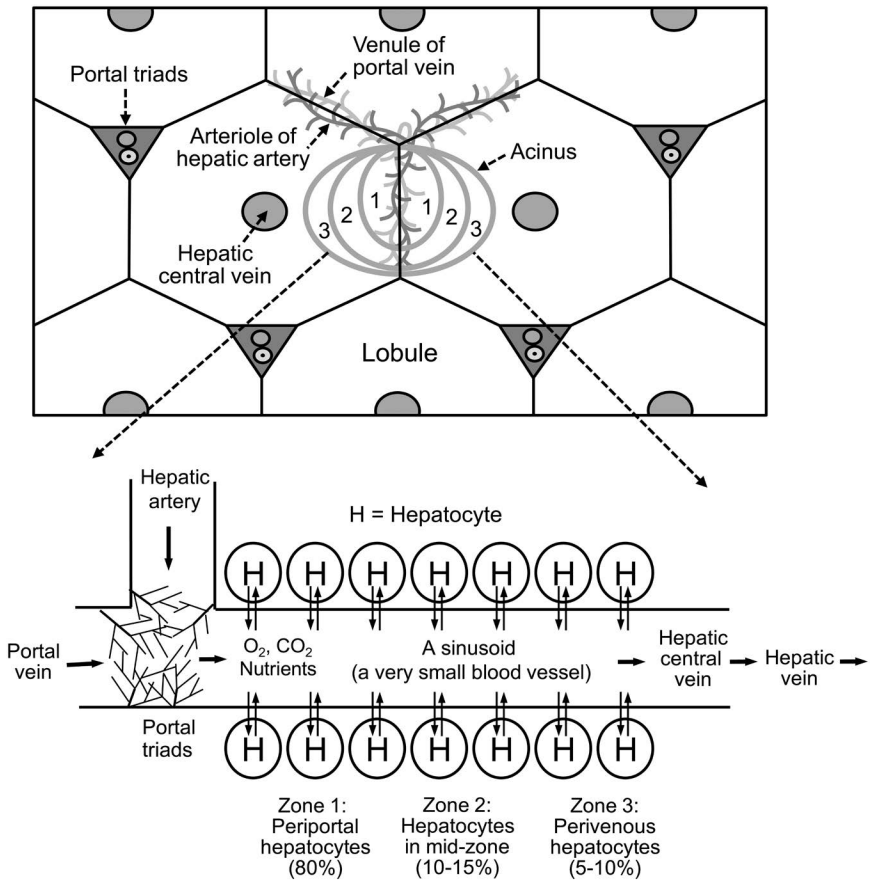


FIGURE 1.23 The hepatic acinus and nutrient exchange through the hepatic sinusoid in the liver. The liver comprises many lobules (hexagonal structures), with the hepatic central vein in the center of each lobule. At the periphery of the lobules are portal triads, which contain branches of the hepatic artery and portal vein to supply blood to the liver. The hepatic acinus, which is oriented around the afferent vascular system, is the functional unit of the liver. The acinus consists of hepatocytes aligned around arterioles of the hepatic artery and venules of the portal vein. The microvasculatures anastomose into sinusoids, which are the sites of the exchange of nutrients, O_2, and CO_2 with hepatocytes. Based on the distance from the portal venules and hepatic arterioles, the acinus is divided into three zones: Zone 1 (periportal hepatocytes, the best oxygenated), Zone 2 (mid-acinar hepatocytes), and Zone 3 (perivenous hepatocytes, poorly oxygenated). Blood leaves the liver through the hepatic central vein.

and perivenous hepatocytes represent ~80%, 10–15%, and 5–10% of total hepatocytes, respectively. These three different types of cells have very different metabolic patterns (Schleicher et al. 2015). This concept of metabolic zonation helps us understand how nutrients are utilized and synthesized in the liver.

Hepatocytes do not possess all the transporters needed for the uptake of physiological substances from the blood, nor do they express all the enzymes needed for nutrient metabolism (Häussinger et al. 1992). In mammals, hepatocytes lack transporters for some molecules (e.g., citrulline), and periportal hepatocytes do not take up glutamate or aspartate from the arterial blood or the portal vein. In addition, mammalian hepatocytes lack enzymes for (a) initiating the catabolism of some amino acids (e.g., branched-chain amino acids) under physiological conditions, (b) the net synthesis of some amino acids (e.g., arginine), and (c) the oxidation of some metabolites (e.g., ketone bodies, urea, and uric acid; Roach et al. 2012; Wu 2022a). However, hepatocytes have a high capacity to oxidize fatty acids and the α-ketoacids of amino acids to CO_2 and H_2O. These cells also play an important role in the interorgan metabolism of nutrients in animals. Furthermore, hepatocytes are capable of synthesizing glucose, glycogen, fats, plasma proteins (e.g., albumin, ferritin, and fibrinogen), lipoproteins, cholesterol, and bile salts, as well as storing glycogen, vitamins (e.g., vitamins A and B_{12}), and minerals (e.g., iron and copper; Jungermann and Keitzmann 1996).

Components of the plasma, including small molecules (e.g., amino acids, glucose, and ammonia) and macromolecules [e.g., albumin, low-density lipoprotein (LDL), and high-density lipoprotein (HDL)] within the hepatic sinusoid, can cross the hepatic endothelium (Hou et al. 2020). This moves molecules through the endothelial cells (for small molecules) and the fenestrae (porous sites between endothelial cells; for macromolecules) into the space of Disse (a location in the liver between a hepatocyte and a sinusoid; Figure 1.24). Subsequently, these substances enter the hepatocyte through its basolateral (sinusoidal) membrane, as reported for humans, pigs, rodents, and zebrafish.

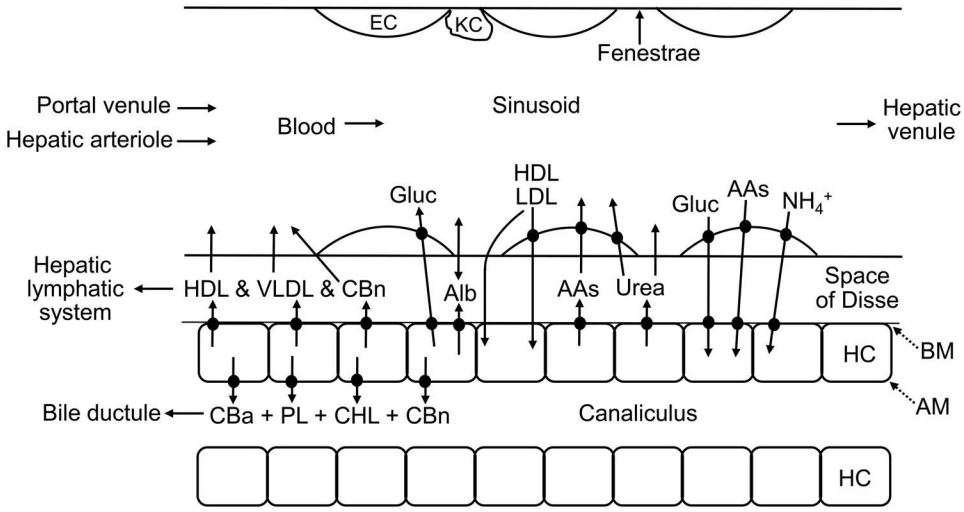


FIGURE 1.24 Uptake and release of substances by hepatocytes aligned with the sinusoid. The hepatic acinus (the functional unit of the liver) consists of terminal branches of the portal vein and hepatic arteries for exchanges of nutrients and gases with hepatocytes and associated cells. Small molecules (e.g., amino acids, glucose, and ammonia) and macromolecules (e.g., albumin, LDL, and HDL) within the hepatic sinusoid (the capillaries in the liver) cross through its endothelium (for small molecules) and its fenestrae (for macromolecules) into the space of Disse. Subsequently, these substances cross the basolateral (sinusoidal) membrane of the hepatocyte into the cell. Likewise, amino acids, glucose, proteins (e.g., albumin) and lipoproteins (e.g., VLDL and HDL), and some conjugated bilirubin are released by the hepatocyte across its basolateral membrane into the space of Disse, and then enter (a) the hepatic sinusoid through the fenestrae in its endothelium and (b) the hepatic lymphatic system. By contrast, glycine- or taurine-conjugated bile acids, phospholipids (e.g., phosphatidylcholine and phosphatidylethanolamine), unesterified cholesterol, and some conjugated bilirubin (a metabolite of heme) are released by the hepatocyte across its apical (canalicular) membrane into the canaliculus formed from adjacent hepatocytes. The bile flows into bile ductules and then bile ducts. The solid circle denotes a transporter, receptor-mediated endocytosis, or receptor-mediated exocytosis. AAs, amino acids; Alb, albumin; AM, apical membrane; BM, basolateral membrane; CBa, conjugated bile acids; CBn, conjugated bilirubin; CHL, cholesterol (unesterified); EC, endothelial cell; HDL, high-density lipoprotein; Gluc, glucose; HC, hepatocyte; KC, Kupffer cell (macrophage in the liver); LDL, low-density lipoprotein; PL, phospholipids; VLDL, very low-density lipoprotein. (Reproduced from Hou et al. 2020, with permission from Springer.)

This process is mediated through specific transporters for amino acids or glucose, receptor-mediated endocytosis for albumin, LDL receptor-mediated endocytosis for LDL, and scavenger receptor class B member 1 (SR-B1)-mediated endocytosis for HDL. Likewise, amino acids, glucose, proteins [e.g., albumin, very-low-density lipoprotein (VLDL), and HDL], and some conjugated bilirubin are released by the hepatocyte across its basolateral membrane into the space of Disse, which flows into (a) the hepatic sinusoid through the fenestrae in its endothelium and (b) the hepatic lymphatic system through its lymphatic capillaries. The non-diffusional transport of these substances at the basolateral membrane of the hepatocyte is mediated through specific transporters for amino acids and glucose, the neonatal crystallizable fragment receptor (FcRn)-mediated exocytosis for albumin, receptor-mediated exocytosis for VLDL and HDL, and multidrug resistance protein 3 for conjugated bilirubin. In contrast, glycine- or taurine-conjugated bile acids, phospholipids (e.g., phosphatidylcholine and phosphatidylethanolamine), unesterified cholesterol, and some conjugated bilirubin (a metabolite of heme) are released by the hepatocyte across its apical (canalicular) membrane into the canaliculus (a duct-like structure) formed from adjacent hepatocytes. The bile salt export pump, ABC transporters (particularly ABC-B4), and multidrug resistance protein 2 are responsible for the canalicular efflux of bile salts, phospholipids, and conjugated bilirubin, respectively. In contrast, ABC-G5/ABC-G8 (as a dimer) and SR-B1 transport unesterified cholesterol from the hepatocyte across its apical membrane into the canaliculus. The bile (e.g., 400–800 ml per day in an adult human) sequentially flows into bile ductules, bile ducts, and the lumen of the duodenum. Note that hepatic stellate cells reside in the space of Disse and play an important role in scar formation in response to liver damage.

The Digestive System in the Pandas as an Omnivore

Molecular studies indicate that the giant panda is a true bear and diverged about 19 million years ago from a common ancestor of the Ursidae family. Anatomically, pandas are carnivores with a relatively short digestive tract (Herring et al. 2021). However, due to the consumption of bamboo as their major natural food for thousands of years, pandas have lost the TAS1R1 gene for umami flavor and evolved to prefer bamboo over meat and other foods. In addition, pandas have retained the digestive system of their carnivore ancestor and thus are still unable to digest cellulose (a main constituent of bamboo) in the foregut. Nonetheless, giant pandas in the wild and captivity prefer to eat primarily

bamboo shoots and leaves but occasionally also ingest other plants (e.g., fruits and vegetables), small animals (including birds and rodents), and carcasses. As much as 98% of the panda's diet consists of the leaves, shoots, and stems of bamboo, a large grass available year-round in much of central China's forested regions. Pandas rapidly pass large quantities of grass through their digestive tracts on a daily basis, spending as much as 16 h per day on eating and frequently eliminating waste up to 50 times per day. Based on these feeding characteristics, giant pandas may be considered either omnivores or primarily herbivores.

Digestive Systems in Carnivorous Mammals

Carnivorous mammals (e.g., dogs and cats) generally have longer canine teeth, a tighter digitation of molars, a shorter digestive tract, a greater stomach volume, and a more rapid rate of food passage through the GIT when compared with other nonruminant mammals such as humans and pigs (He et al. 2024; Vanak and Gompper 2009). The following sections highlight the mouth, stomach, small intestine, and large intestine in dogs and cats.

The Mouth in Dogs and Cats

An adult dog has 42 teeth in its mouth, whereas an adult cat only has 30 teeth but has the same number of incisors and canines as dogs (Table 1.9). Compared with dogs, cats have relatively long canine teeth, as well as shorter incisors and molars and can therefore effectively hunt prey (e.g., rats and mice) and ingest whole tissues (Pekel et al. 2020). In cats, as opposed to dogs, teeth do not play a part in hunting except to dispatch the prey. Cats use sharp claws, which are sharp by remaining retracted when not in use, to capture their prey and then use their long canine teeth to sever the prey's spinal cord with a bite to the back of the neck. The cat's canine teeth have root endings close to the tooth surface, allowing it to "feel" for spaces between vertebrae so that the spinal cord can be severed quickly and with less power than if a crushing bite were used (Lombardero et al. 2021). As with other mammalian carnivores (but unlike omnivores and herbivores), the jaws of dogs and cats only allow for up and down motion, and these animals cannot chew side to side. Therefore, dogs and cats have a lower ability to grind food than most other mammals.

The four pairs of common major salivary glands in dogs and cats located around the jaw, under the tongue, and near the throat are the parotid, mandibular, sublingual, and zygomatic glands (Table 1.9). In addition, the cat has another major pair of molar salivary glands located superficially near the angle of the mandible (the lower-jaw bone) on both sides of the mouth. Hundreds of minor salivary glands exist within the wall of the oral cavity and oropharynx; they are named based on anatomical location (e.g., labial, lingual, buccal, and palatine) and have no connective tissue capsules. As previously noted for omnivores, the saliva of dogs and cats contains lingual lipase, proteases, and lysozymes that have many physiological functions. Interestingly, the enzymatic activity of lysozymes in canine and feline saliva is greater than that in human saliva. The saliva of both dogs and cats has little or no α-amylase activity (He et al. 2024).

Taste buds in the tongue help dogs and cats select food based on its flavor and texture. During evolution, the dog has adapted to omnivorous diets containing taurine-rich meat and starch-abundant plant ingredients. Taste buds of the canine and feline tongues express receptors (transmembrane proteins) to distinguish sour (PKD2L1), umami (mGluR4, Tas1R1 and Tas1R3), bitter (Tas2R), and salty (ENaC) substances. Tas1R1 and Tas1R3 proteins join to form an effective umami receptor. Unlike humans, dogs and cats do not possess very sensitive salt receptors. Interestingly, dogs, but not cats, possess sweet receptors (Tas1R2 and Tas1R3). Thus, dogs are not highly sensitive to dietary salt and consume sweet foods, whereas cats are insensitive to the taste of dietary sugars. Of particular note, dietary salts can substantially enhance canine taste responses to sugars (Kumazawa and Kurihara 1990a), and there is synergism between dietary monosodium glutamate and nucleotides in canine taste nerve responses (Kumazawa and Kurihara 1990b). In contrast to salts and sugars, both dogs and cats are sensitive to the tastes of dietary amino acids (e.g., glutamate, aspartate, glycine, and arginine), other organic acids, and nucleotides. Interestingly, dietary salt had an enhancing effect on the taste of amino acids in dogs but not in rats (Ugawa and Kurihara 1993). The physiologic roles of amino acids other than glutamate and aspartate in the selection of foods remain largely unknown. There are epigenetic effects on taste responses to dietary nutrients in mammals including dogs and cats.

The Stomach in Dogs and Cats

The stomach of dogs consists of distinct proximal and distal regions, whereas cats have a uniform gastric mucosa (Emås and Grossman 1967). The proximal region of the canine and feline stomach expands for food storage so that they can eat a large meal at one time. This allows the partially digested food to remain in the stomach for a prolonged period of time [~4–8 h in dogs (Bourreau et al. 2004) and ~3–6 h in cats (Goggin et al. 1999)], compared to ~ 0.5 h in humans and pigs. In dogs, gastric emptying is slowed by dietary fat, increased by gastrin, and influenced by meal size (He et al. 2024). Bourreau et al. (2004) reported that when adult dogs consumed a meal that met 50% of the daily energy requirements, large-breed dogs (e.g., ~60 kg BW) retained food in the stomach for a longer period and had a lower rate of gastric emptying, compared with small-breed dogs (e.g., 3.5 kg BW). Similarly, there is a strong inverse relationship between BW and gastric emptying time in adult humans weighing 59–93 kg (Lavigne et al. 1978).

The stomach volume of dogs with all BWs is ~90 ml/kg BW, whereas the volume of the feline stomach is reduced with BW, being ~100, 70, 60, and 45 ml/kg BW at the BW of 0.5–0.99, 1.0–1.49, 1.5–3.99, and 4–6 kg, respectively

TABLE 1.10
The Digestive Tract of Dogs, Cats, and Pigs[a]

Animal Species	Saliva pH	GIT WT (% of BW)	GIT Filling (% of BW)	Stomach Fluid pH	Gastric Half-EMPT (min)	SI Length	LI Length	Liver WT (% of BW)	Luminal Fluid pH			SI Volume (% of the GIT)	Retention Time of Food in the GIT (h)
									Duo	Jej	Ile		
Dog	7.34–7.8	6–7[b] 3–4[c]	3–4[b] 10	2.05[e] 1.08– 1.26[f]	72–240	3.9 m per 0.75 m BL	0.5 m per 0.75 m BL	6.82[d] 2.3–3.9	5.7	6.4	6.6	25	23
Cat	7.5	5–6[d] 2–3	--- 3	2.5	22–25[e] 449[f]	1.7 m per 0.5 m BL	0.4 m per 0.5 m BL	4.07[d] 2.46	6.2	6.7	7.0	15	13
Pig	6.0–7.0	2.9[d] 2.3	10 20	2–3[d] 2	160–240	10 m[d] 19 m per 1 m BL	4 m[d] 5 m per 1 m BL	2.7[d] 1.5	6.1	6.6	6.7	51[d] 40	43

Source: He et al. (2024).

[a] The data refer to adult animals, unless indicated otherwise. Animals had a normal birth weight.

[b] Small breed dogs.

[c] Large breed dogs.

[d] Neonate.

[e] Fasted state.

[f] Fed state.

Note: BL, body length; BW, body weight; Duo, duodenum; EMPT, emptying; GIT, gastrointestinal tract; Ile, ileum; Jej, jejunum; LI, large intestine; m, meter; SI, small intestine; WT, weight.

(Bartges 2003). Like other carnivores, the stomach of dogs and cats is relatively simple, and its volume represents 60–70% of the total capacity of the digestive system, which is much greater than the value for nonruminant monogastric mammals. The gastric fluid of dogs (Buddington and Malo 2003) and cats (Adler et al. 2016) contains bacteria (~10^3/ml; primarily Gram positive and aerobic, lactic acid-producing, e.g., *Bifidobacterium*, *Lactobacillus*, and *Streptococcus*), which may help to digest dietary fiber. Compared with adult dogs, adult cats have a higher gastric fluid pH (Table 1.10). The stomach of both dogs and cats secretes gastric lipase, pepsin, HCl, and some mucus to lubricate and protect the stomach wall (Emås and Grossman 1967; Nelson et al. 1977). In both dogs and cats, as in other mammals and in birds, gastric secretion is stimulated by protein intake and the volume of the meal (Wu 2022a). Interestingly, in dogs, gastric lipase is inactive at pH 1.5 and 7.0, active at pH 4 (being 13 times more active on TAGs with long-chain than short-chain TAGs) but has a substantially reduced activity at pH 6 (Carrière et al. 1991). Pancreatic lipase appears to play a major role in the digestion of dietary TAGs by dogs, as their ability to digest medium-chain and long-chain TAGs is reduced by about 25% and 95%, respectively, in the case of pancreatic insufficiency (Rutz et al. 2004). In contrast, canine gastric lipase has a low activity on long-chain TAGs but effectively hydrolyzes medium-chain TAGs. In cats, gastric lipase is primarily localized to the epithelial cells of the gastric mucosa before weaning (Knospe and Plendl 1997) but is present mainly in gastric fluid after weaning (Pansu et al. 1987).

The Small Intestine in Dogs and Cats

Like other carnivores, both dogs and cats have a short GIT. Larger breeds of dogs (>25 kg BW as adults) have a proportionately smaller GIT, comprising 3–4% of BW, compared to 6–7% of BW in smaller breeds (e.g., medium breeds of dogs with 10 to 25 kg BW as adults; small breeds of dogs with <10 kg BW as adults; Meyer et al. 1993). Compared with adult dogs, the small intestine of adult cats has a higher luminal fluid pH, a shorter length, and a smaller volume (Table 1.10). In both dogs and cats, the movement of chyme from the stomach to the small intestine stimulates the secretion of pancreatic juice into the duodenum. The release of CCK from the small intestine is enhanced by partially digested food, fat, and phenylalanine-containing peptides. Pancreatic α-amylase activity is low in cats but high in dogs, whereas the endogenous secretion of N is greater in cats than dogs (Li and Wu 2023). In dogs, the small intestine has a high capacity to absorb both amino acids and glucose. In cats, the small intestine actively absorbs amino acids but has a much lower ability to absorb glucose than in dogs (Batchelor et al. 2011). Aspartate, glutamate, and glutamine are the major sources of energy for the canine and feline small intestines (He et al. 2024).

The number of bacteria (mainly *Streptococci* and *Lactobacilli*) in the fluid of the canine duodenum and jejunum rarely exceeds 10^4/ml (Suchodolski 2011). In the duodenum of healthy dogs, *Firmicutes* is the most abundant microbial species (46.4%), followed by *Proteobacteria* (26.6%), *Bacteroidetes* (11.2%), *Spirochaetes* (10.3%), *Fusobacteria* (3.6%), and *Actinobacteria* (1%; Xenoulis

et al. 2008). In the jejunum of healthy adult dogs, *Proteobacteria* is the most abundant microbial species (46%), followed by *Firmicutes* (15%), *Actinobacteria* (11.2%), *Spirochaetes* (14.2%), *Bacteroidetes* (6.2%), and *Fusobacteria* (5.4%; Suchodolski et al. 2009). The number of bacteria (mainly *E. coli* and anaerobic bacteria) in the canine ileum is ~10^6/ml (NRC 2006). These data extend the report of (Johnston et al. 1993) that, in the canine duodenum, anaerobic bacteria are mainly *Bacteroides spp.*, *Fusobacterium spp.* and *Eubacterium*, whereas aerobic bacteria are mainly *Pasteurella* species. In dogs, the abundance of bacteria increases along the length of the GIT (namely ileum > jejunum > duodenum), and the microbiota in the small intestine inhibits the colonization of pathogenic microorganisms. Compared with dogs, the small intestine of cats contains more bacteria. The duodenal fluid of cats fed a canned diet yields 2.2×10^5 to 1.6×10^8 bacteria, with anaerobic bacteria being 7.5×10^4 to 1.1×10^8 (Johnston et al. 1993). Like canines, the anaerobic bacteria of the feline duodenum are mainly *Bacteroides spp.*, *Fusobacterium spp.* and *Eubacterium*, whereas aerobic bacteria are mainly *Pasteurella* species (Johnston et al. 1993).

The Large Intestine in Dogs and Cats

In dogs and cats, as in other mammals, the major functions of the large intestine are to ferment food that escapes the small intestine to produce various metabolites (including SCFAs, L-lactate, CO_2, H_2S, ammonia, amines, phenols, indoles, and microbial proteins); synthesize and absorb certain vitamins (e.g., B-complex vitamins and vitamin K); absorb H_2O, electrolytes, and SCFAs; regulate intestinal immune cells; produce lactic acid to provide a hostile environment against pathogens; and form and store feces for excretion (Kerns 2005; Wernimont et al. 2020). Compared with omnivores (e.g., pigs), dogs and cats have a relatively short hindgut, including an unsacculated colon and a small cecum, with pH values in the luminal fluid ranging from 5.5 to 7.5, depending on diets. Cats have a less developed cecum than dogs (Griffin 2019). SCFAs are the major sources of energy for canine and feline colonocytes (He et al. 2024).

The large intestine of dogs contains more bacteria than the small intestine. The bacterial counts range from 10^9 to 10^{11}/ml in the colon of dogs, which help to digest fiber and other nutrients (Suchodolski 2011). The main bacteria in their hindgut are *Fusobacteria* (24–40%), *Bacteroidetes* (32–34%), and *Firmicutes* (15–28%), whereas *Proteobacteria* (5–6%) and *Actinobacteria* (0.8–1.4%) are quantitatively minor microbial species (Maskell and Johnson 1993; Suchodolski et al. 2008). For comparison, the main bacteria in the hindgut of healthy pigs are *Firmicutes* (78%), followed by *Bacteroidetes* (14%), *Actinobacteria* (1.6%), and *Proteobacteria* (0.2%; Hoogeveen et al. 2010). Meng et al. (2020) reported that the large intestine of 28-day-old pigs weaned at 21 days of age contained only 0.01% *Fusobacteria*. Thus, the composition of bacteria in the hindgut differs greatly between dogs and omnivores. The large intestine of the cat has the same predominating bacterial species and the same pH as those in dogs (Maskell and Johnson 1993; Suchodolski 2011).

The main stimuli for motility in the large intestine of mammals, including dogs and cats, are distention by its contents and the quantity of undigested material entering the colon. Colonic contents stimulate gut contractions to affect the time of their transit into the rectum. Canine BW has little effect on orocecal transit time, fecal microflora profile, or the absorption of nutrients by the small intestine (He et al. 2024). The feces consists of unabsorbed nutrients (including H_2O and amino acids), sloughed cells, digestive secretions, microbial metabolites, and microbes, and eventually passes into the rectum for excretion via the anal canal. The microflora influences colonic absorption and cell metabolism, as well as fecal quality.

The Liver in Dogs and Cats

Dogs and cats share many common metabolic pathways in the liver but also differ in some aspects. Their livers form glucose, fatty acids, HDL, ketone bodies, albumin, and urea; express phosphoenolpyruvate carboxykinase (a glucogenic enzyme) in both the cytosol and mitochondria; and conjugate bile acids almost exclusively with taurine (Li and Wu 2024). However, hepatic glucokinase activity is present in dogs but absent in cats. In contrast, the hepatic activities of other glycolytic enzymes and rate-controlling glucogenic enzymes are much greater in cats than in dogs. This pattern of hepatic enzyme distribution is consistent with a greater rate of whole-body glucose production in cats than in dogs. Furthermore, most breeds of dogs can synthesize sufficient taurine from methionine or cysteine in the liver, but all breeds of cats have a very limited ability for taurine production. Thus, taurine must be included in feline diets. Finally, cats, unlike dogs, can form felinine and isobuteine (possibly communication signaling molecules) from cysteine, but cannot synthesize (a) arachidonic acid from linoleic acid (an ω6 polyunsaturated fatty acid) or (b) niacin (a water-soluble vitamin) from tryptophan.

The Digestive System in Fish

Fish have a mouth but no salivary glands and thus do not produce saliva. Most fish (including hybrid striped bass and largemouth bass) have teeth to cut their prey and shred food. Fish do not have a muscular tongue that resembles the mammalian tongue, but most fish have a small, thick piece of cartilage called the basihyal (the fish's tongue) on the floor of the mouth. The basihyal aids in protecting blood vessels in the mouth of fish, passing food into the esophagus, and pushing H_2O through gills. Additionally, the basihyal contains tastebuds that can distinguish sweet, sour, salty, and bitter tastes.

Fish have morphologically diverse GITs (Wilson and Castro 2011). For example, channel catfish, rainbow trout, salmonids, and most of the finfish have true stomachs, whereas some fish (e.g., tilapias and lizardfish) have a modified stomach, and certain fish (e.g., zebrafish,

lungfish, and the common carp) have no stomach. The digestive system of fish generally includes the mouth, stomach, intestines, and anus, as well as the hepatopancreas. The mucosa in the stomach of fish has a structure adapted for both food storage and digestion. As in mammals, fish have gastric parietal cells, gastric chief cells, and gastric glands to produce HCl (for converting pepsinogen into pepsin and denaturing dietary protein), pepsinogen, and mucins (for protecting the gastric epithelium from acid destruction). For those fish that do not have a stomach, the anterior intestine performs the function of temporary storage of ingested food. Stomach-less fish (which lack pepsin) are usually herbivores or omnivores that have a nearly mature and slightly alkaline gut. Compared with mammals and birds, fish generally have a shorter and simpler digestive tract, a longer period of food digestion, and a delayed and sustained increase in plasma concentrations of amino acids (Li et al. 2021a).

Secretin (secreted by the S-cells of the duodenum) regulates secretions from the stomach, pancreas, and liver, whereas CCK (secreted by the I-cells of the duodenum) stimulates the secretion of pancreatic digestive enzymes and the release of bile from the gallbladder (for those fish that have this organ). Dietary phospholipids have beneficial effects on stimulating the secretion of peptidases in fish by increasing the level of CCK. The ability of fish to use dietary carbohydrates varies greatly among species. Omnivorous fish tolerate much higher intakes of digestible carbohydrates than carnivores.

The Digestive System in Crustaceans

Examples of this class of animals are crabs, prawns, and shrimp. Note that prawns and shrimp are both decapod crustaceans, as they have exoskeletons and ten legs. Prawns are larger in size, and have larger legs than shrimp, and possess claws on three pairs of their legs. In contrast, shrimp have claws only on two pairs of legs. The digestive tract of crustaceans is essentially an internal tube generally divided into three functional segments (Figure 1.20): (a) the foregut (a tubular esophagus and a stomach for mechanical and extracellular digestion) located in the head region, (b) the midgut (a simple tube with associated ceca and the hepatopancreas for extracellular digestion), and (c) the hindgut (rectum and anus for expelling the undigested food and feces). The esophagus joins the mouth to the stomach [an anterior chamber (the gastric mill apparatus) and a posterior chamber]. The anterior chamber functions in mastication (cutting and grinding) of the ingested food, whereas the posterior chamber keeps food particles from leaving the stomach until the gastric mill has reduced them to a small size (Li et al. 2021b). Secretion of HCl by gastric epithelial cells causes acidic conditions in stomach fluid (pH = 4–5) during digestion and plays a role in the activation of inactive gastric proteases.

Food particles leave the posterior chamber of the stomach and enter the anterior midgut and then the hepatopancreas. The latter is also called the midgut gland or digestive gland (a branching array of blind-ended tubules lined with an epithelium) that connects to the anterior midgut via ducts. The hepatopancreas synthesizes and secretes digestive enzymes and emulsifiers, which flow into the midgut and then retrogradely flow into the stomach. Those digestive enzymes include trypsin, chymotrypsin, aspartate proteinases, collagenases (astacin and brachyurins), cathepsin L, aminopeptidases, carboxypeptidases, nucleases, lipases, esterases (hydrolases that split ester bonds), α-amylase, cellulase, and chitinase (Vogt 2021). Thus, crustaceans can use their own cellulase to digest dietary cellulose. In most crustaceans, the emulsifiers for lipid digestion and absorption are fatty acids (mainly *cis*-5-dodecenoic acid) conjugated with taurine or dipeptides (its terminal amino acid being taurine or cysteine; Vogt 2021). Extracellular digestion of foods occurs primarily within the hepatopancreas and the resultant products (e.g., amino acids, glucose, and lipids) are absorbed across its epithelial layer into the hemolymph. The latter flows to the heart via the circulation system. The midgut plays a relatively minor role in the digestion and absorption of nutrients. Undigested food particles and unabsorbed nutrients from the terminal midgut enter the rectum to form feces, which leaves the gut through the anus.

THE MUSCULAR SYSTEM IN VERTEBRATES

The musculoskeletal system in vertebrates (e.g., mammals, birds, and fish) is composed of skeletal muscles and associated bones and joints (Guyton and Hall 2021; Lopez et al. 2024). This system provides structure, support, and movement to the body. Mesenchymal stem cells are multipotent stem cells that contribute to the development of both bone and skeletal muscle and are responsive to changes in maternal nutrition and endocrine status (Pillai et al. 2016). Collectively, musculoskeletal organs represent 50–60% of total BW in vertebrates.

Skeletal Muscle

Skeletal muscle is a type of striated muscle attached to the skeleton (bone) by tendons (connective tissue) and is the largest tissue in animals, accounting for about 40% and 45% of the BW in the newborn and young adult, respectively (Dyce et al. 1996). In vertebrates, skeletal muscle is composed of multinucleated muscle cells (also known as myocytes, myofibers, or muscle fibers) organized in a cylindrical shape. These cells are formed from the fusion of developmental myoblasts during fetal growth, and their number is fixed at birth (Oksbjerg et al. 2013). Myofibers comprise myofibrils, which contain actin and myosin filaments repeated in a unit called a sarcomere that is necessary for muscle contraction.

The sarcomere is the basic contractile and repeating unit of a muscle fiber (Figure 1.25). Each of the two ends of a

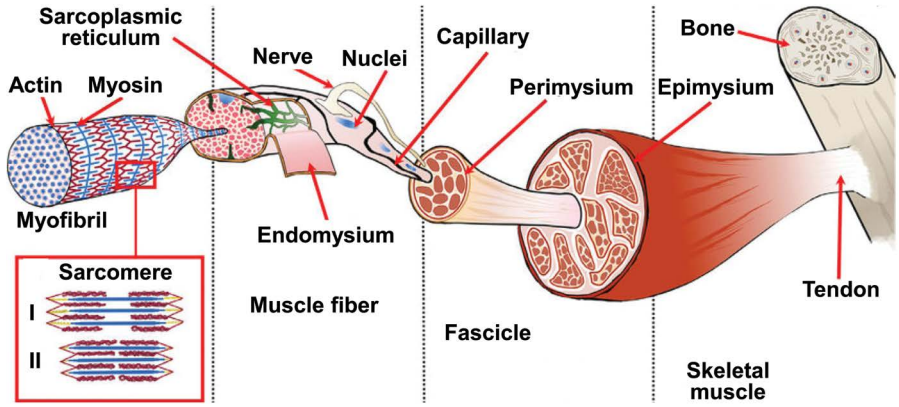


FIGURE 1.25 The structure of skeletal muscle in animals. Skeletal muscle (a striated tissue attached by tendons to bones of a skeleton) consists of multiple fascicles [bundles of multinucleated contractile muscle fibers (muscle cells)] and is ensheathed by a layer of connective tissue called the epimysium. Each fascicle is surrounded by a connective tissue layer called the perimysium. A muscle fiber has a cell membrane (sarcolemma), cytosol (sarcoplasm), sarcoplasmic reticulum (for Ca^{2+} storage and release), mitochondria, lysosomes, and other organelles found in mammalian cells. The sarcolemma is covered by a layer of connective tissue called the endomysium, which contains capillaries and nerves. Muscle fibers contain numerous tubular myofibrils that are composed of repeating sections of sarcomeres (the smallest repeating unit between two Z-lines, also known as Z-bands). Thus, the Z-line defines the lateral boundaries of the sarcomere and anchors its thin titin and nebulin filaments. The sarcomeres contain fibrous proteins: myosin (blue, a thick filament), actin (red, a thin filament), and titin (yellow, a large protein that helps to stabilize and center the thick filament between the thin filaments and to prevent overstretching of the sarcomere). Actin and myosin slide past each other when a muscle relaxes (I) or contracts (II). The Z-line is composed of the overlapping ends of actin filaments crosslinked by 4 to 6 layers of α-actinin. Muscle contractions require ATP and ATPase (bound to myosin), and the resulting tension is transmitted from sarcomere to sarcomere across the Z-line and along the myofibril. [Reproduced from a photo freely available in the public domain (Gotti et al. 2020).]

sarcomere is linked with a Z-line (also known as a Z-band), which anchors the titin and nebulin filaments of the sarcomere. The latter is composed primarily of two main protein filaments (thin actin and thick myosin filaments). Physical tension is generated during a muscular contraction when the myosin heads slide along the actin filaments (the cross-bridge theory). Such tension is transmitted from sarcomere to sarcomere across the Z-line and along the myofibril. This physiological process is triggered by the release of Ca^{2+} from the sarcoplasmic reticulum into the cytosol (sarcoplasm) and powered by ATP hydrolysis. Upon termination of contraction, Ca^{2+} is pumped from the sarcoplasm back into the sarcoplasmic reticulum, resulting in muscle relaxation. Muscle contraction and relaxation are required for the appropriate voluntary and involuntary movements of the body.

The diaphragm, which is a thin skeletal muscle attached to the ribs and sits at the base of the chest, separates the thoracic cavity from the abdominal cavity. This muscle is essential for the respiration of the lungs and thus life. When animals inhale, the diaphragm contracts, resulting in its flattening and the expansion of the thoracic cavity and ultimately creating a vacuum effect that pulls the air into the lungs. When animals exhale, the diaphragm relaxes, pushing gaseous molecules (mainly CO_2) out of the lungs. A substantial (i.e., 50%) loss of protein from the diaphragm, as in cases of cancer and long-term starvation, is incompatible with survival.

Each muscle has a characteristic ratio of type I (slow-twitch) to type II (fast-twitch) myofiber based on histochemical staining reactions for myofibrillar ATPase. Type I myofibers react strongly for acid-stable ATPase but weakly for alkali-stable ATPase, whereas type II myofibers react negatively for acid-stable ATPase but strongly for alkali-stable ATPase. There are three subtypes of type II myofibers (depending on the extent of oxidative or glycolytic capacity) based on their acid liability: IIA: fast-twitch oxidative (showing intermediate oxidative capacity); IIB: fast-twitch glycolytic (showing strongly glycolytic capacity); and IIC: intermediate between slow-twitch and fast-twitch oxidative fibers (accounting for <1% of total myofibers). Overall, type I myofibers have a high number of mitochondria and high oxidative capacity, but low glycogen phosphorylase activity. In contrast, type II myofibers (primarily glycolytic) have a fewer number of mitochondria and lower oxidative capacity but higher glycogen phosphorylase activity compared with type I myofibers. Skeletal muscles rich in type I or type II myofibers have a relatively high or low capillary density, respectively.

Skeletal muscle was traditionally considered to be a relatively inert protein reservoir but is now known to actively participate in intracellular protein turnover, the interorgan metabolism of amino acids, and whole-body homeostasis in mammals and birds and possibly also in other animal species. Compared with the blood, skeletal muscle contains high concentrations of glutamine, glutamate, alanine, taurine, β-alanine, and carnosine to support gluconeogenesis, immune functions, and anti-oxidative reactions in animals (Wu 2022a). In many nonprimate species (e.g., cattle, pigs, and birds), skeletal muscle also contains high concentrations of anserine.

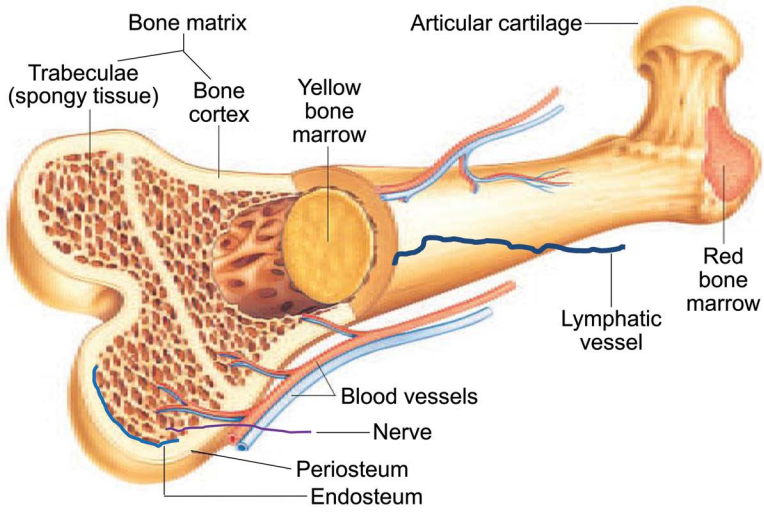


FIGURE 1.26 The bones of vertebrates. Bone is a large and complex organ consisting of specialized connective tissues: bone matrix and bone marrow. The bone matrix includes an outer layer of tissue called bone cortex [covered by the periosteum (a dense vascular layer of connective tissue)] and an inner matrix of tissues called the trabeculae (a lattice-like network of matrix) covered by the endosteum (a thin vascular layer of connective tissue). Both the periosteum and the endosteum contain blood vessels, lymphatic vessels, and nerves that nourish the bone. [Adapted from a photo freely available in the public domain (Walden 2022).]

Bone

Bone is a large and complex organ (12–15% of BW) consisting of bone matrix and bone marrow, which are specialized connective tissues (Figure 1.26). The osteon is the functional unit of bone cortex and is a concentric circle (Figure 1.27). The bone matrix contains about 30% organic substances, 60% minerals, and 10% H_2O (Lopez et al. 2024). Collagen (primarily type 1 collagen), elastin, and ground substances account for about 90%, 1%, and 9% of the organic substances in the bone matrix, respectively. Elastin is about 1000 times more flexible than collagen. The ground substances consist of proteoglycans, including amino sugars (e.g., hyaluronic acid and chondroitin sulfate) that are linked to noncollagenous proteins (e.g., osteocalcin, osteopontin, bone sialoprotein, cadherins, and selectins). Hydroxyapatite crystalline $[Ca_{10}(PO_4)_6(OH)_2]$ is the major form of the calcium phosphate salt in the bone matrix, with the ratio of Ca/P being 2.15 (g/g). Inorganic salts are deposited within the organic framework. Bone mass is dependent on the balance between bone resorption and bone formation.

There are two types of marrow in the bone (yellow bone marrow and red bone marrow). Bone marrow contains blood vessels and sympathetic nerve fibers. The ends of the bone are covered by a smooth white connective tissue called articular cartilage, which forms the joints. In mammals and birds, the bone marrow is the site for the formation and differentiation of blood cells from resident progenitor cells (hematopoiesis; Barminko et al. 2016). In fish, which usually possess no bones, have no medullary cavity (marrow), the kidneys are the primary sites for hematopoiesis (Kobayashi et al. 2016). Analogous to limbs for walking in mammals, the fins (composed of cartilage and related tissues, including mucous connective tissue) in teleost fish provide them with balance, steering, and protection to aid in swimming (Benjamin et al. 1992).

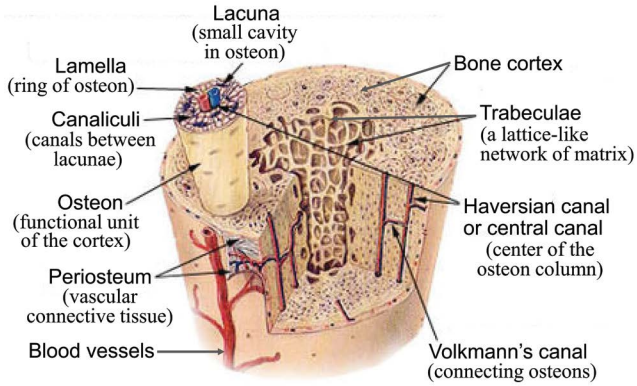


FIGURE 1.27 The bone matrix of vertebrates. The osteon is the functional unit of the bone cortex and is a concentric circle. Each ring of the osteon is made of a calcified collagen matrix called a lamella. There is a small cavity in the osteon called a lacuna, which contains osteocytes. The canals between the lacunae are known as canaliculi, and the osteocytes project into these canals. Running down the center of each osteon column is the central canal, or Haversian canal, which contains blood vessels, nerves, and lymphatic vessels. These vessels and nerves branch off through Volkmann's canals to extend to the periosteum and endosteum. The Volkmann's canals connect the Haversian canals with each other and also with the periosteum. Bone cortex (osteoblasts, osteoclasts, and matrix) and trabeculae (lacunae, osteocytes, red bone marrow, and matrix) compose 80% and 20% of the total bone matrix, respectively. (Adapted from a photo freely available in the public domain from Wikipedia at https://en.wikipedia.org/wiki/Bone)

THE MUSCULAR SYSTEM IN CRUSTACEANS

Like other invertebrates, crustaceans (e.g., shrimp, crabs, and lobsters) have striated and smooth muscles but no inner skeleton. Their movement is not generated by a lever action. However, crustaceans have segmented bodies, jointed legs, and paired limbs (also known as swimmerets) on their abdomen for swimming and/or walking, and many of these animals also have claws for capturing food and defending themselves against predators. Thus, some crustaceans (e.g., shrimp) can swim, whereas others (e.g., lobsters and many crabs) can walk on the ocean floor and on land. Shrimp and other crustaceans have a hard and often transparent exoskeleton (the shell), which is made of chitin and proteins to protect the animals from drying out and provide a hard framework for connection with skeletal muscles. Because of their hard exoskeleton, these animals must molt or shed their old exoskeleton and then produce a new one so that they can grow. This molting period is associated with tremendous stress and survival challenges.

THE RESPIRATORY SYSTEM

O_2 is essential to the life of all animals, but has a low solubility in H_2O. The higher the temperature, the less O_2 is dissolved in H_2O. At standard atmospheric pressure, the solubility of O_2 in H_2O is as follows (mg/L): 20°C, 9.08; 25°C, 8.24; 30°C, 7.54; 37°C, 6.72; and 40°C, 6.41 (EIFAC 1986). Thus, elevations in environmental temperature, which occur seasonally in many regions of the world and also result from global warming, can increase the risk for hypoxia in animals, particularly fish and crustaceans. For comparison, the solubility of CO_2 in H_2O is 23.75 times that of O_2 (Guyton and Hall 2021). Interestingly, at 37°C and standard atmospheric pressure, arterial blood contains about 250 mg O_2/L (i.e., 7.81 mM), which is 39 times the O_2 solubility in H_2O because O_2 has a high affinity for binding oxyhemoglobin [Fe in the +2 redox state (ferrous)], which is found in the erythrocytes of mammals and birds. This supports high rates of oxidative metabolism in animals.

The anatomy of the respiratory system differs markedly among mammals, birds, fish, and crustaceans. However, in all animals, supplying O_2 to and removing CO_2 from the tissues are the two major functions of the respiratory system (Dyce et al. 1996). Secondary functions of the respiratory system include temperature control, the elimination of H_2O, and voice production. In addition, the exhalation of CO_2 helps regulate acid-base balance in the blood. The physiological importance of the respiratory system is epitomized by the very fact that O_2 is vital to animals. An animal may survive for days without H_2O consumption or weeks without food, but its life without O_2 inhalation is measured only in minutes. Mammals, birds, and aquatic animals have evolved complex mechanisms for sensing, adapting to, and surviving environmental hypoxia.

Mammals

In mammals, the respiratory system consists of the nose, the passages [the pharynx, larynx (voice box, a hollow tube), trachea (the hollow tube leading to the lungs), bronchi, and bronchioles], lungs, and diaphragm (a skeletal muscle). The epiglottis in the throat is a layer of elastic cartilage covered with a mucous membrane and is located at the entrance of the larynx, which directs passage from the pharynx to either the trachea or the esophagus (the tube leading to the stomach). Both the trachea and the esophagus are below the larynx and are closely situated in the neck region. When breathing, the epiglottis remains open, allowing air or gas to enter from the pharynx to the larynx and then to the trachea and the lungs. However, when swallowing due to eating, drinking, or saliva flow, the epiglottis moves to cover the larynx, allowing the food or liquid to enter from the pharynx into the esophagus and then to the stomach.

All organs of the respiratory system work together to enable air and gases to get into and out of the lungs (air-filled organs). Specifically, air or gas enters the body through the nose or mouth and, after passing through the larynx, enters the trachea, as described above. The inhaled air or gas is conducted through the trachea and its tubular branches (called bronchi) into the lungs. Within the lungs, the air or gas flows into the smaller branches (bronchioles) of the bronchi and then to the air sacs (the alveoli) surrounded by capillary vessels. In the alveoli, O_2 is exchanged for CO_2 through passive diffusion along concentration gradients between the external gaseous environment and the blood (Dyce et al. 1996). During these processes, O_2 within the air sacs diffuses across the alveolar epithelium into the interstitial space and then into the blood of the capillaries. Within the capillary blood, ~98% of the total O_2 is reversibly bound to hemoglobin contained within erythrocytes for delivery to tissues, and ~2% of the total O_2 is carried as dissolved O_2 in the plasma and erythrocyte H_2O (Guyton and Hall 2021). For comparison, reptiles lack the diaphragm muscle but also use the alveoli of the lungs for gas exchange.

According to the Bohr effect, an increase in dissolved CO_2 in the blood reduces its pH (see below), which decreases the affinity of hemoglobin for O_2 and ultimately causes O_2 to be displaced from hemoglobin into the interstitial space of tissues (Figure 1.28). Meanwhile, in the erythrocytes of the capillary blood, some CO_2 (produced by tissues) reacts directly with the amine radicals of the hemoglobin to form carbaminohemoglobin (CO_2Hb), but most CO_2, along with H_2O, is converted by carbonic anhydrase and also spontaneously into H_2CO_3. The latter is equilibrated with HCO_3^- and H^+. The distribution of CO_2 in the capillary blood is as follows: HCO_3^-, 70%; CO_2Hb, 23%; and CO_2, 7% (Guyton and Hall 2021).

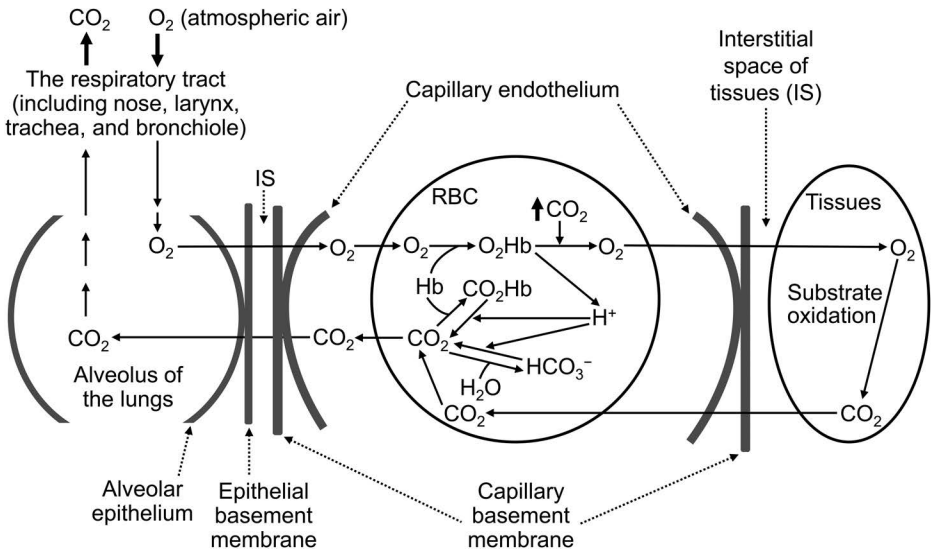


FIGURE 1.28 Roles of the lungs in the respiration of mammals. Ambient air enters the body through the lungs and flows to its air sacs (the alveoli), which are surrounded by blood capillaries. In the alveoli, O_2 is exchanged for blood CO_2 through passive diffusion. According to the Bohr effect, an increase in dissolved CO_2 in the blood reduces its pH, therefore decreasing the affinity of the hemoglobin for O_2 and causing O_2 to be displaced from the hemoglobin into the interstitial space. Thereafter, CO_2 (produced by nutrient oxidation) is released from the capillary blood into the alveoli of the lungs for expiration. IS, interstitial space.

When O_2 binds the hemoglobin, the imidazole group of its histidine residues dissociates more to donate H^+, causing oxygenated hemoglobin to be a stronger acid than the deoxygenated hemoglobin. The more acidic hemoglobin has a lower affinity for CO_2, thereby displacing the CO_2 from the carbaminohemoglobin into the alveoli. The H^+ released from oxygenated hemoglobin reacts with HCO_3^- to form H_2CO_3, which dissociates into CO_2 and H_2O, with CO_2 being released from the capillary blood into the alveoli for expiration. Thus, CO_2 is released from hemoglobin when this protein binds O_2 (the Haldane effect). At the systemic level, the exchange of gases (including O_2) in the lungs is limited by perfusion (i.e., blood flow) and therefore can be modulated by the synthesis of NO from L-arginine in the vasculature.

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

Birds

The respiratory system of birds is among the most efficient respiratory systems in the animal kingdom and is largely similar to that of mammals with the following exceptions (King and Molony 1971). First, birds lack the diaphragm muscle that enables breathing, and instead the expansion of their chest and abdominal cavity generates aspiration into the air sacs. In addition, avian lungs do not inflate and deflate, but their various air sacs are inflated and deflated in sequences. Second, birds have nares but mammals have nostrils; either nares or nostrils can warm air on inhalation and remove moisture on exhalation. Third, birds have nine air sacs located both in front of and behind the lungs, whereas mammals have air sacs within the lungs. Thus, unlike mammals, which perform O_2 and CO_2 exchange in the alveoli, birds have anatomically separate air sacs for gas exchanges between the air capillaries (with a large surface area) and the blood capillaries (connected with the heart). Interestingly, because the lungs of birds are connected to voluminous air sacs by a series of tubes, the total volume of the avian respiratory system is about twice that of mammals of comparable BW. This feature is physiologically important, as birds have higher rates of O_2 consumption at rest than other vertebrates (including mammals) and this rate can increase many times during flight. Fourth, birds inhale and exhale twice for every respiration, whereas mammals inhale and exhale once per respiration. An increase in avian ventilation can result from deeper and more rapid breathing. Finally, the respiratory system of birds is also used for communication through singing songs, with the "voice box" (a membranous structure) located at the lower end of the trachea.

Fish

The respiratory system of fish varies among their diverse species. However, most of them share the same cascade for the transport of gases (including O_2, CO_2, and NH_3): ventilation, gas diffusion, circulation, diffusion to tissues, and cell metabolism. These processes resemble those in mammals and birds, except for the following. First, the gills of fish take up O_2 from the H_2O. O_2-rich H_2O passes through the gill lamellae, where O_2 diffuses into capillaries for binding to oxyhemoglobin (Cech and Brauner 2011). The diffusion of O_2 through cell plasma and basement membranes,

as well as the interstitial spaces of tissues occurs along a concentration gradient. Second, dissolved CO_2 diffuses down its concentration gradient across the gill lamellae into the H_2O. Third, fish excrete ammonia into the surrounding H_2O partly through their gills, thereby sparing the use of energy for converting this nitrogenous metabolite to uric acid, urea, or amino acids. In most fish (e.g., hybrid striped bass, largemouth bass, and zebrafish) that do not contain any lungs, the gills accomplish respiration in these aquatic animals. However, in a few fish (e.g., lungfish) that have both the gills and one or two lungs, these organs carry out gas exchanges. In addition, like amphibians, the skin of fish plays a vital role in gas exchange. Indeed, eels use their skin as a major respiratory surface in addition to their gills.

In teleost fish, the intracellular pH of RBCs is regulated by a plethora of factors, including Cl^-/HCO_3^- and Na^+/H^+ exchanges across the cell membrane, as well as organic phosphate metabolism and cell volume (Nikinmaa 1986). A low pH or elevated CO_2 concentration in the blood reduces not only the affinity of the hemoglobin for O_2 (the Bohr effect) but also the capacity of the hemoglobin to carry O_2 in RBCs (the Root effect), thereby prohibiting complete saturation of the hemoglobin with O_2 even at a high partial O_2 pressure. Interestingly, except for the hemoglobin of a few elasmobranchs (e.g., sharks) that exhibit this characteristic of hemoglobin physiology, the Root effect occurs almost exclusively in teleost fish and is absent from mammals, birds, and crustaceans.

Because neuroepithelial cells are distributed throughout the gills, fish can sense O_2 levels in the H_2O and their blood (Coolidge et al. 2008). These animals can adapt to environmental hypoxia through increases in the lamellar surface areas of the gills, ventilation frequency and volume, the number of erythrocytes, hematocrit and hemoglobin concentrations, and the expression of hypoxia inducible factor 1α. Other adaptations include decreases in heart rate and oxidative metabolism, elevations in glycolysis and lactate production, and the expression of several isoforms of hemoglobin (De Young et al. 1994; Powers 1980). However, like other animals, fish have a limited ability to tolerate hypoxia. Fish reared in a recirculatory aquaculture system do not survive for a prolonged period after the supply of air (O_2) to the water tanks is discontinued (due to either mechanical or electrical outage problems). For example, when air pumps stop working, juvenile hybrid striped bass with BWs of 6.4 and 15 g [reared at the density of 15 fish/tank in 26 L of H_2O with a salinity of 3 ppm and an initial concentration of 8 ppm dissolved O_2 (Jia et al. 2022)] can survive only for 12 and 5 h, respectively.

Crustaceans

Many of the small crustaceans, such as the copepods (1–2 mm long, with a teardrop-shaped body and large antennae) that live in nearly every freshwater and saltwater habitat, have no special respiratory organs. Most copepods also lack gills, and some do not have blood vessels. Interestingly, these animals (being parasites themselves and serving as parasitic hosts) feed directly on the phytoplankton, take up O_2 directly into their bodies, and use maxillary glands as their excretory system. The copepods can also exchange gases through diffusion across their body surface (called the integument).

Like most fish, larger crustaceans (e.g., shrimp, crabs, and lobsters) primarily use gills for respiration. As oxygenated H_2O passes over the gill lamellae, O_2 reversibly binds to the hemocyanins (metalloproteins containing two copper atoms) for transport to tissues via the hemolymph of the circulatory system. Hemocyanin was first discovered in *Octopus vulgaris* by Leon Fredericq in 1878, and the presence of copper in mollusks was first identified by Bartolomeo Bizio in 1833. This protein is composed of at least six subunits, with the molecular weight of each subunit being ~75 kDa. Unlike the hemoglobins in the RBCs of vertebrates, the hemocyanins are not confined to blood cells but are instead suspended in the hemolymph as water-soluble proteins. The oxygenation status of hemocyanins determines their color: the colorless deoxygenated form (Cu^+) and the blue oxygenated form (Cu^{2+}). The hepatopancreas is the site for the synthesis of the hemocyanins in the larger crustaceans.

THE URINARY SYSTEM

Mammals, Birds, and Reptiles

In mammals and birds, the urinary system consists of two kidneys, two ureters, the bladder, and the urethra. Nephrogenesis, which begins at the embryonic stage and is completed at birth, occurs through a series of successive phases: archinephros, pronephros, mesonephros, and metanephros (Li et al. 2020). Mammalian and avian kidneys can regenerate the tubular epithelium of the nephron but cannot form new nephrons during adulthood. In reptiles, there are two pairs of kidneys at birth (the mesonephros and the metanephros). The mesonephros degenerate shortly after birth, but the metanephric kidneys, as in all other amniote vertebrates (including mammals and birds), are retained as the functional kidneys in adults. In contrast to mammals and birds, new nephron formation (nephrogenesis) can occur in adult reptiles.

In mammals, birds, and reptiles, the kidneys are located at the rear of the abdominal cavity and are surrounded by a tough fibrous tissue called the renal capsule (Boron 2004). The kidney is divided into two parts: the outer renal cortex and the inner renal medulla. The kidneys receive blood from a pair of renal arteries, and the blood leaves the kidneys through a pair of renal veins. The rate of blood flow through the kidneys is very high, representing ~ 20% of cardiac output (Dyce et al. 1996).

The functional unit of each kidney is the nephron, which spans across the cortex and medulla (Figure 1.29). The number of nephrons in several species has been determined in some species, such as cattle, 4×10^6; pigs, 1.25×10^6; dogs, 0.415×10^6; and cats, 0.19×10^6 (Reece 1993). The first process in urine formation is glomerular filtration. Filtration takes place when components of blood pass through the glomerular capillary endothelium, the basement membrane, and the Bowman's capsule epithelium into the luminal space of Bowman's capsule in the renal cortex. Glomerular filtrate is an ultrafiltrate of blood and includes H_2O, ions, and small molecules that easily pass through the filtration membrane. However, larger molecules, such as proteins and blood cells, are essentially prevented from passing through the filtration membrane. The amount of filtrate produced every minute (the glomerular filtration rate) is high (e.g., 4 ml/kg BW/min in a 9.5-kg dog; Reece 1993). The second process of urine formation is tubular reabsorption of nutrients and HCO_3^-, which occurs primarily in the proximal tubule (e.g., 65% Na), followed by the loop of Henle (25% Na), the distal tubule (5% Na), and the collecting duct (5% Na). In this process, H_2O, amino acids, glucose, metabolites, HCO_3^-, vitamins, and minerals inside the tubular lumen (glomerular filtrates and derived from the interstitium) pass through the brush border membrane and the basolateral membrane of the tubular epithelial cell via specific transporters into peritubular blood capillaries (Figure 1.30). The remaining portion of the fluid in the tubule is called tubular fluid, which becomes urine. About 99% of the glomerular filtrate is reabsorbed as it passes through the nephron, and the remaining 1% becomes urine. The final process of urine formation is tubular secretion, which involves the transport of some substances (e.g., H^+, minerals, and some waste products) from the peritubular blood capillaries into the interstitial fluid and then to the tubular lumen via tubular epithelial cells (Weiner and Verlander 2011). The urine is collected into the ureters, stored in the bladder, and discharged through the urethra. Note that the basolateral membrane of the renal epithelial cell transports glutamine, ammonia, and urea, but no glutamate, from the interstitium or blood into the cell. Hu et al. (2018) reported that excitatory amino acid transporter (EAAT)-1 is very weakly expressed in the brush-border membrane of the proximal renal tubule, EAAT-2 is absent from the kidneys, and EAAT-3 is highly expressed in the brush-border membrane of the proximal renal tubule but is absent from the basolateral membrane of the whole renal tubule.

In mammals, birds, and reptiles, the primary function of the urinary system is to eliminate H_2O and metabolites (e.g., ammonia, urea, creatinine, nitrite, nitrate, sulfates, and conjugated drugs) from the animal. In healthy animals, the kidneys do not excrete a significant amount of glucose or ketone bodies (Boron 2004). However, with elevated concentrations in blood, they exceed the renal tubular transport capacity and are lost from the body in the urine (e.g., diabetic ketoacidosis). In addition, the kidneys play important roles in regulating (a) whole-body H_2O balance and blood volume, (b) blood pressure, (c) concentrations of electrolytes and metabolites in the blood and urine, and (d) blood pH. The kidneys also participate in interorgan metabolism of amino acids through the endogenous synthesis of arginine from citrulline in adult mammals and glycine production from hydroxyproline (Wu 2022a). Finally, in postnatal mammals and birds, the kidneys also contribute to the regulation of erythropoiesis (the production of erythrocytes) in the bone marrow by releasing a hormone called erythropoietin in response to cellular hypoxia.

Fish

Fish, like amphibians, form the pronephros and the mesonephros during embryonic development, with the latter functioning as the adult kidney (Li et al. 2020). The embryos of these animals usually develop externally and are therefore challenged with environmental pressures to maintain H_2O and salt homeostasis. Thus, the pronephric kidney plays a vital role in osmoregulation during the early stage of development (Davidson 2014). Thereafter, the remnants of their pronephros remain as the most anterior part of the kidney known as the head kidney, and their mesonephros matures to be a secretary and endocrine organ called the hind kidney or adult kidney. Thus, the fish kidney contains two sections: the head kidneys and the hind kidneys, which account for 20% and 80% of the kidney mass, respectively (Martinez 2017).

In contrast to mammals and birds, fish lack bone marrow, lymph nodes, and adrenal glands, but the head kidneys serve as a hematopoietic, lymphoid, and endocrine organ for the production of erythrocytes, molecules involved in innate and adaptive immunities (e.g., cytokines and antibodies), and some hormones (e.g., cortisol, thyroid hormones, and catecholamines). As for the kidneys of other vertebrates, the hind kidney of fish is the primary excretory organ (serving filtration and urine-forming functions), and its functional unit is the nephron (Chambers and Wingert 2020). The hind kidney also plays an important role in osmoregulation in fish. In addition, these aquatic animals use their gills to excrete metabolic products such as ammonia, urea (derived from the hydrolysis of arginine by arginase and the urea cycle), and CO_2. As in reptiles, the mesonephric kidneys of fish continue to generate new nephrons throughout their life, including adulthood when their body mass continues to increase (Davidson 2014).

Crustaceans

Crustaceans have gills in the head to excrete ammonia and other gases such as CO_2 and NO. In addition, these animals possess two different excretory organs: maxillary glands (shell glands) and antennal glands (green glands). These glands open at the base of the maxillae (a pair of mouthparts present) and antennae in the head region, respectively (Walter and Wägele 1990). Both maxillary and antennal glands have the same basic structure (i.e., an end sac and

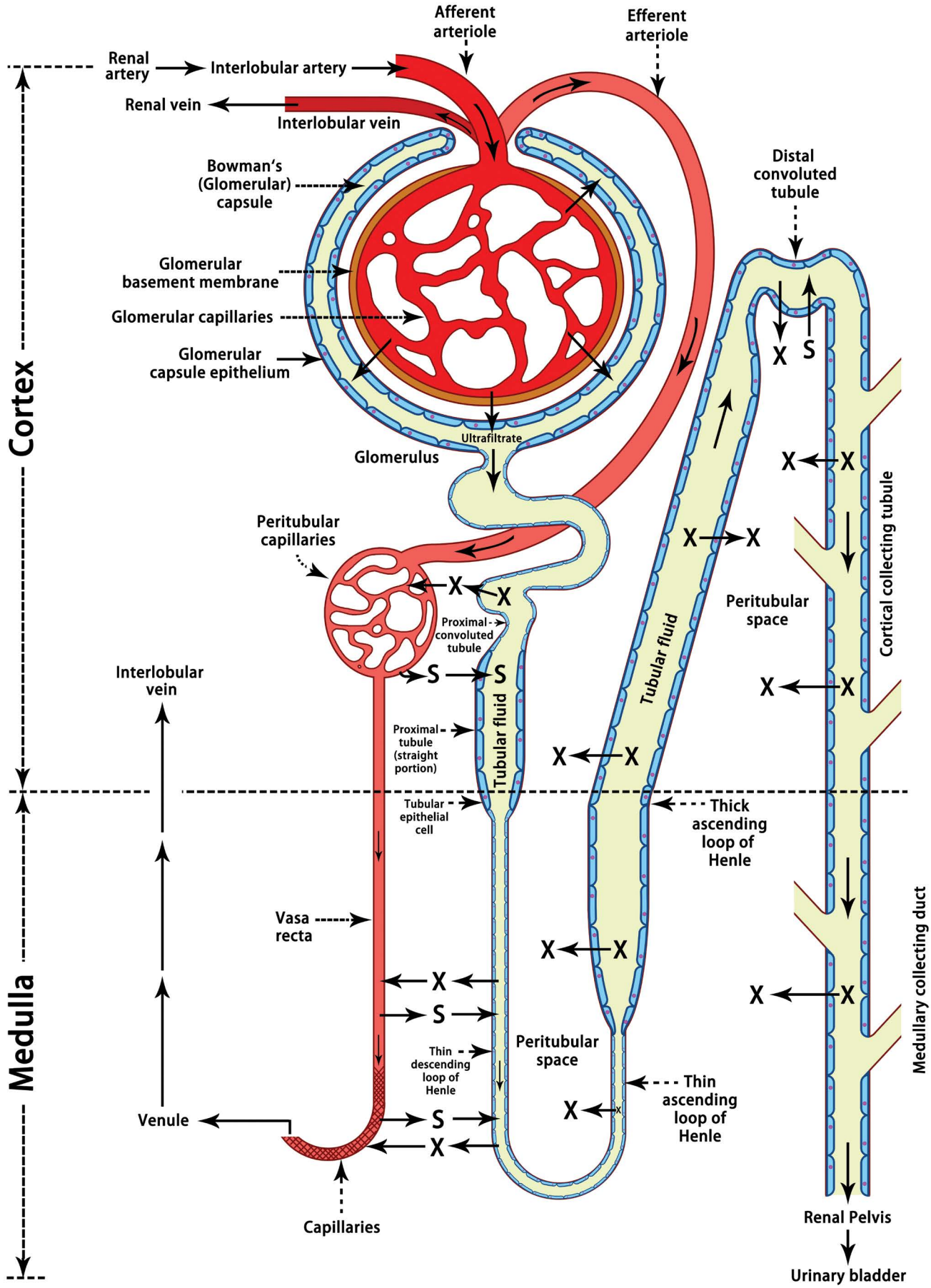

Afferent arteriole
Efferent arteriole
Renal artery
Interlobular artery
Renal vein
Interlobular vein
Bowman's (Glomerular) capsule
Distal convoluted tubule
Glomerular basement membrane
Glomerular capillaries
Glomerular capsule epithelium
Ultrafiltrate
Glomerulus
Cortex
Peritubular capillaries
Proximal convoluted tubule
Cortical collecting tubule
Peritubular space
Tubular fluid
Interlobular vein
Proximal tubule (straight portion)
Tubular epithelial cell
Thick ascending loop of Henle
Vasa recta
Medullary collecting duct
Thin descending loop of Henle
Peritubular space
Thin ascending loop of Henle
Venule
Capillaries
Medulla
Renal Pelvis
Urinary bladder

FIGURE 1.29 The structure of the kidney. The kidney consists of the outer renal cortex and the inner renal medulla. A pair of renal arteries supply blood to the kidneys, and venous blood leaves the kidneys through a pair of renal veins. The functional unit of the kidney is the nephron, which spans the cortex and medulla. Urine formation involves three processes: (a) glomerular filtration of arterial blood, (b) tubular reabsorption of the filtrates into peritubular blood capillaries, and (c) tubular secretion into the interstitial fluid and then to the tubular lumen via tubular epithelial cells. Metabolites are excreted from the body mainly through urine, which is collected into the ureters, stored in the bladder, and discharged through the urethra. The letter "X" represents nutrients being reabsorbed from the renal tubule into the blood in a segment-dependent manner: primarily amino acids, glucose, vitamins, H_2O, NaCl, K^+, and HCO_3^- in the proximal tubule; H_2O in the descending limb; NaCl in the ascending limb of the loop of Henle; H_2O, NaCl, and HCO_3^- in the distal tubule; and NaCl, some urea, and H_2O in the collecting duct. The letter "S" represents substances entering the renal tubule from the blood in a segment-dependent manner: primarily H^+ and NH_3 in the proximal tubule; and H^+ and K^+ in the distal tubule. (Adapted from Dyce et al. 1996.)

a convoluted duct that expands into a bladder before opening to the outside) and play a functional role (e.g., excretion, reabsorption, and osmoregulation) that is analogous to that of the vertebrate kidney. Specifically, the end sac of the maxillary glands allows passive ultrafiltration by hemolymph pressure and the reabsorption of nutrients (e.g., amino acids and glucose) from the primary urine into the hemolymph. The antennal glands are comprised of two anterior parts and a posterior part, with both parts containing the Na^+/K^+-ATPase pump (Tseng et al. 2022). There are three major types of cells in the antennal gland, including coelomic cells, labyrinthine cells, and end-labyrinthine cells. The proximal and distal tubular regions of the antennal glands consist of labyrinthine cells and coelomic cells, whereas the end tubular region of the antennal glands contains all three types of cells, with fewer coelomic cells and more end-labyrinthine cells. The activity of the Na^+/K^+-ATPase pump increases from the proximal to the distal regions of the antennal gland. Thus, the antennal glands play an important role in ion transport for either excretion or reabsorption, depending on physiological and nutritional needs.

THE MALE REPRODUCTIVE SYSTEM

The mammalian male reproductive system consists of (a) two testes (producing sperm and male reproductive hormones), (b) the scrotum (enclosing and protecting the

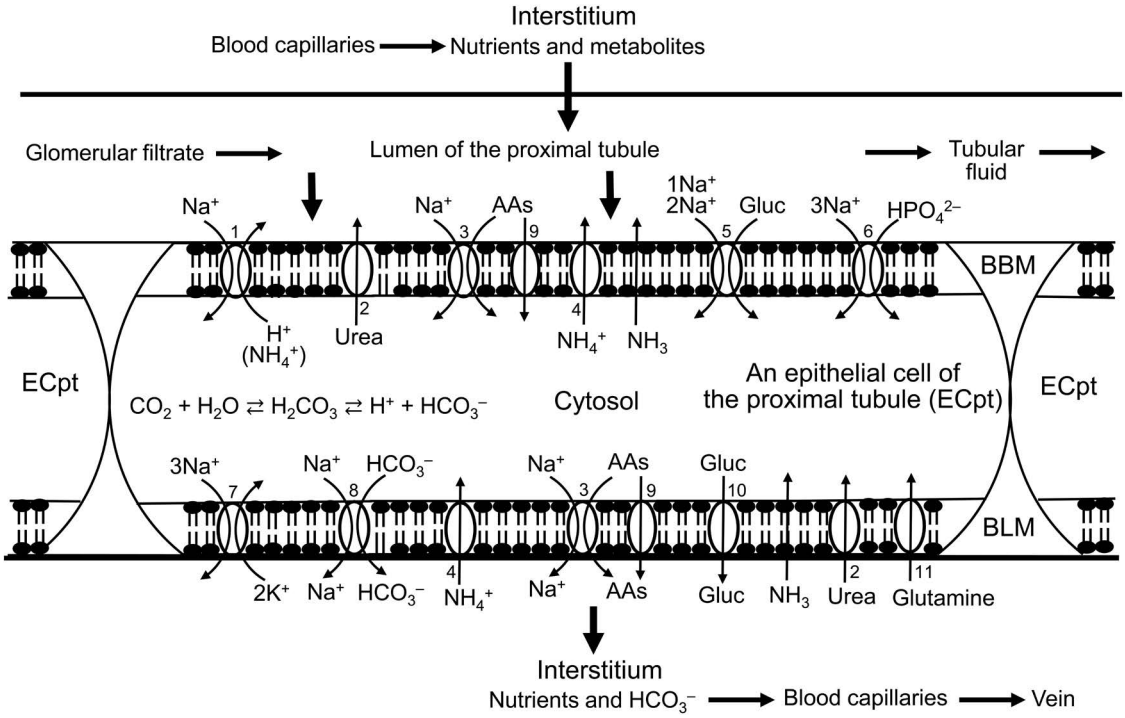


FIGURE 1.30 Transport of sodium, glucose, amino acids, NH_3, and NH_4^+ by proximal renal tubules. Active transport or secondary active transport is indicated by a number: 1, Na^+/H^+ (NH_4^+) exchanger; 2, urea transporters; 3, sodium-dependent amino acid transporters; 4, NH_4^+ transporter; 5, SGLT1 (sodium-glucose linked transporter 1) and SGLT2 (sodium-glucose linked transporter 2); 6, Na^+-phosphate cotransporter; 7, Na^+-K^+-pump; 8, Na^+-bicarbonate cotransporter; 9, sodium-independent amino acid transporters; 10, glucose transporter-2; and 11, glutamine transporters. NH_3 is transported across the cell membrane through simple diffusion. The BLM does not take up glutamate from the interstitium or blood. AAs, amino acids; BBM, the brush-border membrane of the renal epithelial cell; BLM, the basolateral membrane of the renal epithelial cell; Gluc, glucose. (Adapted from Weiner and Verlander 2011; Reece 1993.)

testes), (c) the epididymis (the site for maturation and storage for sperm before they pass into the vas deferens), (d) the vas deferens (the sperm duct), which carries mature sperm from the epididymis to the ejaculatory ducts, (e) the penis (whose erection is dependent on NO-induced vasodilation of blood vessels in the corpora cavernosa), and (f) accessory sex glands (seminal vesicles, the prostate, the Cowper glands, and the urethral glands) that produce fluids to lubricate the reproductive tract and nourish the sperm cells (Dyce et al. 1996). Semen is composed of spermatozoa suspended in fluid from the accessory sex glands called seminal plasma. This fluid has high concentrations of arginine and polyamines that are essential for DNA and protein syntheses. Collectively, these male reproductive organs produce mature sperm and transfer them to the female reproductive tract to enable the fertilization of eggs produced by the ovaries of females.

The male reproductive system in birds is similar to that in mammals (Lake 1971), with the exception of the following aspects: First, the avian testes are located within the body cavity (the anterior abdomen), in contrast to most mammals, whose testes are located outside the abdomen and protected in the scrotum. Second, the ductus epididymis in birds is very short, compared with that in mammals. Third, spermatozoa are stored mainly in the ductus deferens in birds, but in mammals they are stored in the epididymis. Fourth, the accessory sex glands noted previously for mammals are absent in birds. Fifth, after ejaculation, the avian spermatozoa have a longer survival period than their mammalian counterpart. Within the vaginal pouch (uterovaginal gland) and the oviduct, avian sperm cells can remain viable for up to two weeks. These characteristics of the anatomy of the avian male reproductive system may help birds adapt to their behaviors (e.g., long-distance flights) and living environment.

THE FEMALE REPRODUCTIVE SYSTEM

The mammalian female reproductive system consists of the vagina, the uterus, two oviducts, and two ovaries (Dyce et al. 1996). The vagina connects with the cervix (neck portion) of the uterus, while the uterus connects with the oviducts that are in intimate contact with the ovaries. The uterus selectively transports or synthesizes and secretes substances into the uterine lumen that are collectively known as histotroph. Its components include nutrient-transport proteins, ions, mitogens, cytokines, interleukins, enzymes, hormones, growth factors, proteases and protease inhibitors, amino acids and derivatives, fatty acids and derivatives, glucose, fructose, vitamins, and minerals (Bazer et al. 2015). Cervical and uterine secretions help regulate the passage of sperm from the vagina to the oviduct, where the fertilization of the ovum occurs.

Before fertilization, the ovum (egg) is expelled from the ovary, received by the fimbria of the oviduct (fallopian tube), and moved to the mid-portion of the oviduct, where fertilization normally takes place. At the beginning of gestation, histotroph in the uterine lumen provides an environment conducive to supporting the survival, growth, and development of embryos. As the conceptus (embryo/fetus and associated extraembryonic membranes) increases in size, the placenta (amnion, allantois, and yolk sac) develops as a means of meeting the increased need for nutrients (Bazer et al. 2014). The placenta is the organ that transports nutrients, respiratory gases, and the products of metabolism between the maternal and fetal circulations (Dyce et al. 1996). The developing fetus is surrounded by amniotic fluid, which buoys the fetus and allows it to develop symmetrically. Allantoic fluid accumulates due to the transport of H_2O from the mother to the conceptus, and the allantoic sac is also connected to the fetal bladder via the urachus and the placental vasculature that resides within the allantoic membrane. Therefore, in the fetus, nutrients cleared via the fetal kidneys into the bladder can reach the allantoic sac and then reenter the fetal-placental circulation across the allantoic epithelium. The allantoic sac was traditionally considered a reservoir for fetal wastes, but it is now known to be a reservoir of nutrients (e.g., the arginine family of amino acids) and to play an important role in nutrient metabolism (Wu et al. 2006). There is much evidence showing that the successful maintenance of pregnancy requires (a) maintenance of a functional corpus luteum in the ovary to produce progesterone (a steroid hormone) early in gestation and (b) interactions between the conceptus and various uterine cells (e.g., uterine luminal, superficial and glandular epithelia, and stromal cells; Bazer et al. 2010). Coordination among these cells ensures adequate provision of nutrients and O_2 from mother to fetus, as well as conceptus survival, growth, and development.

The avian female reproductive system consists of the vagina, uterus, oviduct, and ovary (Dyce et al. 1996). Birds have a very short uterus known as the shell gland or pouch, in which the egg (the largest cell ever known in the animal kingdom) produced by the ovary is retained during the entire period of shell formation. Interestingly, the female birds of most species (including domestic fowl) have only one functional ovary (the left one), which is covered by the fimbria of the oviduct. The right ovary is present at the embryonic stage, but is vestigial thereafter. The mature ovarian follicle, containing all deposited yolk, ruptures to release the ovum, which falls into the trumpet-like fimbria at the upper end of the oviduct. The ovum remains in the oviduct for 15–20 min, which is the only time it can be fertilized, and then traverses to the magnum, where the ovum remains for ~4 h to acquire the deposition of albumen (egg white) around the yolk (Gilbert 1971). Next, the ovum passes to the isthmus and remains there for 15 min to acquire the shell membrane to protect the embryo. Then, the ovum migrates further to the uterus to acquire the shell and shell pigments during a period of 18–20 h, and finally is expelled via the vagina. The production effort of a hen is remarkable, as it produces many eggs (e.g., 275 eggs each weighing 58 g) per year. In contrast to mammals, avian fertilized eggs are hatched outside the mother. In birds, as

in most reptiles, the embryo and its extraembryonic membranes develop within a shelled egg.

THE ENDOCRINE SYSTEM

The endocrine system consists of glands and organs that synthesize and secrete hormones into the body to be carried towards target organs where they will exert their effects. Endocrine glands in animals include the hypothalamus, pituitary gland (anterior lobe and posterior lobe), pineal gland, adrenal glands (cortex and medulla), thyroid gland, parathyroid glands, pancreas, gonads (testes and ovaries), placenta, GIT, and WAT (Devlin 2011). Hormones (e.g., insulin and glucagon) were traditionally defined as substances that are secreted by specialized endocrine cells and carried through the circulation to target cells at distant body sites (Dyce et al. 1996). Hormones are now more broadly defined as chemical messengers that elicit biological effects in the body (Cooper and Hausman 2016). Thus, paracrine hormones (e.g., fibroblast growth factors and transforming growth factor-β) are substances that are produced and released by cells and then act on neighboring cells. Autocrine hormones (e.g., interleukin-1 and vascular endothelial growth factor) are molecules produced by the cell that bind to plasma-membrane receptors on the same cell.

Let us use secretin (a 27-amino acid polypeptide that was the first hormone to be discovered in 1902) as an example to illustrate how a hormone acts. It is produced and released by the S cells of the duodenum and jejunum, and regulates secretions in the stomach, pancreas, and liver. In the stomach, secretin decreases HCl production by (a) inhibiting the secretion of gastrin by the parietal cells of the stomach, (b) increasing the secretion of HCO_3^- (removing gastric H^+) by the centroacinar cells and the intercalated ducts of the pancreas and from the bile, and (c) delaying gastric emptying into the duodenum (as a feedback inhibition mechanism). In addition, secretin enhances the production of bile by the liver. Furthermore, in coordination with CCK, secretin stimulates the gallbladder to contract to release its stored bile into the lumen of the duodenum. In all the target tissues, secretin binds to its receptors on the plasma membrane, leading to the activation of adenylyl cyclase for the generation of cyclic adenosine monophosphate (cAMP; a cellular secondary messenger) from ATP. Thus, secretin plays an important role in the digestion of nutrients, particularly lipids, proteins, and vitamins.

Understanding the endocrine system is important for studying nutrition because animal metabolism is regulated by hormones, whose concentrations in plasma are affected by the animal's species, sex, developmental stage, diet, disease, toxins, and housing conditions. In general, the hypothalamus secretes releasing or release-inhibiting hormones that control the activity of the anterior pituitary, whose trophic hormones act on target glands or tissues to modulate the release of the final, active hormones in the cascade (Guyton and Hall 2021). For comparison, hormones of the posterior pituitary are synthesized by the hypothalamus and transported to the posterior pituitary for storage prior to their release. Hormones produced by the various endocrine glands and cell types are summarized in Table 1.11. In young mammals such as pigs, physiological concentrations of arginine stimulate the release of insulin, growth hormone, and insulin-like growth factor-I by their respective endocrine glands, while reducing the secretion of glucocorticoids by the adrenal gland (Long et al. 2025).

THE IMMUNE SYSTEM

Animals (particularly aquatic animals) are constantly exposed to various pathogens in the environment. The immune system differs among animal species, but functions to kill pathogenic microorganisms. At birth, the adaptive immune system is largely present but functionally immature (Mowat 1987). The environment, including intestinal microbes, plays an important role in the maturation of the adaptive immune system. The health of all animals critically depends on the dietary intake of energy and nutrients (Li and Wu 2022a,b). Glutamine and glucose are major metabolic fuels for lymphocytes, macrophages, and other cells of the immune system, supporting their integrity and function. Adequate nutrition is essential for the proper development and function of the immune system. Key nutrients include amino acids (particularly arginine, cysteine, glutamate, glutamine, glycine, methionine, proline, taurine, and tryptophan); fatty acids (particularly ω3 and ω6 polyunsaturated fatty acids); minerals (particularly copper, iron, manganese, selenium, and zinc); and vitamins (particularly vitamin A, vitamin C, and vitamin D).

Acquired (Adaptive) and Innate Immune Systems in Mammals and Birds

In mammals and birds, the immune system has two components: the innate immune system (nonspecific) and the adaptive immune system (acquired and specific), which act together to prevent and/or respond to the invasion of various pathogens (Figure 1.31). The gut is the largest lymphoid organ in the body. In mammals, the red bone marrow and the thymus are the primary lymphoid organs, where lymphocytes are produced. By contrast, in birds (e.g., chickens), the bursa of Fabricius, which is connected to the cloaca and the intestinal system, is a primary lymphoid organ and a major site for environmental antigens to stimulate the immune system (Ifrah et al. 2017).

The innate immune system consists of several integral components: (a) physical barriers (e.g., skin, epithelial cell layer in respiratory tract, and GIT); (b) mononuclear phagocytes (e.g., monocytes and macrophages), dendritic cells, polymorphonuclear granulocytes (e.g., neutrophils, eosinophils, and basophils), mast cells, natural killer (NK) cells, and platelets; (c) humoral factors, including collectins, complement proteins, lysozymes, C-reactive proteins, and interferons; (d) antimicrobial peptides in the mucosa

TABLE 1.11
Secretion of Hormones and Their Major Functions in Animals

Endocrine Gland or Exocrine Organ	Hormone	Major Functions
Hypothalamus	Thyrotropin-releasing hormone	↑ Thyroid-stimulating hormone release from anterior pituitary
	Corticotropin-releasing hormone	↑ Release of adrenocorticotropic hormone from anterior pituitary
	GH-releasing hormone	↑ Release of growth hormone from anterior pituitary
	Gn-releasing hormone	↑ Release of FSH and LH from anterior pituitary
	Somatostatin	↓ Secretion of growth hormone and thyroid-stimulating hormone
	Prolactin-releasing hormone	↑ Lactotropes to release prolactin from the anterior pituitary gland
	Dopamine	↓ Prolactin release from the anterior pituitary gland
Anterior pituitary	Growth hormone	↑ Protein synthesis and whole-body lean tissue growth; ↓ WAT
	Prolactin	↑ Milk synthesis (including protein and fat synthesis)
	Thyroid-stimulating hormone	↑ Thyroxine (T_4) and triiodothyronine (T3) synthesis by thyroid gland
	Luteinizing hormone (LH)	↑ Ovulation and development of the corpus luteum in females ↑ Testosterone production by Leydig cells in males; LH acts synergistically with FSH
	FSH	Regulates growth and development of reproductive organs and gametes in males and females; acts synergistically with LH
	Adrenocorticotropic hormone	↑ Production and release of glucocorticoids by adrenal cortex
	ß-Lipotropin	↑ Production of melanin by melanocytes in skin and hair
	β-Endorphin	Acts on cells and neurons to produce analgesic effects
	MSH	↑ Production of melanin by melanocytes in skin and hair; actions in the brain regulate food intake and sexual arousal
	Activin (from gonadotrope cells)	↑ FSH secretion; Has no effect on LH secretion
Posterior pituitary	Vasopressin (ADH)*	↑ Water reabsorption in renal tubules and arterial blood pressure
	Oxytocin*	↑ Contractions of uterus; Causes milk let-down from mammary glands
Pineal	Melatonin	Controls circadian rhythms; an antioxidant
	Serotonin	Neurotransmitter, improvements of mood and behavior, regulation of gut motility and food intake, and anti-inflammatory response
Adrenal cortex	Glucocorticoids[a]	Regulate glucose, protein and lipid metabolism, and gene expression; Play an important role in anti-inflammatory reactions
	Aldosterone[b]	↑ Renal reabsorption of sodium and water
	Adrenal androgens[c]	Regulate the development of sexual organs and characteristics in both males and females
Adrenal medulla	Dopamine	Affects emotion, locomotor activity, and neuroendocrine secretion
Adrenal medulla and NAN in brain	Epinephrine and NEP	Function as hormones to enhance glucose metabolism, heart rate, muscle strength, and blood pressure, and as neurotransmitters in brain
Kidneys	Erythropoietin	↑ Hemoglobin synthesis and bone marrow RBC differentiation
	Renin	Acts as an enzyme that converts angiotensinogen to angiotensin I
Heart atria	Atrial natriuretic factor	↓ Renin secretion and aldosterone release; regulates blood pressure
Thyroid gland	T_3 and T_4	↑ Whole-body energy metabolism; regulate fetal growth and brain development
	Calcitonin	↑ Calcium deposition in bone; ↓ blood calcium concentration
Parathyroid gland	Parathyroid hormone	↑ Renal 1α-hydroxylase activity and blood calcium concentration
Thymus	Thymopoietin (α-thymosin)	↑ T-lymphocyte development; stimulates phagocytes
Pancreas	Insulin (β cells)	↑ Glucose oxidation, protein synthesis, fatty acid synthesis, triacyl- glycerol synthesis and storage, and glycogen synthesis
	Glucagon (α cells)	↑ Glycogen breakdown, glucose synthesis, and lipolysis
	Somatostatin (D cells or δ cells)	↓ Secretion of insulin and glucagon in pancreas (a paracrine effect)
	Pancreatic polypeptide (F-cells)	Regulates pancreatic secretory activity (both endocrine and exocrine); Serves as an antagonist of cholecystokinin; Reduces food intake
Liver	Insulin-like growth factor-I	↑ Muscle protein synthesis; anabolism
Gastrointestinal tract (GIT)	Various hormones (including ghrelin, secretin, and serotonin[d]	Regulate pancreatic secretion, as well as gastrointestinal motility, secretion, and function; modulates appetite
WAT	Various hormones[e]	Regulate fat and glucose metabolism, inflammation, and food intake
Various organs	Prostaglandins	Regulate vasodilation, metabolism, inflammation, and reproduction
Embryo	Chorionic gonadotropin[f]	Pregnancy recognition in some species
Placenta[g]	Placental lactogen[h]	↑ Mammary gland growth and development; acts like GH
Testes	Testosterone (sex hormone)	↑ Development of male reproductive organs; ↑ spermatogenesis; ↑ protein synthesis in skeletal muscle

(*Continued*)

TABLE 1.11 (*Continued*)
Secretion of Hormones and Their Major Functions in Animals

Endocrine Gland or Exocrine Organ	Hormone	Major Functions
Testes	Inhibin (from Sertoli cells)	↓ FSH secretion by antagonizing activin
Ovary	Progesterone (corpus luteum)	Required for embryogenesis and maintenance of pregnancy; stimulates DNA and protein synthesis, as well as growth of the uterine lining ↓ Myometrial contractions; Maintain uterine quiescence
	17-ß-Estradiol (sex hormone)	↑ Development of female reproductive organs and protein synthesis; regulates the estrus and mammary gland development
	Relaxin	↓ Myometrial contractions; maintains uterine quiescence
	Inhibin (from granulosa cells)	↓ FSH secretion by antagonizing activin
	Follistatin	↓ FSH secretion by acting as an activin-binding protein
Various organs[i]	1,25-Dihydroxycholecalciferol	↑ Intestinal absorption of calcium and phosphorus

Source: Cooper and Hausman (2016) and Devlin (2011).

[a] Cortisol is the predominant glucocorticoid in humans and pigs, but corticosterone is the principal glucocorticoid in rats and chickens that lack 11ß-hydroxysteroid dehydrogenase 2 (11ß-HSD2). 11ß-HSD2 converts hormonally active cortisol to inactive cortisone.

[b] An example of mineralocorticoids.

[c] Including dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate, and androstenedione.

[d] Including cholecystokinin (CCK; stimulating gallbladder contraction and release of pancreatic enzymes), gastrin (stimulating HCl secretion by parietal cells), ghrelin, glucagon-like peptide-I (GLP-1; increasing insulin release and decreasing glucagon release), glucose-dependent insulinotropic polypeptide (GIP; also known as gastric inhibitory peptide; acting on pancreatic β-cells to release insulin), and motilin, secretin (stimulating pancreatic acinar cells to release bicarbonate plus water and inhibiting gastric HCl production), serotonin, somatostatin, and substance P (a vasodilator).

[e] Hormones released from white adipose tissue include leptin, adiponectin, resistin, and adipokines.

[f] Present in some species (e.g., horse and humans) but absent in others (e.g., pigs and ruminants).

[g] Placenta is also a source of progesterone and estrogens (estradiol, estrone, and estriol).

[h] Absent in pigs and rabbits.

[i] Predominantly in the kidneys and also in other tissues, such as the bone, placenta and skin.

Note: ADH, antidiuretic hormone; FSH, follicle-stimulating hormone; GH, growth hormone; Gn, gonadotropin; LH, luteinizing hormone; MSH, melanocyte-stimulating hormone; NAN, noradrenergic neurons; NEP, norepinephrine; RBC, red blood cells; WAT, white adipose tissue; ↑, Enhance; ↓, Inhibit; * synthesized in the hypothalamus and stored in the posterior pituitary gland.

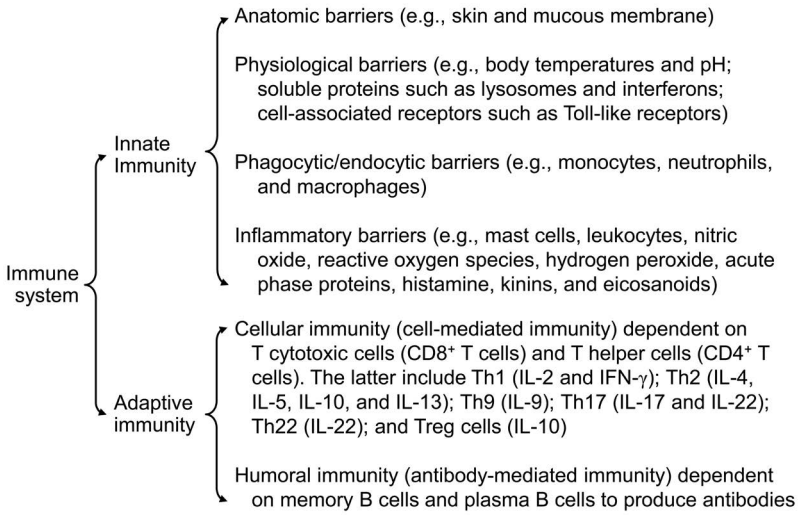


FIGURE 1.31 Immune systems in animals. Mammals, birds, and fish are protected from various pathogens by both innate (natural, nonspecific) and acquired (adaptive, specific) immune systems, which are highly interrelated through various kinds of T- and B-lymphocytes (also called T- and B-cells), as well as cytokines (proteins) and signaling molecules. The innate immune system can rapidly respond to invading microbes, but its major disadvantages include non-specificity and a lack of memory effect. When the invading pathogens are not fully cleared by the innate immune system over a short period of time, the adaptive immune system is activated within days to destroy the infectious agents by producing (a) specific antibodies (humoral immunity) to neutralize pathogenic microorganisms (including viruses) or toxins and (b) cytokines (e.g., interferon-γ) and other cytotoxic proteins by T-lymphocytes (e.g., cytotoxic T-cells; cell-mediated immunity).

and lumen of the small intestine; and (e) neutrophil extracellular traps, comprised of DNA and proteins as major structural components (Iyer et al. 2015; Li et al. 2007). Cell-mediated immune responses, which are directed primarily at intracellular pathogens, involve the destruction of infected cells by cytotoxic T-lymphocytes or the destruction of intracellular pathogens by macrophages activated by helper-1 T-lymphocytes. NO, H_2O_2 and superoxide anion (NADPH-dependent respiratory burst), taurine chloramine, and related oxidants mediate the killing of pathogens by the phagocytes (Li and Wu 2022a). The innate immune system can rapidly respond to invading microbes and is the first line of defense against infections. However, the major disadvantages of this immune system include the lack of both specificity and memory.

The adaptive immune system consists of T-lymphocytes, B-lymphocytes, and humoral factors. Production of antibodies by B-lymphocytes is essential to humoral immunity, which is directed principally at extracellular pathogens. The antibodies act by neutralizing microorganisms (including bacteria and viruses) or toxins by (a) binding to them, (b) activating complement proteins in plasma for the destruction of bacteria by phagocytes, (c) immobilizing bacteria, or (d) opsonizing various pathogens (coating pathogens with opsonins that act as tags to help phagocytes recognize and engulf the pathogens). Host cells infected with pathogens are generally cleared by cytotoxic T-lymphocytes after peptide fragments of pathogens are presented by the major histocompatibility complex on the host cell surface and recognized by T-lymphocytes. In mammals, the red bone marrow is primarily responsible for hematopoiesis and lymphopoiesis, while the thymus is required for T-lymphocyte development. As noted previously, the bursa is the primary lymphoid organ in birds that generates new lymphocytes. Secondary lymphoid organs include the spleen, lymph nodes, and mucosa-associated lymphoid tissues in the gastrointestinal, respiratory, and reproductive tracts. In contrast to the innate immune system, the acquired immune response is highly specific because each lymphocyte only carries surface receptors for a single antigen. In addition, the adaptive immune system becomes effective over several days after initial stimulation and possesses immunological memory through the production of specific antibodies by B-lymphocytes.

Innate and acquired immune systems interact to fight infections. As noted previously, extracellular pathogens are neutralized effectively by antibodies, whereas intracellular pathogens (e.g., viruses and certain bacteria) in infected host cells are generally cleared by cytotoxic T-lymphocytes. When pathogens escape humoral immunity, they are targeted by cell-mediated immunity that involves cytokines [e.g., interferon-γ (IFNγ) and interleukins (IL)] and other cytotoxic proteins produced by T-lymphocytes. In addition to their direct roles in cell-mediated immunity, T-lymphocytes also indirectly contribute to humoral immunity. For example, helper-1 T-lymphocytes induce B-lymphocytes to produce strongly opsonizing antibodies called immunoglobulins (Ig). Moreover, helper-2 T-lymphocytes initiate the humoral response by activating naive B cells to secrete IgM and also induce the production of other antibodies [including IgG1 and IgG3 (mouse), IgG2 and IgG4 (human), as well as IgA and IgE (both mouse and human)] (Chaplin 2010; Nicholson 2016). The innate and acquired immune systems are regulated by an interactive network of chemical communications, which includes the synthesis of antigen-presenting machinery, immunoglobulins, and cytokines (Calder 2006). Both immune systems are highly dependent upon the adequate availability of amino acids for the synthesis of these proteins and polypeptides, and other molecules (e.g., NO, superoxide, hydrogen peroxide, histamine, glutathione, and anthranilic acid), which are of enormous biological importance. Amino acids affect immune responses either directly or indirectly through their metabolites (Wu 2022a). Furthermore, fatty acids, minerals (e.g., iron, copper, zinc, magnesium, and manganese), and vitamins (e.g., vitamins A, E, and C) also play an important role in regulating the production of immunomodulatory substances. Although the immune system is vital to health, it can be dysfunctional under certain conditions (e.g., insulin-dependent diabetes mellitus, rheumatoid arthritis, and asthma), resulting in the development of autoimmune and hypersensitivity diseases. Nutrients (e.g., glycine, tryptophan, and polyunsaturated fatty acids) can be beneficial in preventing and treating immune-related disorders (Li et al. 2007, 2016).

Acquired (Adaptive) and Innate Immune Systems in Fish

Teleost fish have both innate and adaptive (acquired) immune systems, as seen in mammals (Sunyer 2013). Evolutionarily, fish lie in an intermediate step between species with only innate immunity (invertebrates) and species with a fully developed acquired immunity (e.g., mammals). In contrast to mammals, the thymus and the head kidney are the primary lymphoid organs in fish, where the spleen and mucosa-associated lymphoid tissues are secondary lymphoid organs. The innate immune system of teleost fish consists of (a) physical barriers (e.g., skin, the endothelial cell layer in the respiratory tract, and the GIT), mononuclear phagocytes (e.g., monocytes and macrophages that kill ingested pathogens via NO production and respiratory burst), dendritic cells, polymorphonuclear granulocytes (e.g., neutrophils, eosinophils, and basophils), mast cells, and NK cells; and (b) humoral factors, including collections, complements, lysozymes, C-reactive proteins, cytokines; mucins secreted by goblet cells (mucin 2) and absorptive epithelial cells of the intestine (enterocytes, mucin 1), antimicrobial peptides, and alkaline phosphatase released by enterocytes. The mononuclear phagocytes, dendritic cells, and polymorphonuclear granulocytes are in the myeloid lineage, whereas T-lymphocytes, B-lymphocytes, and NK cells are in the lymphoid lineage. The physical barriers and the cellular responses of the innate system provide the

first line of defense against infections. In fish, as in mammals, when the evading pathogens are not fully cleared by the innate immune system over a short period, the adaptive immune system is activated within days to destroy the infectious agents.

The adaptive immune system consists of T-lymphocytes, B-lymphocytes, and humoral factors. Upon activation by pathogens via pathogen presentation, naïve T-lymphocytes differentiate into either helper or cytotoxic effector T-lymphocytes. Helper T-lymphocytes activate other cells of the adaptive system (e.g., B-lymphocytes and macrophages) by secreting cytokines to initiate responses such as antibody-dependent humoral immunity. In fish, as in mammals, cytotoxic T-lymphocytes kill infected cells by releasing two major proteins: perforin and granzymes. Perforin (the pore-forming protein) punches holes in the target-cell membrane, through which the granzymes can enter target cells to induce apoptosis. In fish, as in mammals, the adaptive system recognizes foreign pathogens through B-lymphocyte and T-lymphocyte receptors (Reyes-Cerpa et al. 2012). Upon activation by cytokines released from T-lymphocytes, B-lymphocytes differentiate into plasma cells that produce and release antibodies for humoral immunity. In teleost fish, IgM is generally the primary immunoglobulin that elicits a specific and highly effective humoral response against extracellular pathogens, but IgD and IgT (also known as IgZ) may also be present to play a role in immunity.

In fish, as in mammals, there is intimate interplay between the innate and adaptive immune systems through cytokines and signaling molecules produced by a variety of immunocytes (including macrophages, lymphocytes, granulocytes, and dendritic cells) and epithelial cells upon activation by various pathogens. In fish, the cytokines are classified generally as pro-inflammatory [e.g., tumor necrosis factor (TNF)-α, the IL family of proteins (e.g., IL-1α, IL-1β, IL-6, IL-11, IL-12, IL17, and IL-18)]; regulatory (e.g., IL-2, IL-4, IL-7, IL-15, and IL-21); anti-inflammatory [e.g., IFNα/β, IFNγ, IL-10, IL-20, and transforming growth factor-β (TGF-β)]; and chemokines [e.g., monocyte chemotactic protein-1, IL-8, and cytokeratin-1 (Reyes-Cerpa et al. 2012)]. These molecules modulate immune responses through autocrine or paracrine actions upon binding to their receptors on the cell membrane, thereby guiding the induction of the innate response and the migration of immunocytes to infected tissues, as well as the formation of cytotoxic T-lymphocytes, the activation of B-lymphocytes, and the production of antibodies (Reyes-Cerpa et al. 2012). In contrast to studies with mammals, tools for immunological research in fish are limited (Salinas 2015).

Most pathogens of vertebrates can breach a mucosal barrier (e.g., gastrointestinal, respiratory, and reproductive) to cause disease, making mucosal immunity of crucial importance (Sunnier 2013). In contrast to mammals, teleost fish do not possess Peyer's patches or lymph nodes in the intestine (Salinas 2015). However, these fish do have active and functional mucosa-associated lymphoid tissues, which include the gut-associated lymphoid tissue, skin-associated lymphoid tissue, gill-associated lymphoid tissue, and nasopharynx-associated lymphoid tissue (Gomez et al. 2013). In teleost, the gut-associated lymphoid tissue consists of (a) intraepithelial lymphocytes located among intestinal epithelial cells and (b) lamina propria leukocytes located beneath the epithelium (Salinas 2015). Intraepithelial lymphocytes are mostly T-lymphocytes (primarily γδT-lymphocytes and $CD8^+$ cytotoxic T-lymphocytes, but also some $CD4^+$ helper T-lymphocytes) and, to a much lesser extent, B-lymphocytes (4–5%). The $CD8^+$ T-lymphocytes remove injured enterocytes to maintain the integrity of the mucosal epithelium. Lamina propria leukocytes include a variety of immune cells, such as macrophages, granulocytes, lymphocytes (e.g., with the main $CD4^+$ subsets being Th1, Th2, Th17, and Tregs), plasma cells, and NK cells (Salinas and Parra 2015). Macrophages produce superoxide anion (O_2^- through respiratory burst) and NO (through NO synthase) to kill pathogens, whereas B-lymphocytes (primarily IgD^+/IgM^+ B-lymphocytes), plasma cells, and Ig (e.g., IgM and IgT) defend the gut mucosa from infections, in part via the immune exclusion of luminal bacteria (Gomez et al. 2013). Research on the gut-associated lymphoid tissue of teleost is in its infancy, and therefore this line of research is critical for the development of oral vaccines and the improvement of intestinal health (Parra et al. 2015).

The Innate Immune System in Crustaceans

Aquatic crustaceans are highly susceptible to infections due to their underdeveloped immune responses. Globally, the shellfish industry is challenged by common pathogens, including viruses (e.g., the white spot syndrome virus and the infectious hypodermal and hematopoietic necrosis virus), Gram-negative bacteria (e.g., *Vibrio harveyi*, *Vibrio penaecidia*, and *opportunistic Vibrios*), and protozoa [e.g., *Hematodinium* spp. (species plural, meaning more than one species); Hauton 1992]. In some countries, such as the United States and Canada, other pathogens for crustaceans include the Gram-positive bacterium *Aerococcus viridans var homari* (that infect lobsters).

The innate immune system is primarily responsible for defense against pathogens in crustaceans and can be triggered by substances, including bacterial lipopolysaccharides and peptidoglycans, as well as fungal β-1,3-glucans. Among this large, diverse arthropod taxon of animals, only penaeid prawns (also known as penaeid shrimp) and shrimp are known to possess lymphoid organs. All crustaceans studied to date lack the adaptive immune system. Rather, the hemolymph contains hemocytes and amebocytes, both of which have important roles in innate immune responses. Interestingly, results of recent studies revealed the presence of innate immune memory or trained immunity in animals, including crustaceans (Melillo et al. 2018; Sherwood et al. 2023). Namely, cells, such as those of the myeloid lineage (e.g., monocytes/macrophages and granulocytes) and the lymphoid lineage (e.g., various types of

T-cells) in animals, have an ability to retain the memory of prior pathogen exposure and elicit a broad-spectrum or specific response (e.g., phagocytosis, respiratory burst, or the production of antimicrobial peptides) to a subsequent infection. The underlying mechanisms involve epigenetic modifications to re-program gene expression patterns and metabolism. The innate immune memory is crucial for host defense against pathogens in invertebrates and is also functional in vertebrates.

Hemocytes (both circulating within the haemocoel and embedded within tissues) are phagocytes in invertebrates. These cells also secrete antimicrobial peptides (50–70 amino acid residues with characteristic amino-terminal cysteine rich and carboxy-terminal proline rich domains) and zymogen prophenoloxidase. After zymogen prophenoloxidase undergoes limited proteolysis by serine proteinase of the prophenoloxidase activating system, activated phenoloxidase catalyzes the oxidation of diphenols to quinones for the formation of melanin and its intermediates, which are fungitoxic and fungistatic. In addition, granular hemocytes release peroxinectin (a ~76 kDa cell adhesion molecule), which acts as both an opsonin and a peroxidase. Furthermore, killers of pathogens in crustaceans also include H_2O_2, superoxide anion, and NO (produced by inducible NO synthase). Amebocytes in the hemolymph have a role in the immune system by attaching to bacteria, resulting in the formation of a viscous gel that prevents the bacteria from invading tissues. Finally, as in fish, gut immunity occurs in crustaceans to protect their midgut and gills from infections.

THE INTEGUMENTARY SYSTEM

The integumentary system comprises the skin (the soft outer covering) and its associated structures [including hair (in mammals), scales, feathers, hooves, nails, nerve receptors, and exocrine glands] (Dyce et al. 1996). The skin is also considered a sense organ because nerve endings in the skin, as do those in other parts of the body, transmit sensations to the brain. Hair starts its growth at the bottom of the hair follicle in the skin and then grows out of the skin through its surface. Thus, the integumentary system protects the body from damage, infection, and loss of internal H_2O while contributing to thermoregulation, as well as chemical and physical sensing. Healthy skin and hair should be smooth, elastic, and normally colored. Unhealthy skin and hair result in infections, itchiness, irritation, discomfort, and alterations in the behavior of animals. The health and well-being of animals is critically dependent on the integrity and health of their skin and hair.

The Skin

The skin is a large and complex sense organ in land animals and accounts for 12–24% of BW, depending on species (Connolly and Wu 2024). It consists of three layers: the epidermis, dermis, and hypodermis (Figure 1.32). There are different cell types in the different layers of the skin, such as melanocytes, keratinocytes, and fibroblasts, as well as connective tissue, adipose tissue, nerves, blood vessels (veins and arteries), and lymphatic vessels.

The epidermis is the outermost layer (the surface) of the skin and is an epithelial tissue. The epidermis is composed of 4–5 sublayers of epithelial cells, depending on its location in the body. A layer of water-insoluble lipids covers the epidermis. These lipids consist mainly of TAGs, wax esters, squalene, and LCFAs and, to a much lesser extent, cholesterol, cholesterol esters, and diglycerides. The epidermis is made up of 95% keratinocytes and 5% other cells (i.e., melanocytes, Langerhans cells, Merkel cells, and inflammatory cells; Connolly and Wu 2024). The keratinocytes (held together by sticky proteins called desmosomes) produce keratins and filaggrin. Keratins are cysteine-rich proteins and filaggrin is a serine-rich protein. Keratins are the main component of the epidermis and account for about 95% of epidermal proteins that maintain the physical strength of the skin. Filaggrin promotes the aggregation of keratins and moisturization. In the epidermis, melanocytes convert tyrosine into melanin. The type and amount of melanin (such as eumelanin, pheomelanin, and mixed melanin) determine the color of the skin and hair. Tyrosine, a precursor of melanin, can be generated from phenylalanine in the skin. In the outermost layer of the epidermis, keratinocytes produce *trans*-urocanate from histidine to protect the skin from ultraviolet irradiation.

The dermis (~90% of the skin's weight) contains fibroblasts (the primary cell type) and their secreted products (collagen, elastin, and proteoglycan), hair follicles, sweat glands (present in humans, dogs, and cats but absent from swine and poultry), sebaceous glands, mast cells, lymphocytes, macrophages, white adipocytes, nerves, lymphatic vessels, and blood capillaries (Lai-Cheong and McGrath 2021). The hair follicles are epidermal invaginations that project further into the dermis. In addition, blood vessels in the dermis extend to the hypodermis. Arteries provide nutrients and O_2 to the skin, whereas veins remove metabolites from the skin. The lymphatics help to drain excess fluid from tissues in the skin back into the blood circulation. The sebaceous glands produce sebum through enzyme (protein)-based reactions. The sebum is a mixture of oily and waxy substances. These substances include TAGs, squalene, free cholesterol, and fatty acids, as well as the esters of glycerol, cholesterol, and wax. Sebum is secreted into the hair follicle through the sebaceous duct and then appears on the surface of the skin through the hair's growth. Collagen accounts for 70% of DM in the skin's dermis, 90% of total proteins in the skin, and 75% of the skin's DM, and helps to maintain the toughness and strength of the skin (Lai-Cheong and McGrath 2021). Proteoglycans confer both viscosity

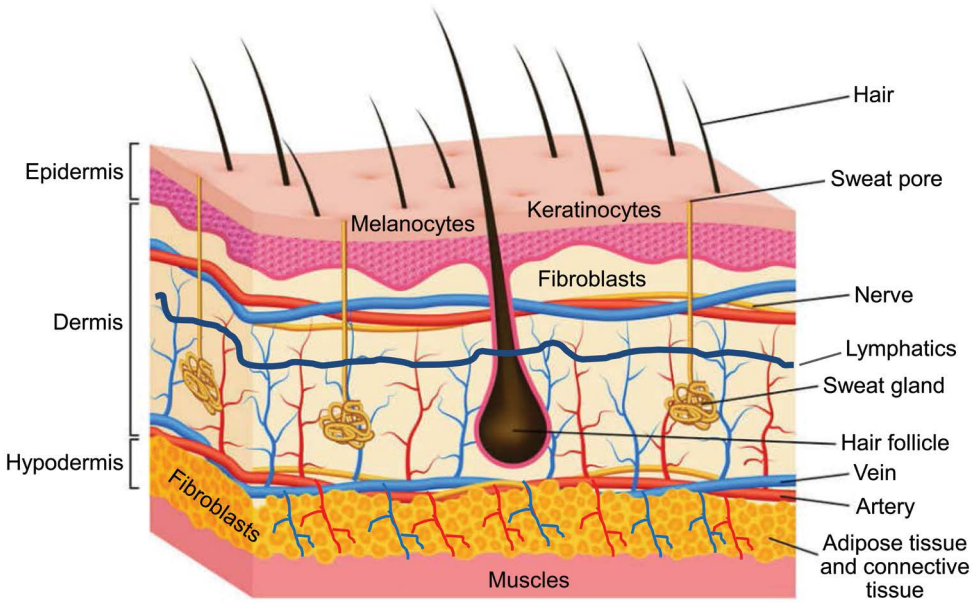


FIGURE 1.32 The structure of skin in animals. There are three layers of tissues in the skin: the epidermis, dermis, and hypodermis. The epidermis (outermost layer) is made up of 95% keratinocytes (producing keratins) and 5% other cells (including melanocytes that form melanin). The dermis accounts for ~90% of the skin's weight, with fibroblasts as the primary cell type for synthesizing and releasing collagen (70% of DM in the dermis), elastin (5% of DM in the dermis), and proteoglycan. Hair follicles project from deep in the dermis through the surface of the epidermis and regulate the growth of hair through interactions with hormones, neuropeptides, and immune cells. Whether sweat glands occur in the dermis depends on animal species; they are present in humans, dogs, and cats, but absent in swine and poultry, for example. Ruminants lack the eccrine type of sweat gland that is present in humans and therefore rely mainly on other methods (e.g., panting, water, or seeking shade) for heat dissipation. The sebaceous glands produce sebum (a mixture of oily and waxy substances) that is secreted into hair follicles through the sebaceous duct. The hypodermis (the innermost layer of the skin) connects the dermis layer of the skin to muscles and is composed of white adipose tissue (the major tissue for energy storage) and fibroblasts (forming collagen as their primary protein). Blood vessels provide nutrients and O_2 to the skin, whereas lymphatic vessels aid in draining excess fluid from tissues in the skin back into the blood circulation. [Reproduced from a photo freely available in the public domain (Buka 2022).]

and hydration to the dermis. Elastin (accounting for 5% of DM in the skin's dermis and 2–4% of the skin's DM) provides normal elasticity and flexibility in the skin. In other words, the elastin protein allows the skin to retain its firmness and return to its original position after being stretched. The dermis also contributes nutritional support to the skin, immune responses, thermoregulation, and the sensation of physical contact.

The hypodermis (the innermost layer of the skin) is connected to the muscle layer. WAT is the major tissue in the hypodermis, where connective tissue (containing fibroblasts, sweat glands, nerves, macrophages, lymphatic vessels, and blood vessels) is also present. The hypodermis helps to store energy and connects the dermis layer of the skin to muscles in the body. Because the hypodermis contains fibroblasts, this layer of the skin, like the dermis, produces collagen. Collagen is the major protein in the hypodermis. The hypodermis provides insulation, thermoregulation, and protection to the body.

Overall, the skin guards the underlying muscles, bones, ligaments, and internal organs while providing the first line of physical barriers from the external environment (including H_2O, water-soluble toxic chemicals, ultraviolet-visible irradiation, pathogens, and mechanical pressure). In addition, the skin helps regulate body temperature, prevent external H_2O from entering the animal, and protect animals from dehydration. Furthermore, the skin is a major component of innate immunity in animals, particularly fish and crustaceans.

The Hair

The hair is a unique connective tissue in mammals and accounts for an appreciable proportion of their BW, depending on species, breed, and age [e.g., for adults, 0.12% in beef cows (McGregor and Graham 2010), 2.0% in dogs (Mundt and Stafforst 1987) and cats (Hendriks et al. 1997), ~4% in Suffolk sheep, ~5% in Rambouillet sheep, ~8% in adult Merino sheep)]. The hair consists of two distinct parts: the hair follicle and the hair shaft (Figure 1.33). The hair follicle is the part of the hair embedded within the skin, and is composed of many different cell types, including keratinocytes, melanocytes, fibroblasts, and stem cells. These cells constitute the hair root, the dermal papilla, and the connective tissue. At the base of the hair root is the germinal matrix (also known as the

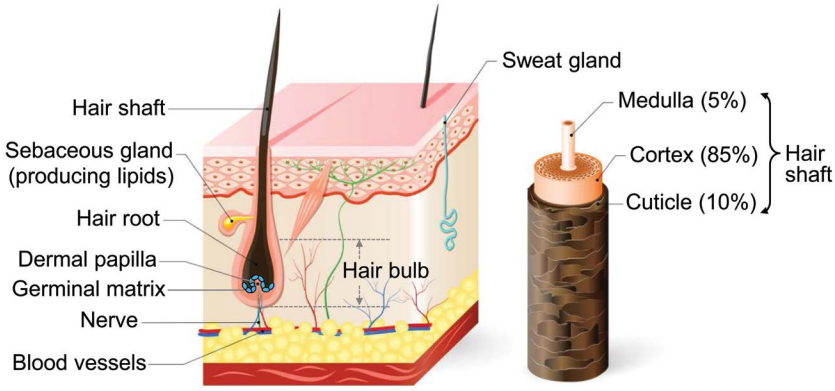


FIGURE 1.33 Structure of hair in mammals. Hair is composed of two parts: the hair follicle (embedded within the skin) and the hair shaft. The hair follicle is composed of epidermal (epithelial) and dermal (mesenchymal) compartments, which are separated by a basement membrane. Dermal and epithelial cells are inducers and responders, respectively, in the process of hair formation. The dermal layer consists of two compartments: the dermal papilla (located at the base of the hair follicle) and dermal sheath (a connective tissue lining the epithelium of the hair follicle). At the base of the hair root, there is a germinal matrix (also known as the hair matrix). Below the germinal matrix is a dermal papilla containing blood vessels, fibroblasts, and multi-potent stem cells to regulate hair growth and development. The lower part of the hair root and dermal papilla constitute the hair bulb. The hair shaft (made of highly keratinized cells) is the visible part of the hair above the skin and is composed of 95% keratin. There are three layers in the hair shaft: cuticle, cortex, and medulla, and their major cell types are cuticle, cortical, and medullary keratinocytes, respectively. [Reproduced from a photo freely available in the public domain (Rejuvenate 2019).]

hair matrix). The latter consists primarily of trichocytes, and to a lesser extent, other epithelial cells and melanocytes (the cells that synthesize and release melanin). Trichocytes are also known as hair keratinocytes, and they are specialized epithelial cells that primarily form the hair and produce the keratin protein in the hair. There are 3 types of trichocytes (medullar, cortical, and cuticle keratinocytes) that are derived from the follicle bulb stem cells. The ratio of hair keratinocytes to melanocytes in the hair bulb is 5:1, which is greater than the ratio of keratinocytes to melanocytes in the epidermis.

Below the germinal matrix is the dermal papilla, which contains blood vessels, fibroblasts, and multipotent stem cells to regulate hair growth and development. The lower part of the hair root (including the germinal matrix layer) and dermal papilla constitute the hair bulb. The hair shaft is the visible part of the hair that is above the skin and is composed of 95% keratin. The hair shaft is made of highly keratinized cells, formed by the proliferation and differentiation of the germinal matrix cells in the hair follicle. The hair shaft consists of 3 layers. The medulla forms the central core of the hair, which is surrounded by the cortex. The cortex is a layer of keratinized cells that is covered by an outer layer of keratinized cells known as the cuticle. Their major cell types are medullar, cortical, and cuticle keratinocytes, respectively.

The hair is made up of ~90% keratin in mammals and contains keratin-associated proteins, such as elastin (produced by fibroblasts). The elastin helps anchor hair follicles and allows the hair to remain flexible and avoid breakage. Cysteine is the most abundant amino acid in hair proteins, followed by serine and glycine. The hair has various physiological and social functions, such as serving as an insulating layer between the skin and the external environment, protecting animals against external factors, pulling up sebum (that is produced in the dermis layer) to the surface of the skin, social significance in interactions among animals, and contributing to thermoregulation.

Sense Organs

There are five senses: sight (eye), hearing (ear), taste (tongue), smell (nose), and touch (skin). The retina is a thin layer of tissue that lines the inner surface of the back of the eyeball and is located near the optic nerve. This tissue consists of photoreceptor cells (rods and cones), as well as glial and neuronal cells, to convert light energy into electrical impulses for initiating the vision process. In vertebrates, the nose is the primary organ of smell and is also an entrance to the respiratory tract. This organ consists of the olfactory epithelium, which is a specialized epithelial tissue inside the nasal cavity that contains olfactory receptor cells (neurons). Olfactory receptors (also known as odorant receptors; members of the class A rhodopsin-like family of G-protein-coupled receptors) are expressed in the plasma membrane of olfactory receptor cells and are responsible for the detection of odorants that give rise to the sense of smell. Activated olfactory receptors trigger nerve impulses, which transmit information about odor to the central nervous system (brain). Interestingly, fish can sense the smell of amino acids dissolved in H_2O (Caprio and Byrd 1984). Some microbial

metabolites, such as dimethylamine and trimethylamine with a strong odor, stimulate the olfactory bulb of fish and are used as feed attractants. The presence of dimethylamine and trimethylamine in fishmeal contributes to the preference of fish for this high-protein foodstuff. The tongue is a muscular organ within the cavity of the vertebrate mouth and is almost completely covered by a layer of mucosa. Much of the tongue's surface is covered by taste buds housed in numerous lingual papillae. Taste receptor cells are located on the taste buds of the tongue and connect with nerves that detect and transmit taste signals to the brain, giving rise to five distinct tastes (i.e., sweet, sour, bitter, salty, and umami) noted previously (Wu 2020a). The ear, which is the organ of hearing and in mammals also balance, consists of the outer ear (the pinna and the ear canal), the middle ear (including the tympanic cavity and the three ossicles), and the inner ear (the semicircular canals, the utricle and saccule, and the cochlea for hearing). Of note, a signaling cascade enables hearing and balance via a transmembrane channel-like protein located in the inner ear present in mammals, birds, fish, amphibians, and reptiles. This protein forms a sound- and motion-activated pore that allows the conversion of sound or head movement into nervous signals that are sent to the brain.

Sense organs send information from the external environment (including food and temperature) to the brain through the peripheral nervous system, allowing animals to react in a timely manner; there are specialized sensory neurons (also called receptors) for specific stimuli (Dyce et al. 1996). Examples of these receptors include photoreceptors (vision receptors), mechanoreceptors (hearing and touch receptors), and chemoreceptors (taste receptors and smell receptors or olfactory sensory neurons). When sensory receptors are activated, the electrical potential causes depolarization in the membrane to transduce the respective stimuli across the cell membrane to an attached neuron (Sporns et al. 2004). The electrochemical information is collected by the dendrites of the neuron, transferred through its cell body, and then brought to the axon. This allows the electrochemical signal to be transmitted to the brain for processing and integration. Neurotransmitters used by sense organs to transmit information to the brain for integration and interpretation are either amino acids (e.g., glutamate, aspartate, and glycine) or their metabolites (e.g., NO, GABA, and serotonin; He and Wu 2020). Thus, the eyes, ears, tongue, nose, and skin provide the senses of sight, hearing, smell, taste, and touch, respectively, to aid survival, development, learning, and adaptation.

Sensory capacities differ among animal species and individuals due to different genomes and epigenetic alterations. Receptor-mediated mechanisms are responsible for the chemosensory transduction of five basic taste qualities [sweet, sour, bitter, umami (meaty, broth-like, or savory taste), and salty]. Taste receptors for sweet, bitter, and umami stimuli are G-protein-coupled receptors (Wu 2020a). Specifically, the gene *Tas1R* encodes for sweet and umami receptor proteins, which have three subunits, Tas1R1, Tas1R2, and Tas1R3. Three known umami substances are glutamate, 5′-inosine monophosphate, and 5′-guanosine monophosphate. Bitter receptors are Tas2Rs (or T2Rs) and belong to the taste receptor family 2 proteins. Taste receptors for sour and salty substances are H^+ and Na^+ ion channels, respectively. These physiologic responses help humans and animals avoid foods with strong bitter or sour tastes to protect against poisons. The development and health of sense organs are particularly vital for aquatic animals because they are constantly exposed to H_2O, potentially harmful chemicals, and predators in their natural living environment.

OVERVIEW OF METABOLIC PATHWAYS

Major Metabolic Pathways and Their Significance

In all animals, dietary macronutrients undergo oxidation to form CO_2 and H_2O, with the concomitant release of chemical energy to support various biological processes. Of note, the French chemist, Antoine Lavoisier (1743–1794), is often considered the founder of nutritional sciences because of his seminal contribution to the development of modern chemistry and his discovery of the role of O_2 in the oxidation of nutrients. It is now known that many series of biochemical reactions are responsible for both the oxidation and catabolism of nutrients. Although these metabolic pathways are complex, they are the foundation of life (Brosnan 2005; Srere 1987; Watford 1991), and it is important that nutritionists understand the features and physiological significance of the pathways and their interrelationships.

Metabolic control of nutrient utilization is cell specific (Fell 1997). A metabolic pathway can be defined as a series of enzyme-catalyzed reactions in which a substance is either degraded to simpler products or synthesized from simpler precursors. A pathway can be linear (such as proline synthesis), cyclic (such as the Krebs cycle), or spiral (fatty acid synthesis). Major metabolic pathways and their physiological significance are summarized in Table 1.12. A physiologically useful definition of a metabolic pathway is a series of enzyme-catalyzed reactions, initiated by a flux-generating step and ending with the formation of products. Such a definition indicates that a metabolic pathway may span more than one tissue. This is graphically illustrated by the interorgan metabolism of amino acids (Wu 2022a). Of note, the pathway for arginine synthesis in most adult mammals is considered to be initiated in the small intestine by the formation of citrulline from glutamine or proline into citrulline and to end in the kidneys and other cell types (e.g., endothelial cells and macrophages) by the conversion of citrulline into arginine (Glutamine → → Citrulline → → Arginine). The central role of the Krebs

TABLE 1.12
Major Pathways for Nutrient Metabolism and Their Physiological Significance

Nutrient	Pathway	Site	Major Functions
All	Krebs cycle[a]	Mitochondria	Oxidation of glucose-, fatty acid-, and AA-derived acetyl-CoA to produce CO_2, water, NADH, ATP, and GTP; bridges the metabolism of glucose, fatty acids, and AAs; anaplerosis
	Respiratory chain	Mitochondria	Major source of energy in mitochondrion-containing cells
	Glucose-alanine cycle	Cytosol of liver and skeletal muscle	Skeletal muscle synthesizes alanine from glucose-derived pyruvate and AAs, and releases alanine, which is taken up by liver for glucose production; transports nitrogen in a nontoxic form from skeletal muscle to the liver
	One-carbon metabolism	Cytosol and Mit in most cells	Provides the methyl group for the methylation of DNA, RNA, protein and AAs, and for the conversion of homocysteine into methionine; produces thymidine and purines for nucleic acid synthesis
Glucose	Glycolysis	Cytosol of all cells	Conversion of glucose into pyruvate and lactate; produces 3-phosphoglycerol to provide glycerol backbone for triglycerides, glycolipids, and phospholipids; generates ATP under anaerobic conditions in all cells; the sole source of energy in mitochondrion-free cells
	Glycogen synthesis	Cytosol of most cells (particularly liver, heart and skeletal muscle)	Conversion of glucose into glycogen for storage in the fed state; regulates blood glucose concentration; aids in controlling osmolality in cells by converting monomers to polymers
	Glycogen degradation	Cytosol of most cells (particularly liver, heart and skeletal muscle)	Liver: provides glucose rapidly in response to needs; muscles: converts glucose into glucose-1-P, which enters glycolysis as glucose-6-P; muscles: provides pyruvate and lactate
	Gluconeogenesis	Liver and kidneys (mainly the cytosol but also Mit in some species due to PEPCK location)	Converts noncarbohydrate substances into glucose; regulates glucose homeostasis (particularly in neonates, strict carnivores, and ruminants, and in all animals during starvation);[b] helps regulate blood pH by consuming H^+
	Pentose cycle	Cytosol of all cells	Serves as the major source of cellular NADPH; provides ribose-5-P for purine and pyrimidine synthesis
	Hexosamine	Cytosol of all cells	Conversion of fructose-6-P and glutamine into glucosamine-6-P, a precursor for synthesis of all amino sugars and glycoproteins
Fatty acids	Fatty acid synthesis	Cytosol of most cells[c]	Conversion of glucose and AAs into fatty acids, which serve as precursors for synthesis of TAGs; prevents the loss of water-soluble energy substrates from the body
	TAG synthesis	Cytosol and Mit of most cells[c]	Conversion of fatty acids and glycerol into fats for storage; prevents the toxicity of free fatty acids
	TAG-FA cycle	Most cells; Mainly in WAT	Regulates the balance of TAGs in the body
	Cholesterol synthesis	Cytosol and Mit of liver	Synthesis of cholesterol from acetyl-CoA; cholesterol is required to form plasma and subcellular membranes, bile acids, bile alcohols, and steroid hormones
	Lipoproteins	Small intestine and liver	Synthesis of chylomicrons in the small intestine, VLDL in the liver, and HDL in peripheral tissues; release HDL
	β-Oxidation[d]	Mitochondria primarily in the liver, heart, and skeletal muscle of animals	Converts fatty acids into acetyl-CoA for oxidation via the Krebs cycle; provides acetyl-CoA for ketogenesis during fasting, lactation and late pregnancy, and for the activation of hepatic pyruvate carboxylase in liver
	HMG-CoA cycle	Mit of hepatocytes in liver; ruminal epithelium	Formation of acetoacetate (a ketone body) from acetyl-CoA; help regulate the availability of acetyl-CoA in cells
	Ketogenesis	Mit of liver, colonocytes and ruminal epithelium	Conversion of acetyl-CoA into ketone bodies as metabolic fuels for the brain and other extrahepatic tissues when blood glucose concentration is low; limited in pigs
AAs	Urea cycle	Mit and cytosol of liver, enterocytes in mammals	Converts ammonia and bicarbonate into urea for the detoxification of ammonia in the liver (mainly) and enterocytes
	Protein synthesis	Mainly the cytosol and Mit of all cells except for mature RBCs	Synthesis of secretory and membrane proteins in RER, cellular proteins on ribosomes, and some (~13) proteins in Mit; helps control osmolality in cells by converting monomers to polymers

(*Continued*)

TABLE 1.12 (*Continued*)
Major Pathways for Nutrient Metabolism and Their Physiological Significance

Nutrient	Pathway	Site	Major Functions
	Proteolysis	Mainly the cytosol and lysosomes of all cells	Degrades proteins to release AAs; regulate cellular protein concentration; removes aged, oxidized, and damaged proteins
	Protein turnover	Most cells except for the lack of protein synthesis in mature RBCs	Regulates the balance of proteins in cells, the release of secretory proteins from cells, endogenous provision of AAs, as well as metabolism, immune response, and cell growth
	Ubiquitin cycle	Cytosol of all cells	Protein degradation by ubiquitin-dependent proteasomes
	AA synthesis	Cytosol and/or Mit of cells	Forms AAs in a cell-dependent manner to compensate for a deficiency of AAs in diets; fulfil specific physiological needs (e.g., glucose-alanine cycle and malate-aspartate shuttle)
	AA degradation	Cytosol and/or Mit of cells	Degradation of AAs in a cell-dependent manner to regulate the homeostasis of AAs in the body
	Uric acid synthesis	Mainly the liver of most animals (e.g., birds and mammals)	Converts ammonia and carbons (from formate, glycine and bicarbonate) into uric acid for detoxification of ammonia in birds; purine degradation in mammals
	Synthesis of nucleotides	Liver and other tissues	Production of RNA and DNA for protein synthesis; essential components of genetic material
	Synthesis of other substances	Liver and kidney, and many types of cells	Provides carnitine, carnosine, glutathione, creatine, inositol, acetylcholine
Vitamins (Vit)	Production of Vit B_3 and D_3	Liver, kidney, skin, bone and/or small intestine	Synthesizes vitamins B_3 and D_3 from tryptophan and cholecalciferol, respectively, in a species-dependent manner
	Formation of Vit A from β-carotene	Enterocytes of all species except for cats	Converts β-carotene to retinol for supporting the functions of the eyes, epithelial cells, and reproductive systems; antioxidant
	Synthesis of Vit C	Liver of most species (including pigs and rats)[e]	Converts glucose to Vit C for supporting antioxidative function, as well as the activities of prolyl oxidase and lysyl oxidase
	Vit K cycle	Cytosol of the liver	Regenerates a reduced form of Vit K from oxidized Vit K

[a] The Krebs cycle provides: (a) oxaloacetate for gluconeogenesis; (b) succinyl-CoA for heme synthesis; (c) α-ketoglutarate for the synthesis of glutamate and glutamine; and (d) citrate for the transport of acetyl-CoA from the mitochondria to the cytosol for fatty acid synthesis. Enzymes of the Krebs cycle are present in the cytosol of prokaryotes (e.g., bacteria and archaea) that lack the mitochondria and other organelles (e.g., a nucleus), and acetyl-CoA can be oxidized to CO_2 in these microbes in the presence of O_2.

[b] Carnivores ingest a limited amount of carbohydrates from their diets, whereas ruminants absorb little or no glucose in the small intestine.

[c] The major sites of lipogenesis are: liver in chickens, humans, and adult rats; white adipose tissues in pigs and ruminants; and both liver and white adipose tissues in cats, dogs, mice, rabbits, and young rats.

[d] β-Oxidation occurs in the cytosol of prokaryotes (e.g., bacteria and archaea) that lack mitochondria and other organelles (e.g., a nucleus).

[e] Humans and other primates, as well as guinea pigs and fruit bats cannot synthesize vitamin C.

Note: AA, amino acid; FA, fatty acid; HDL, high-density lipoprotein; HMG, 3-hydroxy-3-methylglutaryl-CoA; Mit, mitochondria; PEPCK, phosphoenolpyruvate carboxykinase; RBCs, red blood cells; TAGs, triacylglycerols; Vit, vitamin; VLDL, very low-density lipoprotein; WAT, white adipose tissue.

cycle is to bridge glucose, fatty acid, and amino acid metabolism (Figure 1.34).

CHARACTERISTICS OF METABOLIC PATHWAYS

Enzyme-Catalyzed Reactions

Enzymes are biological catalysts. They are responsible for nearly all reactions in cells. An example is the conversion of D-glucose into D-glucose-6-phosphate (D-glucose-6-P) by hexokinase in the cytosol.

$$\text{Glucose} + \text{ATP} \rightarrow \text{Glucose-6-P} + \text{ADP} + \text{H}^+$$
$$\left(\text{Hexokinase; } \text{Mg}^{2+}\text{as a cofactor for the enzyme}\right)$$

Most enzymes are proteins, while a few of them are now known to be RNA (Devlin 2011). The significance of enzymes in living organisms is more than their roles in making biochemical reactions possible. The existence of enzymes not only enhances the rates of metabolic pathways but also enables their precise regulation to meet physiological needs. As a result of this regulation, individual reactions and metabolic pathways can be integrated into an exquisite metabolic system that functions effectively in the whole organism to maintain homeostasis (Brosnan 2005; Schimke and Doyle 1970). It should be borne in mind that most, but not all, enzyme-catalyzed reactions follow Michaelis-Menten kinetics in a hyperbolic relationship (Figure 1.35). The mathematical

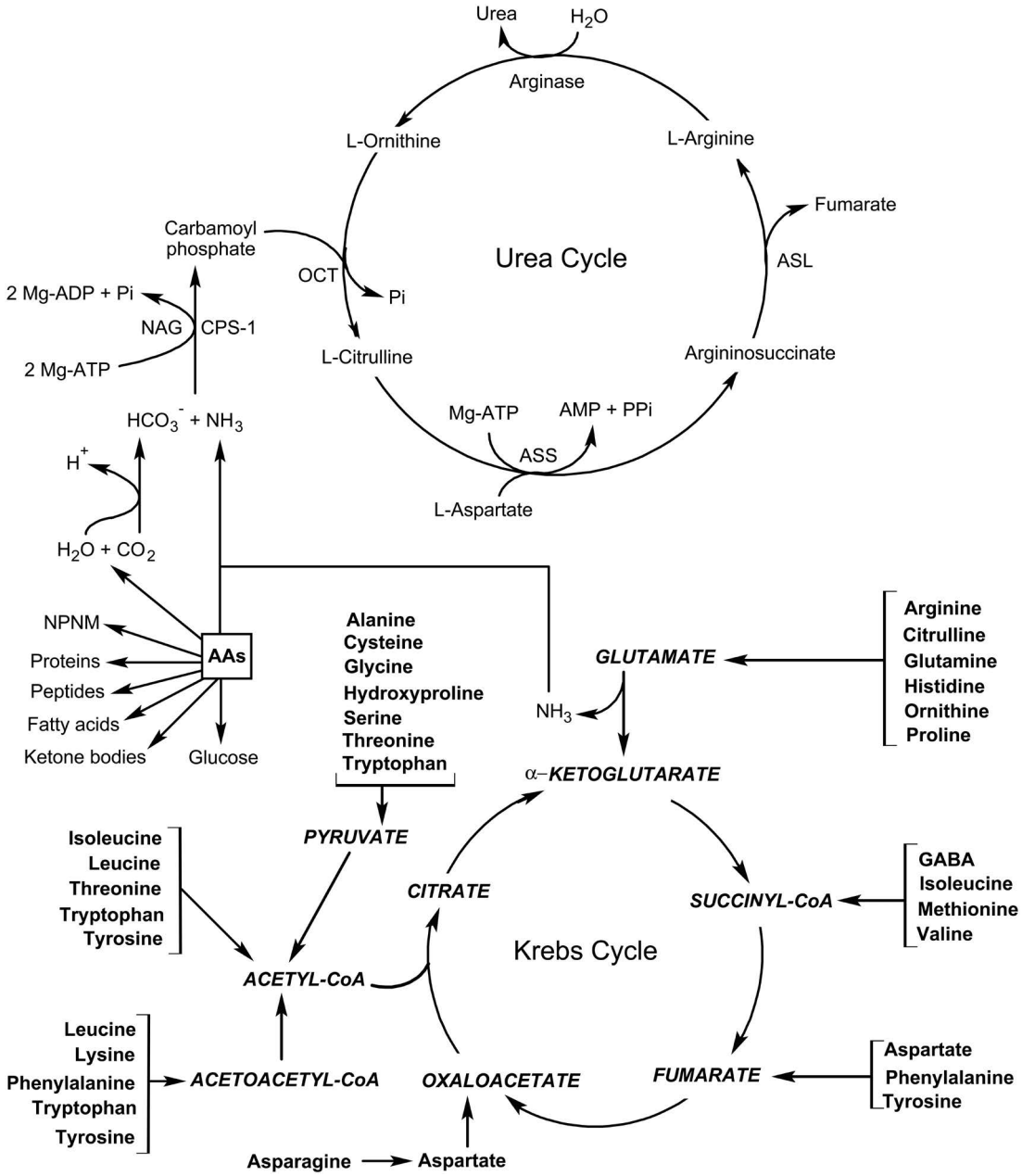


FIGURE 1.34 Interrelationships among the metabolism of glucose, amino acids, and fatty acids in animals. The Krebs cycle plays a central role in bridging the pathways for nutrient metabolism. In animals, amino acids are utilized to produce proteins (including enzymes), small peptides, other nitrogenous metabolites (e.g., nitric oxide, creatine, carnitine, and ammonia), fatty acids, and glucose. Ammonia plays an important role in bridging the Krebs cycle with the urea cycle. AAs, amino acids; ASL, argininosuccinate lyase; ASS, argininosuccinate synthase; CPS-1, carbamoylphosphate synthetase-I; GABA, γ-aminobutyrate; NAG, *N*-acetylglutamate; NPNM, non-peptide nitrogenous metabolites; and OCT, ornithine carbamoyltransferase. (Adapted from Rezaei et al. 2013.)

relationship between initial rate (i.e., catalytic activity) and substrate concentration can be described by the following equation:

$$V_i = \frac{V_{max}[S]}{K_m + [S]} \quad \frac{1}{V_i} = \frac{K_m + [S]}{V_{max}[S]} \quad \frac{1}{V_i} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{[S]}{V_{max}[S]}$$

where V_{max} is the maximum rate of reaction, K_M is the Michaelis constant, and [S] is substrate concentration. Some enzymes may show an allosteric characteristic that has more complex sigmoid kinetics than the Michaelis-Menten kinetics (Devlin 2011).

Catabolism of a substrate is enhanced with an increase in its concentrations until the reaction velocity reaches a maximum. A high capacity for the degradation of nutrients in animals provides a biochemical basis for a high, safe level of its dietary supplementation. Similar principles apply to the formation of a product from its precursors in

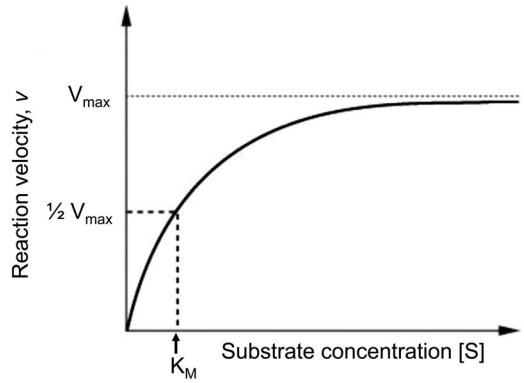


FIGURE 1.35 A hyperbolic relationship exists between the substrate concentration and reaction velocity in an enzyme-catalyzed reaction. Most enzymes exhibit Michaelis-Menten kinetics. The initial velocity of the reaction increases with increasing substrate concentration and reaches the maximum value (V_{max}) when the enzyme is saturated with the substrate. The equation, $v = V_{max}$ [S]/(K_M + [S]), is known as the Michaelis-Menten equation, where v is reaction velocity and K_M is the substrate concentration [S] at which the velocity of the reaction is one-half the maximum value (i.e., ½ V_{max}). A K_M value may indicate the physiological relevance of an enzyme-catalyzed reaction in animals.

animals. This also means that there are limits to nutrient catabolism and synthesis in animals. For example, substrate availability and enzyme activity limit amino acid synthesis in pigs and thus their growth, development, lactation, and reproduction (Hou et al. 2016). Therefore, providing enough pre-formed nutrients or their immediate precursors in diets may be necessary for maximum growth and production performance of farm animals, as well as their optimal health and well-being.

Enzymes are classified as (a) oxidoreductases that catalyze oxidation-reduction reactions (e.g., lactate dehydrogenase and glyceraldehyde-3-P dehydrogenase), (b) transferases that catalyze the transfer of a chemical group from one molecule to another (e.g., hexokinase and aminotransferase), (c) hydrolases that catalyze essentially irreversible hydrolysis reactions (e.g., arginase and glutaminase) in which a bond is broken by H_2O, (d) lyases that usually catalyze carbon-carbon bond cleavage (e.g., aldolase and decarboxylases), (e) isomerases that catalyze the intramolecular transfer of functional groups (e.g., amino acid racemases and glucose-6-P isomerase), (f) ligases that catalyze the joining of two molecules by forming a new chemical bond (e.g., argininosuccinate synthetase and aminoacyl tRNA synthetase, and (g) hydratases that catalyze the addition or removal of H_2O and include both dehydratases (that remove H_2O) and hydrases (that add H_2O) in biochemical reactions (Devlin 2011). Regardless of the types of reactions, enzymes contain substrate-binding sites. Allosteric enzymes also have additional sites (allosteric sites) for the binding of an activator or inhibitor. In a catalytic reaction, an enzyme may utilize a coenzyme, a cosubstrate (a second substrate; e.g., ATP for protein kinase), or a cofactor. A coenzyme is a small organic molecule that is often a derivative of a vitamin (e.g., NADH and FAD), whereas a cofactor is either an inorganic metal ion (e.g., Mn^{2+} or Mg^{2+}) or an organic factor (e.g., *N*-acetylglutamate or L-lactate) that binds to the enzyme. In all enzyme-catalyzed reactions, an important kinetic variable is K_M, which refers to the substrate concentration [S] at which the velocity of the reaction is one-half the maximum value (V_{max}). The K_M value may suggest the physiological relevance of an enzyme-catalyzed reaction, such as the affinity of the enzyme toward its substrate(s) and the possible occurrence of the reaction under physiological conditions.

Biochemical studies have revealed the following general mechanisms for regulating enzyme activity in animals:

a. concentrations of substrates and cofactors for enzymes in plasma and cells: based on the Michaelis-Menten kinetics;
b. allosteric regulation: the control of enzyme activity by an effector molecule that binds to a regulatory site on the enzyme other than its active (substrate-binding) site; effectors that enhance or decrease enzyme activity are referred to as allosteric activators or inhibitors, respectively;
c. concentrations of nonallosteric activators and inhibitors in plasma and cells that are either endogenous or exogenous, such as Ca^{2+} and calmodulin for activating constitutive NO synthase, as well as competitive, uncompetitive, or noncompetitive inhibitors;
d. covalent modifications of intracellular enzymes: biochemical alterations of an enzyme through the addition or removal of chemical groups (e.g., the phosphorylation and dephosphorylation of protein; the phosphorylation and dephosphorylation mechanism does not involve a change in the amount of the enzyme protein and allows for rapid activation or inactivation of an enzyme;
e. reduction-oxidation (i.e., redox) potential in the cytosol and mitochondria: ratios of NADH/NAD^+, NADPH/$NADP^+$, or reduced glutathione/oxidized glutathione;
f. acyl-CoA potential in the cytosol and mitochondria: ratios of acyl-CoA/CoA;
g. quantity of enzymes (e.g., proteins) in plasma and cells: the release of enzymes (e.g., arginase) by injured tissues (e.g., the liver) can deplete extracellular substrates (e.g., arginine in plasma); long-term regulation of enzyme activity is achieved through changes in gene expression and the regulation of intracellular protein turnover (protein synthesis, protein degradation, or both);
h. blood and intracellular pH, as well as temperatures in animals: factors that affect the chemical structures of enzymes; each enzyme has its optimal pH and temperature; and

i. ion concentrations in plasma and cells: factors that affect the chemical structures of enzymes; some ions are cofactors for a specific enzyme. Nutrients and hormones can affect one or more of the factors listed above and therefore metabolic pathways in the body.

Animals contain endogenous inhibitors or activators of enzymes under both physiological and pathological conditions. Inhibitors reduce enzyme activity through one of the following modes: (a) competitive inhibition (an inhibitor with a structure similar to that of the substrate binds to the enzyme) in which K_M is increased but V_{max} is not altered; (b) noncompetitive inhibition (an inhibitor binds both the free enzyme and the enzyme-substrate complex) in which K_M is not altered but V_{max} is decreased; and (c) uncompetitive inhibition (an inhibitor binds only the enzyme-substrate complex) in which both K_M and V_{max} are decreased; this kind of enzyme inhibition is very rare. Knowledge of enzyme inhibition can greatly facilitate the development of a means to overcome the inhibition. For example, L-N^G-monomethylarginine inhibits NO synthase in cells through competitive inhibition, which can be alleviated by increasing the concentration of the enzyme substrate, L-arginine (Alderton et al. 2001). In contrast, lactate inhibits proline oxidase in pig enterocytes through noncompetitive inhibition, which is ameliorated by removing extracellular lactate (Dillon et al. 1999).

Although it has long been stated that a metabolic pathway is limited by a single enzyme, the concept of distributed metabolic control has gained growing recognition over the past three decades (Fell 1997). Accordingly, metabolic control is distributed throughout the metabolic sequence or resides in one or more of the reactions. For example, control points for glycolysis can be plasma glucose concentration, ATP needs, glucose transport, hexokinase, 6-phospho-fructokinase-1, enolase, and pyruvate kinase, but not 6-phospho-fructokinase-1 alone. This ensures that metabolic chaos will not occur in animals (Brosnan 2005).

Intracellular Compartmentalization of Metabolic Pathways

Enzymes and thus metabolic pathways are localized in different organelles of the cell. An excellent example of the intracellular compartmentalization of a metabolic pathway is the urea cycle. It involves both mitochondria and the cytosol. Within a compartment, enzymes are either loosely or closely associated, and they sequentially pass intermediates along the pathway (Ovadi and Saks 2004). In the hepatic urea cycle, the physiological significance of the intracellular compartmentalization is evident in that the ammonia produced in the mitochondrion and ornithine are locally converted to citrulline, a nontoxic product, for export to the cytosol. In all mammalian and avian cells studied, citrulline and aspartate are efficient substrates for the formation of arginine in the cytosol. When such a metabolic pathway is well organized in a sequential association, it is called a metabolon (Srere 1987). Advantages of a metabolon include (a) facilitation of the transfer of intermediates between enzymes to maintain a high concentration of substrate in the catalytic site to enhance the efficiency of product formation and (b) fewer side reactions (chemical or enzymatic) when intermediates are not free within cells.

Cell-, Zone-, Time-, Age-, and Species-Dependent Metabolic Pathways

Another feature of metabolism is its occurrence in a cell-, age-, time-, or species-specific manner. In mammals, gluconeogenesis occurs only in the liver and kidney. Even within the same organ, cells do not have the same metabolic pathways, as graphically illustrated by the metabolic zonation of the liver (Häussinger et al. 1992). Specifically, the liver exhibits remarkable heterogeneity of its hepatocytes along the porto-central axis in the ultrastructure and expression of enzymes, resulting in different metabolic patterns within different zones of the liver lobules (Table 1.13). This spatial organization of various metabolic pathways (known as metabolic zonation) provides the biochemical basis for (a) adaptation of the liver to changes in nutritional, physiological, and pathological conditions and (b) prevention of futile cycles (Schleicher et al. 2015). In addition, circadian rhythms (also known as biological clocks) regulate hormonal secretions and nutrient metabolism in diurnal species (e.g., birds, mammals, and fish; Tripathi et al. 2025). Thus, the rate of whole-body energy metabolism in animals varies remarkably among different times of the day, independent of feed intake (Assaad et al. 2014). Moreover, temporal differences in the expression of enzymes are also notable within the same animal, as indicated by the finding that intestinal arginine degradation is extensive in postweaning pigs but is negligible in preweaning pigs (Wu and Morris 1998).

A well-known difference in species-dependent metabolism is the conversion of ammonia and CO_2 into urea that takes place in mammals but not in birds. In addition, cows synthesize large amounts of glucose from propionate in the liver (Thompson et al. 1975), but this pathway of gluconeogenesis is insignificant in nonruminants (Bondi 1987). Furthermore, in sheep, which possess high arginase activity to degrade arginine in the placentomes, citrulline is unusually abundant in allantoic fluid during early and mid-gestation (e.g., up to 10 mM) because of increased transfer from mother to fetus to conserve the arginine carbons (Kwon et al. 2003). By contrast, in pigs that express little arginase activity in placentae, arginine is particularly abundant in allantoic fluid, primarily due to its direct transport from mother to fetus (Wu et al. 1996). These examples illustrate the complexity of metabolism among animal species in their strategies to maximize survival and the efficiency of nutrient utilization. Understanding cell- or tissue-specific metabolic pathways is essential in appreciating the

TABLE 1.13
Primary Localization of Metabolic Pathways in Periportal or Perivenous Hepatocytes of the Mammalian Liver[a]

Periportal Hepatocytes	Perivenous Hepatocytes
Amino acid (AA) metabolism	Amino acid (AA) metabolism
AA uptake and degradation (except glutamate, aspartate and histidine)	Glutamate, aspartate, and α-ketoglutarate uptake[b]
Glutaminase[b]	Ornithine aminotransferase
Histidine, glycine and serine degradation[b]	Glutamine synthetase[b]
Urea cycle and gluconeogenesis from AAs	Glutathione *S*-transferase Mu class subunits 2, 3, 6
Lipid metabolism	Glutathione synthesis (mainly)
ATP-citrate lyase	Lipid metabolism
Cholesterol synthesis (mainly) and uptake	Bile acid synthesis (mainly) and bile reserve
Fatty acid oxidation (mainly)	Esterification of free fatty acids
Bilirubin production and excretion	Fatty acid synthesis
Bile excretion and the uptake of bile salts	Long-chain fatty acid elongation
Ketogenesis[c]	The pentose cycle
Hepatic lipase	Triacylglycerol synthesis
Glucose metabolism	Very-low-density-lipoprotein synthesis
Glucose release	Glucose metabolism
Gluconeogenesis	Glucose uptake
Glycogen degradation to glucose	Glycolysis
Glycogen synthesis from lactate and AAs	Glycogen degradation to pyruvate
Other metabolic pathways	Glycogen synthesis from glucose
Carbonic anhydrase V	Other metabolic pathways
Oxidative energy metabolism[d]	Carbonic anhydrase II and III
Glutathione peroxidation	Xenobiotic metabolism and detoxification[e]
	Taurine synthesis from cysteine (mainly)

Source: Braeuning et al. (2006), Häussinger et al. (1992), and Schleicher et al. (2015).

[a] Except for those pathways indicated with a superscript letter b, most pathways are unequally distributed along the acinus (metabolic zonation), with highest activities in the indicated hepatocytes.

[b] Exclusively located in the indicated hepatocytes.

[c] This pathway is highly active in most animals during starvation but is limited in pigs.

[d] Including the Krebs cycle and the electron transport system.

[e] Including monooxygenation, glucuronidation, and mercapturate formation

coordination of fuel utilization for the precise regulation of homeostasis in animals.

Disturbance in homeostasis for a prolonged period may result in disease and even death (Wu 2020b). A reduction in blood pH from 7.4 to 7.1 is fatal, as shown in untreated insulin-dependent diabetic animals, including humans (Marliss et al. 1982). This is because low pH denatures protein, is deleterious to cell structure, and inhibits enzyme activity. The severe adverse outcomes are the dysfunction of multiple organs, such as the impaired function of the respiratory center in the brain, as well as cardiac and renal failure.

BIOLOGICAL OXIDATION IN ANIMAL CELLS

Redox State and Substrate Oxidation

In chemistry, oxidation is defined as the removal of electrons from an electron donor (called the reductant), and reduction as the gain of electrons by an electron acceptor (the oxidant). In an oxidation-reduction reaction, an oxidant and its reductant form a redox couple. In the reversible reaction catalyzed by L-lactate dehydrogenase (a cytosolic enzyme), L-lactate is oxidized to pyruvate and NAD^+ is reduced to NADH and therefore an [L-lactate]/[pyruvate] ratio is a useful indicator of the cytosolic redox state. In addition, D-ß-hydroxybutyrate dehydrogenase (a mitochondrial enzyme) converts D-ß-hydroxybutyrate plus NAD^+ into acetoacetate plus NADH, and therefore a [D-ß-hydroxybutyrate]/[acetoacetate] ratio is a useful indicator of the mitochondrial redox state.

$$\text{L-Lactate} + \text{NAD}^+ \rightleftarrows \text{Pyruvate} + \text{NADH} + \text{H}^+ \left(\text{Cytosol}\right)$$

$$\text{D-β-Hydroxybutyrate} + \text{NAD}^+ \rightleftarrows \text{Acetoacetate} + \text{NADH} + \text{H}^+ \left(\text{Mitochondria}\right)$$

Dietary nucleotides [e.g., NAD and nicotinamide mononucleotide (NMN)] undergo extracellular degradation to nicotinamide riboside and nicotinamide in the lumen of the small intestine. These products, along with dietary

nicotinamide and nicotinic acid, as well as their corresponding nucleosides, enter enterocytes via transporters and then the blood circulation. Animal cells (including enterocytes) can readily hydrolyze nicotinamide and nicotinic acid nucleosides to nicotinamide and nicotinic acid, which are substrates for the synthesis of NAD^+ in the cytosol (Nikiforov et al. 2011). In addition, nicotinamide and nicotinic acid are substrates for the cytosolic formation of their corresponding mononucleotides, which are transported into the mitochondria for the generation of NAD^+ via NMN adenylyltransferase (Nikiforov et al. 2011). This metabolic pathway for mitochondrial NAD^+ synthesis plays an important role in maintaining mitochondrial NAD^+ homeostasis because animal cells cannot directly transport NAD^+ from the cytosol into the mitochondria due to a lack of its transporters in the inner mitochondrial membrane (Palmieri 2013). By contrast, plants and yeast express specific transporters to move NAD^+ from the cytosol into the mitochondria (Nikiforov et al. 2011).

In animal cells containing mitochondria, the conversion of food energy (i.e., chemical energy in amino acids, lipids, and carbohydrates) into biological energy (primarily as ATP and, to a lesser extent, GTP and UTP) occurs through well-coordinated metabolic pathways. The major biochemical reactions are (a) generation of acetyl-CoA from glucose via glycolysis (pyruvate production), fatty acids via β-oxidation, and amino acids via interorgan cooperation, which will be discussed in other chapters; (b) oxidation of acetyl-CoA via the Krebs cycle to form GTP, CO_2, H_2O, $NADH + H^+$, and $FADH_2$; (c) oxidation of $NADH + H^+$ and $FADH_2$ in the presence of O_2 to produce H_2O in the electron transport system (respiratory chain); and (d) the synthesis of ATP from ADP and Pi by ATP synthase via the proton gradient in the mitochondrial respiratory chain (Devlin 2011). In the liver, the mitochondrial respiratory chain occurs primarily in periportal hepatocytes. As noted previously, the Krebs cycle and the electron transport system are two major metabolic pathways for biological oxidation in animals.

The Krebs Cycle in Mitochondria

In 1937, Hans A. Krebs published a landmark paper describing the role of citric acid in the oxidation of acetate (as acetyl-CoA) in homogenates of pigeon breast muscle. This pathway was named "the Krebs cycle" after Dr. Krebs and is also known as the citric acid cycle or the tricarboxylic acid cycle. In this cycle, acetyl-CoA combines with oxaloacetate (OAA) to form citrate, which is then converted sequentially into isocitrate, α-ketoglutarate, succinate, fumarate, L-malate, and OAA (Figure 1.36). L-Malate, citrate, and α-ketoglutarate are the intermediates of the Krebs cycle that can exit the mitochondrial matrix into the cytosol. In animal cells, the mitochondrial matrix contains all the enzymes of the Krebs cycle except for succinate dehydrogenase, which is bound to the inner mitochondrial membrane. A series of eight enzyme-catalyzed reactions in the Krebs cycle generate GTP (equivalent to ATP in high-energy phosphate bonds), CO_2, H_2O, $NADH + H^+$, and $FADH_2$. These two reducing equivalents ($NADH + H^+$ and $FADH_2$) are then fed into the electron transport system in the inner mitochondrial membrane for oxidation, producing (a) ATP from ADP + Pi and (b) H_2O from $2H^+$ and $½O^-$.

It should be borne in mind that the Krebs cycle oxidizes acetyl-CoA only, with no net changes in any intermediates of the cycle. Net oxidation of these intermediates occurs through their conversion into L-malate, which exits the mitochondrion into the cytosol to produce pyruvate. Pyruvate is then transported into the mitochondrial matrix for oxidation to acetyl-CoA by pyruvate dehydrogenase. In addition to its functions of oxidizing acetyl-CoA (a common metabolite of fatty acids, amino acids, and glucose) and generating ATP, the Krebs cycle provides four- and five-carbon precursors for the synthesis of glucose and certain amino acids (e.g., aspartate, glutamate, asparagine, glutamine, alanine, and proline), as well as $NADH + H^+$ for many biochemical reactions. Under metabolic conditions that favor lipogenesis, citrate exits the mitochondrion to the cytosol to provide acetyl-CoA for FA synthesis. Thus, the Krebs cycle is the central pathway that bridges the metabolism of fatty acids, amino acids, and glucose in animals.

The activity of the Krebs cycle is controlled by many factors to meet the cell's needs for ATP and substrates involved in various synthetic reactions (Figure 1.37). These factors include:

a. supply of acetyl-CoA from glucose-, glycerol-, amino acid-derived pyruvate, and from the oxidation of fatty acids;
b. availability of OAA and thus its precursors (e.g., glucose and glucogenic amino acids) for reacting with acetyl-CoA as well as O_2;
c. availability of NAD^+ and FAD as coenzymes for dehydrogenases;
d. availability of minerals (e.g., Fe^{2+}, Mg^{2+}, and Mn^{2+}) as cofactors for Krebs cycle enzymes; (e) activity of the electron transport system to oxidize $NADH + H^+$ and $FADH_2$ for the regeneration of NAD^+ and FAD, respectively;
e. rates of ATP synthesis (determined by metabolic demands for ATP) and therefore the supply of ADP, Pi, and O_2 to the mitochondria;
f. integrity of mitochondrial membranes to prevent any uncontrolled exchange of substances across them;
g. quantity of Krebs cycle enzymes and the phosphorylation state of α-ketoglutarate dehydrogenase;
h. concentrations of iron, copper, Ca^{2+}, and lactate, as well as the ratio of ATP/ADP and GTP/GDP; and
i. numerous endogenous and exogenous inhibitors of Krebs cycle enzymes (e.g., an inhibition of aconitase by H_2O_2, NO, and fluoroacetate; of α-ketoglutarate dehydrogenase complex by arsenite; and of succinate dehydrogenase by malonate)

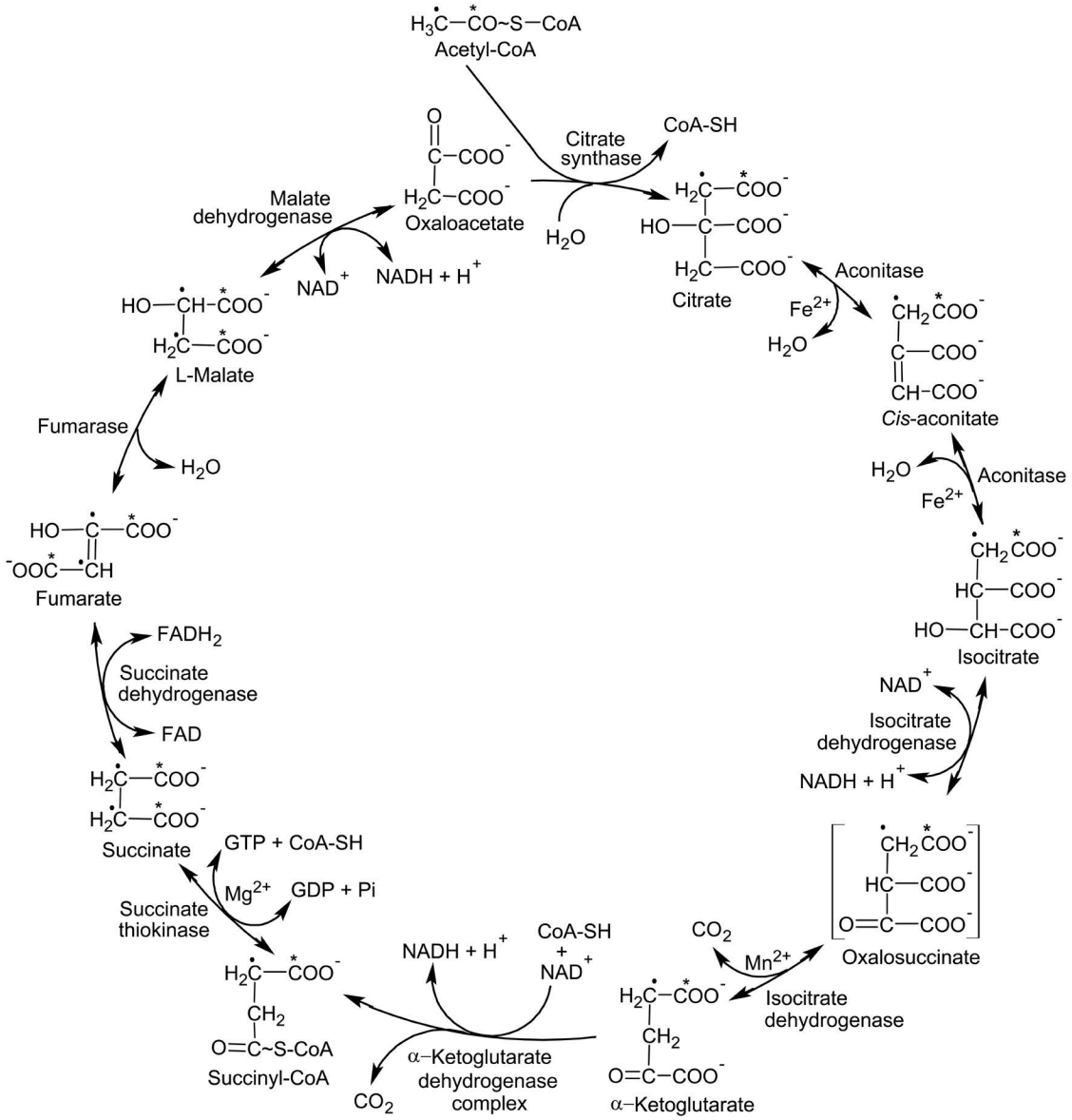


FIGURE 1.36 The Krebs cycle for oxidation of acetyl-CoA to CO_2, water, NADH + H^+, and $FADH_2$. The carboxyl and methyl carbons of acetyl-CoA are shown with labels designated by the symbols "*" and ".", respectively. In the first turn of the cycle, two molecules of CO_2 are produced, which are derived from the oxaloacetate portion of citrate but not the acetyl-CoA that immediately enters the cycle. Because succinate is a symmetrical compound and because succinate dehydrogenase does not differentiate between its two carboxyl groups, the "randomization" of labeling occurs at this step such that all four carbons of succinate, fumarate, malate and oxaloacetate are labeled after one turn of the cycle. The carboxyl and methyl carbons of acetyl-CoA are completely lost as CO_2 after two and fifteen turns of the cycle, respectively. (Reproduced from Wu 2022a, with permission from CRC Press, Boca Raton, FL.)

and the mitochondrial electron transport system (e.g., inhibition of complexes I, III, and IV by oligomycin and uncouplers, and additional inhibition of complex IV by H_2S, CO, and cyanide). Thus, the Krebs cycle is regulated by both intrinsic and extrinsic factors.

The Electron Transport System in Mitochondria

NADH and $FADH_2$ generated from the oxidation of fatty acids, amino acids, and glucose in the mitochondrial matrix are oxidized to NAD^+ and FAD via the electron transport system (Figure 1.38). The discovery of respiratory-chain enzymes led to the award of the Nobel Prize in Physiology or Medicine to Otto H. Warburg in 1931. Note that the NAD^+ molecule has one net negative charge, and the superscript "+" denotes the positive charge on the N atom in its nicotinamide ring. The reduction-oxidation reactions involving quinone, NAD^+, FAD, and Fe^{2+} are shown in Figure 1.39. Electrons flow from low- to high-reduction potential carriers (a set of proteins containing redox centers). This respiratory chain is localized in the inner

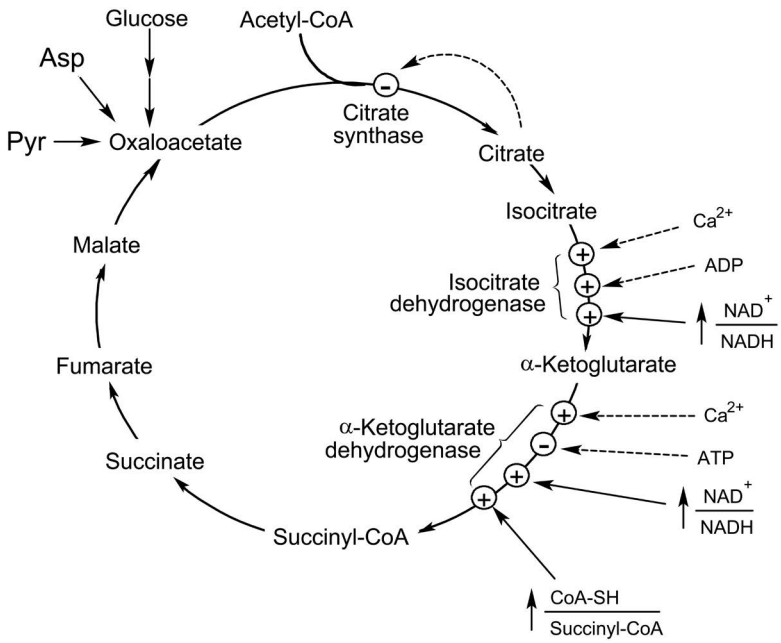


FIGURE 1.37 Regulation of the Krebs cycle by intrinsic and extrinsic factors. The intrinsic factors include components of the cycle itself. The extrinsic factors include the supply of acetyl-CoA and the activity of the electron transport system, as well as associated enzymes and substrates (e.g., O_2 and phosphate provision).

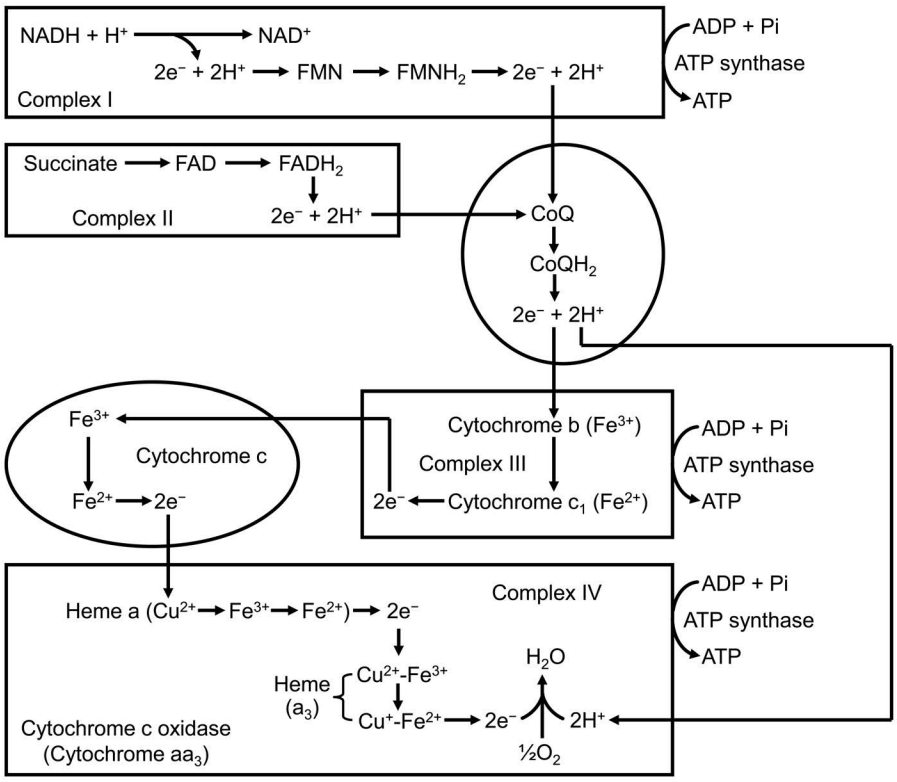


FIGURE 1.38 The mitochondrial electron transport system (the respiratory chain). NADH and $FADH_2$ are generated from the oxidation of fatty acids, amino acids, and glucose in the mitochondrial matrix. These reducing equivalents are oxidized to NAD^+ and FAD with the formation of H_2O via the electron transport system, which is localized in the inner mitochondrial membrane and consists of complexes I to IV. The energy released from the oxidation reactions is used in the formation of the proton gradient that drives the synthesis of ATP from ADP and Pi by ATP synthase (also known as complex V). (Adapted from Dai et al. 2013.)

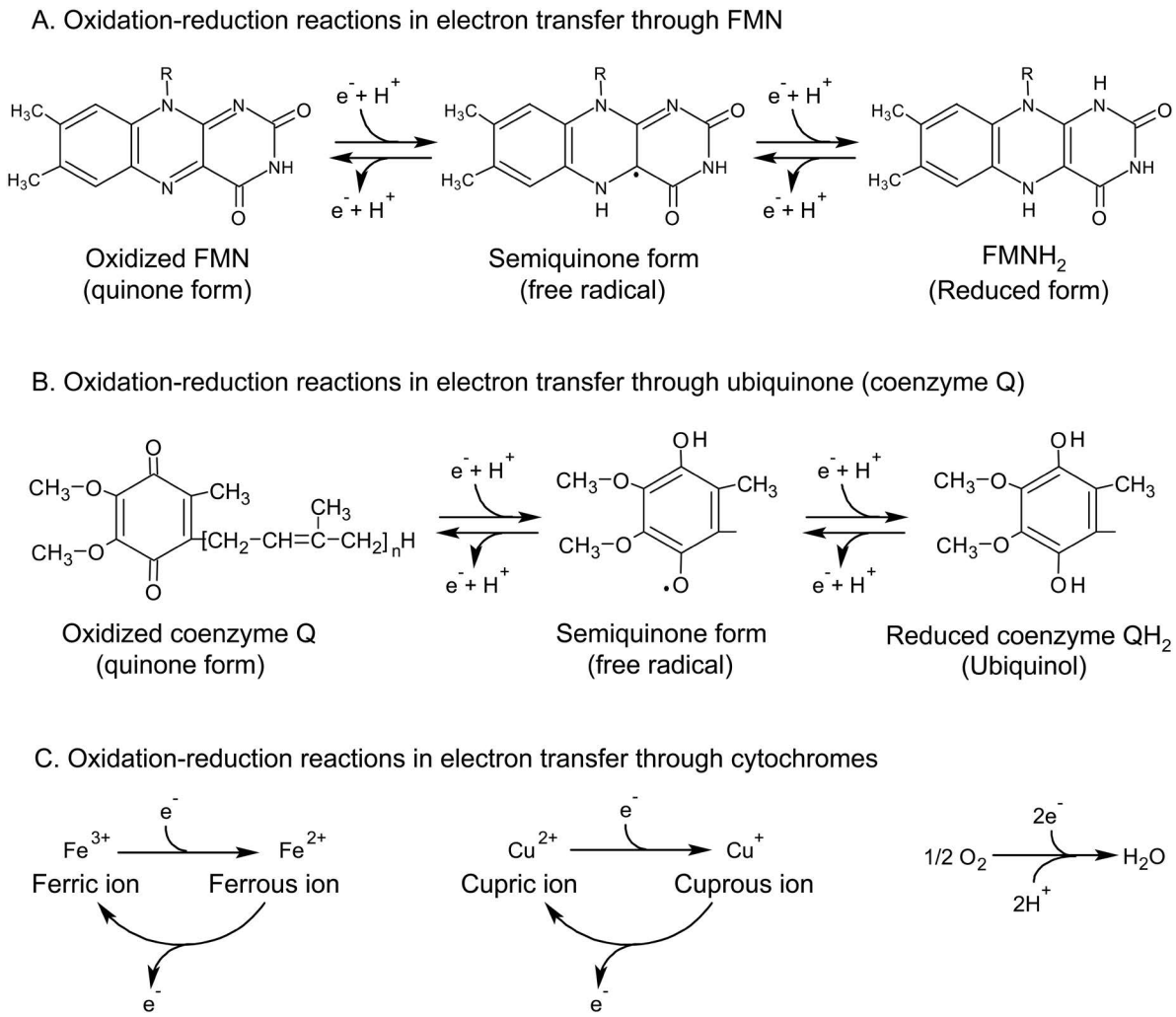


FIGURE 1.39 The reduction-oxidation reactions involving quinone, NAD^+, FAD, and Fe^{2+} in the mitochondrial electron transport system. Oxidation is defined as the removal of electrons from an electron donor (called the reductant), and reduction as the gain of electrons by an electron acceptor (called the oxidant). The tendency of an electron donor to give up its electrons to an electron acceptor is called the oxidation-reduction potential. The reductant of a redox pair with a large negative potential will release its electrons more easily than a redox pair with smaller negative or positive potential.

mitochondrial membrane and consists of complexes I through IV (see below for the oxidation of substrates). Complexes I, III, and IV also act as proton pumps to translocate 4 electrons from the mitochondrial matrix to the intermembrane space, generating the proton-motive force across the inner mitochondrial membrane (Figure 1.40).

The energy from the electrochemical gradient is used to drive the synthesis of ATP from ADP and Pi by ATP synthase (also known as complex V). The ATP synthase complex consists of two domains: F_0 and F_1. In mammals, birds, fish, and other animals, the F_0 domain is an integral protein within the inner mitochondrial membrane and contains a proton channel, whereas the F_1 domain (which has ATP synthase activity) is a peripheral protein that is located in the mitochondrial matrix but is bound to the inner mitochondrial membrane. Thus, the F_0 domain provides a pore for the passage of protons from the intermembrane space into the F_1 domain in the mitochondrial matrix (Figure 1.40). The F_0 and F_1 domains are linked by central and peripheral stalks consisting of proteins. The discovery of the enzymatic mechanisms for ATP synthesis led to the award of the Nobel Prize in Chemistry to Paul D. Boyer and John E. Walker in 1997.

Complex I: Oxidation of NADH by coenzyme Q (CoQ), forming ubiquinol (reduced CoQ).
Complex II: Oxidation of $FADH_2$ by CoQ, forming reduced CoQ (ubiquinol).
Complex III: Oxidation of reduced CoQ (ubiquinol) by cytochrome bc_1, regenerating CoQ.
Complex IV: Oxidation of reduced cytochrome c_1 by O_2 (molecular oxygen), forming H_2O.

Complex I (NADH dehydrogenase) is also known as NADH-CoQ reductase and contains FeS centers. This complex uses NADH + H^+ as substrates, as well as FMN and

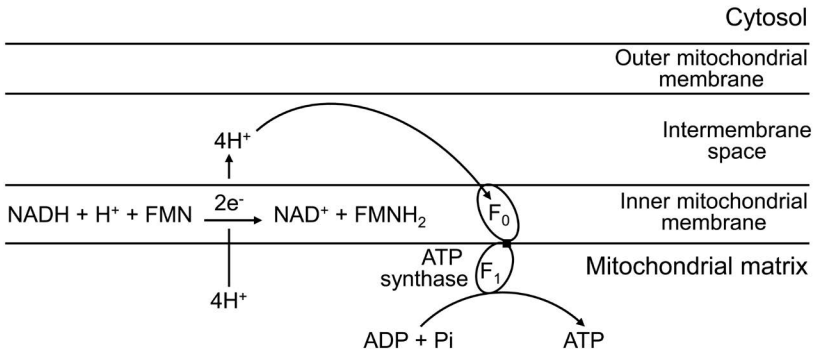


FIGURE 1.40 Translocation of protons in complex I of the mitochondrial electron transport system and mitochondrial ATP synthesis in animals. During the oxidation of NADH + H^+ and $FADH_2$, complex I acts as a proton pump to translocate 4 electrons from the mitochondrial matrix to the intermembrane space. This generates the proton-motive force across the inner mitochondrial membrane to drive the synthesis of ATP from ADP and Pi by ATP synthase (also known as complex V). The ATP synthase complex consists of two domains: F_0 (an integral protein containing a proton channel) and F_1 (a peripheral protein containing ATP synthase activity). Electrons pass through the F_0-F_1 complex, resulting in ATP synthesis. (Adapted from Devlin 2011.)

coenzyme Q [CoQ, also called ubiquinone (Q), a lipid-soluble mobile carrier] as its coenzymes. Note that NADH + H^+ are generated from NAD^+ and a reductant (e.g., succinate) by an enzyme (e.g., succinate dehydrogenase) in the mitochondrion (Figure 1.41). Complex I transfers 2 electrons and 2 protons from NADH + H^+ to FMN and then from $FMNH_2$ (1 electron and 1 proton at a time) to CoQ, forming the reduced form of CoQ ($CoQH_2$), ubiquinol. Thus, FMN is a two-electron acceptor and $FMNH_2$ is a one-electron donor. The energy released during the transfer of electrons and protons from NADH + H^+ to CoQ is used by Complex I to pump 4 protons (H^+) from the mitochondrial matrix into the intermembrane space, generating an electrochemical gradient (proton-motive force) across the inner mitochondrial membrane, as noted previously. This makes the intermembrane space more acidic and the mitochondrial matrix more alkaline. The 4 protons that are translocated from the mitochondrial matrix into the intermembrane space move through the proton channel in the F_0 domain (the inner mitochondrial membrane) of ATP synthase to the F_1 domain (the mitochondrial matrix) of ATP synthase, causing a conformational change in the F_1 domain (Devlin 2011). This results, sequentially, in the synthesis of 1 ATP molecule from 1 ADP molecule and 1 Pi molecule, as well as the release of ATP from the F_1 domain into the mitochondrial matrix. This process of ATP synthesis, called chemiosmosis, was proposed by Peter D. Mitchell in 1961 and is now widely accepted in biochemistry.

Complex II (succinate dehydrogenase) is also known as succinate-CoQ reductase, and it contains FeS centers and

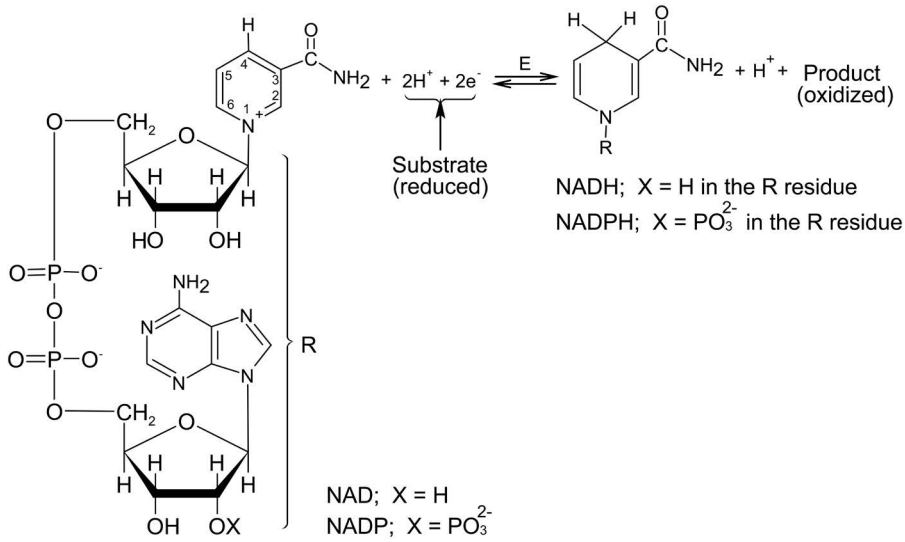


FIGURE 1.41 The role of NAD^+ and $NADP^+$ in reduction-oxidation reactions. NAD^+ or $NADP^+$ accepts a proton to be reduced to NADH or NADPH, respectively. The NAD^+ molecule has one net negative charge, and the superscript "+" denotes the positive charge on the nitrogen atom in its nicotinamide ring. Likewise, the $NADP^+$ molecule has three net negative charges, and the superscript "+" denotes the positive charge on the nitrogen atom in its nicotinamide ring.

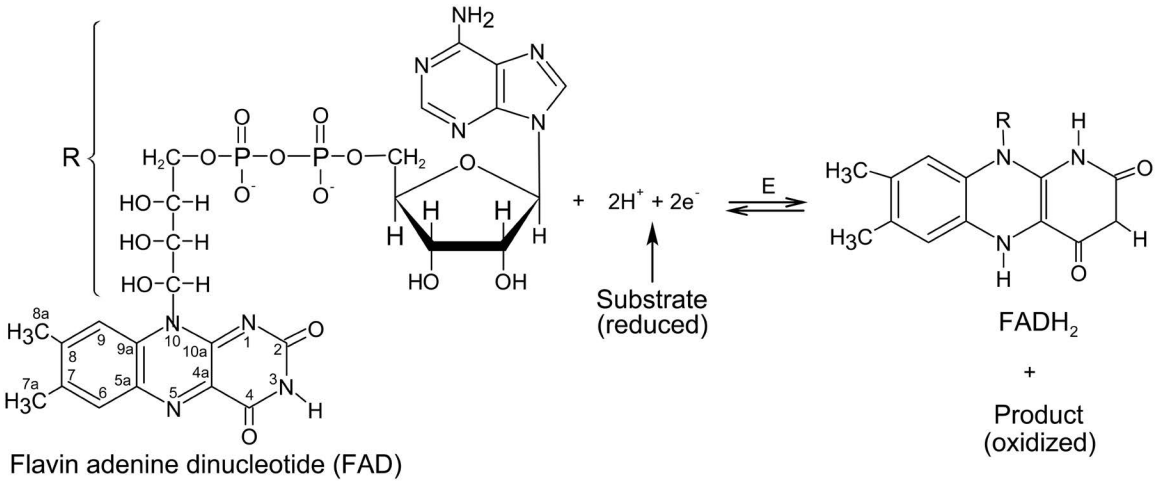


FIGURE 1.42 The role of FAD in reduction-oxidation reactions. FAD accepts two protons and two electrons from a reductant (e.g., succinate) to be reduced as $FADH_2$. The β-oxidation of fatty acids and the oxidation of acetyl-CoA in the mitochondrion produce $FADH_2$. This reducing equivalent is oxidized via the respiratory chain to FAD, with the generation of H_2O and ATP.

covalently bound FAD. This enzyme oxidizes succinate to fumarate, with the transfer of 2 electrons and 2 protons to FAD to form $FADH_2$ (Figure 1.42). The $FADH_2$ donates the electrons and protons to CoQ via the FeS centers, producing ubiquinol. The free energy released during these oxidative reactions is not sufficient to pump protons from the mitochondrial matrix into the intermembrane space. Thus, complex II is not a proton pump, does not generate an electrochemical gradient across the inner mitochondrial membrane, and does not synthesize ATP.

Complex III (cytochrome bc_1) is also known as cytochrome c reductase (CoQ-cytochrome c reductase). This complex contains cytochrome b (with a heme group), cytochrome c_1 (a water-soluble, mobile electron carrier), and Rieske FeS protein. The globular head of the Rieske protein moves between cytochromes b and c_1. Ubiquinol is the donor of 2 electrons and is oxidized to ubiquinone (ubiquinone/ubiquinol; $E_0' = +90$ mV). Note that each cytochrome can accept only 1 electron at a time. The 2 electrons released from the oxidation of ubiquinol (QH_2) are split, with one electron being transferred to the Rieske Fe_2-S_2 cluster and the other electron to cytochrome b within the same enzyme complex. Energy from the reduction of the Rieske Fe_2-S_2 cluster drives the reduction of cytochrome b (see below). This coupled process is called electron bifurcation, which enables an energetically unfavorable (endergonic) reaction by its coupling with an energetically favorable (exergonic) reaction (Brandt 1996). Thus, through the Rieske Fe_2-S_2 cluster, electrons can flow from reduced CoQ (ubiquinol) to cytochrome b. Cytochrome b passes the electron to cytochrome c_1. Overall, complex III transfers 2 electrons and 2 protons from ubiquinol to cytochrome c_1 (QH_2 + 2 Ferricytochrome c_1 → Q + 2 Ferrocytochrome c_1 + 2 H^+). As in complex I, the energy released from the oxidative reactions drives the translocation of 4 protons from the mitochondrial matrix into the intermembrane space, generating an electrochemical gradient across the inner mitochondrial membrane to synthesize 1 ATP molecule from 1 ADP molecule and 1 Pi molecule.

$$\text{Rieske } 2Fe^{3+}\text{-}S_2\text{cluster} + e^- \rightarrow \text{Rieske } Fe^{3+} / Fe^{2+}\text{-}S_2\text{cluster}$$
$$\left(E_0' = +285 \text{ mV; exergonic, favorable/ spontaneous reaction}\right)$$

$$Fe^{3+}\text{-cytochrome b} + e^- \rightarrow Fe^{2+} - \text{cytochrome b}$$
$$\left(E_0' = -90 \text{ mV; endergonic, unfavorable/ non-spontaneous reaction}\right)$$

$$\text{Rieske } 2Fe^{3+}\text{-}S_2\text{cluster} + 2\ e^- + Fe^{3+}\text{-cytochrome b} \rightarrow \text{Rieske } Fe^{3+} / Fe^{2+}\text{-}S_2\text{cluster} + Fe^{2+}\text{-cytochrome b}$$
$$\left(E_0' = +375 \text{ mV}\right)$$

$\left[E_0'\right.$ = standard redox potential; positive or negative values indicate spontaneous or non-spontaneous reactions, respectively.$\left.\right]$

Complex IV is also known as cytochrome c oxidase or cytochrome aa_3. It consists of 13 subunits, with 3 and 10 subunits being encoded by mitochondrial DNA and nuclear DNA, respectively. Cytochrome c oxidase contains cytochromes a and a_3, as well as two copper centers, C_{UA} and C_{UB}. Complex IV transfers 2 electrons and 2 protons from the reduced cytochrome c to ½ O_2 as the final oxidant of NADH + H^+ and $FADH_2$ (i.e., the terminal acceptor of the 2 electrons from NADH + H^+ or $FADH_2$) to produce H_2O. The energy released during the oxidative reactions is used by complex IV to pump 4 protons from the mitochondrial matrix to the intermembrane space. However, 2 of the 4 protons leak from the intermembrane space into the cytosol to generate superoxide anion (O_2^-), while the remaining 2 protons form an electrochemical gradient across the inner mitochondrial membrane. Thus, only 2 protons translocate from the intermembrane space into the F_0-F_1 complex of

ATP synthase, and the electrochemical potential is sufficient to synthesize only ½ mol of ATP from ½ mol of ADP and ½ mol of Pi. Due to the release of 2 electrons from the mitochondrion to the cytosol per NADH or $FADH_2$ molecule oxidized, the rate of superoxide production (and hence oxidative stress) is positively correlated with the rate of ATP production in cells (and hence dietary energy intake).

The energetic efficiency of electron transport for ATP production is approximately 80%. Specifically, the oxidation of 1 mol of NADH + H^+ produces 2.5 mol of ATP from 2.5 mol of ADP and 2.5 mol of Pi through the mitochondrial electron transport system (1, 1 and 0.5 mol of ATP at complexes I, III, and IV, respectively). This gives a P/O ratio of 2.5 (mol of ATP synthesized per mol of [O] consumed) for the transport of 2 electrons and 2 protons from NADH + H^+ to ½ O_2, rather than a theoretical P/O ratio of 3 (Hinkle 2005). Oxidation of 1 mol of $FADH_2$ does not involve complex I and thus produces 1.5 mol of ATP from 1.5 mol of ADP and 1.5 mol of Pi through the electron transport system (1 and 0.5 mol of ATP at complexes III and IV, respectively). This gives a P/O ratio of 1.5 for the transport of 2 electrons and 2 protons from $FADH_2$ to ½ O_2, rather than a theoretical P/O ratio of 2 (Hinkle 2005). Oxidation of 1 mol of acetyl-CoA via the Krebs cycle and the respiratory chain generates 10 mol ATP in cells with mitochondria from the production of 3 mol of NADH + H^+ (equivalent to 7.5 mol of ATP), 1 mol $FADH_2$ (equivalent to 1.5 mol of ATP), and 1 mol of ATP (catalyzed by succinate thiokinase).

ATP Transfer between the Mitochondria and the Cytosol

ATP is transferred from the mitochondria to the cytosol via (a) phosphocreatine–creatine shuttle; (b) ADP/ATP exchange through adenine nucleotide translocator (ANT; also known as adenine nucleotide translocase or ADP/ATP carrier) in the inner mitochondrial membrane; and (c) passages through the VDAC that resides in the outer mitochondrial membrane (Schlattner et al. 2006; Walzel et al. 2002; Wallimann et al. 2011). The phosphocreatine–creatine shuttle involves the following steps: (a) creatine in the cytosol enters the mitochondrial intermembrane space via the VDAC, and ATP in the mitochondrial matrix moves into the mitochondrial intermembrane space via the ANT; (b) mitochondrial creatine kinase, which is located within the mitochondrial intermembrane space adjacent to the outer surface of the inner mitochondrial membrane, converts creatine and ATP into phosphocreatine and ADP; and (c) phosphocreatine diffuses across the outer mitochondrial membranes into the cytosol via the VDAC (Figure 1.43). In response to metabolic needs for ATP, cytosolic creatine kinase converts phosphocreatine and ADP into creatine and ATP.

Recent results from studies using ATP imaging technologies indicate that ATP may be transferred from the cytosol into the mitochondria in mammalian cells (White et al. 2023). Specifically, when HEK293T cells were treated with atractyloside (an ANT inhibitor) in normoxia, the import of ADP from the cytosol to the mitochondria and the export of ATP from the mitochondria to the cytosol were reduced, confirming an important role for the ANT in ADP and ATP exchange between the cytosol and the mitochondria. In response to hypoxia that inhibited mitochondrial substrate oxidation, atractyloside increased mitochondrial ATP concentration and concomitantly decreased cytosolic ATP concentration. Thus, under conditions of limited mitochondrial ATP production, the ATP generated from glucose via glycolysis can be transferred from the cytosol into the mitochondria for both metabolic utilization by this organelle and the maintenance of mitochondrial membrane integrity. This adaptation is biologically significant in mammalian embryos, tumors, and exercise.

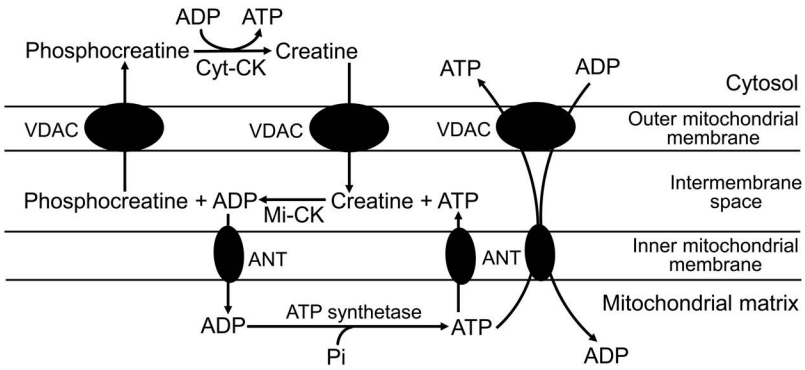


FIGURE 1.43 Transfer of ATP from the mitochondria to the cytosol in animal cells. ATP exits the mitochondria into the cytosol via (a) phosphocreatine–creatine shuttle; (b) ADP/ATP exchange through adenine nucleotide translocator (ANT; also known as adenine nucleotide translocase or ATP/ADP carrier) in the inner mitochondrial membrane; and (c) passages through the voltage-dependent anion channel (VDAC) that resides in the outer mitochondrial membrane. Creatine and phosphocreatine can readily diffuse across the outer mitochondrial membrane. In the phosphocreatine–creatine shuttle, mitochondrial creatine kinase (Mi-CK) located within the mitochondrial intermembrane space generates phosphocreatine and creatine from creatine and ATP. In response to metabolic needs for ATP, cytosolic creatine kinase (Cyt-CK) converts phosphocreatine and ADP into creatine and ATP.

Uncouplers of Oxidative Phosphorylation within the Mitochondrial Electron Transport System

Uncoupling protein-1 (UCP-1) uncouples oxidative reactions from ATP synthesis. This protein is uniquely expressed in BAT (Wu et al. 2012). UCP-1 is localized exclusively in the inner mitochondrial membrane and provides a pore for the movement of electrons from the intermembrane space to the mitochondrial matrix, such that the electrons will not pass through the F_0-F_1 complex of ATP synthase. As a result, the mitochondrial electron transport is uncoupled from ATP synthesis, and the energy from the oxidation of NADH + H^+ and $FADH_2$ is released as heat. Thus, mitochondrial electron transport may proceed without ATP production, although O_2 is consumed by cells. In many mammals (including humans, rats, mice, cattle, and sheep), BAT starts to develop in fetuses during the mid- or last-trimester of gestation and is abundant in newborns, with most BAT being located near the kidneys, heart, and neck. The oxidation of fatty acids in BAT plays a major role in nonshivering thermogenesis in the newborn of these species, allowing them to keep warm, particularly during exposure to a cold environment (Cannon and Nedergaard 2004). BAT can also oxidize glucose, glutamine, and leucine (Tekwe et al. 2023). Of note, some animal species (e.g., pigs and chickens) do not contain BAT at any stage of their life cycle (Satterfield and Wu 2011).

In addition to UCP-1, mammals also express UCP-2 in the WAT, stomach, spleen, and lung, and UCP-3 in the skeletal muscle, heart, liver, WAT, BAT, spleen, and thymus (Mailloux and Harper 2011). The major function of UCP-2 and UCP-3 (mitochondrial membrane transporters) in these tissues appears to be an inhibition of the mitochondrial production of reactive oxygen species, but not for the purpose of promoting thermogenesis. In addition, some chemicals (e.g., proton ionophores) can serve as uncouplers of mitochondrial oxidative phosphorylation by reducing proton concentration in the inner mitochondrial membrane (Table 1.14). Specifically, these substances, which are lipophilic (e.g., 2,4-dinitrophenol-O^-), accept protons from the intermembrane space (where proton concentration is relatively high) to form protonated molecules (e.g., 2,4-dinitrophenol-OH). The protonated molecules rapidly diffuse into the mitochondrial matrix, where the protons that have been picked up within the intermembrane space are dissociated due to a relatively low H^+ concentration. Through the addition of a proton to an uncoupler in the inner mitochondrial membrane (known as protonation), the electrons that are translocated from the mitochondrial matrix into the intermembrane space during the oxidation of NADH + H^+ and $FADH_2$ do not enter the F_0-F_1 complex of ATP synthase, resulting in no ATP production.

TABLE 1.14
Uncouplers of Oxidative Phosphorylation and Inhibitors of the Electron Transport System Block ATP Synthesis from ADP and Inorganic Phosphate in Mitochondria

Molecule	Action	Comment
UCP-1	Uncoupler of oxidative phosphorylation	Uniquely expressed in BAT in many animals
DNP	Uncoupler of oxidative phosphorylation	Synthetic substance
Dicumarol	Uncoupler of oxidative phosphorylation	Naturally occurring anticoagulant
Rotenone	Inhibitor of complex I	Common insecticide found in roots of plants
Ptericidin	Inhibitor of complex I	Synthetic substance
Amobarbital	Inhibitor of complex I	Synthetic substance
Mercurials	Inhibitor of complex I	Mercury derivatives (toxic substances)
Demerol	Inhibitor of complex I	Also known as *meperidine, s*ynthetic substance
Malonate	Inhibitor of complex II	Binds to the active site of the target enzyme
Carboxin	Inhibitor of complex II	A synthetic pesticide
TTFA	Inhibitor of complex II	A synthetic chelating substance
Antimycin	Inhibitor of complex III	Produced by *Streptomyces* bacteria
Dimercaprol	Inhibitor of complex III	Treatment of metal toxicity through chelation
Cyanide (C≡N)	Inhibitor of complex IV	Synthetic substance
Azide ($N=N^+=N$)	Inhibitor of complex IV	Synthetic substance
H_2S	Inhibitor of complex IV	A metabolite of cysteine; synthetic substance
CO (high dose)	Inhibitor of complex IV	A metabolite of heme; synthetic substance
NO (high dose)	Inhibitor of complexes I, II, III and IV	A metabolite of arginine; synthetic substance
DCCD	Inhibitor of ATP synthase	Covalent bonding to carboxyl groups of protein
Oligomycin	Inhibitor of ATP synthase	Binds F_0 and blocks movement of e^- into F_0-F_1

Source: Cooper and Hausman (2016) and Devlin (2011).

Note: BAT, brown adipose tissue; CO, carbon monoxide; DCCD, dicyclohexylcarbodiimide; DNP, 2,4-dinitrophenol; H_2S, hydrogen sulfide; NO, nitric oxide; TTFA, 2-thenoyltrifluoroacetone; UCP-1, uncoupling protein-1.

In addition to the endogenous and exogenous uncouplers of oxidative phosphorylation, some toxic substances can also block ATP synthesis from ADP and Pi by directly inhibiting enzymes or proteins in the electron transport system. These substances are listed in Table 1.14. Caution should be taken to ensure that such toxic chemicals will not be part of any diets for animals. Note that while physiological levels of CO and NO stimulate energy metabolism in organisms, high concentrations of these gases reduce ATP production by inhibiting complexes I to IV (Dai et al. 2013). This further highlights the importance of a balanced diet in the health and well-being of mammals, birds, fish, and other species.

BIOLOGICAL OXIDATION IN PROKARYOTES: ELECTRON BIFURCATION AND ELECTRON CONFURCATION

Electron Bifurcation in Prokaryotes

As noted previously, electron bifurcation (ubiquinone-based) occurs in animal cells, such as electron transfer within Complex III of the mitochondrial electron transport system. Electron bifurcation in prokaryotes was discovered by Thauer's group in 2008, based on the coupled reduction of crotonyl-CoA and oxidized ferredoxin with NADH catalyzed by the soluble butyryl-CoA dehydrogenase/Etf complex (a flavoprotein containing FAD as a prosthetic group) from *Clostridium kluyveri* (Li et al. 2008). Such a biochemical process is now known to occur commonly in the cytosol and cell (plasma) membrane of anaerobic bacteria (both Gram-positive and negative) and archaea (e.g., those in the rumen; Figure 1.44). Electron donors are usually NAD(P)H, dissolved H_2, reduced ferredoxin, reduced coenzyme F_{420} ($F_{420}H_2$), and formate. Electron acceptors may be $NAD(P)^+$, methylene-tetrahydrofolate, pyruvate, coenzyme M-S-S-coenzyme B (heterodisulfide), crotonyl-CoA, ubiquinone, oxidized ferredoxin, and oxidized flavodoxins (FMN-containing proteins; Buckel and Thauer 2018). Aerobic microbes generally do not utilize electron bifurcation in metabolism to a significant extent.

$$\text{Crotonyl-CoA} + 2\text{NADH} + 2\text{H}^+ + 2\text{Fd}_{ox} \rightarrow \text{Butyryl-CoA} + 2\text{NAD}^+ + 2\text{Fd}_{red}^-$$

$$\left[\text{Fd}_{red}^- = \text{reduced ferredoxin; Fd}_{ox} = \text{oxidized ferredoxin}\right]$$

Electron bifurcation plays an important role in microbial nutrient metabolism and energy conservation. The coupling of an energetically favorable reaction with an energetically unfavorable one is more economical in the use of biological energy than the hydrolysis of ATP that must first be synthesized at <100% efficiency. Let us take, as an example, the electron-bifurcating (Fe-Fe) hydrogenase complex (a flavoprotein containing FAD as a prosthetic group) that catalyzes the oxidation of H_2 to $2H^+ + 2e^-$ in anaerobic bacteria. The two electrons from the same donor (H_2) are split, with 1 electron being transferred to a high-potential acceptor [$NAD(P)^+$] and the other electron to a low-potential acceptor (oxidized ferredoxin) within the same enzyme complex. In this process, the energetically favorable (exergonic) reduction of $NAD(P)^+$ by H_2 is used to drive the energetically unfavorable (endergonic) reduction of oxidized ferredoxin by H_2, ultimately yielding $2H^+ + 2e^-$ (Constant and Hallenbeck 2019). Such a chemical transformation does not need the hydrolysis of ATP to provide energy nor generate heat (the dissipation of biological energy), thereby helping conserve energy in anaerobic microbes. The two separate nonenzymatic reactions and the hydrogenase-catalyzed electron bifurcation are outlined as follows:

$$\text{NAD(P)}^+ + \text{H}_2 \rightarrow \text{NAD(P)H} + \text{H}^+ (E_0' = +450 \text{ mV at pH 7.0; exergonic, energetically favorable reaction})$$

$$2\,\text{Fd}_{ox} + \text{H}_2 \rightarrow 2\,\text{Fd}_{red}^- + 2\,\text{H}^+ (E_0' = -398 \text{ mV at pH 7.0; endergonic, energetically unfavorable reaction})$$

$$2\,\text{Fd}_{ox} + \text{NAD(P)}^+ + 2\,\text{H}_2 \rightarrow 2\,\text{Fd}_{red}^- + \text{NAD(P)H} + 3\,\text{H}^+ \left(E_0' = +848 \text{ mV at pH 7.0; energetically favorable reaction}\right)$$

[Fd_{red}^- = reduced ferredoxin; Fd_{ox} = oxidized ferredoxin; ΔG = change in Gibbs free energy]

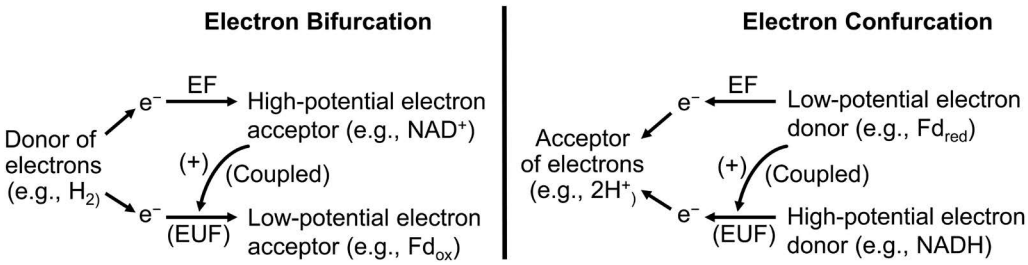


FIGURE 1.44 Electron bifurcation and electron confurcation in prokaryotes. In electron bifurcation, two electrons from the same donor are split and then transferred to separate acceptors within the same enzyme complex. The energy from the energetically favorable reaction (EF) for high-potential electron acceptor drives the energetically unfavorable (EUF) reaction for low-potential electron acceptor. The reversal of electron bifurcation is called electron confurcation, where two electrons from two different donors conjugate within the same enzyme complex. In electron confurcation, the energy from the energetically favorable reaction for a low-potential electron donor drives the energetically unfavorable reaction for a high-potential electron donor. A high-potential electron acceptor readily accepts an electron and is easily reduced, whereas a low-potential electron donor readily donates an electron and is easily reduced.

Electron Confurcation in Prokaryotes

The reverse process of electron bifurcation is called electron confurcation, where 2 electrons from different donors (1 e^-/donor) are combined to reduce a higher potential electron acceptor, effectively "fusing" the electrons together (Müller et al. 2018). Like electron bifurcation, electron confurcation is also a biochemical mechanism for energy conservation. Let us again use, as an example, the electron-bifurcating (Fe-Fe) hydrogenase that also reversibly catalyzes the reduction of $2H^+ + 2e^-$ to H_2 in anaerobic bacteria (Jay et al. 2020). Namely, 1 electron from reduced ferredoxin and 1 electron from NADPH flow to the hydrogenase, where the energy from the reduction of 1 H^+ in the energetically favorable (exergonic) oxidation of reduced ferredoxin is used to drive the energetically unfavorable (endergonic) reduction of another H^+ by NAD(P)H, ultimately generating H_2. Like electron bifurcation, electron confurcation itself does not directly result in ATP synthesis by microbes.

$$2\ Fd_{red}^- + 2\ H^+ \rightarrow 2\ Fd_{ox} + H_2$$
$$(\Delta G = -19.3\ \text{kJ/mol}\ H_2;\ \text{energetically favorable reaction})$$

$$NAD(P)H + H^+ \rightarrow NAD(P)^+ + H_2$$
$$(\Delta G = 9.8\ \text{kJ/mol}\ H_2;\ \text{energetically unfavorable reaction})$$

$$2\ Fd_{red}^- + NAD(P)H + 3\ H^+ \rightleftharpoons 2\ Fd_{ox} + NAD(P)^+ + 2\ H_2$$
$$(\Delta G = -9.5\ \text{kJ/mol}\ H_2;\ \text{energetically favorable reaction})$$

[Fd_{red}^- = reduced ferredoxin; Fd_{ox} = oxidized ferredoxin; ΔG = change in Gibbs free energy; 25°C, pH 7.0]

Ruminal methanogens oxidize dissolved hydrogen gas (H_2) and use its electrons to reduce CO_2 to CH_4 through a series of enzyme-catalyzed reactions. The energy released from this process is captured to synthesize ATP from ADP and Pi by ATP synthase. Methane formation consumes about two-thirds of the electrons generated from ruminal fermentation (Leahy et al. 2022). Energy from these electrons and associated substances could be converted into usable organic compounds rather than methane. Thus, regulating electron flow within the rumen in favor of syntheses of SCFAs, amino acids, and proteins likely plays an important role in reducing methane emissions from ruminants.

BIOLOGICAL OXIDATION IN PROKARYOTES: ELECTRON TRANSPORT CHAINS IN THE PLASMA (CELL) MEMBRANE OF PROKARYOTES

Catabolism of carbohydrates (e.g., starch, glucose, galactose, and fructose), glycerol, pyruvate, and lactate by bacteria and archaea (e.g., those in the rumen) produces ATP and NADH. In addition, these cells metabolize fatty acids to yield both $FADH_2$ and NADH. Furthermore, due to their lack of mitochondria, prokaryotes contain enzymes of the Krebs cycle in the cytoplasm that generate ATP, GTP, $FADH_2$, and NADH from amino acids and related metabolites (e.g., oxaloacetate, succinate, and α-ketoglutarate). In bacteria and archaea, some NADH reacts with pyruvate to form NAD^+ and lactate, whereas some NADH and nearly all $FADH_2$ are oxidized to NAD^+ and FAD, respectively, via cell membrane-bound electron transport chains (Figure 1.45). This is analogous in many aspects to the electron transport system in the mitochondria of eukaryotes. Thus, electron carriers (e.g., quinones, flavines, heme, Fe-S clusters, and nitrate, reduced ferredoxin, and H_2) and ATP synthase (extending into the cytoplasm) play important roles in microbial electron transport within the cell membrane. The membrane-bound ATP synthase consists of A_0 and A_1 domains in archaea, but F_0 and F_1 domains in bacteria.

In prokaryotic cell membranes, electrons from substrates enter the diverse electron transport chains at the points of dehydrogenase, quinone (e.g., ubiquinone or menaquinone), and cytochrome (Britton et al. 2024; Muras et al. 2019). Of note, electrons from NADH are transferred to (a) cell membrane-associated proteins called Rnf protein complexes (ferredoxin:NAD^+ oxidoreductases) in many bacterial species (e.g., *Rhodobacter capsulatus* and *Fusobacterium nucleatum*) and some archaea (e.g., *Methanosarcina acetivorans*) and (b) caldariellaquinone (a type of quinone) in certain archaea (e.g., the genus *Sulfolobus*). Some membrane-bound dehydrogenases (e.g., NADH dehydrogenase) and reductases (e.g., nitrate reductase) are also proton pumps, whereas most membrane-bound oxidases [e.g., Rnf protein complexes and Na^+-translocating NADH:quinone oxidoreductase (Na^+-NQR)] are also Na^+ pumps. Thus, under both aerobic and anaerobic conditions, the electrochemical potential generated through the electron transport within the cell membrane can be coupled with the export of Na^+ from bacteria and archaea to the cell exterior. Finally, the terminal reaction of oxidation is catalyzed by either class-I oxidases (cytochrome oxidases) or class-II oxidases (quinol oxidases). Cytochrome bc_1 is a proton pump found in many, but not all, bacteria. Therefore, in prokaryotic electron transport chains, intracellular protons or Na^+ ions can be pumped across the cell membrane to create an electrochemical gradient that drives the synthesis of ATP from ADP plus Pi by ATP synthase.

Electron acceptors include O_2 (only for aerobic microbes), as well as N (nitrate or nitrite), S (sulfate or sulfite), and carbon (fumarate or carbonate) compounds, depending on the environment and microbial species. In the presence of O_2 as the ultimate electron acceptor, both H_2O and ATP are produced ($½\ O_2 + 2\ H^+ \rightarrow H_2O$) by aerobic microbes. However, in the absence of O_2 (e.g., in microbes of the rumen and intestine), H^+ serves as the ultimate electron acceptor, and hydrogenases reduce protons (H^+) to H_2. The latter is subsequently utilized by methanogens to generate CH_4 and ATP via a series of biochemical reactions.

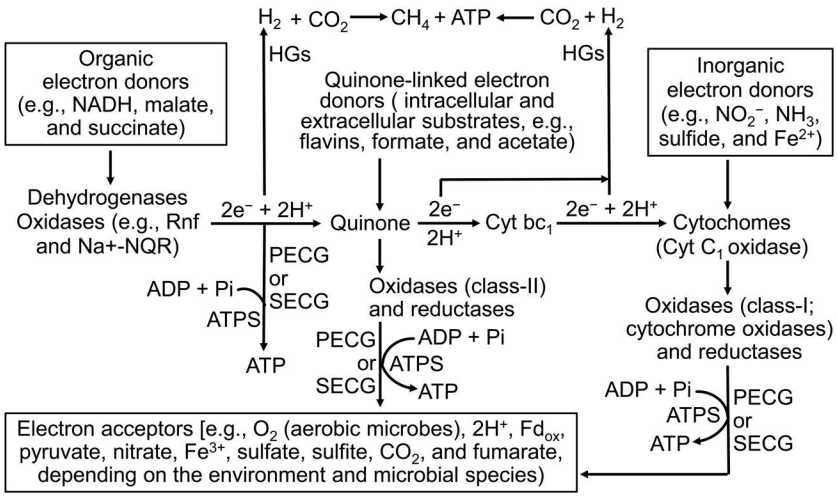


FIGURE 1.45 Diverse sources of substrates for ATP production in bacteria and archaea via cell membrane-bound electron transport chains. Electrons from various substrates enter electron transport chains within the cell membrane at the points of dehydrogenases, quinone, and cytochromes. The terminal reaction of oxidation is catalyzed by either class-I oxidases (cytochrome oxidases) or class-II oxidases (quinol oxidases). Reductases include nitrate reductase, sulfate reductase, and fumarate reductase. Some membrane-bound dehydrogenases (e.g., NADH dehydrogenase) and reductases (e.g., nitrate reductase) are also proton pumps, whereas most membrane-bound oxidases [e.g., Rnf protein complexes and sodium-translocating NADH:quinone oxidoreductase (Na^+-NQR)] are also Na^+ pumps. Thus, protons or Na^+ ions are pumped across the cell membrane to create an electrochemical gradient that drives the synthesis of ATP from ADP plus Pi by ATP synthase (ATPS). Electron acceptors vary, depending on the environment and microbial species. Both H_2O and ATP are produced by aerobic microbes in the presence of O_2. In contrast, under anaerobic conditions (e.g., in the rumen), hydrogenases (HGs; prototypical hydrogenases, bifurcating hydrogenases, and energy-converting hydrogenases) reduce protons to H_2. During the formation of methane from H_2 and CO_2, ATP is produced by methanogens. Fd_{ox}, oxidized ferredoxin; PECG, proton electrochemical gradient; SECG, sodium electrochemical gradient.

SUMMARY

Nutrients are substances required by animals to maintain life, grow, develop, lactate, and reproduce. Proximate analysis of foodstuffs traditionally provides useful information on their content of H_2O and DM, including CP, crude fat, minerals, crude fiber, and other carbohydrates. Modern analytical methods have made it possible to determine amino acid composition in proteins, free fatty acids and derivatives, simple and complex sugars, macro- and micro-elements, as well as water- and lipid-soluble vitamins. To date, more than 40 nutrients are known to be essential for mammals, birds, fish, and crustaceans. Under the control of the nervous and endocrine systems, as well as the influence of sense organs, animals consume food and H_2O to meet their nutrient requirements. The physiological state of the animal (e.g., pregnant, lactating, suckling, and weanling) must be considered when classifying a nutrient as nonessential, essential, or conditionally essential.

Utilization of dietary nutrients (including biological oxidation of nutrients for ATP production via the Krebs cycle and the electron transport system in the mitochondria) depends on well-coordinated biochemical and physiological processes. They include (a) digestion and absorption in the GIT; (b) the transport of absorbed nutrients through the circulatory system (including the blood capillaries); (c) the uptake of excess interstitial fluid by the lymphatic vessels for return to the blood circulation; (d) the provision of O_2 by the respiratory system, (e) enzyme-, coenzyme- and cofactor-dependent metabolism of nutrients via a series of complex pathways (e.g., glycolysis, gluconeogenesis, the Krebs cycle, the urea cycle, intracellular protein turnover, β-oxidation, ketogenesis, hexosamine synthesis, one-carbon unit transfer, and the mitochondrial electron transport system); (f) the tubular reabsorption of nutrients and bicarbonate from the lumen of renal tubules (e.g., proximal tubule) into the blood in the kidneys; and (g) the excretion of metabolites through the kidneys, lungs, and skin. In animal cells, the metabolic pathways are compartmentalized (e.g., cytosol, nucleus, mitochondrion, ribosome, and lysosome) in a cell-, tissue-, species-, and age-specific manner. Their regulation by various hormones (e.g., insulin, glucagon, growth factors, thyroid hormones, glucocorticoids, progesterone, estrogen, and adiponectin) is essential to animal growth (primarily musculoskeletal growth and fetal growth), immunity, health, and survival. In prokaryotes, electron bifurcation, electron confurcation, and cell membrane-bound electron transport chains play important roles in nutrient metabolism (including the generation of H_2 and CH_4 under anaerobic conditions), Na^+ export, redox coupling, ATP production, and energy conservation. In animals, deficiencies of nutrients cause a wide array of metabolic diseases. Therefore, adequate knowledge of

principles of animal nutrition is required for the development of balanced diets that enhance the efficiency of modern livestock, poultry, fish, and crustacean production worldwide, as well as the health and well-being of all animals (including humans).

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2 Chemistry of Carbohydrates

Carbohydrates are widely distributed organic substances in nature (Sinnott 2013). In the nineteenth century, chemists thought that carbohydrates were the hydrates of carbon and had the empirical formula of $C_m(H_2O)_n$, where m is ≥3 and m may be different from n. This was found to be true for the simple sugars known at that time and for their covalently linked dimers and polymers. In 1891, Emil Fisher established the structures of monosaccharides (glucose, fructose, mannose, and arabinose) and discovered that these compounds contain hydroxyl groups and carbonyl groups. Between 1920 and 1930, W. Norman Haworth identified that a hexose primarily formed a six-membered ring (pyranose) but could also form a five-membered ring (furanose), with each ring structure containing an oxygen atom. Subsequently, it was observed that some substances [e.g., deoxyribose ($C_5H_{10}O_4$) and pyruvic acid ($C_3H_4O_3$)] with the chemical properties of carbohydrates could be derived from polyhydroxy aldehydes or ketones, but they did not have the formula of $C_m(H_2O)_n$. To date, carbohydrates are defined as polyhydroxy aldehydes, polyhydroxy ketones, or their derivatives (Sinnott 2013). Examples of carbohydrates are hexoses, starch, cellulose, glycogen, chitins (in the exoskeletons of animals), and murein (in the bacterial cell wall), as well as sugar alcohols (alditols), deoxy sugars, amino sugars, and sugar phosphates (Robyt 1998). Therefore, based on modern definitions, not all carbohydrates contain hydrogen and oxygen in the ratio of 2:1, and some of them even contain nitrogen (N), sulfur, or phosphorus.

Plants capture sunlight energy to convert water (H_2O) and CO_2 into glucose ($6CO_2 + 6H_2O + 2870\ kJ \rightarrow C_6H_{12}O_6 + 6O_2$). Glucose is then used to form starch and other complex carbohydrates. This transformation of energy from sunlight into carbohydrates is essential for food production, and the released oxygen molecule is vital for animal life (Demura and Ye 2010). In the ecosystem, omnivores and herbivores consume plants as their major sources of monosaccharides, disaccharides, starch, and fiber (plant material composed of complex nonstarch carbohydrates and lignin) to produce meat (containing glycogen) and milk (the source of lactose). In contrast, glycogen is the main carbohydrate in the natural diets of carnivores. Lactose is the principal carbohydrate for preweaning mammals.

Carbohydrates can be classified as simple (e.g., monosaccharides and disaccharides) or complex (e.g., molecules containing three or more units of monosaccharides). Carbohydrates comprise up to 75% of the dry matter (DM) in plant-sourced feedstuffs and constitute the greatest proportion of the diets consumed by animals other than carnivores (Pond et al. 2005). In contrast, relatively small amounts of glucose and glycogen are present in the bodies of animals, where they fulfill essential functions as metabolic fuels and synthetic precursors. Complex carbohydrates occur in the cell walls of microbes and plants and in animals. Legumes (e.g., soybeans, lentils, chickpeas, mung beans, and white lupins) contain water-soluble tri- and tetra-oligosaccharides. In all plants, water-insoluble carbohydrates, particularly cellulose and hemicellulose, are responsible for their structural stability and mechanical firmness, whereas the water-soluble carbohydrates (e.g., glucose and starch) serve as energy reserves (Robyt 1998). In most plants, the polysaccharides of cell walls are mainly cellulose, hemicelluloses (e.g., xyloglucans, mannans, and glucuronoarabinoxylans) and, to a lesser extent, pectin. However, arabinoxylan and β-glucan are the major polysaccharides in the cell walls of some plants, such as wheat and related grains such as barley, oats, and rye (Lafiandra et al. 2014). Hemicelluloses coat and cross-link cellulose fibrils, either directly or via a pectin layer. Fermentable fiber is the fiber that is resistant to digestion and absorption in the small intestine but is broken down partially or completely by microbes in the large intestine (Gidenne 2015; Turner et al. 2013). This chapter highlights the chemistry of carbohydrates relevant to animal nutrition.

GENERAL CLASSIFICATION OF CARBOHYDRATES

Overview

In nutrition, carbohydrates are classified into five groups: (a) monosaccharides (also known as simple sugars), (b) disaccharides (containing 2 monosaccharide units), (c) oligosaccharides (containing 3–10 monosaccharide units), (d) polysaccharides (containing >10 monosaccharide units), and (e) conjugated carbohydrates. The latter substances are covalently bound to lipids or proteins to form glycolipids or glycoproteins, respectively. Water-soluble monosaccharides (e.g., glucose and fructose), disaccharides (e.g., sucrose and maltose), and oligosaccharides that have a sweetness flavor are collectively called sugars. Mono-, di-, and oligo-saccharides are soluble in 50% (v/v) ethanol, but polysaccharides precipitate out of the solution.

Based on the composition of constituent monosaccharides, polysaccharides are subdivided into homo-polysaccharides (containing only one type of monosaccharide) and hetero-polysaccharides (containing more than one type of monosaccharide). Based on the types of polymers, polysaccharides are also subdivided into starch and nonstarch polysaccharides (NSPs). NSPs are among the most complex carbohydrates and include water-soluble and insoluble polysaccharides, primarily present in plant cell walls. The building blocks of NSPs are pentoses (arabinose and xylose), hexoses (glucose, galactose, and mannose), 6-deoxyhexoses (rhamnose and fucose), and uronic

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acids [glucuronic acid (GlcA), galacturonic acid (GalA), and their 4-*O*-methyl esters] (Bach Knudsen 2023). Polysaccharides that contain amino sugars are called glycosaminoglycans and are also known as mucopolysaccharides. Proteoglycans are large molecules in which glycosaminoglycans are covalently linked to a protein core and are important components of the extracellular matrix of all multicellular organisms.

In laboratory analyses, upon hydrolysis by strong acids or specific enzymes, oligosaccharides and polysaccharides are broken down into monosaccharides. Carbohydrates of nutritional interest are summarized in Table 2.1. Their content in plant-sourced feedstuffs is affected by many factors, including species, breed, environment, season, and maturity (Mertens 2003; Villalba et al. 2021). Dietary intake of carbohydrates affects animal growth and health. For example, high-starch diets result in ruminal acidosis in ruminants (Wu 2020) and intestinal dysfunction in cats (Li and Wu 2024). In addition, nonstructural carbohydrate (NSC) fractions (sugars + fructans + starch) in diets may play a role in equine diseases related to glucose intolerance and inflammation (e.g., insulin resistance and laminitis), as feeding seed grains (e.g., oats, barley, wheat, and corn) with high starch content to horses can induce laminitis (Garner et al. 1975). In contrast, small amounts of forage pellets with low amounts of NSC (on a DM basis), such as timothy hay (9.5% CP and 10% NSC), alfalfa hay (16.3% CP and 9.8% NSC), timothy-alfalfa hay (17.2% CP and 9.8% NSC), and Bermudagrass (9.8% CP and 10–12% NSC), can be used to manage horses (particularly aged adults) that are sensitive to glucose intolerance and laminitis (Kagan et al. 2011; Macon et al. 2024).

D- and L-Configurations of Carbohydrates

The smallest carbohydrate has three carbons. If a carbohydrate has at least one asymmetric carbon or chiral center, it will have D- and L-configurations according to the absolute

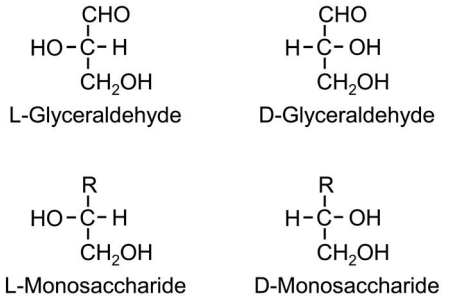
TABLE 2.1
Classification of Carbohydrates

Classification	Examples
Simple Carbohydrates	
Monosaccharides	
Trioses ($C_3H_6O_3$)	Glyceraldehyde and dihydroxyacetone
Tetroses ($C_4H_8O_4$)	Erythrose
Pentoses ($C_5H_{10}O_5$)	Arabinose, xylose, xylulose, ribose, ribulose, and 5-deoxy-ribose
Hexoses ($C_6H_{12}O_6$)	Glucose, fructose, galactose, and mannose
Heptoses ($C_7H_{14}O_7$)	Sedoheptulose, mannoheptulose (in avocados), and L-glycero-D-manno-heptose
Disaccharides	Sucrose (in plants), lactose (in milk), maltose, isomaltose, cellobiose, and trehaloses[§]
Complex Carbohydrates	
Oligosaccharides[a]	
Trisaccharides	Raffinose, kestose,[†] maltotriose (three units of glucose), planteose, and melezitose (also known as melicitose), and panose (synthesized by microbes)
Tetrasaccharides	Stachyose, nystose, and lychnose (1-α-galactosyl-raffinose)
Pentasaccharides	Verbascose and β-fructofuranosylnystose
Polysaccharides[b]	
Homoglycans[c]	Pentosans $(C_5H_8O_4)_n$, e.g., arabans and xylans Hexosans $(C_6H_{12}O_6)_n$, e.g., starch, cellulose, callose, levans,[‡] mannans, and glycogen
Heteroglycans[d]	Hemicelluloses, pectins, exudate gums, seaweed polysaccharides (algin, carrageenans, agar), aminopolysaccharides (e.g., chondroitin and hyaluronic acid), and sulfated polysaccharides (e.g., chondroitin sulphate)
Fructans	Agavin, graminan, inulin, levan, and neo-levan
Conjugated carbohydrates	
Glycolipids[e]	Glyceroglycolipids and sphingolipids
Glycoproteins[f]	Mucins, immunoglobulins, and membrane-bound hormone receptors

[a] Containing 3 to 10 units of monosaccharides, including fructo-oligosaccharides and mannan-oligosaccharides.
[b] Containing more than 10 units of monosaccharides.
[c] Containing more than 10 units of a single type of monosaccharides.
[d] Containing more than 10 units of different types of monosaccharides.
[e] Monosaccharides or oligosaccharides covalently bound to a lipid moiety.
[f] Monosaccharides or oligosaccharides covalently bound to a protein moiety.

Note: [§] Including α,α-trehalose, α,β-trehalose, and β,β-trehalose; [†] fructooligosaccharides (e.g., 1-kestose, 6-kestose, and neokestose); [‡] fructans that are polysaccharides.

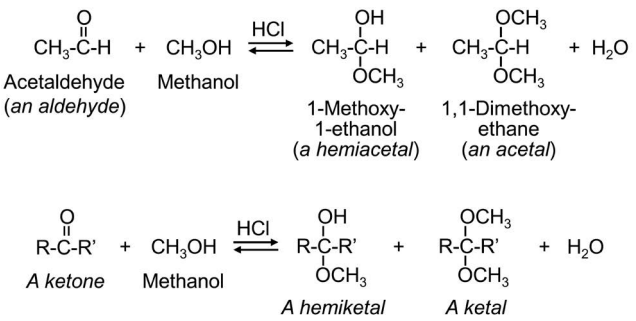
configuration of glyceraldehyde, as defined by Emil Fischer in 1885. If there are two or more chiral centers in a molecule, the arrangement at the highest number chiral center (e.g., the carbon attached to the –OH group in the monosaccharide shown here) is used to specify the configuration. The D- and L-structures are the mirror images of each other and are therefore called enantiomers. For example, D-galactose is the mirror image (i.e., enantiomer) of L-galactose. Most naturally occurring carbohydrates have only the D-configuration, but some of them have both the D- and L-structures. The absolute D- and L-configuration should not be confused with "*d*" (for *dextrorotatory*) if the compound rotates plane-polarized light to the right or "*l*" (for *levorotatory*) if the compound rotates plane-polarized light to the left. For example, although D-glucose rotates plane-polarized light to the right, D-fructose rotates plane-polarized light to the left.



R = Remaining part of a monosaccharide; R ≠ H.

Cyclic Hemiacetate (Aldose) and Hemiketals (Ketoses)

Carbohydrates contain important functional groups: aldehyde (–CHO), hydroxyl (–OH), and carbonyl (–C=O). They exist mainly as cyclic hemiacetal, acetal, ketal, or hemiketal compounds. A hemiacetal (*hemi* meaning "half" in Greek) contains a hydroxyl group (–OH) and an alkoxyl group ($-OCH_3$). In an acetal, two alkoxyl groups ($-OCH_3$) are bonded to the same carbon atom. When an aldehyde (e.g., acetaldehyde) reacts with methanol in the presence of HCl, a hemiacetal (e.g., 1-methoxy-1-ethanol), an acetal (1,1-dimethoxy-ethane), and H_2O are produced. However, when the carbonyl compound is a ketone instead of an aldehyde, the products are a hemiketal, a ketal, and H_2O. Thus, hemiacetals and hemiketals are compounds that are derived from aldehydes and ketones, respectively.



(R may be equal to R'. However, R or R' cannot be a hydrogen atom.)

These concepts of hemiacetals and hemiketals can be explained with aldose and ketose sugars (Bruice 2011; Ege 1984). In an aldose sugar (e.g., D-glucose), a stable cyclic hemiacetal is formed by an attack from the –OH group bonded to the fifth carbon (C-5) on the aldehyde carbon (C-1), leading to intramolecular cyclization (pyranose with a 6-member ring). The cyclic form of D-glucose is called D-glucopyranose. In a ketose sugar (e.g., D-fructose), a stable cyclic hemiketal is formed by an attack from the –OH group bonded to the sixth carbon (C-6) on the ketone carbon (C-2), resulting in intramolecular cyclization (pyranose with a 6-member ring). However, when the ketone carbon of D-fructose is attacked by the –OH group bonded to the fifth carbon (C-5), a stable cyclic furanose (a 5-member ring) is generated. Thus, the cyclic form of D-fructose is called either D-fructopyranose or D-fructofuranose. In an aqueous solution, the ratio of fructopyranose to fructofuranose is 3:1. Note that aldoses and ketoses undergo these transformations spontaneously without enzyme catalysis.

MONOSACCHARIDES

Definition

Monosaccharides are divided into aldoses (also known as polyhydroxy aldehydes) and ketoses (also known as polyhydroxy ketones) according to whether they contain an aldehyde or ketone group (Sinnott 2013). In organisms, most monosaccharides exist only in the D-configuration (e.g., D-glucose, D-fructose, and D-ribose), but some of them occur in both the D- and L-configurations (e.g., lactate, arabinose, and galactose). Animal cells produce only L-lactate (a 3-carbon metabolite) from D-glucose and other substrates. In contrast, bacteria synthesize both D-lactate and L-lactate, depending on the cell type. L-Arabinose and L-galactose are constituents in plants. A monosaccharide with a free aldehyde group or a free ketone group is a reducing sugar, which reduces another compound, with itself being oxidized, such that the carbonyl carbon of the reducing sugar is converted into a carboxyl group. Ketoses (e.g., D-fructose) are not reducing sugars unless they are isomerized to their aldose form (e.g., the isomerization of D-fructose to D-glucose under alkaline conditions). All monosaccharides are highly soluble in H_2O. Monosaccharides commonly present in plant cell walls are hexoses (D-glucose, D-galactose, and D-mannose), pentoses (L-arabinose and L-xylose), 6-deoxyhexoses (L-rhamnose and L-fucose), and uronic acids [D-GlcA, D-GalA, L-iduronic acid (L-IdoA), D-mannuronic acid (D-ManA), or their methyl ethers]. Note that D-GlcA was first isolated from the urine of animals. In nature, most physiological sugars are of the D-configuration.

Chemical Representation of Monosaccharide Structures

Open-Chain Form

A monosaccharide can be present in a linear (open-chain) form, a Haworth projection, or a chair form (Figure 2.1). The open-chain form and the ring forms of

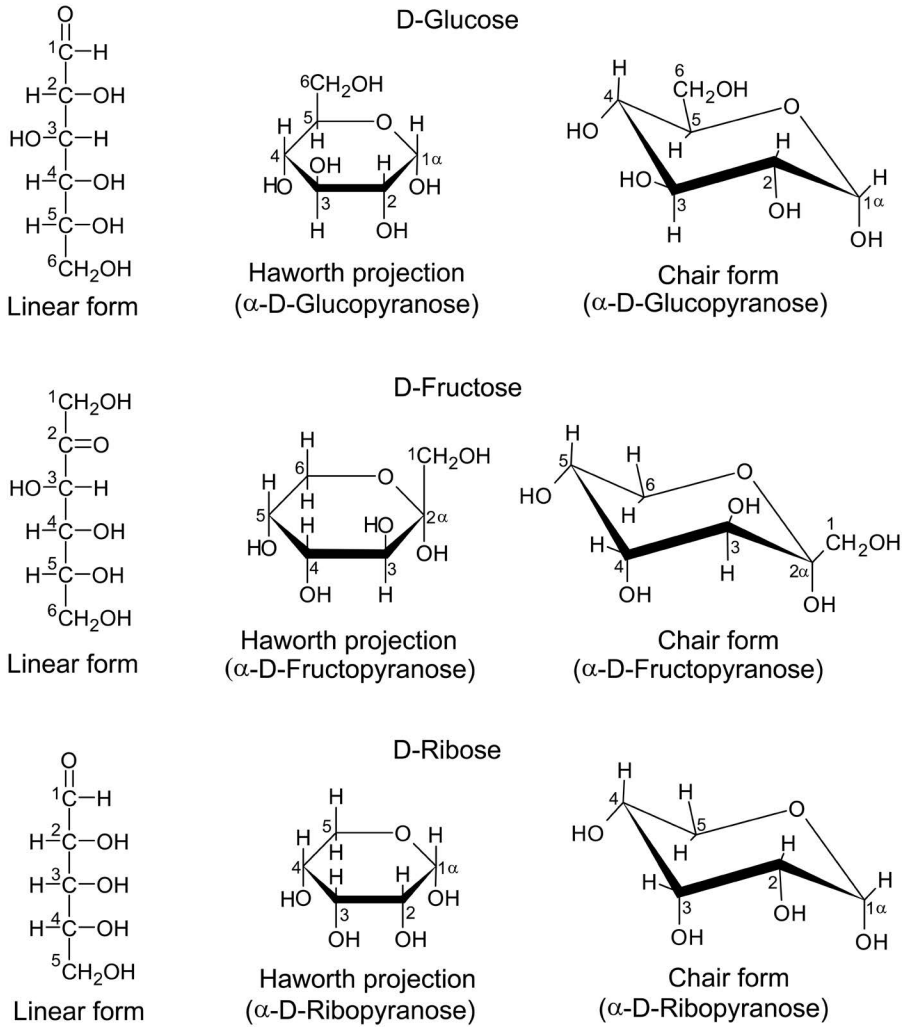


FIGURE 2.1 Chemical structure of a monosaccharide in a linear (open-chain) form, a Haworth projection, or a chair form. The open-chain form represents only small amounts of monosaccharides compared with the ring forms, but participates in many of the reactions involving C-1. When a hemiacetal or hemiketal ring is formed, anomerization (also known as mutarotation) occurs at C-1 to form an α- or β-anomer. In the α-anomer, the –OH group at C-1 is facing down in the six-membered ring. In the β-anomer, the –OH group at C-1 is facing up in the six-membered ring. Crystalline α-D-hexose (e.g., α-D-glucose) is an α-D-pyranose (the Haworth form), such as α-D-glucopyranose.

a monosaccharide can be spontaneously interconverted in H_2O. Although the open-chain form represents only small amounts of nearly all monosaccharides in a solution (e.g., 0.002% for D-glucose, 0.02% for D-galactose, 0.05% for D-ribose, and 0.8% for D-fructose; Table 2.2), many of the reactions involving C-1 take place in the open-chain form (Robyt 1998). Because the free aldehyde group of D-glucose in the open-chain form is chemically active (e.g., reactive with the ε-NH_2 group of lysine, either free or protein-bound to form a covalent bond), the minute concentration of the monosaccharide in this configuration is of physiological importance for minimizing adverse protein glycation. Thus, among all the aldohexoses (allose, altrose, glucose, mannose, gulose, idose, galactose, and talose), glucose has the lowest proportion of straight-chain structure and, thus, free aldehyde groups (Angyal 1984). This may be a major reason why glucose was favored as the principal monosaccharide during the evolution of both plants and animals (Brosnan 2005).

Cyclic Hemiacetal or Hemiketal Form

According to the Haworth projection, a monosaccharide can exist as a six-membered ring (pyranose) or a five-membered ring (furanose) structure (Figure 2.2). Monosaccharides exist primarily in the pyranose form rather than the furanose form (Table 2.2). For example, D-fructose (which has a ketone group) in solution forms a six-membered cyclic hemiketal called D-fructopyranose, with its hydroxyl group on the C-6 carbon attacking the C-2 ketone carbon to form an oxygen-linked bridge. When the hydroxyl group on the C-5 carbon

TABLE 2.2
Distributions of Various Structural Forms of Monosaccharides at Equilibrium in Water[a]

		Pyranose Form		Furanose Form		
Carbohydrate	**Temp (°C)**	**α**	**β**	**α**	**β**	**Open-Chain Form**
D-Allose	31	14	77.5	3.5	5	0.01
D-Arabinose	31	60	35.5	2.5	2	0.03
D-Fructose	31	2.5	65	6.5	25	0.8
D-Galactose	31	30	64	2.5	3.5	0.02
D-Glucose	31	38	62	0.5	0.5	0.002
Glycero-D-Gal	22	28	67	2	3	0.01
Glycero-L-Gal	22	36	57	3	4	0.02
D-Idose	31	38.5	36	11.5	14	0.2
D-Mannose	44	65.5	34.5	0.6	0.3	0.005
D-Psicose	27	22	24	39	15	0.3
D-Rhamnose	44	65.5	34.5	0.6	0.3	0.005
D-Ribose	31	21.5	58.5	6.5	13.5	0.05
D-Sorbose	31	93	2	4	1	0.25
D-Tagatose	31	71	18	2.5	7.5	0.3
D-Talose	22	42	29	16	13	0.03
D-Xylose	31	36.5	63	0.3	0.3	0.002

Source: Angyal (1984).

[a] The distribution values of monosaccharides are expressed as % (mol/mol).

Note: Gal, galactose; Temp, temperature.

of the open-chain D-fructose attacks its C-2 ketone carbon, D-fructofuranose with a 5-membered ring is formed. In contrast to D-fructose, D-glucose (which has an aldehyde group) in solution forms a six-membered cyclic hemiacetal called D-glucopyranose when its hydroxyl group on the C-5 carbon attacks the C-1 aldehyde carbon to form an oxygen-linked bridge. In the conversion of the open-chain D-glucose into D-glucofuranose with a 5-membered ring, the hydroxyl group on its C-4 carbon attacks the C-1 aldehyde carbon. For both D-fructose and D-glucose, the pyranose form is

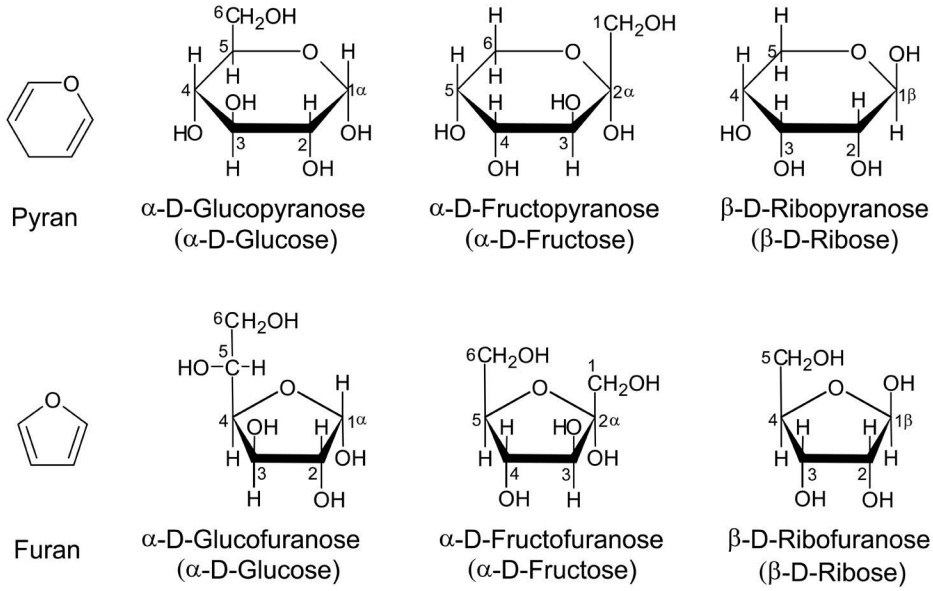


FIGURE 2.2 The Haworth projection of a monosaccharide as a six-membered ring (pyranose) or a five-membered ring (furanose) structure. For a monosaccharide, the pyranose form is more stable than the furanose form. Examples are α-D-glucose, α-D-fructose, and β-D-ribose.

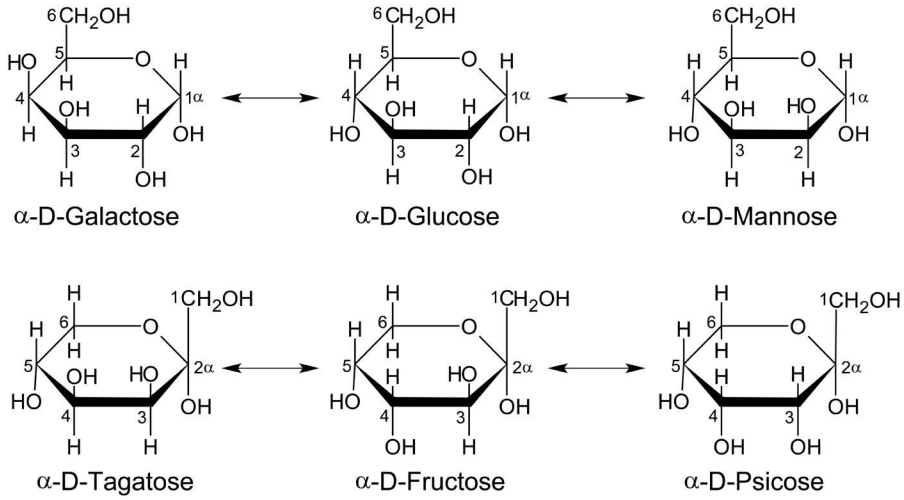


FIGURE 2.3 Epimerization of monosaccharides in solution. In carbohydrates, this reaction refers to the formation of isomers at a carbon other than C-1, resulting in the generation of a different substance. Epimerization can be either spontaneous (generally a very slow process) or catalyzed by enzymes (epimerases). For example, α-D-galactose and α-D-mannose are formed from α-D-glucose in animal and plant cells. Note that a C-3 epimer of D-glucose is D-allose, a rare sugar in certain plants.

thermodynamically more stable than the furanose form. Thus, in an aqueous solution, D-glucopyranose (or D-fructopyranose) is much more abundant than D-glucofuranose (or D-fructofuranose; Table 2.2).

Epimerization of monosaccharides refers to the formation of stereoisomers at a carbon other than C-1 and, therefore, different substances (Figure 2.3). For example, D-mannose and D-galactose are formed from the epimerization of D-glucose at carbon 2 and carbon 4, respectively, and these three sugars are epimers. Epimerization can be spontaneous (generally a slow process) or catalyzed by enzymes (epimerases) and bases (e.g., in alkaline solution). Epimerization differs from mutarotation (generally a fast process) not only in the rates of spontaneous reactions but also in the chemical mechanisms. Specifically, epimerization is the formation of stereoisomers (called epimers) at one chiral center of a monosaccharide.

When a hemiacetal or hemiketal ring is formed spontaneously by intramolecular cyclization in a solution, anomerization occurs at C-1 through acid and base catalysis at a low rate, forming an α- or β-anomer, such as α-D-glucose and β-D-glucose. This process is called mutarotation (with examples for D-glucose, D-fructose, and D-ribose in Figure 2.4) and can be greatly facilitated by specific mutarotases (e.g., galactose mutarotase). Thus, mutarotation involves the formation of a hemiacetal or hemiketal ring and anomers from the intramolecular cyclization of a monosaccharide and a subsequent change in its specific rotation. In the α-anomer of the D-isoform of monosaccharides (e.g., D-glucose and D-galactose), the –OH group at C-1 will be facing down in a six- or five-membered ring. For the β-anomer of the D-isoform of monosaccharides, the –OH group at C-1 will be facing up in the six-membered ring. By contrast, in the α- and β-anomers of the L-isoform of monosaccharides (e.g., L-iduronate and L-galactose), the anomeric carbon has the hydroxyl group pointing upwards and facing down, respectively.

At equilibrium in H_2O, the β-anomer of monosaccharides is more abundant than the α-anomer for D-glucose, D-fructose, D-galactose, D-xylose, or D-ribose, but the opposite is true for D-mannose and D-rhamnose (Table 2.2). For example, in an aqueous solution, the hemiacetal ring of D-glucose opens to bring about changes in the position of –H and –OH groups on carbon 1 via the acyclic aldehyde form; this results in a mixture of α-D-glucopyranose (~38%), β-D-glucopyranose (~62%), α and β anomers of glucofuranose (<0.3%), and the acyclic aldehyde form (<0.01%) at equilibrium. Thus, when pure α-D-glucose (with a specific rotation of +112.2°) is dissolved in H_2O, β-D-glucose (with a specific rotation of +18.7° in the pure form) is generated, resulting in a solution of mixed glucose anomers with a specific rotation of +52.7° at equilibrium. In addition, when pure α-D-fructose (with a specific rotation of –133.5°) is dissolved in H_2O, β-D-fructose (with a specific rotation of +78° in the pure form) is generated, resulting in a solution of mixed fructose anomers with a specific rotation of –92.4° at equilibrium. Of note, the equilibrium of hexoses between α- and β-pyranose forms in H_2O is not affected by changes in temperature of the solution, but the proportion of furanose forms is increased with the increasing temperature (Angyal 1984). A hemiacetal is in chemical equilibrium with the open-chain aldehyde, and a hemiketal is in chemical equilibrium with the open-chain ketone. A monosaccharide with a hemiacetal or a free aldehyde is a reducing sugar.

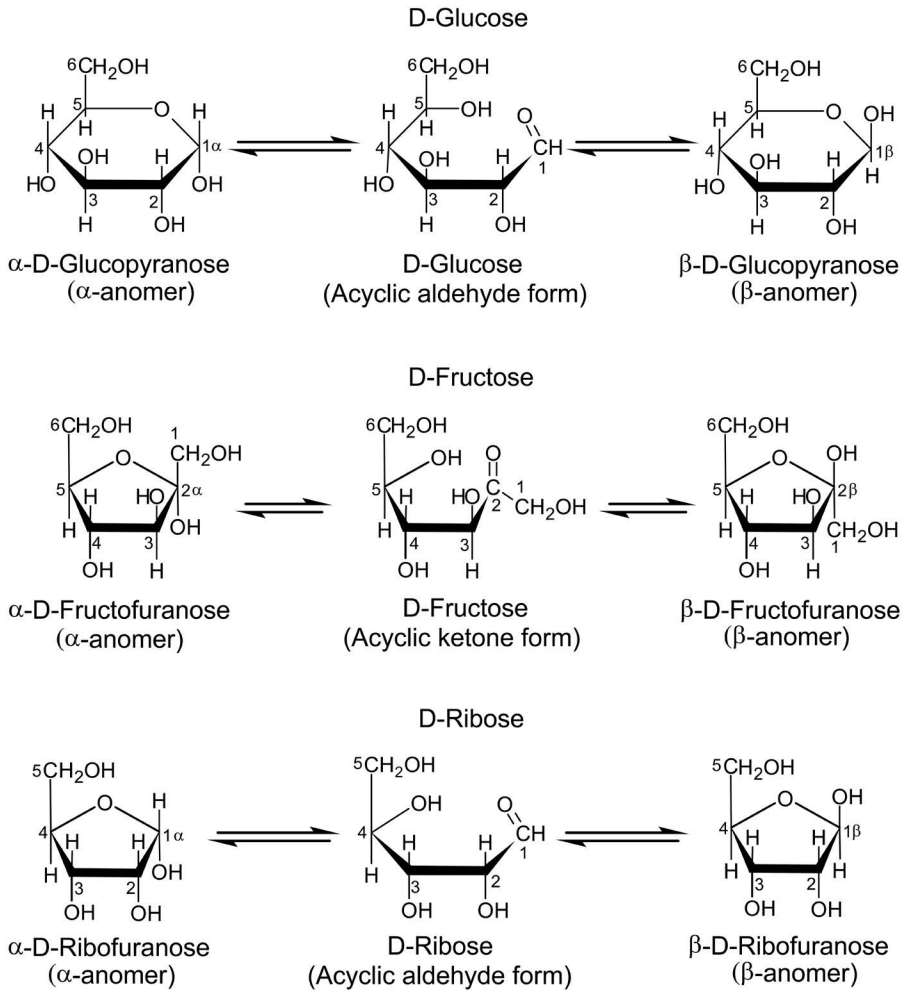


FIGURE 2.4 Mutarotation of monosaccharides (e.g., D-glucose, D-fructose and D-ribose) in a solution. Intramolecular cyclization of an open-chain monosaccharide results in the generation of a ring structure and an α- or β-anomer. This reaction spontaneously proceeds rapidly at pH 7.0 and can also be catalyzed by mutarotase. The α- or β-anomers are at chemical equilibrium in the solution. In the case of D-glucose, the aldehyde group (–CHO) reacts with the hydroxyl group (alcohol) at C-5 to form a hemiacetal at C-1. In the case of D-fructose, the ketone group (–C=O) reacts with the hydroxyl group (alcohol) at C-5 to form a hemiketal at C-2. The hydroxyl group at the hemiacetal or hemiketal carbon is most reactive in monosaccharides.

Glucose and Fructose in Plants

Monosaccharides are usually present in low concentrations in plant products (e.g., vegetables, fruits, and forages; Bach Knudsen 2023). Glucose and fructose have different chemical structures but the same molecular formula of $C_6(H_2O)_6$. Glucose and fructose are usually the two most abundant monosaccharides in plants, with the amount of glucose generally being greater than that of fructose (Robyt 1998). Glucose is the basic unit of starch and cellulose, which are usually the main storage forms of monosaccharides in cell contents and walls of plants, respectively. Of note, fructose is the only widely spread ketohexose of nutritional and physiological significance in nature, and it occurs in many plants (particularly green plants), honey, trees, vine fruits, flowers, berries, tubers, and most root vegetables (Rumessen 1992). In addition, fructose is a component of the sweet disaccharide (sucrose) and polysaccharides called fructosans or fructans in plants.

Total amounts of glucose or fructose differ markedly among plants and with their developmental stage (Table 2.3). Forage plants contain 1–3% glucose plus fructose on a DM basis. In contrast, green plants contain much smaller amounts of free hexoses (glucose and fructose) than starch because hexoses are linked to form polysaccharides (starch, cellulose, and hemicellulose) that are quantitatively the most important constituents of plant-sourced feeds (McDonald et al. 2021).

TABLE 2.3
Composition of Monosaccharides, Disaccharides, Oligosaccharides, and Starch in Plant-Sourced Feedstuffs[a]

Feedstuff	DM	Glucose	Fructose	Sucrose	Raffinose	Stachyose	Verbascose	Starch
				% (as-fed basis)				
Alfalfa hay	89.4	0.18	0.16	2.66	0.03	0.03	0.00	1.0–2.2
Alfalfa meal	90.0	0.28	0.25	1.17	0.18	0.00	0.00	6.12
Barley (grain)	89.0	0.15	0.10	2.0	0.23	0.002	0.00	60.5
Barley (hulled)	89.0	0.21	0.14	1.07	0.45	0.09	0.00	52.2
Bermudagrass	89.4	0.54	0.54	2.32	2.15	ND	ND	0.89
Canola (rapeseed) meal	89.0	0.11	0.11	5.16	0.36	1.07	0.00	1.60
Coconut cake	90.0	0.25	0.22	10.2	0.18	0.18	0.00	0.90
Corn (grain)	89.0	0.36	0.25	1.2	0.18	0.09	0.01	61.4
Corn bran	88.7	0.16	0.15	1.86	0.35	0.09	0.00	33.4
Corn flour	89.0	0.18	0.16	0.53	0.09	0.00	0.00	80.3
Corn gluten (feed)	89.0	0.28	0.25	2.23	0.45	0.18	0.00	25.1
Cottonseed cake	89.0	0.07	0.06	0.89	3.47	1.25	0.09	1.60
Cottonseed meal	91.0	ND	ND	1.46	3.20	1.19	0.18	1.74
Faba bean (mature)	90.0	trace	trace	2.43	0.36	1.44	3.06	36.6
Grass hay[b]	84.2	3.28	2.44	1.01	VL/ND	VL/ND	VL/ND	0.7
Linseed meal	89.0	0.12	0.10	2.49	0.89	0.18	0.00	2.40
Molasses (cane)	76.8	4.06	6.20	37.5	0.02	0.00	0.00	0.25
Molasses (beet)	77.6	0.22	0.23	47.3	0.47	ND	ND	0.06
Oats (hulled)	89.0	0.06	0.11	0.98	0.27	0.18	0.00	41.7
Oats (grain)	89.0	0.05	0.09	0.64	0.19	0.03	0.00	55.8
Oat bran	90.6	0.07	0.05	1.99	0.33	0.19	0.03	43.8
Oat flour	89.7	0.06	0.03	0.45	0.18	0.07	---	80.0
Oat hay	89.2	0.27	0.24	4.25	0.65	0.34	0.05	11.2
Palm cake	90.0	0.16	0.13	1.53	0.18	0.00	0.00	0.99
Peas (field)	89.0	0.12	0.39	2.67	0.45	2.05	1.96	40.4
Rice (milled)	90.0	0.04	0.03	0.14	0.02	0.00	0.00	73.5
Rye (grain)	89.0	0.08	0.10	1.70	0.09	0.002	0.00	54.6
Rye bran	89.0	0.04	0.05	3.47	2.14	0.53	0.00	7.74
Rye middlings	89.0	0.20	0.24	2.94	1.25	0.36	0.00	32.8
Soybean (grain)	87.0	0.43	0.21	4.45	0.34–0.61	1.34–4.26	0.13	0.52
Soybean hulls	89.1	0.11	0.13	0.78	0.17	0.38	0.03	5.2
SBM (48% CP)	90.0	0.04	0.03	7.0	1.0	4.7	0.30	1.89
SBM (45% CP)	93.9	0.00	0.00	7.10	0.77	4.88	0.00	1.89
Sorghum (grain)	89.0	0.09	0.09	0.85	0.11	0.00	0.00	67.1
SPC	92.6	0.00	0.00	0.67	0.46	0.91	0.00	1.89
Sunflower cake	90.0	0.20	0.17	3.24	1.26	0.27	0.00	0.89
Wheat (grain)	89.0	0.13	0.13	0.98	0.36	0.18	0.00	57.9
Wheat bran	89.2	0.09	0.05	2.68	1.07	0.36	0.00	19.8
Wheat flour	90.0	0.04	0.03	0.72	0.30	0.27	0.00	73.8
Wheat middlings	86.5	0.31	0.37	1.92	0.92	0.00	0.00	20.3
White lupins	89.0	0.15	0.13	2.14	0.89	4.72	1.25	1.25

Source: Bach Knudsen (1997), Cardoso et al. (2021), Casas and Stein (2024), Ermawar et al. (2015), Henry and Saini (1989), INRA (2024), Ispiryan et al. (2020), Kagan et al. (2011, 2014), Koehler and Wieser (2013), Lazaridou and Biliaderis (2007), Li et al. (2023), Lim et al. (1992), Long et al. (2022), MacArthur and D'Appolonia (1979), Müller et al. (2016), Murai et al. (2024), NRC (2012), Palmonari et al. (2020), Prieto-Santiago et al. (2022), Shelton and Lee (2000), and USDA (2018).

[a] Plants contain sugars (e.g., galactose and myo-inositol) other than those shown in this table. Concentrations of carbohydrates in plant-sourced feedstuffs can vary substantially, depending on origin, maturity, and processing.

[b] Consisting of 50% timothy (*Phleum pratense*), 40% meadow fescue (*Festuca pratensis*), and 10% red clover (*Trifolium pratense*).

Note: DM, dry matter; ND, not detected; SBM (48% CP), soybean meal (47.7% crude protein, dehulled); SBM (45% CP), soybean meal (44.6% crude protein, expelled); SPC, soy protein concentrate; VL, very low.

Glucose and Fructose in Animals

α-D-Glucose is the main product of starch digestion in the gastrointestinal tract (GIT) of nonruminants and is produced from glucogenic substrates in both ruminants, and nonruminants. Glucose is the predominant hexose in the blood of healthy gestating mammals and postnatal animals, which ranges from 2–15 mM, depending on species, developmental stage, and nutritional state (Bergman 1983b). When dietary intake of fructose and its disaccharides or polymers is low or negligible, fructose is virtually absent from the plasma of postnatal mammals, birds, or fish. Likewise, D-fructose is present only as a minor sugar in fetal fluids of humans (e.g., 0.05–0.08 mM; Trindade et al. 2011), dogs, cats, guinea pigs, rabbits, rats, and ferrets (Wang et al. 2016). In contrast, large amounts of fructose are present in the semen of males (Akhter et al. 2014; Baronos 1971) and in the fetal fluids (including fetal blood) of ungulates (such as cattle, sheep, and pigs) and whales (Table 2.4). For example, the concentrations of fructose in the semen of cattle and sheep are 29 and 14 mM, respectively, in comparison with 3 mM glucose. In sheep, the concentration of fructose (up to 32 mM) is 30 times that of glucose in the allantoic fluid during pregnancy (Kim et al. 2012). Different distributions of glucose and fructose in the fluids of livestock species and other mammals likely have important physiological significance (Bazer et al. 2024).

Wang et al. (2013) reported that the free aldehyde group of glucose (22.6 mM) in the open-chain form nonenzymatically glycated serum albumin (1.5 mM, close to physiological concentration in blood) via the covalent bond, as occurs in diabetic patients. Interestingly, fructose (a ketose, 22.6 mM) did not react with the protein. Much of the evidence shows that nonenzymatic glycation, which is driven by high glucose concentrations, can result in the abnormal structure and function of proteins (Cao et al. 2015). Thus, the presence of much larger amounts of fructose than glucose in fetal fluid and blood helps to minimize protein glycation in the conceptus. This protective mechanism would be of physiological importance for embryonic and fetal survival and growth. Therefore, D-fructose has been added to the culture medium of embryos (Tsujii et al. 2008).

TABLE 2.4
Concentrations of D-Glucose and D-Fructose in the Semen and Other Physiological Fluid of Males and in Fetal Blood and Fluids[a]

Semen, Blood, or Fetal Fluid (Day of Gestation)	Volume	D-Glucose (mM)	D-Fructose (mM)
	Cattle		
Semen	5.0 ml (1.2×10^9 sperm/ml)	3.0	29
Fetal blood (Day 260)	13% of fetal body weight	1.5	3.5
Fetal allantoic fluid (Day 100)	480 ml	1.7	18
Fetal amniotic fluid (Day 100)	1025 ml	2.2	3.7
	Pigs		
Semen	300 ml (0.2×10^9 sperm/ml)	0.17	0.72
Fetal blood (Day 103)	11% of fetal body weight	2.1	4.6
Fetal allantoic fluid (Day 30)	200 ml	1.8	6.5
Fetal amniotic fluid (Day 30)	1.5 ml	1.7	4.2
	Sheep		
Semen	1.5 ml (2.0×10^9 sperm/ml)	3.0	14
Fetal blood (Day 76)	13% of fetal body weight	1.1	5.6
Fetal allantoic fluid (Day 80)	115 ml	1.1	32
Fetal amniotic fluid (Day 80)	432 ml	0.56	9.0
	Human		
Semen	3.4 ml (0.10×10^9 sperm/ml)	5.7	15
Fetal blood (full term)	11% of fetal body weight	3.6	0.08
Fetal amniotic fluid (full term)	500–600 ml	2.17	0.18

Source: Bacon and Bell (1948), Baronos (1971), Bazer et al. (1988), Bazer et al. (2012), Bearden and Fuquay (1980), Britton (1962), Gorodeski et al. (1979), Hitchcock (1949), Li et al. (2005), Schirren (1963), and Trindade et al. (2011).

[a] Trace amounts of fructose are present in maternal blood or adult-male blood.

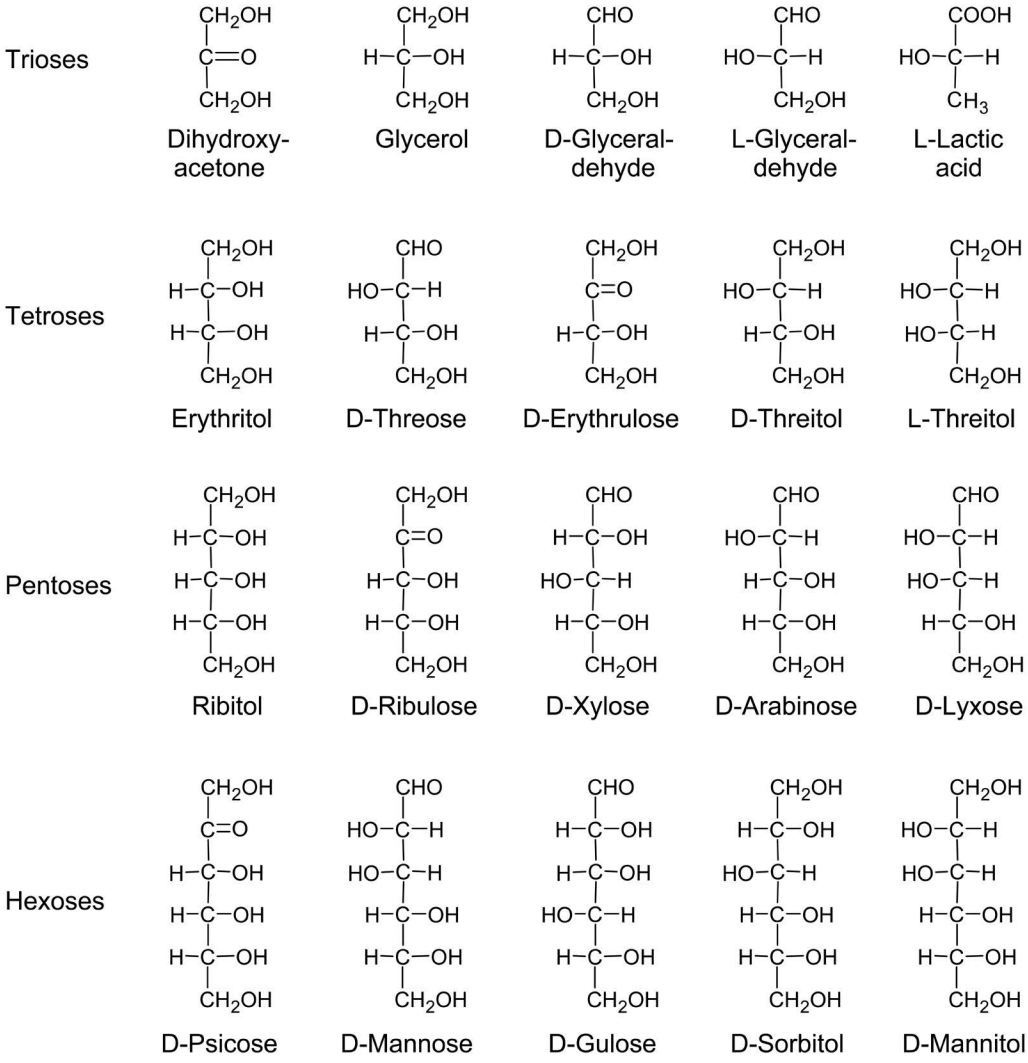


FIGURE 2.5 Structures of select trioses, tetroses, pentoses, and hexoses in nature. All of these monosaccharides are present in plants, but the trioses and D-sorbitol also occur in animals as glucose metabolites. Compounds that have a plane of symmetry in their structure (e.g., erythritol, ribitol, and galactitol) or have no asymmetric carbon (e.g., dihydroxy-acetone, glycerol, and pyruvate) are optically inactive, and do not have D- or L-configurations.

Other Monosaccharides in Plants and Animals

The structures of select monosaccharides in nature, trioses, tetroses, pentoses, and hexoses (e.g., galactose), are illustrated in Figures 2.5 and 2.6. They are all present in plants for the synthesis of polysaccharides. Some of these monosaccharides (the trioses, D-sorbitol, and myo-inositol) also occur in animals. Of particular note, D-sorbitol (2.2 mM) is much more abundant than D-fructose or D-glucose in horse semen, whereas inositol (a metabolite of D-glucose) has a concentration of 29 mM in boar semen (Table 2.5) and 50 mM in the epididymal luminal fluid of male rats (Hinton et al. 1980). Ribose is present in all living cells as a component of ribonucleic acid, certain vitamins, and energy-rich compounds (e.g., ADP and ATP). Xylose and arabinose (aldopentoses), which contain an aldehyde (–CHO) functional group, occur in a polymerized form in xylans (xylose polymers), arabinans (arabinose polymers), and hemicellulose. Furthermore, both plants and animals contain heptoses with seven carbon atoms.

Plants also contain rare sugars, including hexoses (e.g., D-allose, D-psicose, D-sorbose, and D-tagatose) and pentoses (e.g., D-lyxose, D-xylulose, L-xylulose, and D-xylitol) with potential nutritional and industrial applications (Mijailovic et al. 2021). Interestingly, these monosaccharides are not found in animals. D-Allose is a C-3 epimer of D-glucose and occurs in the leaves of the African shrub *Protea rubropilosa*. D-Allose has the same molecular

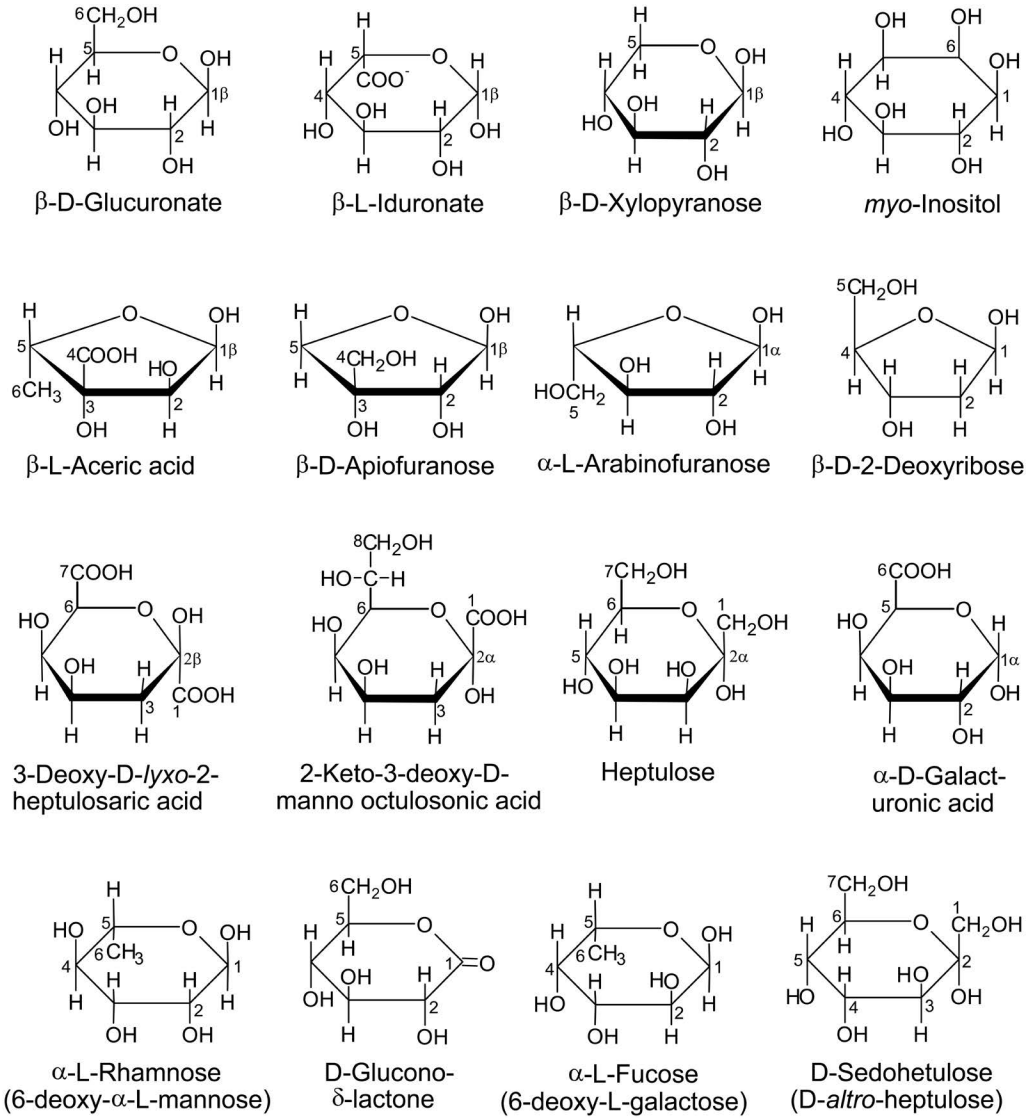


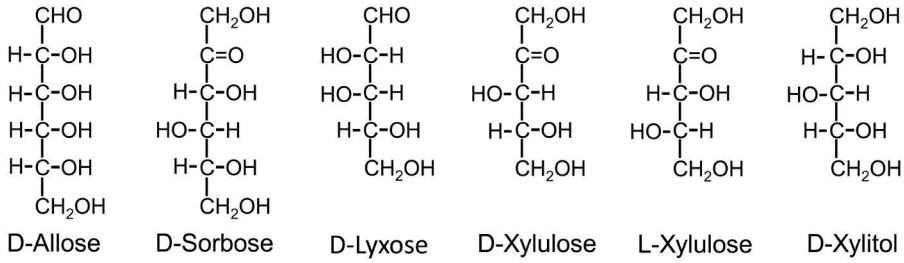
FIGURE 2.6 Haworth structures of select hexoses (6-carbon molecules) and heptoses (7-carbon molecules) in nature. All of these monosaccharides are present in plants. Myo-inositol also occurs in animals and microbes as a metabolite of D-glucose.

TABLE 2.5
Concentrations of D-Sorbitol, Inositol, Citric Acid, Glycerylphosphorylcholine (GPC), Plasmalogen (PLG), and Minerals in Livestock Semen

Animal Species	D-Sorbitol (mM)	Inositol (mM)	Citrate (mM)	Lactate (mM)	GPC (mM)	PLG (mg/mL)	Na (mM)	K (mM)	Cl (mM)	Ca (mM)	Mg (mM)
Cattle	4.1	2.0	38	1.0	14	0.6	100	36	51	6.5	3.7
Sheep	4.0	0.67	7.3	1.2	64	3.8	83	23	58	5.7	4.3
Pig	0.66	29	6.8	2.4	6.8	2.0	106	16	93	3.1	4.5
Horse	2.2	1.7	1.4	2.3	2.7	0.6	123	15	76	5.0	2.1

Source: Abdel-Rahman et al. (2000), Bearden and Fuquay (1980), Cragle et al. (1958), Erb et al. (1959), Evans et al. (1980), Kareskoski and Katila (2008), Mann et al. (1963), and Rodríguez et al. (2013).

weight as D-glucose and possesses sweet, anti-oxidative, anti-inflammatory, and possibly anti-obesity properties. Extracts from the freshwater alga *Ochromas malhamensis* contain the sugar allose, but its absolute configuration is unknown. D-psicose (also known as D-allulose) is a C-3 epimer of D-fructose and, therefore, has the same molecular weight as D-fructose. D-Psicose was first identified in wheat in the 1940s and subsequently found in certain foods (e.g., sugar cane, syrups, beet molasses, and coffee). This monosaccharide has antioxidative and possibly anti-diabetes effects in animals; its addition to foods can help to prolong their shelf lives.



Rare hexoses and pentoses in plants

Simple Aminosugars as Monosaccharides in Plants and Animals

Some monosaccharides (2-amino-2-deoxysugar) are amidated by the amino group from glucosamine-6-P, which is formed from L-glutamine and D-fructose-6-P. Examples of simple amino sugars are shown in Figure 2.7. Among over 60 amino sugars present in nature, *N*-acetyl-D-glucosamine (the main component of chitin) is the most abundant (Denzel and Antebi 2015). Glucosamine-6-P, which is synthesized from L-glutamine and fructose-6-P, is an important amino sugar in the metabolism and physiology of both plants and animals. Other significant amino sugars include *N*-acetyl-glucosamine, sialic acid, and aminoglycosides. Some aminoglycosides are also antimicrobial compounds.

DISACCHARIDES

Definition

Disaccharides consist of two units of monosaccharides, which link together via a glycosidic bond and the elimination of one H_2O molecule: $2C_6H_{12}O_6 \rightarrow C_{12}H_{22}O_{11} + H_2O$. The linkage may involve (a) the α- or β-hydroxyl group at

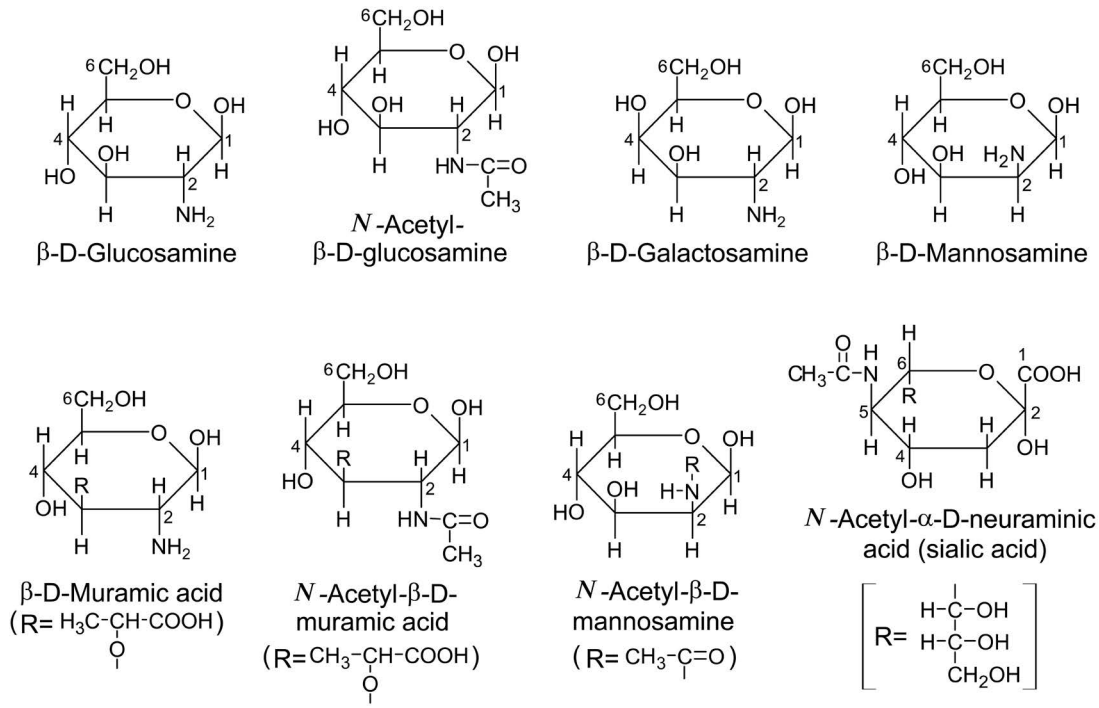


FIGURE 2.7 Structures of select simple and complex amino sugars in nature. All of these amino sugars are present in plants. Sialic acids, as well as α-anomers of D-glucosamine and N-acetyl-D-glucosamine also occur in animals and microbes as metabolites of fructose-6-phosphate and L-glutamine.

C-1 of the hemiacetal or at C-2 of the hemiketal and (b) the α-hydroxyl group in C-2, C-3, C-4, or C-6 of a monosaccharide. In forming a disaccharide from two monosaccharides, when the C1 of the first monosaccharide is α-D-glucose, the resulting glycosidic bond has a prefix of glucosyl "α-1." The word "glucosyl" is often omitted in writing. For example, the C1 of α-D-glucose (the first monosaccharide) and the C2 of β-D-fructose (the second monosaccharide) form an α-1,2 glycosidic linkage in the resulting disaccharide (sucrose).

Glycosides are usually nonreducing carbohydrates, but a few disaccharides (e.g., cellobiose, melibiose, and turanose) still have reducing abilities. Most naturally occurring disaccharides are found in plants, but lactose is synthesized by the mammary glands of lactating mammals (Rezaei et al. 2016). The structures of select disaccharides are shown in Figure 2.8. Chemically, a glucosyl β-1,4 linkage is very different from a galactosyl β-1,4 linkage and holds important nutritional implications. For

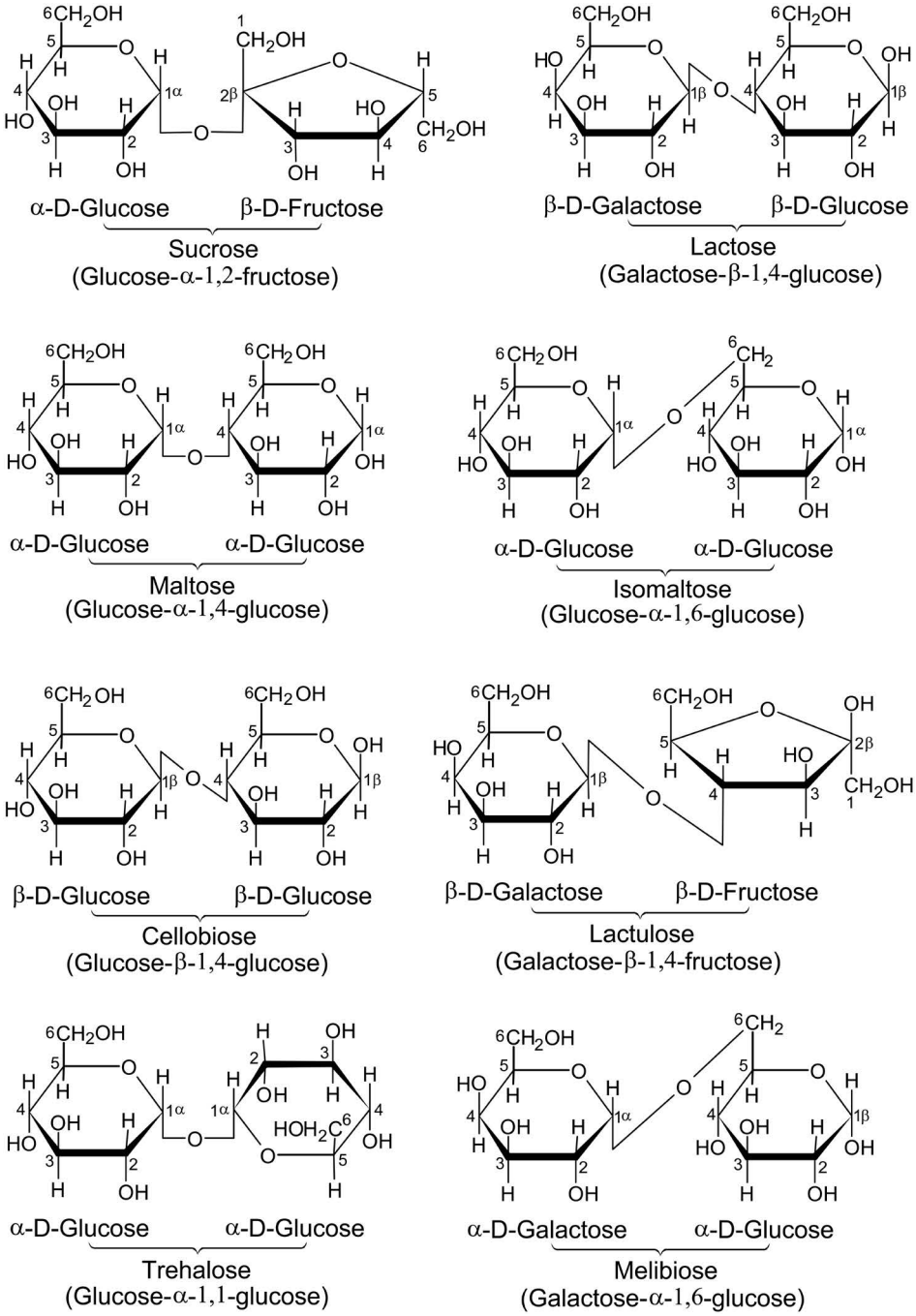


FIGURE 2.8 Structures of select disaccharides in nature. All of these disaccharides, except for lactose, are present in plants. Lactose is the most abundant carbohydrate in the milk of livestock species, dogs, rats, mice, and humans but is absent from plants. For comparison with cellobiose, laminaribiose [a product of hydrolysis of (1,3;1,4)-β-D-glucan; not shown in the figure] is a disaccharide of β-D-glucose residues linked via the β-1,3-glycosidic bond.

example, the small intestine of most mammals produces lactase to hydrolyze lactose (a disaccharide with galactosyl β-1,4 linkage), but its mucosal cells do not express enzymes to break down glucosyl β-1,4-linked disaccharides. Likewise, all animal cells do not metabolize disaccharides with glucosyl β-1,4 linkages. In contrast, bacteria in the digestive tract of animals possess enzymes to degrade disaccharides with α-1,4 or β-1,4 linkages.

Disaccharides (e.g., maltose and lactose) spontaneously undergo mutarotation in H_2O at a low rate as for monosaccharides, and this chemical process can be greatly facilitated by specific mutarotases. The mutarotation of a disaccharide occurs in a monosaccharide unit where its anomeric carbon is not involved in a glycosidic linkage. The proportions of anomers vary among disaccharides. Thus, when pure α-D-maltose (with a specific rotation of +168°) is dissolved in H_2O, β-D-maltose (with a specific rotation of +112° in the pure form) is generated, resulting in a solution of mixed maltose anomers with a specific rotation of +136° at equilibrium. In addition, when pure α-D-lactose (with a specific rotation of +89.4°) is dissolved in H_2O, β-D-lactose (with a specific rotation of +35.0° in the pure form) is generated, resulting in a solution of mixed lactose anomers with a specific rotation of +50.6° at equilibrium. Note that sucrose does not have anomeric forms because its anomeric carbons are involved in a glycosidic linkage.

Cellobiose

Cellobiose (the fundamental repeating unit of cellulose) is a reducing sugar and consists of two β-D-glucose molecules linked by the β-1,4 glycosidic bond. The nomenclature of this glycosidic bond refers to the configuration of the anomeric carbon of the first monosaccharide (i.e., β-D-glucose); the number of the anomeric carbon (C-1) of the first monosaccharide; and the number of the carbon (C-4) of the second monosaccharide (i.e., β-D-glucose) to which the first monosaccharide is linked. Cellobiose can be synthesized from two units of β-D-glucose or obtained by the enzymatic or acidic hydrolysis of cellulose and cellulose-rich materials (e.g., cotton and paper). Cellobiose has eight hydroxyl (–OH) groups, one acetal linkage, and one hemiacetal linkage, which provide strong inter- and intra-molecular hydrogen bonds (Robyt 1998).

Lactose

Lactose is a disaccharide formed from β-D-galactose and α-D-glucose via a galactosyl β-1,4-linkage, which is different from glucosyl β-1,4-linkage in cellobiose and dietary fiber. Lactose was first isolated in 1633 and identified as a sugar in 1780. This disaccharide is present only in the milk of mammals (Table 2.6) and is the most abundant carbohydrate in the milk of land-based animals (Rezaei et al. 2016). Of note, the milk of most marine mammals

TABLE 2.6
Composition of Lactose and Total Carbohydrates (TC) in the Mature Milk of Mammals[a]

Animal Species	Lactose	TC	Animal Species	Lactose	TC	Animal Species	Lactose	TC
Antelope	42	47	Fox	47	50	Pronghorn	40	43
Baboon	60	77	Giant panda	12	15	Rabbit	18	21
Bat	34	40	Giraffe	34	40	Rat	30	38
Bear (black)	3.0	27	Goat (domestic)	43	47	Reindeer	28	35
Bear (grizzly)	4.0	32	Goat (mountain)	28	32	Rhesus monkey	70	82
Bear (polar)	4.0	30	Gorilla	62	73	Rhinoceros	66	72
Beaver	17	22	Guinea pig	30	36	Sea lion	0.0	6.0
Bison	51	57	Hamster	32	38	Seal (fur)	1.0	24
Buffalo	40	47	Horse (domestic)	62	69	Seal (gray)	1.0	26
Blue whale	10	13	Human	70	80	Seal (harp)	8.9	23
Camel	49	56	Kangaroo	<0.01	47	Seal (hooded)	0.0	10
Cat (domestic)	42	49	Lion	27	34	Sheep (domestic)	48	55
Chimpanzee	70	82	Llama	60	66	Sperm whale	20	22
Cow (domestic)	49	56	Mink	69	76	Squirrel (gray)	30	34
Coyote	30	32	Mithun	45	54	Tree shrew	15	20
Deer	26	30	Moose	33	38	Water buffalo	48	55
Dog (domestic)	33	38	Mouse	30	36	Water shrew	1.0	30
Dolphin	10	11	Mule	55	62	White whale	2.0	18
Donkey (Ass)	61	68	Musk ox	27	33	Wolf	32	35
Elephant	51	60	Opossum	16	20	Yak	50	54
Ferret	38	44	Peccary	66	71	Zebra	74	82
Fin whale	2.0	26	Pig (domestic)	52	58	Zebu-Brahman	47	54

Source: Fonseca (2017), Mech et al. (2008), and Rezaei et al. (2016).

[a] Values are expressed as g/kg of whole milk.

contains little or no lactose, and these animals may have no tolerance to this disaccharide (Geraci 1981). However, lactose is the predominant sugar in dolphin milk at mid-lactation. Lactose is present at very low concentrations in the milk of bears and monotremes (e.g., platypus and echidna). Globally, most human adults exhibit lactose maldigestion due to their reduced capacity to hydrolyze lactose in the GIT, but they have greater tolerance for the consumption of fermented dairy products than those that are unfermented (Heaney 2013). This digestive problem can also occur in some laboratory, companion, or farm animals, such as monkeys, dogs, cats, calves, foals, and goats (Gaschen and Merchant 2011).

Maltose and Isomaltose

Maltose consists of two α-D-glucose joined together via an α-1,4 linkage and thus is also called α-maltose. This disaccharide is the fundamental repeating unit of starch and glycogen (Lu and Sharkey 2006). The difference in the configuration of the glycosidic link between maltose and cellobiose (the β-1,4 bond) is responsible for their different digestibilities in nonruminants. Isomaltose, a product of the hydrolysis of starch and glycogen, is similar to maltose but has an α-(1,6)-linkage, instead of the α-(1,4)-linkage in maltose. Thus, isomaltose is also called α-isomaltose.

Sucrose

The word "sucrose" was coined in 1857 by the English chemist William Miller from the French *sucre* and the generic chemical suffix *-ose* for sugars in English. Sucrose is composed of α-D-glucose and β-D-fructose with an α-1,2-glycosidic linkage. The nomenclature of this covalent bond indicates the configuration of the anomeric carbon (α) of the first monosaccharide (i.e., α-D-glucose), the number of the anomeric carbon (C-1) of the first monosaccharide, and the number of the carbon (C-2) of the second monosaccharide (i.e., β-D-fructose) to which the first monosaccharide is linked. The correct specification of the configuration of the anomeric carbon is critical. Amounts of sucrose vary markedly among feedstuffs (Table 2.3). Sucrose is usually the most abundant sugar in plant products (e.g., vegetables, fruits, and forages). Of note, sucrose is very abundant in sugar beets and sugar cane and is present in larger amounts (2–8% of DM) than monosaccharides in herbage (Sinnott 2013). In addition, sucrose and fructans are prominent components of fresh grasses. The concentration of disaccharides in plants increases during the day as a result of photosynthesis and decreases during the night (Ruan 2014). Success in conserving herbage (including grasses) as silage depends on the presence of readily fermentable carbohydrates (sugars, starches, or fructosans).

α,α-Trehalose

Trehalose (also known as mycose or tremalose) is a disaccharide of two α-D-glucose molecules formed by an α,α-1,1 glucoside bond (Lunn et al. 2014). The nomenclature of this glycosidic bond refers to the configuration of the anomeric carbon (α) of the first monosaccharide (i.e., α-D-glucose) and the second monosaccharide (i.e., α-D-glucose); the number of the anomeric carbon (C-1) of the first monosaccharide; and the number of the carbon (C-1) of the second monosaccharide to which the first monosaccharide is linked. α,α-Trehalose was originally discovered in 1832 in an ergot of rye and subsequently isolated from trehala manna. This disaccharide is produced by fungi [including yeasts (single cell organisms)], plants, and invertebrate animals (e.g., insects). Of note, it is also a major energy source for insects, as it is typically their primary "blood" (hemolymph) sugar.

OLIGOSACCHARIDES

Definition

Oligosaccharides consist of three to ten units of monosaccharides, which are linked via glycosidic bonds. The formation of each glycosidic linkage results in the elimination of one H_2O molecule, as noted for the formation of a disaccharide. Fructo-oligosaccharides (FOSs) consist of entirely or predominantly fructose units linked via the β-2,1-glycosidic bond. Mannan-oligosaccharides (MOSs), which are commonly derived from the partial hydrolysis of mannan polysaccharides present in yeast and plants, are made up mainly of mannose units linked by α-1,6- (in yeast) or β-1,4- (in plants) glycosidic bonds. α-1,6- and β-1,4-linked MOSs are called α-MOSs and β-MOSs, respectively. Galacto-oligosaccharides (GOSs) are composed of galactosyl residues and often a terminal glucose residue linked via the β-1,2-, β-1,3-, β-1,4-, or β-1,6-glycosidic bond. Xylo-oligosaccharides (XOSs) comprise primarily xylose units linked via the β-1,4-glycosidic bond. Nearly all naturally occurring oligosaccharides are found in plants. However, human, elephant, and bear milks are exceptions, as they contain relatively high levels of oligosaccharides. Examples for oligosaccharides in plant-sourced feedstuffs are provided in Table 2.3. The major sources of carbohydrates and energy in swine and poultry diets vary with countries due to local cultivation and availability. Examples are corn in the Midwestern United States, South America, and Asia; sorghum in the central Great Plains states of the United States (particularly Kansas, Oklahoma, Texas, Nebraska, and South Dakota); wheat and barley (and to a lesser extent, rye) in Europe; and cassava in Africa.

For nonruminants, oligosaccharides are further subdivided into digestible and nondigestible groups (Bach Knudsen 2023). Bacteria in the digestive tract of animals possess enzymes to hydrolyze all oligosaccharides with α-1,4 or β-1,4 linkages. However, animal cells do not produce the enzymes necessary to hydrolyze either α-1,6-galactosyl oligosaccharides (e.g., stachyose, raffinose, and verbascose) or β-1,4-glucosyl oligosaccharides (e.g., products of microbial cellulose hydrolysis).

TABLE 2.7
Major Sources of Complex Carbohydrates and Phenolic Polymers in Diets for Animals

Carbohydrate	Major Sources
Oligosaccharides	
Raffinose†	Bermudagrass, cottonseed, legumes,[a] rye bran and middlings, sunflower seed, sugar beets, wheat bran and middlings, and white lupins
Melezitose†	Insects and the sweet exudates of many trees
Panose†	Honey, microbes (which synthesize panose), and grain (e.g., rice) wine
Stachyose†	Canola (rapeseed) meal, cottonseed cake, and legumes[a]
Verbascose†	Legumes[a]
FOS†	Many plant-sourced foods, such as the Jerusalem artichoke, tequila agave, bananas, onions, chicory root, garlic, asparagus, jícama, leeks, legumes, wheat, and barley. The Jerusalem artichoke, its relative yacón, and tequila agave have high amounts of FOS among cultured plants.
Milk-borne OS†	Fucosylated OS (e.g., 2′-fucosyllactose) and nonfucosylated OS (e.g., lacto-*N*-tetraose, lacto-*N*-neotetraose, and sialylated OS). Some of them are galactose-OS.
Homopolysaccharides	
Starch†	Plants (grains)
Glycogen†	Animals (mainly muscle and liver)
Chitin	Animal shells.
Cellulose‡	A major nonstarch polysaccharide in plant cell walls
Hemicellulose[b],‡	A major nonstarch polysaccharide in plant cell walls
Arabinan§	Plant cell walls at very low concentrations
Galactan§	Plant cell walls usually at very low concentrations but a prominent polysaccharide in lupins
(1,3;1,4)-β-DG§	Plant cell walls; most abundant in barley, followed by oat, and is least in wheat, sorghum, and rice
Levans†	Non-structural polysaccharide in plants; a fructan in grasses, herbage, tubers, and sugar beets
Pure mannans‡	Plant cell walls; rich in palm seeds, including ivory nuts
Chitin‡	Exoskeletons of crustaceans; yeast, green algae, and brown and red seaweeds
Heteropolysaccharides	
Pectin§	A major nonstarch polysaccharide in plant cell walls
SHPS‡	Connective tissues of animals; in meat and bone meal, poultry by-product meal, and related products
Agar¶	Cell walls of red-purple seaweed (red algae)
Algin	Cell walls of brown algae; its sodium form is soluble in water.
Carrageenans	Cell walls of red algae; soluble in hot water
Phenolic polymers	
Lignins‡	Plant cell walls; associated with cellulose and hemicellulose
Tannins§	Plant cell walls; relatively abundant in trees, nuts, legumes, and fruits

[a] Including soybeans, lentils, chickpeas, mung beans, and white lupins.

[b] A group of heteropolysaccharides containing β-1,4 linked D-galactopyranosyl units in the main chain.

Note: DG, D-glucans; OS, oligosaccharides; SHPS, sulfated heteropolysaccharides; †, soluble in water; ‡, insoluble in water; §, soluble in water when isolated from plant cell walls; ¶, insoluble in cold or warm (e.g., 20–40°C) water but soluble in boiling water.

Both FOSs and MOSs are prebiotics commonly used in animal feeds. The major sources of oligosaccharides in animal diets are summarized in Table 2.7.

Trisaccharides

Trisaccharides are composed of three units of monosaccharides with two glycosidic bonds for linkages. These compounds most often occur in plants and are negligible in animals. Similar to disaccharides, each glycosidic bond in a trisaccharide is formed between any hydroxyl groups of monosaccharides, with the removal of one H_2O molecule per glycosidic linkage (Robyt 1998). Raffinose (also called melitose) [α-D-galactopyranosyl-(1,6)-α-D-glucopyranosyl-(1,2)-β-D-fructofuranoside] is a nonreducing trisaccharide present in plants, particularly sugar beets and legumes such as soybeans and green beans (Sengupta et al. 2015). Despite the presence of one fructose unit in its structure, raffinose is not an FOS. Melezitose is a nonreducing trisaccharide in the sweet exudates of many trees. Of note, a trisaccharide (3'-galactosyl-lactose), which consists of β-D-galactopyranosyl-(1,3)-β-D-galactopyranosyl-(1,4)-β-D-glucose, is a major oligosaccharide in the milk of some mammals, such as the tammar wallaby and the gray kangaroo (Messer et al. 1980). The structures of select trisaccharides in plants

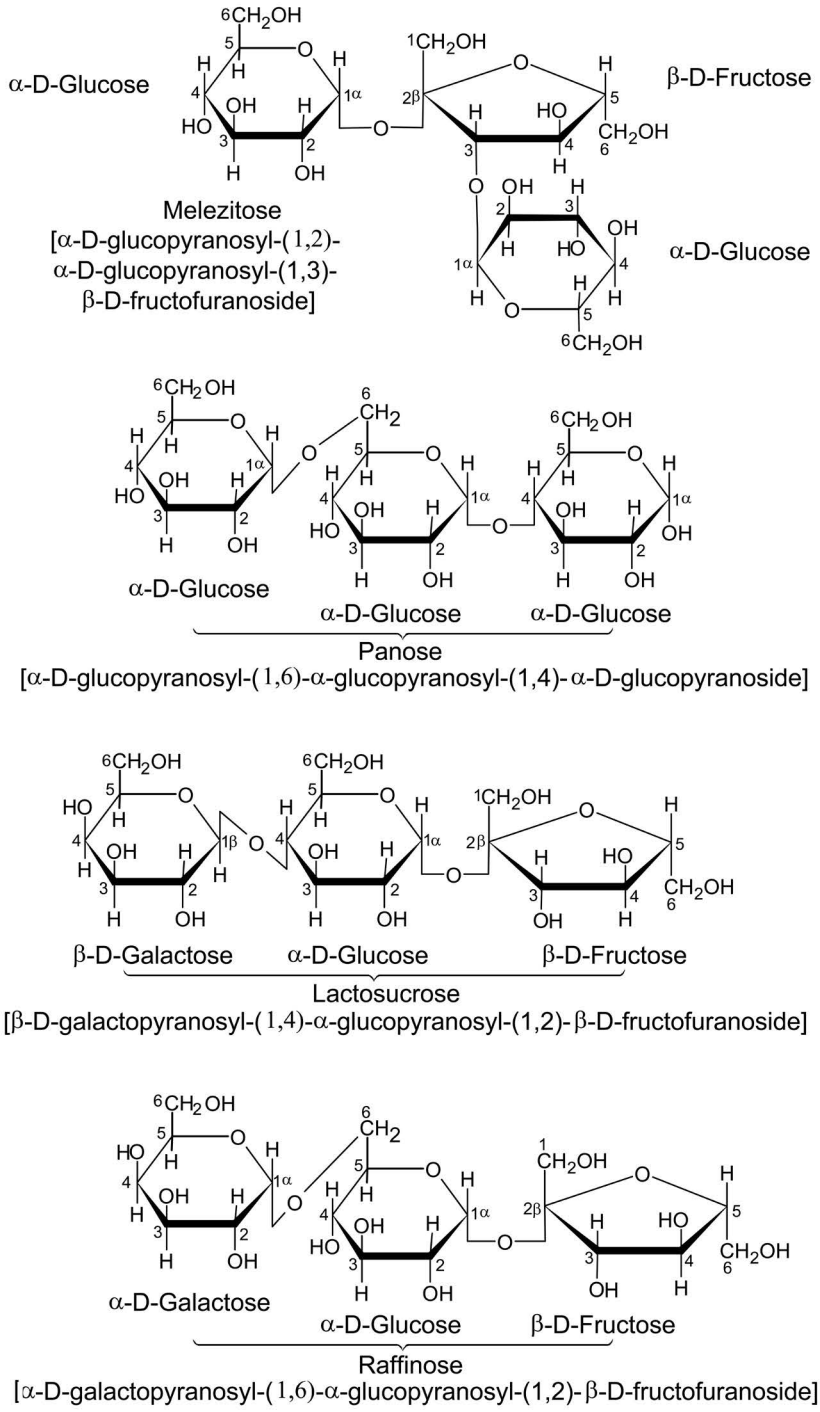


FIGURE 2.9 Structures of select trisaccharides in plant-sourced feedstuffs. All of these trisaccharides occur in plants. Raffinose (nondigestible in the small intestine) is an important component of carbohydrates in legumes. Except for melezitose, these substances are not present in animals. Melezitose is also produced by many species of insects as part of their honeydew.

are shown in Figures 2.9 and 2.10. They include FOSs, such as 1-kestose, 6-kestose, and neokestose, which are useful dietary prebiotics for animals and have a potent anti-inflammatory effect (Lee et al. 2015).

Tetrasaccharides

Plants also contain tetrasaccharides. A tetrasaccharide consists of four units of monosaccharides with three glycosidic bonds connecting them. Stachyose [α-D-galactopyranosyl-(1,6)-α-D-galactopyranosyl-(1,6)-α-D-glucopyranosyl-(1,2)-β-D-fructofuranoside] is a tetrasaccharide (Figure 2.11) that is widely present in plants, including legumes such as green beans, soybeans, and other beans (Hagely et al. 2013). This oligosaccharide is composed of two α-D-galactose units, one α-D-glucose unit, and one β-D-fructose unit, which are linked sequentially as galactose(α-1,6)galactose(α-1,6)

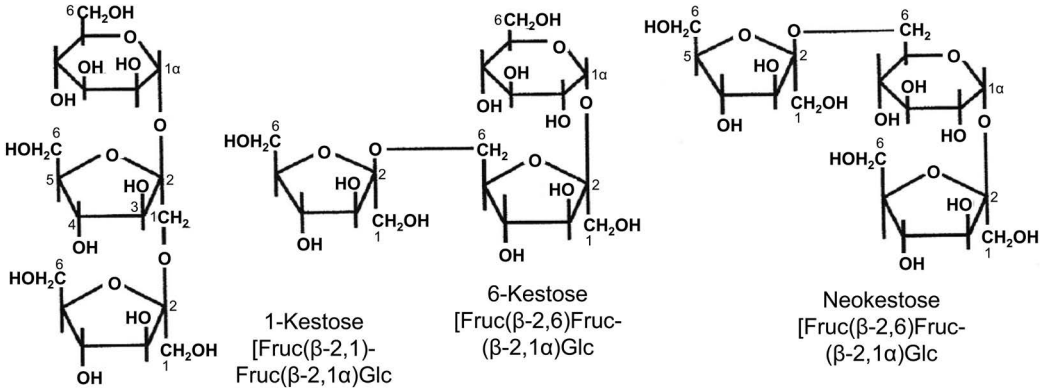


FIGURE 2.10 Structures of 1-kestose, 6-kestose, and neokestose in plants. All these three trisaccharides are composed of one β-D-fructose unit and one sucrose unit (α-D-glucose-1,2-β-D-fructose) but differ in the way a β-D-fructose unit is linked to sucrose. In 1-kestose and 6-kestose, a β-D-fructose unit is attached to C-1 and C-6 of the β-D-fructose moiety of the sucrose unit via β-2,1- and β-2,6-glycosidic linkages, respectively. In neokestose, a β-D-fructose unit is attached to C-6 of the α-D-glucose moiety of the sucrose unit via a β-2,6-glycosidic linkage.

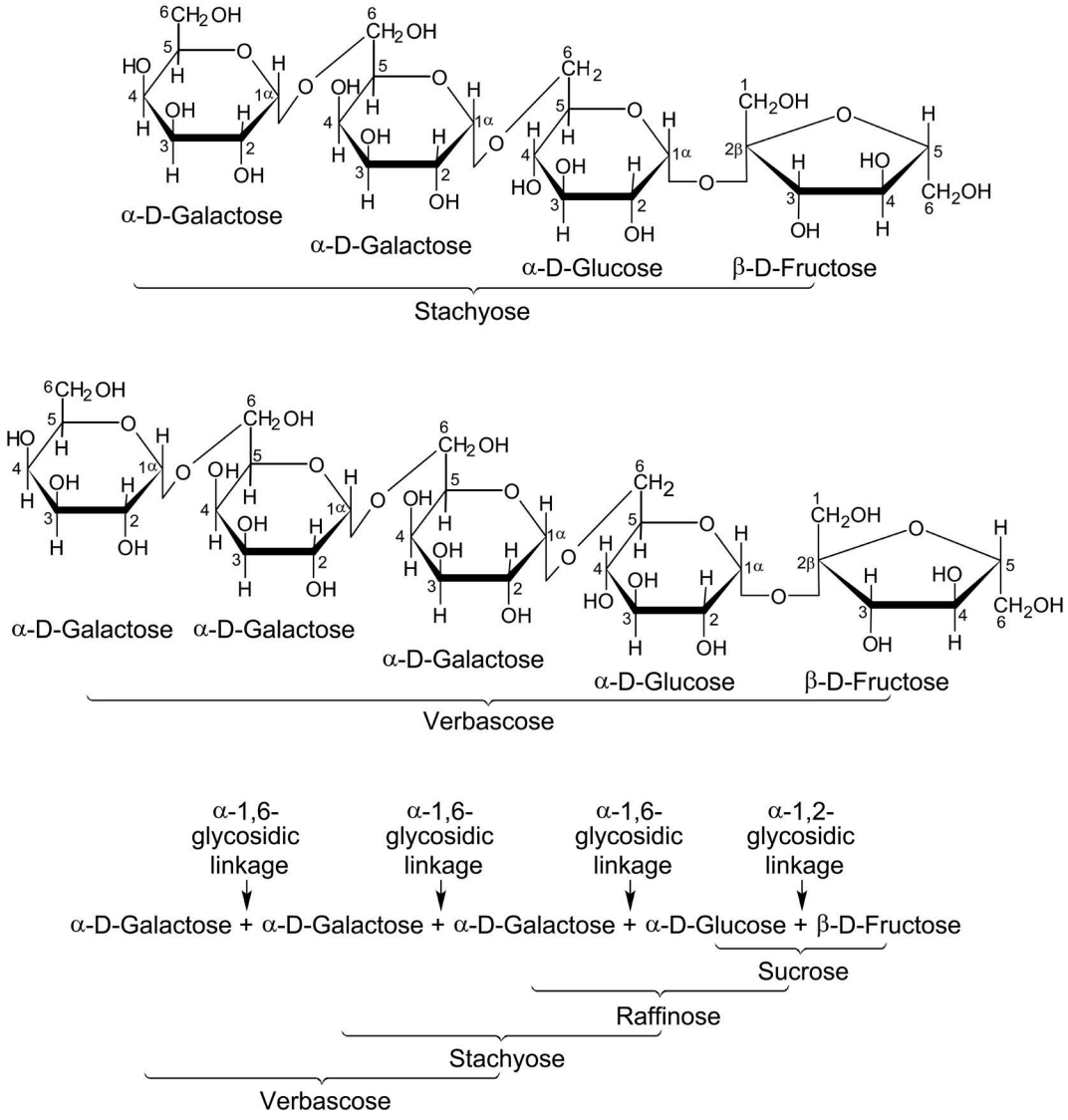


FIGURE 2.11 Structures of select tetra- and penta-saccharides in plant-sourced feedstuffs. Stachyose (a tetrasaccharide) and verbascose (a pentasaccharide) are soluble dietary fibers that are indigestible in the small intestine. These oligosaccharides are widely present in plants, particularly legumes, but are absent from animals. Bacterial enzymes can hydrolyze tetra- and penta-saccharides into constituent monosaccharides.

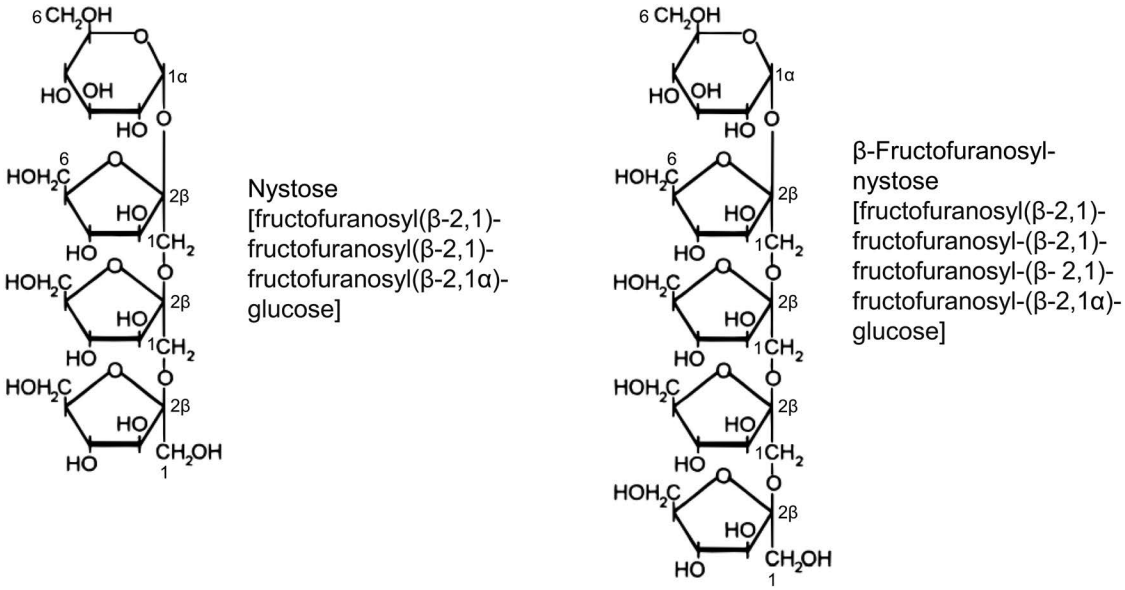


FIGURE 2.12 Structures of nystose and β-fructofuranosylnystose (oligosaccharides) in plants. β-Fructofuranosylnystose contains one more β-D-fructose unit than nystose. In both sugars, two β-D-fructose units are linked together via a β-2,1 glycosidic bond, and the last β-D-fructose unit is attached to one α-D-glucose unit via a β-2,1α glycosidic bond.

glucose (α-1,2)fructose. Another tetrasaccharide in plants is nystose (an FOS composed of 3 fructose molecules and 1 glucose molecule; Figure 2.12), which is a useful dietary prebiotic for intestinal health in animals (Bali et al. 2015).

Pentasaccharides

A pentasaccharide consists of five units of monosaccharides with four glycosidic bonds for linkages. Verbascose [α-D-galactopyranosyl-(1,6)-α-D-galactopyranosyl-(1,6)-α-D-galactopyranosyl-(1,6)-α-D-glucopyranosyl-(1,2)-β-D-fructofuranoside] (Figure 2.11), a major pentasaccharide, is common in many legumes (Peterbauer et al. 2002). Like raffinose and stachyose, verbascose is a water-soluble dietary fiber that is undigestible in the small intestine of animals. All of these oligosaccharides may make intestinal digesta and stools softer. In the large intestine, they are degraded by bacterial enzymes to fatty acids, which are primarily short-chain fatty acids that are beneficial for colon health (Turner et al. 2013). Another pentasaccharide is β-fructofuranosylnystose (an FOS composed of 4 fructose molecules and 1 glucose molecule; Figure 2.12), which is a useful dietary prebiotic for animals and has a potent anti-inflammatory effect in the intestine (Bali et al. 2015).

Other Oligosaccharides

The milk of mammals (e.g., humans, pigs, and ruminants) contains many kinds of oligosaccharides that are largely resistant to digestion by animal enzymes. They consist of fucosylated oligosaccharides (including 2′-fucosyllactose), nonfucosylated neutral oligosaccharides (including lacto-*N*-tetraose [Gal(β-1,3)GlcNAc(β-1,3)Gal(β-1,4)Glc] and lacto-*N*-neotetraose [Gal(β-1,4)GlcNAc(β-1,3)Gal(β-1,4)Glc)], and sialylated oligosaccharides. These carbohydrates account for 35–50%, 42–55%, and 12–14% of total oligosaccharides in term human milk, respectively (Smilowitz et al. 2014). The precursors of milk oligosaccharides are glucose, galactose, *N*-acetylglucosamine, *N*-acetylgalactosamine, fucose, *N*-acetylneuraminic acid, and *N*-glycolylneuraminic acid through the actions of specific glycosyltransferases (Poulsen et al. 2019). Some milk oligosaccharides are galacto-oligosaccharides, which consist of some β-1,6-galactosyl residues linked to a terminal glucose unit via an α-1,4-bond and are produced from lactose by the transglycosylating activity of β-galactosidase. They consist of a number of β-1,6-linked galactosyl residues linked to a terminal glucose unit via an a-1,4-bond.

Oligosaccharides are the third-most abundant organic matter in human mature milk (10–20 g/L) after lactose and fats, but present in cow's mature milk at concentrations of only 0.2–0.3 g/L (Durham et al. 2023; Smilowitz et al. 2014). For comparison, porcine colostrum and mature milk contain 12.0–12.2 and 6.8–7.0 g/L oligosaccharides, respectively (Difilippo et al. 2016). Milk oligosaccharides are prebiotics that confer many health benefits, such as stimulating the growth of beneficial bacteria in the gut, promoting the development of the brain, and exerting anti-pathogenic activity by preventing the binding of pathogens to intestinal epithelial cells (Smilowitz et al. 2014).

POLYSACCHARIDES

Polysaccharides include homopolysaccharides (e.g., chitin, glycogen, and starch) and heteropolysaccharides (e.g., arabinogalactan, hyaluronic acid, and pectin; Figure 2.13). In recent years, there has been growing interest in the nutritional

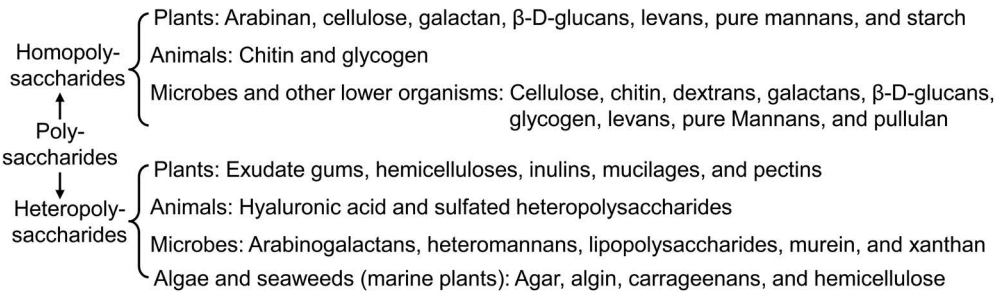


FIGURE 2.13 Classification of polysaccharides in plants, animals, microbes, and other organisms. These carbohydrates are divided into poly- and hetero-polysaccharides and have different chemical properties. Hemicellulose is a group of heteropolysaccharides (including arabinogalactans, heteromannans, mixed-linkage β-glucans, xylans, and xyloglucans) and occurs in the cell walls of plants and some yeasts. Cellulose, hemicellulose, and pectin are primarily present in plant cell walls and the major components of nonstarch polysaccharides. Starch and glycogen are the primary forms of α-D-glucose storage in plants and animals, respectively. Chitin is a polymer of β-D-glucose in the exoskeletons of invertebrates and in fungi and algae. Heteropolysaccharides are resistant to degradation by digestive enzymes in animals.

roles of NSPs from plants, algae, and seaweed in intestinal and metabolic health (Anwar et al. 2022; Choi et al. 2024). In these food sources, NSPs include homo- and hetero-polysaccharides other than starches and generally contain linear β-glucans and/or heteropolysaccharides in β-glucosidic linkages.

NSPs are abundant in the cell walls of legumes, cereals, forages, and roughages and are also present in marine plants. NSPs are classified as soluble or insoluble according to their solubility in H_2O. Water-soluble NSPs include pectin (when isolated from the cell wall), inulin, xanthan gum, alginate (as sodium salt), and carrageenans (as sodium salt), whereas water-insoluble NSPs include cellulose, hemicellulose, resistant starch, chitin, and lignin. In most plant-sourced products, insoluble NSPs are much more abundant than soluble NSPs (Table 2.8). The composition of this class of complex carbohydrates varies with feedstuffs. For example, NSPs comprise mainly arabinoxylans, other hemicelluloses, and cellulose in corn (49%, 29%, and 22%, respectively) and sorghum (43%, 32%, and 25%, respectively). By contrast, cellulose, pectins, and β-mannans are predominant NSPs in soybean meal; cellulose, β-glucans, and arabinoxylans in barley and oats; arabinoxylans, cellulose, and β-glucan in rye and wheat (Bach Knudsen 1997; Jaworski et al. 2015).

Soluble and insoluble NSPs have different nutritional and physiological effects in animals (Grabitke and Slavin 2009). Water-soluble NSPs (e.g., arabinoxylans and β-glucans) give rise to viscosity in an aqueous solution. Although NSPs, along with lignin, are commonly called dietary fiber, this term is a misnomer because they are not all fibrous in chemical structure. As parts of dietary fiber, NSPs can beneficially modulate the microbial composition and activity of the GIT.

The term "dietary fiber" is derived from the literature of human nutrition, but over the past decades its definition has gradually been modified to include an increasing number of components. The Codex Alimentarius definition of dietary fiber is as follows: "Carbohydrate polymers (and lignin) with three and more monomeric units which are neither digested nor absorbed in the human small intestine" (Codex 2010). In some countries, even nondigestible oligosaccharides are included in the term dietary fiber. Thus, dietary fiber is a nutritional, but not a chemical, definition.

HOMOPOLYSACCHARIDES: HOMOPOLYSACCHARIDES IN PLANTS

Definition

Homopolysaccharides (homoglycans) are polysaccharides consisting of a single type of many monosaccharide molecules joined together through glycosidic linkages. Depending on the monosugar, a homopolysaccharide may be called glucan (consisting of D-glucose); fructan (consisting of D-fructose); galactan (consisting of D-galactose); arabinan (consisting of D-arabinose); pure mannan (consisting of D-mannose); or xylan (consisting of D-xylose). Plant-sourced homopolysaccharides include starch, cellulose, levans, galactans, and β-D-glucans (Robyt 1998). Glycogen serves to store glucose and energy in animals, whereas other homopolysaccharides fulfill structural functions in the cell wall of plants. Animal cells can hydrolyze homopolysaccharides with an α-1,4 linkage, but not those with a β-1,4 linkage. However, cellulose, levans, galactans, and β-D-glucans can be extensively degraded by enzymes released from bacteria present in the rumen of ruminants and in the large intestine of all animals (Pond et al. 2005).

Arabinan

Arabinan has a complex branched structure consisting of D-arabinose residues linked via α-1,3, α-1,5, and β-1,2 glycosidic bonds (Chandrasekaran 1998). Pure arabinan is present in the plant cell wall at a very low concentration. Highly purified linear 1,5-α-L-arabinan can be obtained from sugar beets. Arabinan is soluble in water when isolated from plant cell walls.

TABLE 2.8
Composition of Complex Carbohydrates and Organic Acids in Select Feedstuffs

	Carbohydrates (% of DM)			Sugars	Starch	Pectins	OA
Feedstuff	NDF[a]	NFC[b]	NSC[c]	% of NFC			
Alfalfa hay	43.1	22.0	12.5	11.8	7.3	56.4	0.00
Alfalfa meal	44.0	21.9	9.7	11.9	31.0	54.8	0.91
Alfalfa silage	51.4	18.4	7.5	0.0	24.5	33.0	42.5
Barley (grain)	23.2	62.0	61.0	9.1	81.7	9.2	0.0
Beet pulp	47.3	36.2	19.5	33.7	1.8	64.5	0.0
Canola (rapeseed) meal	31.6	32.7	12.1	32.1	4.9	13.8	17.1
Corn (shelled, HM)	13.5	71.8	70.6	2.8	95.5	0.34	0.0
Corn bran	44.2	42.2	38.0	6.6	82.9	10.4	0.1
Corn gluten meal	4.1	21.4	19.9	0.0	93.0	1.9	0.0
Corn grain (ground)	13.1	70.7	68.7	19.1	78.1	2.8	0.0
Corn silage (30–35% DM)	44.2	41.0	34.7	0.0	71.3	0.0	28.7
Cottonseed (whole)	48.3	10.0	6.4	21.0	25.0	0.0	30
Cottonseed meal (low fiber)	27.0	20.6	8.5	25.2	16.0	0.0	26.7
DDGS (corn; 6–9% oil)	34.2	18.1	12.5	9.4	59.7	0.0	30.9
Grass hay (Chazy, NY)	64.4	19.2	11.9	51.0	10.9	26.0	12.0
Linseed meal (>5% oil)	23.7	26.2	11.5	16.4	27.5	2.0	17.2
Mixed hay (mainly grass)	60.9	16.6	13.6	35.4	15.2	49.4	0.0
Molasses (sugar cane)	0.8	73.1	63.5	85.2	0.45	0.27	11.8
Molasses (beet cane)	0.8	71.2	62.7	87.2	0.11	0.09	11.7
Oats (grain)	35.5	49.8	42.4	3.2	81.5	1.4	0.60
Oat bran	15.8	65.9	51.5	4.6	73.3	4.1	4.4
Oat flour	9.5	86.3	81.0	0.99	92.7	0.58	0.23
Oat hay	61.7	31.9	22.5	20.4	39.2	7.8	0.63
Rice (grain, polished)	4.8	93.1	86.2	0.75	91.8	2.6	0.09
Rice bran (11–20% fiber)	34.4	32.6	25.3	8.6	68.7	8.0	7.7
Rye (grain)	13.9	82.9	70.4	4.3	75.0	9.5	0.24
Rye bran	29	49.2	42.8	15.2	57.7	0.30	1.93
Soybean meal, 48% CP	9.6	34.4	17.2	28.2	28.2	43.6	0.0
Soybean hulls	66.6	14.1	5.3	18.8	18.8	62.4	0.0
Sorghum (grain)	11.0	79.3	76.5	1.8	93.9	Low	1.25
Wheat (grain)	13.9	74.7	70.4	4.3	88.1	0.34	1.35
Wheat bran	45.2	45.5	33.8	15.8	50.8	7.7	11.6
Wheat middlings	33.7	53.5	43.1	15.9	60.4	4.6	8.6

Source: Bader et al. (2019), Bailoni et al. (2005), Chatterton et al. (2006), Dann et al. (2007), Hahn et al. (1990), Houston et al. (1963), INRA (2024), Lim et al. (1992), Maner (1985), National Research Council (NRC) (2001), Prasadi and Joye (2020), Palmonari et al. (2020), Salvati et al. (2024), and Stevenson et al. (2012).

[a] Neutral detergent fiber (NDF). NDF includes mainly cellulose + hemicellulose + lignin.

[b] Nonfiber carbohydrates (NFC) are calculated by difference [i.e., 100 – (% NDF + % CP + % Fat + % Ash)] or direct analyses. NFC = Sugars + Starches + Fructans + β-Glucans + Pectins + Any other carbohydrates that are soluble in neutral detergent solution + Water-soluble organic acids (e.g., pyruvate, lactate, malate, oxaloacetate, and α-ketoglutarate).

[c] Nonstructural carbohydrates (NSC). NSC = Sugars + Starches + Fructans.

Note: CP, crude protein; DDGS, distiller's dried grain with solubles; DM, dry matter; HM, high moisture; OA, water-soluble organic acids (including lactic acid and short-chain fatty acids) that are neither amino acids nor medium- and long-chain fatty acids.

Cellulose

Cellulose is a linear polymer of β-1,4-linked D-glucose units and has no branching chain (Figure 2.14). The numbers of polymerized glucose molecules in cellulose may range from 900 to 2000. Cellulose is the major structural component of cell walls in higher plants and the most abundant biopolymer on the planet. This homopolysaccharide amounts to ~20–40% of the DM in green plants (including grasses and bamboo), and constitutes the bulk of flax (80%), jute (60–70%) and wood (40–50%; Robyt 1998). In plant tissues, cellulose forms fibers that are composed of crystalline microfibrils held together by strong hydrogen

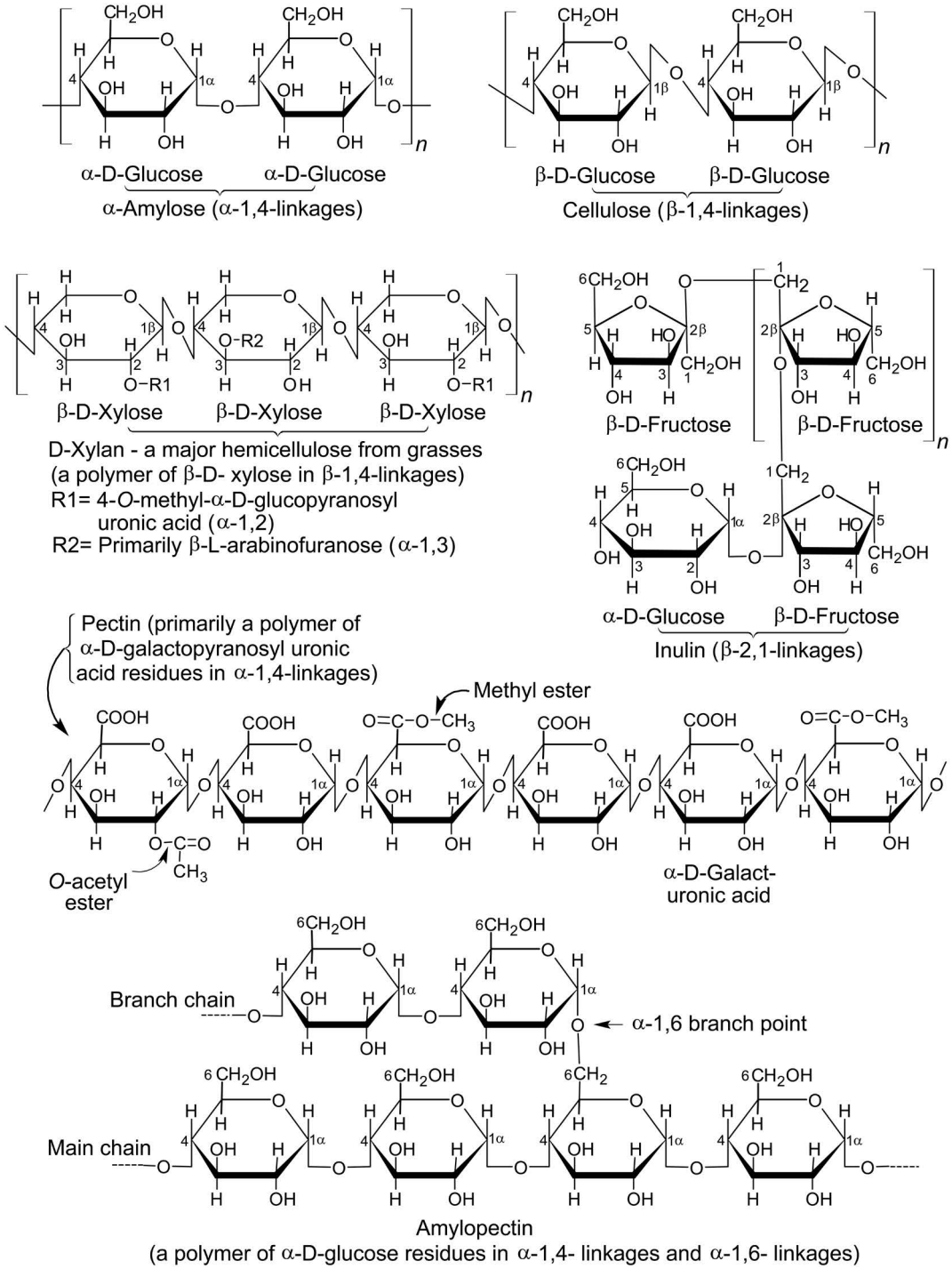


FIGURE 2.14 Structures of select homopolysaccharides and heteropolysaccharides in plants. Amylose is a polymer of α-1,4-linked glucose residues without any branch, and amylopectin is a polymer of α-1,4-linked glucose residues branched by α-1,6-linkages. Starch is a mixture of amylose and amylopectin. In contrast, cellulose is a polymer of β-D-glucose with β-1,4-linkages. Starch and cellulose are homopolysaccharides. In contrast, D-xylan and inulin (heteropolysaccharides) consist of more than 10 units of different types of monosaccharides. None of these polysaccharides are produced by animals.

bonds (Li et al. 2015). The crystalline microfibrils are encircled by a largely amorphous matrix of hemicellulose, lignin (cross-linked phenol polymers with a high molecular weight), and some proteins on the cell walls. The different types of polysaccharides, lignin, and proteins are held together on the plant cell walls by hydrogen bonding and van der Waals forces (Li et al. 2015). This chemical structure contributes most to the rigidity and strength of higher plants. In addition, cellulose is virtually insoluble in H_2O, ethanol, and dilute or concentrated acid solutions, has very low solubility in a dilute alkaline solution at 25°C, and is highly resistant to hydrolysis by a concentrated acid (e.g.,

2 M H_2SO_4) even at 100°C. In contrast, cellulose is completely dissolved in a 10% NaOH solution at 10°C (Kamide et al. 1984) and completely hydrolyzed by 12 M (i.e., 72%) H_2SO_4 at 25°C (Camacho et al. 1996). Nutritionally, this homopolysaccharide is indigestible to mammal-, bird-, or fish-sourced digestive enzymes. However, crustaceans can synthesize cellulase to degrade dietary cellulose.

Galactan

Pure galactan (also called galactosan) is a linear chain of β-D-galactose residues linked via the β-1,4-glycosidic bond in higher plants and macroalgae (Arifkhodzhaev 2000). This homopolysaccharide is present in plant cell walls at a very low concentration, except that galactan is a prominent polysaccharide in lupins (Al-Kaisey and Wilkie 1992). Interestingly, the galactose residues of galactan are primarily linked via the α-1,6-glycosidic bond in *Anogeissus latifolia* but via the α-1,3-glycosidic bond in acacia trees. Some galactose residues of galactan may be sulfated. Galactan is soluble in water when isolated from plant cell walls.

β-D-Glucans [β-1,3-D-glucan and (1,3;1,4)-β-D-glucan]

β-D-Glucans are homopolysaccharides containing a backbone of β-D-glucose units linked via either a β-1,3 bond or both β-1,3 and β-1,4 glycosidic bonds (Robyt 1998). Both β-1,3-D-glucan and (1,3;1,4)-β-D-glucan comprise only β-D-glucose but no other types of sugars. Unlike cellulose, plant β-D-glucans are characterized by the presence of β-1,3-glycosidic linkages in the main chains. In some plants, β-D-glucans may have side chains consisting of a various number of β-D-glucose units linked via β-1,4 or possibly β-1,6 glycosidic bonds (Farrokhi 2006). Examples of plant β-D-glucans are callose (β-1,3-D-glucan) and (1,3;1,4)-β-D-glucan (also known as β-1,3- and β-1,4-D-glucan). The contents of β-D-glucans, which are present mainly in the cell walls of grasses and cereal grains, are shown in Table 2.3.

Callose

Callose is a β-1,3-D-glucan (i.e., β-1,3 glycosidic bonds in the main chain) with some β-1,6-branches (Chen and Kim 2009). Its side chains, which consist of β-1,6-D-glucose units, are linked to the main chain via β-1,6 bonds. Callose exists in the cell walls of a wide variety of higher plants (e.g., barley, cotton, rice, soybean, tomato, and wheat) and plays important roles during a variety of development processes and in response to multiple biotic and abiotic stresses. Like cellulose, callose is insoluble in H_2O but is soluble in a dilute alkaline solution.

(1,3;1,4)-β-D-Glucan

(1,3;1,4)-β-D-glucans (also known as mixed-linkage β-glucans) occur at various concentrations in the cell walls of the Poaceae family of flowering plants, including cereal crops (e.g., barley, corn, oats, rice, rye, sorghum, and wheat); legumes (e.g., alfalfa, clover, peanut, peas, and soybean); and some grasses (e.g., bamboos, bermudagrass, sugarcane, and tall fescue). Of note, (1,3;1,4)-β-D-glucan is a major polysaccharide in the cell wall of horsetail (a land plant) outside the Poales. In cereal grains, (1,3;1,4)-β-glucans are unsubstituted and unbranched homosaccharides composed of 1,000 or more glucosyl residues linked by both β-1,3 and β-1,4 bonds (Murphy et al. 2021). In these molecules, the β-1,3-linkages are commonly separated by two or three β-1,4-linkages, and longer segments of consecutively β-(1→4)-linked glucosyl residues are less frequent (Bach Knudsen 2014). Thus, these NSPs are polymers of β-(1,4)-glucans with interspersed β-(1,3)-linkages that introduce a "link" into the linear β-(1,4) backbone. This prevents the molecule from self-associating and thus precipitating, and is the reason that very large polymers remain in water and generate viscous solutions within the lumen of the GIT (Campbell and Bedford 1992). The ratio of (1,4)- to (1,3)-β-D-glucose residues in (1,3;1,4)-β-glucans ranges from 2.2 to 2.6:1 in most cereals but is 1.15:1 in sorghum endosperm (Ermawar et al. 2015). Among the cereals studied, (1,3;1,4)-β-glucan is most abundant in barley, followed by oat, and is least abundant in wheat, sorghum, and rice. In barley, (1,3;1,4)-β-glucan comprises 70% (w/w) of the cell wall and 2.5–11.3% of grain (Lazaridou and Biliaderis 2007). However, low amounts of (1,3;1,4)-β-glucan are present in the grain of sorghum, ranging from 0.16%–0.27% (Ermawar et al. 2015). Cereal (1,3;1,4)-β-D-glucans are highly soluble in H_2O and the small intestine and can affect intestinal-luminal viscosity and the reabsorption of bile acids. β-D-Glucans are well fermented in the large intestine of ruminants and nonruminants.

Levans

Levan is a nonstructural, water-soluble homopolysaccharide consisting of D-fructose molecules and is a type of fructan present in many plants, including grasses, herbage, tubers, and sugar beets (Benkeblia 2013). By definition, fructans are a group of fructose-based oligo- and polysaccharides. Levans contain main chains of 100–200 D-fructofuranose units in β-2,6 glycosidic linkages (Bali et al. 2015). These homopolysaccharides also have branch chains of D-fructofuranose residues in β-2,1 glycosidic linkages. The number of D-fructofuranoses ranges from one to four. Levans can serve as storage for carbohydrates instead of starch in some special species of plants and are indigestible to animal-sourced digestive enzymes (van Arkel et al. 2013).

Pure Mannans

Plant mannans are classified as pure mannans, glucomannans, galactomannans, and galactoglucomannans based on their composition and structures (Buckeridge et al. 2000; Srivastava and Kapoor 2017). All of these polysaccharides have β-1,4-linked mannose units in their main chains and are hemicelluloses. Except for pure mannans (also known as linear mannans) without any side chain, other mannans have frequent branches. In some species of algae (e.g., *Codium fragile* and *Acetabularia crenulate*), mannan microfibrils replace cellulose as the dominant structural component of their cell walls (Mackie and Preston 1968).

TABLE 2.9
Mannans (Polymers Containing D-Mannose as a Major Principal Sugar) in Plants and Yeast

Mannan	Backbone Residues	Substitution in Backbone	Chemical Properties	Presence in Plants or Yeasts
		β-Mannan in plants		
Pure β-mannan (homomannan)	(β-1,4)-D-mannose	None	100–2,500 residues; soluble in boiling water at neutral pH; precipitates with ethanol	Certain algae; rich in palm seeds, including ivory nuts
Glucomnnan	(β-1,4)-D-glucose and D-mannose; partially acetylated	None	100–5,000 residues; viscous; soluble in water at neutral pH	Monocot seeds; orchid stems; wood; *Amor- phophallus* corms
Galactomannan	(β-1,4)-D-mannose	(α-1,6)-D-galactose	1,000–10,000 residues; viscous; soluble in water at neutral pH	Legume seeds
Galactoglucomannan[a]	(β-1,4)-D-glucose and D-mannose	(α-1,6)-D-galactose on D-glucose or D-mannose residues	100–200 residues; soluble in water at neutral pH	Softwood; *Asparagus* seeds; *Arabidopsis* seed mucilage; ripe kiwifruit
		α-Mannan in yeasts		
Pure α-mannan[b]	(α-1,6)-D-mannose	None	Soluble in boiling water at neutral pH	Cell walls of yeasts
D-Galacto-α-D-mannan	(α-1,6)-D-galactose and D-mannnose	*N*-acetyl-D-GlcN	Viscous; soluble in water at neutral pH	Cell walls of yeasts
D-Xylo-α-D-mannan	(α-1,6)-D-xylose and D-mannnose	*N*-acetyl-D-GlcN	Viscous; soluble in water at neutral pH	Cell walls of yeasts

[a] Possibly containing a small amount of D-xylose and D-arabinose.
[b] Containing α-1,2 and α-1,3-linked mannose side chains.
Note: D-GlcN, D-glucosamine.

Plant β-mannans (β-1,4-linkage) are structurally distinct from α-linked mannans in microbes. Heteromannans are an important part of hemicellulose in plants and the main structural components of seeds and softwoods (Table 2.9).

Pure mannans in plant cell walls are linear homopolysaccharides of D-mannose linked via the β-1,4 glycosidic bond and are insoluble in H_2O at 25°C and neutral pH (Voiniciuc 2022). Some mannose residues may be acetylated or sulfated. Of interest, pure mannans account for as much as 60% of palm seeds, including the ivory nut (*Phytelephas macrocarpa*; Melton et al. 2009). Pure mannans bind cellulose in the plant cell wall, serve to store free carbohydrates in plants, and modulate plant growth and development. In addition to higher plants, pure mannan occurs in green algae that belong to the *Codiaceae and Dasycladaceae* families (Frei and Preston 1968).

Starch

Starch (α-glucan) is synthesized by all plants as a water-insoluble semi-crystalline macromolecule assembled in the form of spherical granules (Keeling and Myers 2010). It is the major homopolysaccharide in plants, particularly in grains, seeds (that may contain up to 70% on an as-fed basis), cereal byproducts, and tubers (with ~30% starch on the DM basis), but its content differs markedly among feedstuffs (Tables 2.3 and 2.8). The two major starches in plants are amylose and amylopectin (Figure 2.14), which account for 20% and 80%, respectively, of dietary starch (Smith et al. 2005). Amylose is a long linear chain in which 250–300 units of D-glucose are joined by α-1,4 linkage and generally has no branches. In contrast, amylopectin is a glucose polymer with a backbone of α-1,4-linked glucose units and α-1,6 branching chains that occur every 24–30 units of α-D-glucose. Thus, starch is a mixture of amylose and amylopectin, both of which consist of α-D-glucose residues. The ratio of amylose to amylopectin varies with the sources of starch but is usually 1:3 (g/g).

Starches from different sources of plants have different structures. For nonruminants, starch is subdivided into three types: rapidly digestible, slowly digestible, and resistant starch based on the rates of their in vitro enzymatic digestion. Rapidly digestible starch has high amylopectin content and high digestibility, such as the starch in the waxy corn (found in China in 1909) containing 100% amylopectin and 0% amylose), freshly cooked starch, and white bread. Slowly digestible starch generally possesses more amylopectin than amylose; such starch is found in most raw cereals (dent corn varieties, barley, wheat, and rice), whose semi-crystalline structure renders it less accessible to digestive enzymes. Amylopectin/Amylose ratios are affected by plant source (e.g., cereals, legumes, tubers) and processing. The true digestibility of raw starch in the small intestine of postweaning pigs and post-hatching chickens are relatively high.

Resistant starch generally has high amylose content and relatively low digestibility, such as high-amylose corn, high-amylose wheat, legumes, and bananas (Zhang and Hamaker 2009). Resistant starch is further classified as physically inaccessible (RS1; e.g., in legumes and whole or partially milled grains), resistant starch granule (RS2; e.g., in bananas, raw potatoes, or high amylose corn), retrograded starch (RS3;

e.g., starches in potato and bread that are first cooked and then recoil after cooling), and chemically modified starch (RS4; many types of starch with different cross-linking due to human processing). The rate of digestion of the resistant starches in the small intestine of nonruminants (e.g., pigs) differs among their types, with RS1 resisting digestion, RS2 being slowly digested, and RS3 and RS4 being difficult to digest. In their natural state, tuber and grain starches (e.g., in potatoes) exist in a water-insoluble granular form (Sinnott 2013), which resists digestion in the small intestine of nonruminants (Pond et al. 2005). Such starch-containing foods must be cooked before they can be more efficiently utilized by chickens and pigs. The cooking of foods markedly aids in their digestion by animals through breaking down (gelatinizing) and solubilizing starch granules. In general, amorphous or completely dispersed starches are highly susceptible to digestive enzymes. Using young pigs [~7 kg body weight (BW)] in studies, Yin et al. (2010) reported that the digestibility of native resistant starch in the different segments of the small intestine within 3 hours post-feeding was: anterior jejunum, 30%; posterior jejunum, 46%; anterior ileum, 54%; and posterior ileum, 67. Uncooked natural RS may be considered as a prebiotic.

HOMOPOLYSACCHARIDES: HOMOPOLYSACCHARIDES IN ANIMALS

Definition

As in plants, homopolysaccharides in animals are composed of a single type of monosaccharide joined through glycosidic linkages. In contrast to plants, the monosaccharide unit can be D-glucose or its derivative, *N*-acetyl-glucosamine. To date, glycogen and chitin are the only homopolysaccharides known to be present in animals.

Chitin

Animal-sourced chitin is a water-insoluble long-chain polymer of primarily *N*-acetyl-glucosamine molecules in β-1,4 glycosidic linkages. Chitin occurs in the exoskeletons of crustaceans (e.g., crabs, lobsters, and shrimps), insects, the radulae of molluscs, as well as the beaks and internal shells of squid and octopuses (Younes and Rinaudo 2015). Note that chitin is not present in the structure of an avian beak, which is composed mainly of the keratin protein.

The structure of animal-sourced chitin is the same as that of cellulose in plants, except that the C-2 of the D-glucopyranose residue in cellulose is substituted with

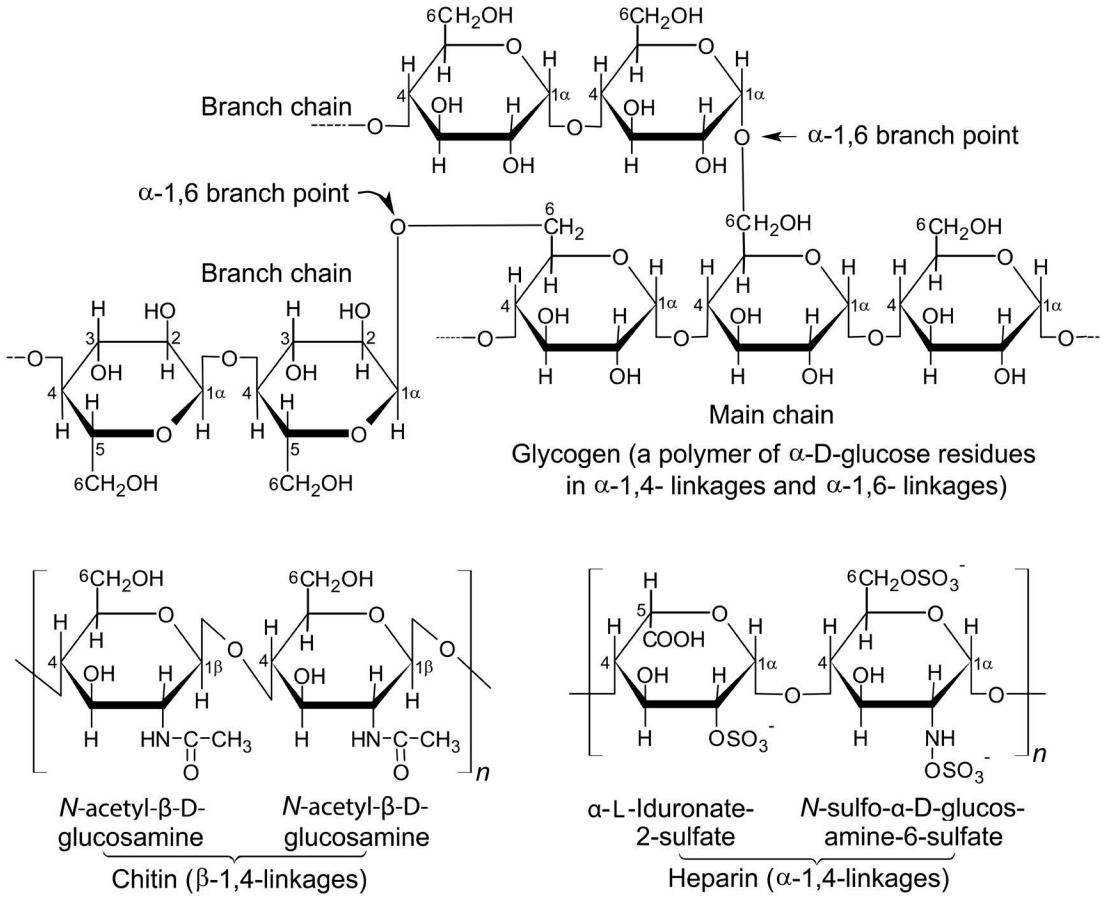


FIGURE 2.15 Structures of select homopolysaccharides and heteropolysaccharides in animals. Glycogen is the major homopolysaccharide in mammals, birds, and fish, and is also present in shrimp and insects. It is a polymer of α-D-glucose with α-1,4-linkages in the main chain and α-1,6-branching linkages. Chitin, which is a polymer of α-D-glucosamine molecules, is a main homopolysaccharide in many aquatic animals. Heparin is a heteropolysaccharide in animals and contains α-1,4- and β-1,4-linkages. Glycogen is synthesized mainly in the cytosol of liver and muscle, whereas heparin is formed in the rough endoplasmic reticulum of mast cells.

an *N*-acetyl-amino group (Figure 2.15). Chitin also has the same structural and protective functions in animals as cellulose does in plants. In the presence of a strong alkali (e.g., sodium hydroxide), chitin undergoes partial deacetylation to form water-soluble chitosan composed of *N*-acetyl-D-glucosamine (also called 2-amino-2-deoxy-β-D-glucose; Sinnott 2013). In animals (e.g., pigs, humans, birds, and fish), both the gastric juice and the lumen of the small intestine contain chitinase to degrade chitins.

Glycogen

Glycogen is a polymer of α-D-glucose in α-1,4 glycosidic linkages on the main chain with α-1,6 branch linkages (Roach et al. 2012). It has a molecular weight of ~10^8, a mass equivalent to 6×10^5 glucose residues. However, the precise molecular weight of glycogen is not known. Glycogen resembles amylopectin in structure (Figure 2.15) but has a higher degree of branching than amylopectin. Specifically, branching occurs in glycogen approximately every 8 to 12 glucose residues. Due to the presence of –OH groups, glycogen is associated with H_2O molecules in the ratio of 1:3 (g/g). Thus, the deposition of 1 g glycogen in the body results in the gain of 4 g BW.

Glycogen occurs in all animal cells (Table 2.10). It is the only homopolysaccharide in mammals, the principal homopolysaccharide in birds and fish, and a quantitatively significant homopolysaccharide in insects and crustaceans (e.g., crabs, lobsters, and shrimps) with extensive exoskeletons. In mammals, birds, and fish, glycogen is present primarily in the liver, as well as skeletal and cardiac muscles. For example, the liver of animals contains ~1.5 to 8% glycogen; calves, 2–5%; adult cattle, 1.5–4%; hens, 3–4%; geese, 4–6%; and skeletal muscle, 0.5–1% (Bergman 1983a,b; McDonald et al. 2021). In resting and well-nourished horses, skeletal muscle contains up to 4% glycogen. In animals, ~80% of total glycogen is present in skeletal and cardiac muscles, ~15% in the liver, and ~5% in other tissues.

Glycogen serves as the major form of glucose storage in animals, which helps to minimize the negative impacts of high glucose concentrations on osmolarity. However, excess storage of glycogen in the liver and skeletal muscle causes abnormalities in their structures and functions (Li et al. 2022). Because of its limited reserve in the body, glycogen can only provide enough glucose to sustain a starving animal for up to 12 h. However, glycogen is the immediate source of glucose when animals have an urgent need for

TABLE 2.10
Amounts of Glycogen in the Liver and Muscle (Skeletal Muscle Plus Heart) of Animals in the Postabsorptive State

				Glycogen in Tissue			
Animal Species	Body Weight (kg)	Tissue Type	Tissue Weight (kg)	Amount (g)	Percent of Tissue Weight (%)	Percent of Body Weight (%)	Energy (kcal)
Cat	4	Muscle	1.8	19.8	1.1	0.50	81.2
		Liver	0.10	4.9	4.9	0.123	20.1
Cattle	500	Muscle	240	4800	2.0	0.96	19680
		Liver	7.5	375	5.0	0.075	1538
Chicken	2	Muscle	0.96	11.5	1.2	0.58	47
		Liver	0.04	1.8	4.5	0.09	7.4
Dog	30	Muscle	13.5	230	1.7	0.77	943
		Liver	1.06	45.6	4.3	0.15	187
Horse	425	Muscle	212	4452	2.1	1.05	18253
		Liver	6.8	370	5.4	0.087	1517
Human	70	Muscle	35	245	0.70	0.35	1005
		Liver	1.5	72	4.8	0.10	295
LMB[a]	0.0264	Muscle	0.0119	8.08	0.068	0.031	33.1
		Liver	0.000465	0.050	10.8	0.19	0.205
Pig	70	Muscle	35	263	0.75	0.38	1078
		Liver	1.5	71	4.7	0.10	291
Rabbit	2.6	Muscle	1.17	7.6	0.65	0.29	31.2
		Liver	0.098	2.94	3.0	0.11	12.1
Rat	0.30	Muscle	0.135	1.76	1.3	0.59	7.2
		Liver	0.00825	0.182	2.2	0.061	0.75
Sheep	55	Muscle	26	260	1.0	0.47	1066
		Liver	0.83	38	4.6	0.07	156

Source: Barrett et al. (1994), Bergman (1983a,b), Fyfe et al. (1992), Li et al. (2020a,b), McDonald et al. (2021), and McDonough and Southard (2017).

[a] Juvenile largemouth bass (LMB) were fed a diet containing 10% starch and 10% lipids (Li et al. 2020a,b).

glucose under physiological and stressful conditions (e.g., exercise and exposure to a cold environment).

HOMOPOLYSACCHARIDES: HOMOPOLYSACCHARIDES IN MICROBES AND OTHER LOWER ORGANISMS

Cellulose

Some bacteria contain cellulose, which is a linear polymer of β-1,4-linked D-glucose units as in plants (Römling and Galperin 2015). These bacteria belong primarily to the genera *Acetobacter*, *Sarcina ventriculi,* and *Agrobacterium*, including (a) the N-fixing plant symbiont *Rhizobium leguminosarum*, (b) soil bacteria *Burkholderia spp.* and *Pseudomonas putida*, (c) plant pathogens *Dickeya dadantii* and *Erwinia chrysanthemi,* (d) tumor-producing *Agrobacterium tumefaciens*, *Escherichia coli,* and (e) *Salmonella enterica*. In natural habitats, the bacteria synthesize cellulose to form protective envelopes or biofilms. In addition, cellulose occurs in the cell walls of green, brown, and red macroalgae (seaweed), contributing to their structural support and rigidity.

Chitin

Chitin is one of the most abundant biopolymers in nature (Kou et al. 2022). In addition to animals, chitin is also present in the cell walls of fungi (including yeasts), green algae, as well as brown and red seaweed (types of algae). As in animals, such as crustaceans and insects, chitin in microbes and marine plants is a long-chain linear polymer of *N*-acetyl-glucosamine in β-1,4 glycosidic linkages (Egusa et al. 2015). Because of its intermolecular hydrogen bonding, chitin is insoluble in H_2O. In these lower organisms, chitin functions to support their cell structures and facilitate biomineralization in the cell membrane, thereby providing rigidity, strength, and protection from environmental stresses. Chitin is the precursor of chitosan through deacetylation.

Dextrans

Dextrans are synthesized from sucrose by many different bacteria (Sinnott 2013). These carbohydrates have continuous α-1-6-linked D-glucopyranose units in the main chains, as well as branch linkages of α-D-glucopyranose residues via α-1,2, α-1,3, or α-1,4 bonds (Figure 2.16). The length of

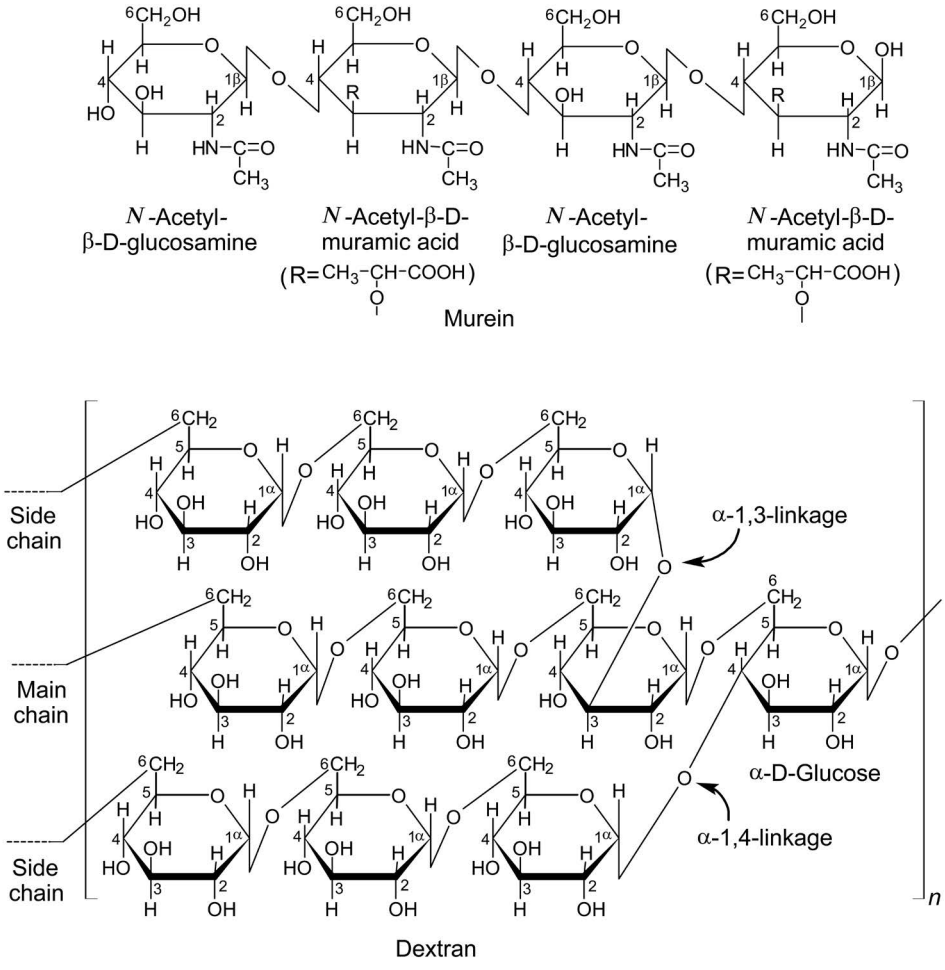


FIGURE 2.16 Structures of select homopolysaccharides and heteropolysaccharides in microbes. Dextrans are homopolysaccharides made by many different bacteria, whereas murein is a heteropolysaccharide in the cell walls of all known bacteria. When the R group is linked with a peptide (e.g., 4 amino acids), murein is a peptidoglycan. Lysozymes can hydrolyze β-1,4-bonds in murein.

the branch chains varies with the species of bacteria. These polysaccharides are absent from green, brown, and red macroalgae. Dextrans are soluble in H_2O and can reduce blood viscosity when fed to animals.

Galactans

Galactans occur in microbes. Kavitake et al. (2016) identified a novel galactan (a homopolysaccharide of α-1,6-galactose units) produced and released by *Weissella confusa* KR780676 (a lactic acid bacterium) from an acidic, cereal-legume-based, fermented food. This exopolysaccharide is soluble in H_2O and has desirable emulsifying, H_2O-holding, textural and sensorial, aflatoxin B_1-binding, antioxidant, and probiotic properties. Galactans that consist of approximately 30 units of D-galactose with alternating β-1,5 and β-1,6 glycosidic linkages constitute the side chains of microbial arabinogalactans. In addition to microbes, galactans and their sulfated derivatives occur in green, brown, and red macroalgae.

β-D-Glucans

β-D-Glucans occur in the cell walls of microbes (including bacteria, yeast, and other fungi) and algae (Farrokhi et al. 2006). All microbial β-D-glucans are polymers of β-D-glucose and have a β-1,3-linked linear backbone, with variances in structure that depend on the presence or absence of glycosidic side branches. Bacterial (1,3)-β-D-glucans are unbranched, but fungal (including yeast) and algal (1,3)-β-D-glucans contain β-1,6 branching chains. For example, laminarian (a water-soluble β-glucan extracted from the cell wall of *Eisenia bicyclis*, a brown macroalgae) has a β-1,3-linked backbone of β-D-glucose units and β-1,6 branching chains. In contrast, curdlan, a (1,3)-β-D-glucan isolated from *Agrobacterium* (a genus of Gram-negative bacteria), has no branching chain (Murphy et al. 2021). In addition to (1,3)-β-D-glucans, bacteria, fungi, and macroalgae also contain (1,3;1,4)-β-glucans (homopolysaccharides). β-D-Glucans have anti-inflammatory, anti-apoptotic, antitumor, antioxidant and anticoagulant properties (Chang et al. 2021). Some yeast (e.g., *Saccharomyces cerevisiae*)-based products may contain as high as 27.5% β-glucans (Van den Abbeele et al. 2020). Intake of yeast β-glucans through fermented foods may be beneficial for the composition and activity of intestinal microbes.

Glycogen

In addition to animals, glycogen is also present in bacteria, fungi (including yeast and the multicellular species of the fungi kingdom), protozoa, and blue-green algae (Roach et al. 2012). In these lower organisms, glycogen is also composed of D-glucose linked via α-1,4-glycosidic bonds on the main chain, as well as branch points where D-glucose molecules are joined to the main chain via α-1,6-linkage. The average length of the chain is 8–12 and 11–12 glucose units in bacteria and yeasts, respectively. Glycogen biosynthesis in nonanimal species is a major strategy for the storage of glucose in response to excess nutrient supply and for coping with starvation.

Levans

Levans are fructose polymers (fructans) containing mainly β-2,6 linkages in microbes (including bacteria and fungi), as in plants (Srikanth et al. 2015). Bacterial and fungal levans have branch chains of D-fructofuranose residues in β-2,1 glycosidic linkages, which allow for the formation of methyl ethers and a spherical shape. These polysaccharides are synthesized from sucrose by two Gram-positive bacteria genera, *Bacillus* and *Streptococcus*. Bacterial levans have the same chemical structures as plant levans. In microbes, levans contribute to the formation of the extracellular polysaccharide matrix.

Pure Mannans

Pure mannans in microbes and other lower organisms are homopolysaccharides of D-mannose (Table 2.9). Their structures vary by microbial species and are highly branched (Chandrasekaran 1998). In yeasts, pure mannan has α-1,6 linkages in the main chain, as well as α-1,2 and α-1,3 branches, and these structures differ from those in the pure mannan of plants but are similar to those of carbohydrates in mammalian glycoproteins. The structure of mannan in *Saccharomyces cerevisiae* differs from that of *Candida albicans* by the side-chain structure and substitution frequency. Some bacteria (e.g., marine bacterium *Edwardsiella tarda*) also produce mannans with α-1,3 linkages in the main chain, as well as α-1,2 and β-1,6 branches (Guo et al. 2010).

Pullulan

Pullulan is made by many species of fungi, including yeasts (Cheng et al. 2011). It is a linear chain consisting of α-D-glucopyranose residues in α-1,4 and α-1,6 glycosidic linkages in the ratio of 2:1. In this structure, three α-D-glucose molecules in maltotriose are linked via α-1,4, and the maltotriose units are joined end to end by an α-1,6 glycosidic linkage. Pullulan has no branch chains and is highly soluble in H_2O (Robyt 1998). Bacteria and algae do not synthesize pullulan.

HETEROPOLYSACCHARIDES: HETEROPOLYSACCHARIDES IN PLANTS

Definition

Heteropolysaccharides (heteroglycans) are polysaccharides consisting of two or more different types of monosaccharides. The content of heteropolysaccharides in plants varies by their species and developmental stages. Therefore, the amounts of complex carbohydrates differ markedly among feedstuffs for animals (Table 2.11). Plant-sourced heteropolysaccharides include arabinogalactan, exudate gums, hemicelluloses, inulins, mannans, mucilages, and pectins, as well as conjugated carbohydrates (e.g., glycolipids and glycoproteins). The major sources of heteropolysaccharides in animal diets are summarized in Table 2.7.

TABLE 2.11
Content of Nonstarch Polysaccharides (NSPs) and Lignin in Plant-Sourced Products

Feedstuff	Water-Soluble NSPs				Water-Insoluble NSPs	Total NSPs	Lignin
	Fructans	β-Glucans	Others[a]	Total			
	% (on a dry-matter basis)						
Agave (2–3 yr old) leaf	4.2	ND	8.3	12.5	49.3	61.8	11.0
Alfalfa (lucerne) hay	1.2	ND	12.4	13.6	46.1	59.7	7.6
Alfalfa meal	0.6	ND	7.7	8.3	25.2	33.5	12.8
Artichoke tubers	68.0	4.7	4.0	76.7	9.0	85.7	1.1
Apple fiber	ND	ND	13.9	13.9	48.7	62.6	26.8
Bamboo meal	---	---	---	3.9	44.8	44.9	19.0
Barley (hulled)	0.4	4.2	5.6	10.2	13.1	23.3	3.5
Barley (dehulled)	0.5	4.4	5.0	9.9	7.7	17.6	1.9
Barley hull meal	0.7	1.6	2.0	4.3	45.9	50.2	11.5
Canola (rapeseed) meal	ND	0.05	5.45	5.5	17.5	23.0	13.4
Chicory	44.7	0.35	5.0	50.1	30.7	80.8	14.9
Coconut cake	ND	ND	3.2	3.2	39.0	42.2	6.6
Corn (grain)	0.6	0.1	0.9	1.6	8.8	10.4	1.1
Corn bran	0.4	0.2	3.2	3.8	32.3	36.1	2.5
Corn flour	0.2	0.1	0.8	1.1	1.3	2.4	0.4
Corn gluten (feed)	0.2	0.2	3.4	3.8	31.7	35.5	3.2
Corn meal (feed)	0.2	0.1	1.0	1.3	14.7	16.0	1.8
Cottonseed cake	ND	ND	6.1	6.1	19.5	25.6	8.3
Cottonseed meal	ND	ND	6.6	6.6	21.7	28.3	9.2
Faba beans	ND	ND	5.0	5.0	14.0	19.0	2.0
Grass hay (timothy)	1.0	0.1	5.3	6.4	62.2	68.6	4.6
Grass meal (first cut)	6.0	ND	3.8	9.8	32.7	42.5	16.2
Kidney beans	0.61	ND	2.29	3.0	4.0	7.0	0.2
Lindseed meal	ND	ND	13.8	13.8	11.2	25.0	11.9
Oats (grain)	0.2	5.5	0.9	6.6	8.4	15.0	2.5
Oats (rolled)	0.1	4.2	4.8	9.1	4.3	13.4	1.5
Oats (hulled)	0.3	2.8	4.0	7.1	19.2	26.3	6.6
Oat flour	0.1	0.56	3.94	4.6	9.5	14.1	0.6
Oat hull meal	0.2	1.4	1.3	2.9	49.1	52.0	14.8
Oat meal (feed)	0.2	4.2	4.2	8.6	4.7	13.3	1.9
Palm cake	ND	ND	3.2	3.2	43.4	46.6	13.6
Palm kernel expeller	ND	ND	7.7	7.7	42.3	50.0	9.1
Peas (field)	ND	ND	5.2	5.2	12.9	18.1	1.2
Rice (grain, white)	ND	0.50	3.9	4.4	5.4	9.8	0.8
Rice (brown)	0.26	0.40	0.14	0.8	6.0	6.8	2.0
Rice bran	0.05	0.39	4.26	4.7	46.7	51.4	6.8
Rye (grain)	3.1	1.6	4.2	8.9	11.0	19.9	2.1
Rye bran	2.3	4.5	6.3	13.1	36.0	49.1	6.8
Rye middlings	2.2	3.7	6.2	12.1	22.6	34.7	3.9
Soybean (grain)	ND	0.8	1.4	2.2	7.1	9.3	1.2
Soybean hulls	ND	1.8	6.6	8.4	64.4	72.8	2.3
Soybean meal (48% CP)	ND	0.38	6.3	6.7	15.4	22.1	1.6
Soy protein concentrate	ND	0.26	8.1	8.4	9.7	18.1	0.8
Sorghum (grain)	0.67	0.22	0.82	1.71	7.21	8.92	1.1
Sugar beet fiber	ND	ND	40.7	40.7	37.2	77.9	3.5
Sunflower cake	ND	0.02	5.68	5.7	25.9	31.6	13.3
Tomato fiber	0.2	ND	6.5	6.7	76.0	82.7	12
Wheat (grain)	1.5	0.8	2.5	4.8	9.4	14.2	1.9
Wheat bran (for humans)	2.6	2.8	3.5	8.9	49.6	58.5	6.5
Wheat bran (for pigs)	2.0	2.4	2.9	7.3	34.5	41.8	7.5
Wheat flour	1.6	0.4	1.6	3.6	2.0	5.6	0.0
Wheat middlings	2.3	2.6	7.1	12.0	12.0	24.0	1.1
White beans (dried raw)	ND	ND	4.3	4.3	13.4	17.7	1.2
White lupins	ND	ND	13.4	13.4	27.0	40.4	1.2

Source: Bach Knudsen (1997), Bouaziz et al. (2014), Chawla and Patil (2010), Corbin et al. (2015), Díaz et al. (2022), Haldar et al. (2025), Herrera et al. (2010), INRA (2024), Jones et al. (2020), Nwafor et al. (2017), Yu et al. (2016), and Zimmermann et al. (2023).

[a] Including pectins.

Note: CP, crude protein; DM, dry matter; ND, not detected; ---, data are not available.

Exudate Gums

Exudate gums are produced by plants (e.g., trees) either spontaneously or after mechanical injury to seal wounds in their bark. Most of these heteropolysaccharides are soluble in H_2O, and they all have complex structures with emulsifying, stabilizing, thickening, and gel-forming properties. Their main chains are composed of D-galactopyranosyl units linked with β-1,3 and β-1,6 bonds, as well as D-glucopyranosyl uronic acid units linked with β-1,6 bonds (Aspinall 1967). The main chains carry three kinds of branched trisaccharides consisting of α-L-rhamnopyranose, β-D-glucuronic acid, β-D-galactopyranose, and α-L-arabinofuranose with 1,3-, 1,4-, and 1,6-glycosidic linkages. Exudate gums also contain α-L-rhamnopyranosyl units at the nonreducing ends. Chemical breakdown of exudate gums is limited in the small intestine of nonruminants (Pond et al. 2005). Thus, gums are indigestible to animal-sourced enzymes but are fermented to varying degrees by the microbiota of the large intestine.

Hemicelluloses

Hemicellulose is a group of heteropolysaccharides containing β-1,4-linked sugar units in the main chain. They are associated with cellulose and pectin in plant cell walls and have highly branched chains. The major sugar residues in hemicelluloses are two pentoses (α-L-arabinose and β-D-xylose) and three hexoses (β-D-mannose, α-D-galactose, and β-D-glucose), but these polysaccharides also comprise uronic acid-modified sugars (α-D-4-O-methyl-D-glucuronic, α-D-galacturonic, and α-D-glucuronic), as well as a small amount of hexose sugars (α-L-fucose, L-rhamnose, and D-fructose). Hemicelluloses include arabinogalactan (galactose in the main chain and arabinose in side chains), heteromannans (D-mannose), mixed-linkage β-glucans (D-glucose), xylans (D-xylose), and xyloglucans (D-glucose in the main chain and xylose plus galactose in side chains), based on their chemical composition. Xylans and heteromannans are the most and the second most abundant hemicellulose in nature, respectively, as structural and storage polysaccharides in higher plants, algae, and seaweed.

Hemicelluloses have some unique chemical properties. Just like cellulose, high-molecular-weight hemicelluloses (e.g., 100–200 monomer units) are generally insoluble in H_2O and dilute acid solutions. However, lower-molecular-weight hemicelluloses (e.g., ≤70 monomer units) that are rich in glucose, xylose, and galactose are soluble in H_2O at elevated temperatures (e.g., 80°C; Peng et al. 2019). In addition, low- or high-molecular-weight hemicelluloses are soluble in a dilute alkaline solution, which differentiates them from cellulose (Scheller and Ulvskov 2010). Furthermore, low- or high-molecular-weight hemicelluloses are less resistant to chemical degradation than cellulose. Thus, hemicelluloses can be hydrolyzed by a relatively mild acid solution, but the hydrolysis of cellulose requires a concentrated acid as noted previously.

Hemicellulose is indigestible to animal-sourced enzymes in the GIT. However, bacteria in the large intestine of nonruminants are able to ferment hemicelluloses more extensively than cellulose (McDonald et al. 2021). In ruminants, most hemicelluloses are digested in the rumen just like cellulose, and some escape the rumen to be further degraded in the large intestine. Some hemicelluloses (e.g., arabinoxylan and heteromannans) are useful prebiotics for both ruminants and nonruminants (including humans, pigs, and poultry).

Heterogalactans (Including Arabinogalactans)

Heterogalactans are polymers of β-D-galactopyranosyl residues in the main chain linked to β-D-galactopyranosyl and/or α-D-arabinopyranosyl residues as major sugars in side chains (Arifkhodzhaev 2000). The side-chain monosaccharides are usually bonded to small amounts of hexoses and pentoses, as well as their uronic acid derivatives. In higher plants, arabinogalactans are the most common type of heterogalactan and are further classified as arabino-4-galactan and arabino-3,6-galactan. These highly complex polysaccharides are soluble in H_2O and have been obtained from apple fruit, aquatic moss, broad-bean leaves, coffee beans, coniferous woods, rapeseed, Saguaro cactus cortex, Siratro leaves, soybean seeds, sycamore, and tomato (Fincher et al. 1974). Arabinogalactan is a major component of many gums (including gum arabic and gum ghatti) and is usually attached to proteins (8% of the proteoglycan by weight) to form an arabinogalactan-protein complex (Fincher et al. 1974). This proteoglycan contains 90% glycan and acts as an intercellular signaling molecule and a glue to heal plant wounds.

Arabino-4-galactan (Type-I Arabinogalactans)

Arabino-4-galactan (type-I arabinogalactans) comprises the β-1,4-glycosidic backbone of D-galactopyranosyl residues that are substituted commonly at the C-2 position and branched via (2,1)-linkages with either β-D-galactopyranosyl or α-D-arabinopyranosyl residues. Varying types of branching have been reported in some plants (Arifkhodzhaev 2000). For example, in potato, branching with a β-D-galactopyranosyl or α-D-arabinopyranosyl unit occurs at the C-3 and C-6 positions of different D-galactopyranosyl residues in the main chain. In the leaves of *Symplocos spicata*, α-1,6-branching occurs with α-D-arabinopyranosyl residues at the C-6 position of the main chain's D-galactopyranosyl residues. Depending on the species of plants, the side chains of arabino-4-galactans contain various types of β-1,3-, β-1,4-, or β-1,6-linked sugars, including arabinose, galactose, glucose, rhamnose, and xylose residues, as well as GalA and GlcA units (Arifkhodzhaev 2000). In ginseng roots, arabino-4-galactans have highly branched β-1,6-D-galactans at the *O*-3 position of the main chain's D-galactopyranosyl units or a trace amount of branched β-1,4-D-galactan at the *O*-6 position of the main chain's D-galactopyranosyl units (Cui et al. 2021). Some arabinose and galactose residues of arabino-4-galactans may be methylated.

Arabino-3,6-galactan (Type-II Arabinogalactans)

Arabino-3,6-galactan (type-II arabinogalactans) is characterized by a main chain of β-(1,3)-linked D-galactopyranosyl residues, with branching commonly at one or more of their C-6 positions via the β-1,6-bond. The side chains of arabino-3,6-galactans consist of various sugar units, including α-D-arabinopyranose, β-L-arabinopyranose, α-L-arabinofuranose, α-L-rhamnopyranose, and GalA residues. For example, α-L-arabinofuranosyl-(1,3)-α-L-arabinofuranosyl units and α-L-rhamnopyranosyl-(1,3)-α-L-arabinofuranosyl units occur in the flowers of *Calendula officinalis*. In some plants (e.g., *Odina wader* and *Lannea grandis*), besides the β-1,6-linkage between the main chain and side chains, β-1,3- and β-1,4-branching also occur with D-galactopyranosyl, GalA, or α-L-arabinofuranosyl residues at the C-3 and/or C-4 positions of the main chain's D-galactopyranosyl units. These side chains can be further branched. Some GlcA residues of arabino-3,6-galactans may be methylated.

Heteromannans (Glucomannans, Galactomannans, and Galactoglucomannans)

In addition to the pure mannans noted previously, some mannans exist in plants with β-1,4-D-mannose backbones and the branches of other carbohydrates. When some C-6 carbons of the mannose backbone are attached to β-D-glucose via a β-1,6- or α-1,6-glycosidic bond or to D-galactose via an α-1,6-glycosidic bond in side chains, the mannan-based heteropolysaccharides are called glucomannans or galactomannans, respectively (Moreira and Filho 2008). If the side-chain D-galactose residues are also linked with D-glucose residues, the mannan-based heteropolysaccharides are called galactoglucomannans. Glucomannan contains 8% β-D-glucose and is acetylated at the *O*-2 and/or *O*-3 positions. In contrast, galactomannans have variable amounts of α-D-galactose residues in their side chains. For example, the ratio of mannose to galactose is 3.5:1 for locust bean gum; 3:1 for tara (*Caesalpinia spinosa*) gum; and 1.5:1 for guar gum (Melton et al. 2009). High amounts of β-mannans, which are commonly found in legumes, are present in soybean meal and soybean hulls; they have antinutritive properties (e.g., reducing nutrient digestion) but can confer benefits to the intestinal health. Mannose is the main monosaccharide in the hemicellulose of plant seeds, endosperms, fruits, and softwood.

Xylans (Arabinoxylan and Glucuronoarabinoxylan)

Xylan is an extensively studied hemicellulose. The main chain of xylan is composed primarily of D-xylopyranoses linked in β-1,4 bonds and has 4-*O*-methyl-D-glucopyranosyl uronic acid units linked in α-1,2 bonds (Figure 2.14). The side chains of xylan may contain D-xylose, D-galactose, fucose, and other saccharides. D-xylose is the main monosaccharide in the hemicellulose of grasses and hardwood. Arabinoxylan and Glucuronoarabinoxylan are two specific types of xylan.

Arabinoxylan is the primary hemicellulose xylan (Figure 2.17) and a major polysaccharide in the cell walls of wheat, barley, oats, and rye grains. Arabinoxylan also occurs in grasses. In these plants, arabinoxylan comprises a backbone of β-D-xylopyranosyl residues linked through β-1,4 glycosidic linkages. Some –OH groups at C-2 or C-3 positions in β-D-xylopyranosyl residues are substituted with α-L-arabinopyranosyl residues. The –OH group at the C-3 position in α-L-arabinopyranosyl residue may be substituted with ferulic acid. Oxidation of ferulic acids yields

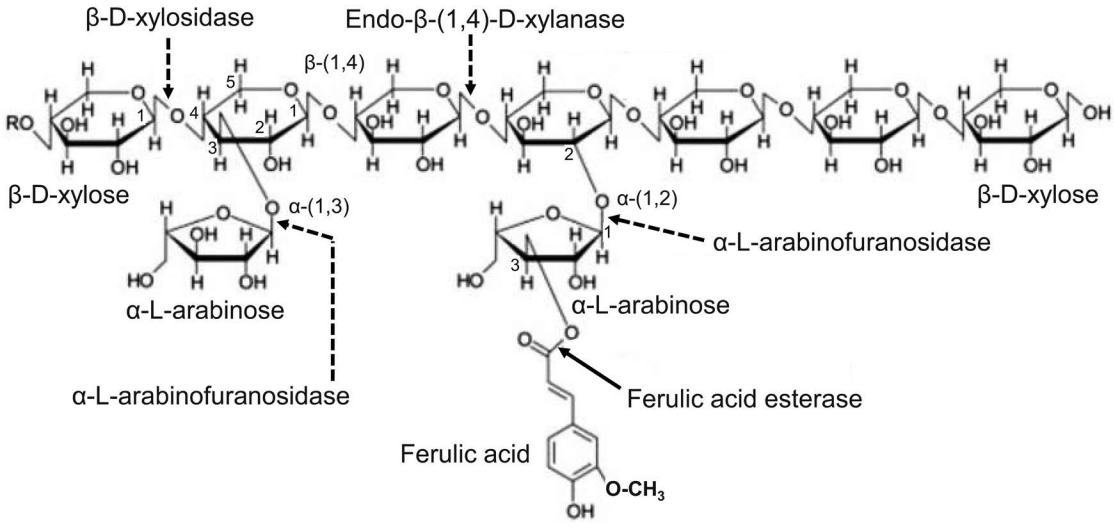


FIGURE 2.17 Structure of arabinoxylan (a heteropolysaccharide) in plants. Arabinoxylan is composed of the β-D-xylose backbone and α-L-arabinose side chains. The –OH group at C-3 in α-L-arabinose residue may be esterified with ferulic acid. Arabinoxylan is a type of hemicellulose in the cell walls of many plants, particularly wheat and corn. Arabinoxylan is hydrolyzed by microbial enzymes: endo-β-1,4-D-xylanase (yielding esterified XOSs or feruloylated oligosaccharides), β-D-xylosidase (generating xylose units), α-L-arabinofuranosidase (forming arabinofuranosides), and ferulic acid esterase (releasing ferulic acid).

dehydrodiferulates (ferulate dimers) as cross-links either between adjacent arabinoxylan chains or with lignin. Thus, arabinoxylans are based on a xylan backbone with varying degrees of arabinose substitution. In both backbone and side-chain monosaccharide residues, additional modifications may include acetylation, substitution with galactose and glucuronic acid, and, in the case of α-L-arabinopyranosyl residues, also substitution with *p*-coumaric acid instead of ferulic acid. Note that the arabinoxylans occur in the common oilseed meals.

Glucuronoarabinoxylan has a main chain that is often heavily substituted with GlcA residues. In xyloglucan (a hemicellulose in all vascular plants), most glucose residues in its β-1,4-linked glycosidic backbone are substituted with β-1,6 linked xylose side chains, with the xylose residues being extensively capped with a galactose residue and occasionally a fucose residue.

Xyloglucan

Xyloglucan is a branched heteropolysaccharide consisting of β-1,4-linked D-glucose units in the main chain with α-(1,6)-linked D-xylopyranosyl, β-1,2-linked D-xylopyranosyl, α-1,2-linked L-arabinofuranosyl, and α-1,3-linked L-arabinofuranosyl substituents (Figure 2.18). The main chain is branched via α-(1,6)-linkages, at the C6 position of D-glucose residues, with α-(1,6)-D-xylopyranosyl units. Some of the α-D-xylose residues are linked with additional sugar units in the side chain, including β-D-galactose (pyranose), α-L-galactose (pyranose), α-D-xylose (pyranose), α-L-arabinose (pyranose), α-L-arabinose (furanose), α-L-fucose (pyranose), and β-D-GalA in various proportions that depend on plant species (Schultink et al. 2014; Vincken et al. 1997). For example, the side chains of xyloglucan contain both α-L-fucose and α-L-galactose residues in some plants (e.g., in jojoba seeds) but lack fucosyl residues (e.g., in tamarind seeds) in some plants. Xyloglucan is the major hemicellulose in the cell walls of most land plants, where it cross-links cellulose microfibrils to form a strong but extensible network. Xyloglucan can also occur as a storage amyloid in seeds.

Inulins

Inulins are fructans in the roots and tubers of many plants and in some species of algae (Apolinário et al. 2014). These polysaccharides contain 20–30 D-fructofuranose units exclusively in β-2,1 glycosidic linkages. The C-2 position of their terminal D-fructofuranose units is linked with a D-glucopyranose. Inulin is soluble in H_2O and has a molecular weight of 3,000–5,000 Da. Inulins serve as the storage form of carbohydrates instead of starch in certain plants (Robyt 1998). Animal-sourced digestive enzymes do not degrade inulins.

Mucilages

Mucilages are gelatinous substances of nearly all plants (including legumes or seaweed) and some microbes. These complex carbohydrates are very similar to gums and contain polysaccharides that are linked to proteins via covalent bonding. The structures of mucilages are unknown, but their hydrolysis generates pentoses and hexoses, including arabinose, galactose, glucose, mannose, rhamnose, and xylose (Hamman et al. 2015). The most common acids in mucilages are uronic acids (e.g., GalA), but other acids (e.g., sulfonic acids) are also present in the polysaccharides. In plants, there are pectic, cellulose, and hemicellulose types of mucilages based on their chemical composition.

Pectins

In plants, pectins are present primarily in the cell wall but also occur in the spaces between the cell wall and intracellular membranes. Pectins are associated with other cell wall components (e.g., cellulose, hemicellulose, and lignin) to form a gel-like network that provides structural support and flexibility to the plant cell wall (Atmodjo et al. 2013). Although the fine structures of various pectic constituents or their interactions are largely known, it is likely that these complex macromolecules are covalently linked to each other. Pectins extracted from plant cell walls share common chemical properties: (a) being soluble in H_2O at neutral pH; (b) having a water-holding capacity; (c) forming gels either in the presence of Mg^{2+} or Ca^{2+} or, at an acidic pH, in the

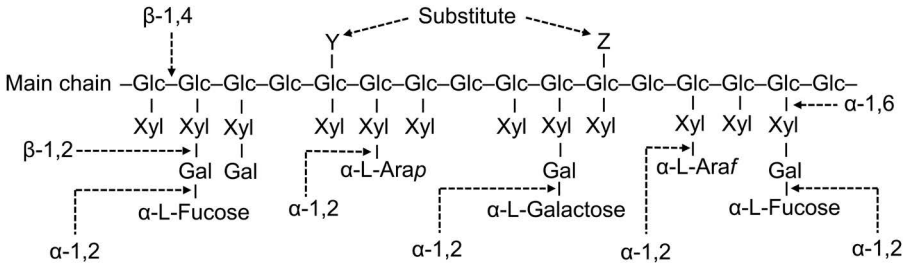


FIGURE 2.18 Structures of xyloglucan in higher plants. The main chain of xyloglucan comprises β-1,4-linked glucose units, is substituted with xylose or arabinose residues, and is branched with α-1-6-linked xylose residues. Depending on plant species, xylose units in the side chains are linked with other sugars via varying glycosidic linkages. Abbreviations: α-L-Ara*f*, α-L-arabinose (furanose); α-L-Ara*p*, α-L-arabinose (pyranose); Gal, β-D-galactose (pyranose); Glc, β-D-glucose (pyranose); Xyl, α-D-xylose (pyranose); Y, xylose (pyranose) and arabinose (furanose) residues linked to the main chain's β-1,4-glucose units via β-1,2 and α-1,2 (or α-1,3) bonds, respectively; and Z, xylose (pyranose) residue linked to the main chain's β-1,4-linked glucose unit via the β,1-2 bond.

presence of high concentrations of sugar; and (d) stabilizing emulsion.

The structures of pectins are more uniform than those of hemicelluloses. All pectins are composed of a main chain (homogalacturonan) of D-GalA (pyranose form) units linked with α-1,4 bonds, as well as various side chains consisting of up to 16 different monosaccharides (including arabinose, fucose, galactose, mannose, rhamnose, and xylose) linked via up to 20 different types of bonds (Le Normand et al. 2021). Several side chains of pectin have been reported, which include rhamnogalacturonan (types I and II), arabinogalactans (types I and II), and xylogalacturonan. Type-I rhamnogalacturonan contains a backbone of up to 100 repeats of the disaccharide of α-L-rhamnopyranose linked to the 4-position of α-D-GalA, which is in turn linked to the 2-position of the α-L-rhamnopyranose. Thus, the main chain comprises repeating (1→2)-α-L-rhamnopyranosyl and (1→4)-α-D-GalA disaccharide units. The side chains of type-I rhamnogalacturonan are mainly linked to the 4-*O*-rhamnose units and contain neutral sugars in such polysaccharides as arabinans, galactans, and arabinogalactans. Type-II rhamnogalacturonan consists of a backbone of α-1,4-D-GalA units linked to four different side chains consisting of up to 13 monosaccharides (including D-GalA, L-rhamnose, L-arabinofuranose, L-arabinopyranose, and L-fucose). As noted previously, arabino-4-galactan and arabino-3,6-galactan are also known as types I and II arabinogalactan, respectively. Xylogalacturonan comprises a main chain of α-(1,4)-D-GalA units containing substitution with β-D-xylose at the *O*-3 position, as well as the side chains of β-D-xylose units at the C-3 site of the main chain's D-GalA residue.

The average molecular weight of pectin is 100,000 Da. In plants, many of the carboxyl groups of the GalA units are esterified with methanol (-CH_3OH) to form methoxy pectins. Thus, pectins are classified as high- versus low-methoxy types (Bailoni et al. 2005). In addition, some carboxyl groups of the GalA units are neutralized with calcium or magnesium, and some pectins also have 2-*O*-acetyl, 3-*O*-acetyl, and α-L-rhamnopyranosyl groups on the D-GalA units. The presence of acetyl groups confers emulsion-stabilizing properties to pectins. Note that molasses from sugarcane and beet cane contains pectins with significant amounts of arabinans in the side chains (Palmonari et al. 2020). Chemically, highly methoxylated pectins form gels along with high sugar concentrations under acidic conditions, and low methoxy pectins form gels by interactions with divalent cations (e.g., Ca^{2+} or Mg^{2+}).

In the pectin molecule, the C-1 hydroxyl group of the first monosaccharide above the plane of its ring reacts with the C-4 hydroxyl group of the second monosaccharide below the plane of its ring to form the glycosidic linkage (Robyt 1998). This cross-planar bond is not hydrolyzed by animal-sourced enzymes. However, pectins are hydrolyzed by microbial enzymes in the rumen and thus are well utilized by ruminants. In both ruminants and nonruminants, some pectins are also extensively fermented in the large intestine. Of note, pectins generate methanol and acetate through the hydrolysis of their methoxy groups and acetyl esters in the GIT of mammals, respectively (Siragusa et al. 1988). Furthermore, in nonruminants (including pigs and humans), dietary pectins increase the viscosity and volume of stool. These effects provide a metabolic basis for the physiological functions of pectins, such as improving gut health, preventing constipation, binding toxins, holding H_2O in the lumen of the intestine, and reducing mild to moderate diarrhea in animals.

HETEROPOLYSACCHARIDES: HETEROPOLYSACCHARIDES IN ANIMALS

Definition

Heteropolysaccharides in animals usually contain amino, sulphate, or carboxyl groups. They are important structures in the extracellular matrix and cell membranes (Sinnott 2013). Examples of these substances are glycosaminoglycans, which consist of repeating disaccharide units of uronic acids and 2-acetamido-2-deoxy sugars (Figure 2.19). Such heteropolysaccharides (e.g., hyaluronic acid, chondroitin sulfates, dermatan sulfate, heparan sulfate, heparin sulfate, keratan sulfate, and chitosan) are widely present in animals, and most of them are galactans. Those molecules fulfill such important biological functions as: (a) constituents of the organic matter of bones, connective tissue, or cell membrane substances with blood group specificity; (b) anticoagulants; (c) stabilizers, cofactors, and coreceptors for growth factors, cytokines, and chemokines; (d) cell signaling, proliferation, and differentiation; (e) regulators of enzyme activity, cell metabolism, and immune responses; (f) angiogenesis (the formation of new blood vessels from existing ones); (g) wound healing; and (h) targets for bacterial, viral, and parasitic virulence factors for attachment, recognition, invasion, and immune system evasion (Trowbridge and Gallo 2002). The heteropolysaccharides are degraded by exoglycosidases and endoglycosidases to release *N*-acetyl-glucosamine, which is subsequently converted into glucosamine-6-P, fructose-6-P, and pyruvate in animals (Wu 2022). Glycosaminoglycans are degraded by animal-sourced digestive enzymes.

Hyaluronic Acid

Hyaluronic acid (also called hyaluronan) is a water-soluble nonsulfated heteropolysaccharide (Viola et al. 2015). The sodium salt of hyaluronic acid is called sodium hyaluronate. Hyaluronic acid has a linear chain of the repeating disaccharide units of D-GlcA and D-*N*-acetyl-glucosamine linked via alternating β-1,3 and β-1,4 glycosidic bonds. Hyaluronic acid can be 250 to 25,000 disaccharide repeats in length. Of note, it is a primary glycosaminoglycan that is synthesized by placental cells and present in the placental stroma. This substance plays an important role in (a) embryonic survival, growth, and development; (b) lubrication of the joints; (c) angiogenesis; and (d) support of connective tissue in the skin, blood vessels, and bones. In addition, many other cell types, including fibroblasts (e.g., in the dermis and chicken combs) and synoviocytes, also synthesize hyaluronic acid from glucose and glutamine and contain this heteropolysacchatide.

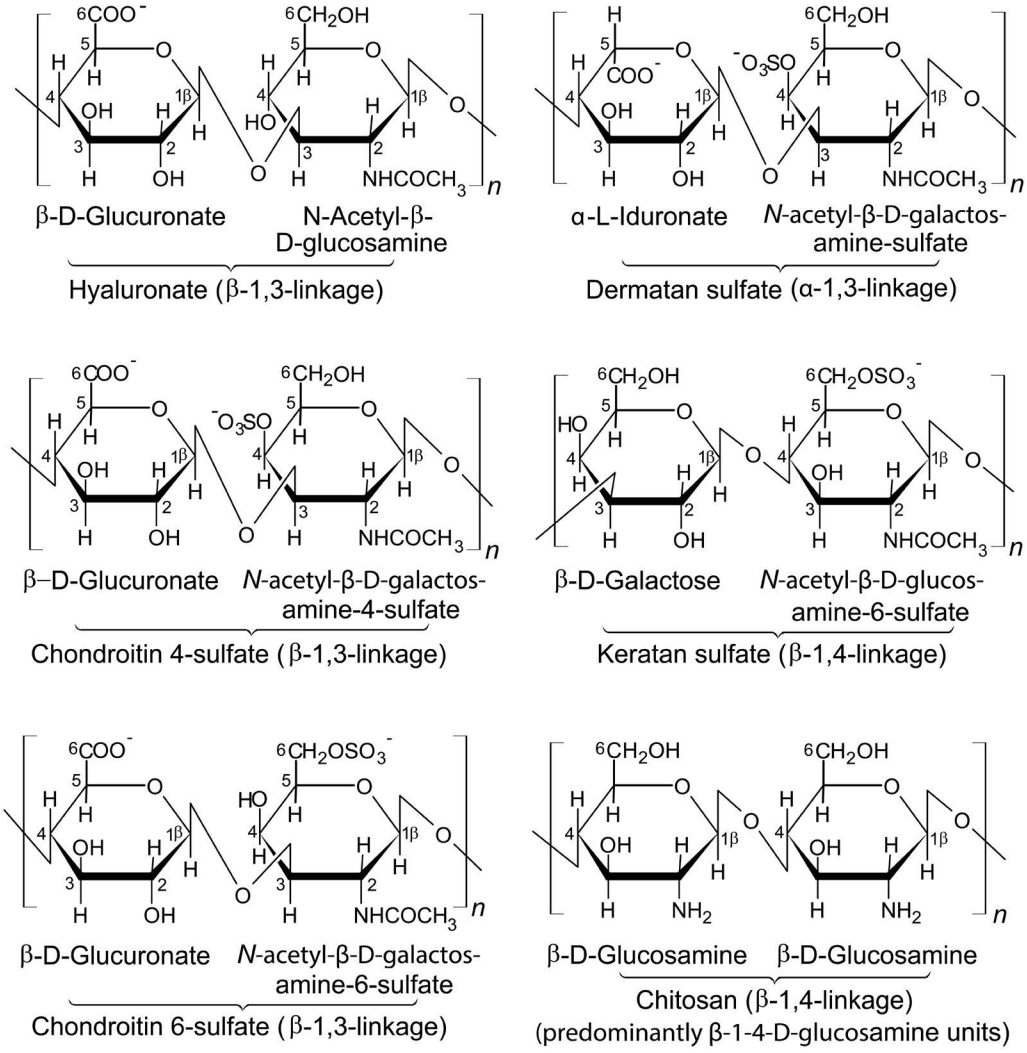


FIGURE 2.19 Glycoaminoglycans in animals. These heteropolysaccharides are long, unbranched polysaccharides consisting of a repeating disaccharide unit. They are widely present in mammals, birds, and fish. Except for keratan, the repeating unit contains an amino sugar (*N*-acetylglucosamine or *N*-acetylgalactosamine) and a uronic sugar (GlcA or iduronic acid) or galactose. GlcA and galactose are derivatives of D-glucose.

Sulfated Heteropolysaccharides

Sulfated heteropolysaccharides are abundant components in connective tissue, including chondroitin sulfates, dermatan sulfate (also known as chondroitin sulfate B or β-heparin), keratan sulfate, heparin, and heparan sulfate (Mikami and Kitagawa 2013). Chondroitin sulfates have a repeating disaccharide unit of β-D-GlcA and sulfated *N*-acetyl-D-galactosamine in β-1,3 and β-1,4 glycosidic linkages, and are major components in cartilage. Interestingly, Pacific white shrimp (*Litopenaeus vannamei*) contain a highly sulfated chondroitin consisting of 2,3-di-*O*-sulfo GlcA and *N*-acetyl-β-D-galactosamine-4-sulfate (Cavalcante et al. 2018), and the major repeating disaccharide of this substance is shown in Figure 2.20. Dermatan sulfate (first isolated from the pig skin in 1941) is a macromolecule with a repeating disaccharide unit of α-L-iduronic acid and sulfated *N*-acetyl-D-galactosamine in α-1,3 and β-1,4 glycosidic linkages, which is present mainly in the skin. Keratan sulfate is highly unique with the basic repeating disaccharide of β-D-galactose and sulfated *N*-acetyl-glucosamine in β-1,4 glycosidic linkages and is found mainly in the erythrocytes, cornea, cartilage, and bone.

Heparin was discovered in 1916 and has been used as an anticoagulant since 1935. It is synthesized in the rough endoplasmic reticulum of connective tissue-type mast cells and released by these cells to the blood. The major repeating disaccharide in heparin is α-L-iduronate-2-sulfate and *N*-sulfo-α-D-glucosamine-6-sulfate in α-1,4 glycosidic linkages. Note that the α-linkage of L-iduronate is analogous to the β-linkage in chondroitin 4- or 6-sulfates. As a water-soluble macromolecule, heparin is present in many tissues, such as the heart, blood, intestine, lungs, and skin.

Heparan sulfate (discovered in 1948) has the most common repeating disaccharide unit of GlcA linked to sulfated

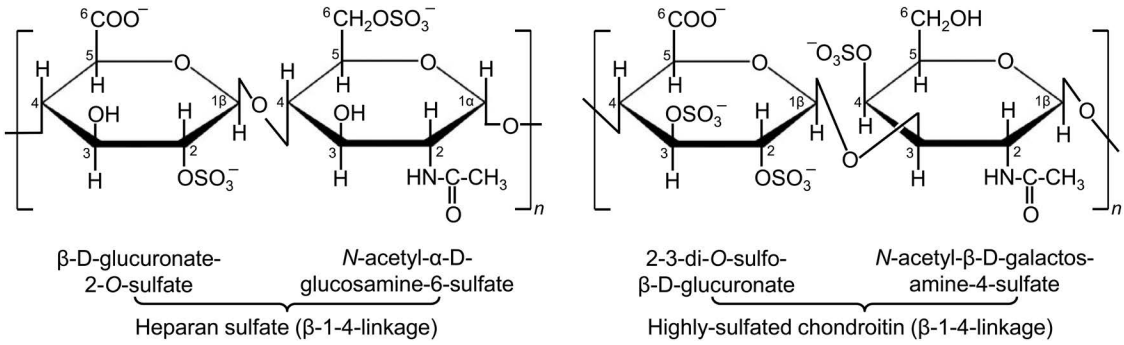


FIGURE 2.20 Heparan sulfate and highly sulfated chondroitin in animals. Both substances are derivatives of D-glucuronic acid and possess *N*-acetyl and sulfate groups. Heparan sulfate occurs in all tissues of terrestrial and aquatic animals. Pacific white shrimp (*Litopenaeus vannamei*) contain a highly sulfated chondroitin consisting of 2,3-di-*O*-sulfo glucuronic acid and *N*-acetyl-β-D-galactosamine-4-sulfate. Heparan sulfate is formed in the rough endoplasmic reticulum of mast cells.

N-acetyl-glucosamine via β-1,4 bonds in a linear chain (Figure 2.20) and is present on cell surfaces and in the extracellular matrix of all animal tissues. Heparan sulfate is synthesized in the rough endoplasmic reticulum by most, if not all, cell types in animals as a water-soluble macromolecule (Li and Kusche-Gullberg 2016); it is the second most abundant glycosaminoglycan in placental tissue. The 5-epimerization of β-D-GlcA residues on heparan sulfate to α-L-iduronic acid, which is catalyzed by a C-5 epimerase, converts heparan sulfate into heparin (Robyt 1998).

Chitosan is formed from chitin through the enzyme-catalyzed deacetylation of most *N*-acetyl-glucosamine moieties to glucosamine groups (Kou et al. 2022). Thus, the major repeating disaccharide unit in chitosan is the two identical β-D-glucosamine molecules joined together in the β-1,4 linkage. However, some *N*-acetyl-glucosamine moieties remain in chitosan. The molar fraction of the repeating units with amino groups in chitosan indicates the extent of its deacetylation. Because of the presence of the ionizable $-NH_2$ group, chitosan with a greater degree of deacetylation has a higher H_2O solubility and stronger biological effects (e.g., antibacterial, antifungal, antitumor, antioxidant, anti-inflammatory properties, coagulation, and wound healing; Younes and Rinaudo 2015).

HETEROPOLYSACCHARIDES: HETEROPOLYSACCHARIDES IN MICROBES

Microbes synthesize and release many types of heteropolysaccharides, including arabinogalactans, heterogalactans, murein, and xanthan. In addition, Gram-negative bacteria synthesize and release lipopolysaccharides (LPSs). By contrast, bacteria, fungi, and protozoa are not known to produce hemicelluloses as those found in plants. However, all these microbes can secrete hemicellulases to degrade heteropolysaccharides, including hemicelluloses.

Arabinogalactans

In microbes, arabinogalactan is a heteropolysaccharide of arabinose and galactose residues solely in the furanose configuration (Knoch et al. 2014). The linear galactan portion of the microbial arabinogalactan consists of about 30 galactose residues with alternating β-1,5 and β-1,6 glycosidic linkages. The arabinan chain, which is about 30 arabinose residues linked via α-1,3, α-1,5, and β-1,2 glycosidic bonds, is attached at three branch points within the galactan chain (residues 8, 10, and 12) and is usually capped with mycolic acids. Arabinogalactan is a major structural component of the microbial cell wall and, like other microbial heteropolysaccharides, is indigestible to animal-sourced digestive enzymes.

Heterogalactans

There is little or no heterogalactan in bacteria. In fungi, heterogalactans have a common backbone of α-(1,6)-linked D-galactopyranose residues that are substituted at the C-2 position and branched with either β-D-mannopyranosyl or α-L-fucopyranosyl residues. Side chains may include arabinose, fructose, mannose, galactose, or glucose units. Most of the side chains are terminated with L-fucose or D-mannose residues. A model system for these chemical studies is the fruit bodies of Lentinus edodes, which is a type of edible mushroom native to Japan and China (Shida et al. 1975).

Heteromannans

There is little or no heteromannan in bacteria. However, this heteropolysaccharide is present in fungi along with β-1,3-glucans and β-1,6-glucans. The major heteropolysaccharides in the cell wall of yeast also include heteromannans, which glycosylate proteins. The main backbone of heteromannans in yeast consists of either α-1,6-linked D-mannose residues or β-1,4-linked D-mannose residues, and the mannans are either linear or branched polysaccharides. A *Saccharomyces cerevisiae*-based product may contain as high as 22.5% MOSs (Abbeele et al. 2020). The consumption of yeast and mannans through fermented foods may be beneficial for the composition and activity of intestinal microbes.

Lipopolysaccharides

LPSs, also known as lipoglycans or endotoxins, are examples of glycolipids present in the outer membrane of Gram-negative bacteria (Gnauck et al. 2016). These complex molecules consist of a lipid moiety linking via the α-2,6 bond to a polysaccharide core composed of an inner core and an outer core (Figure 2.21). Common saccharides in the inner core are 3-deoxy-D-mannooctulosonic acid [also known as keto-deoxy-octulosonate (KDO)] and heptose. The outer core contains a repetitive glycan polymer (composed of β-D-Galactose, β-D-galactosamine, β-D-glucosamine, and β-D-glucose) as the *O*-antigen. In addition, α-D-glucosamine is attached to a lipid moiety via covalent bonding. Because of these unique chemical structures, LPSs are strong stimuli of immune responses in animals.

Murein

Murein is the major component of complex carbohydrates in the cell walls of microbes (Fiedler et al. 1973). It is a linear chain of alternating β-1,4-linked *N*-acetyl-glucosamine and *N*-acetyl-muramic acid (amino sugar) residues. Murein is cross-linked to short peptides to form a water-insoluble peptidoglycan, which supports the shape and rigidity of bacteria. Note that muramic acid is formed from lactate and glucosamine (a metabolite of glutamine and fructose-6-P).

Xanthan

Xanthan is produced by Gram-negative bacteria. It is composed of a repeating pentasaccharide unit containing two D-glucose residues, two D-mannose residues, and one D-GlcA residue (Becker et al. 1998). The two D-glucose are joined by the β-1,4-glycosidic bond. A trisaccharide is attached to every other D-glucose residue in the main chain via an α-1,3 linkage. This results in a repeating unit of pentasaccharide. Of note, the special trisaccharide is a D-mannopyranosyl unit linked, via a β-1,4 bond, to D-glucopyranosyl uronic acid that joints, via a β-1,2 linkage, with a D-mannopyranosyl residue. Xanthan is also highly soluble in H_2O.

HETEROPOLYSACCHARIDES: HETEROPOLYSACCHARIDES IN ALGAE AND SEAWEED (MARINE PLANTS)

Most heteropolysaccharides in algae and seaweed consist of a galactan backbone linked with other sugars, such as D-glucuronic acid, D-mannuronic acid, and L-guluronic acid. Thus, these substances are galactan-based heteropolysaccharides. Some of them are sulfated, and others may contain pyruvate and minerals (e.g., sodium). In contrast to microbes, algae and seaweed can synthesize and release not only cellulose but also hemicellulose and other heteropolysaccharides (Khan et al. 2018). Structural

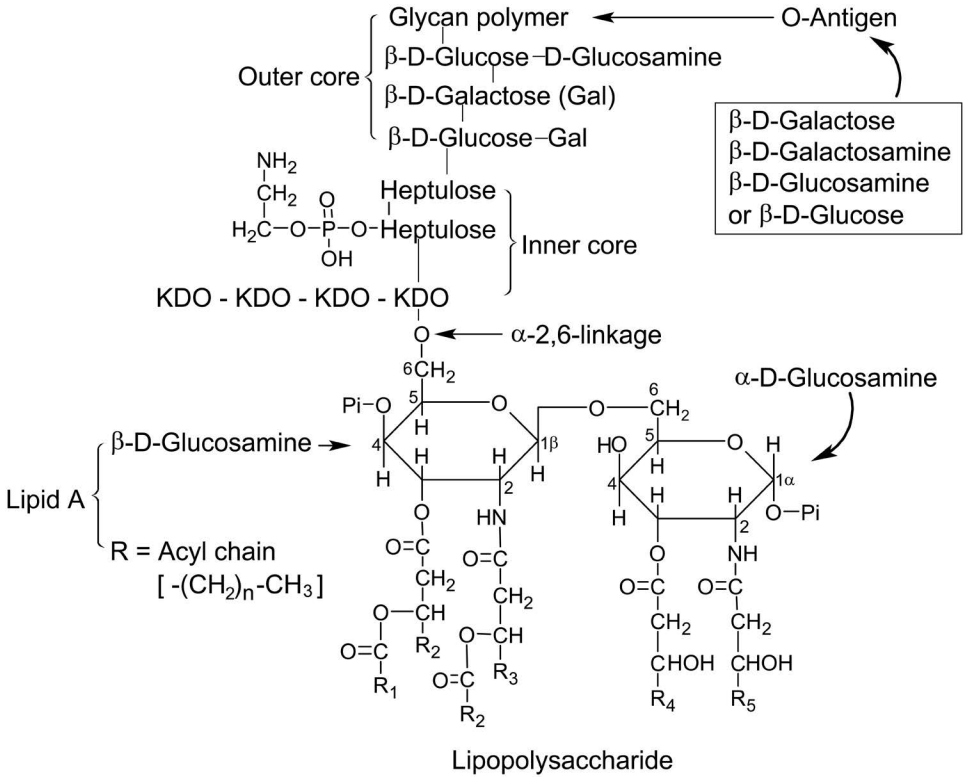


FIGURE 2.21 Structure of lipopolysaccharide (LPS) in the outer membrane of Gram-negative bacteria. LPS (an endotoxin) is a complex carbohydrate that contains sugars, α-D-glucosamine, and lipids. The chemical structures and biological activities of LPS vary by the bacterial species or strains.

heteropolysaccharides in algae and seaweed are not degraded by animal-sourced digestive enzymes.

Agar (or Agar-Agar)

Agar is a mixture of two linear-chain polysaccharides (agaran and agaropectin) and is prepared from the cell walls of red-purple seaweed (also known as red algae; Mišurcová et al. 2012). Agar is not soluble in cold or warm (e.g., 20–40°C) water, but is soluble in boiling water. The chemical structure of agaran is similar to that of κ-carrageenan and *l*-carrageenan, except that (a) the first residue in the repeating disaccharide unit has no sulfated α-D-galactopyranose, (b) the second residue in the repeating disaccharide unit has the L-configuration instead of the D-configuration, and (c) ~20% of the α-D-galactopyranose residues have a 2-*O*-methyl group (Robyt 1998). Because the 3,6-anhydro-galactopyranose in agaran has the L-configuration, it is similar to the 3,6-anhydro-α-D-galactopyranose residue in κ- and *l*-carrageenans, and to the L-guluronic acid residue in alginic acid. Thus, agaran consists of repeating units of β-1,3-linked-D-galactopyranose and α-1,4-linked 3,6-anhydro-L-galactopyranose. In contrast, agaropectin is a water-soluble sulfated polysaccharide containing 3–10% sulfate as ester sulfate, D-glucuronic acid, and small amounts of pyruvic acid (Robyt 1998). The proportion of agaran and agaropectin varies with the species of seaweed. Like other heteropolysaccharides in fungi, algae or seaweed, agar may have a beneficial role as a prebiotic in animal nutrition (Mišurcová et al. 2012; Rajapakse and Kim 2011).

Algin (Alginic Acid)

Large amounts of algin are present in the cell walls of brown seaweed, which amount to 20–40% of their masses. Approximately 15–20% of algin is localized in the cell wall, and 80–85% of algin is present between cells. Algin is composed of uronic acid (Robyt 1998), which can exist both in the acid form (–COOH), which is not water-soluble, and in the sodium form (–COONa) as sodium alginate, which is water-soluble (Tavassoli-Kafrani et al. 2016). Algin is a linear polysaccharide chain consisting of β-D-mannopyranosyl uronic acid and α-L-gulopyranosyl uronic acid in both β-1,4- and α-1,4-linkages, with the ratio of D-mannuronic acid to L-guluronic acid being 2:1 for most algins (Robyt 1998). Thus, there are two types of uronic acid in algin: mannuronic acid and guluronic acid derivatives.

Carrageenans

Carrageenans are linear chains of D-galactans consisting of sulfated D-galactopyranose and its derivatives in β-1,4- and α-1,3-linkages (Tavassoli-Kafrani et al. 2016). They are the major polysaccharides in the cell walls of red seaweed (types of red algae). Carrageenans are composed of three linear D-galactans: κ-carrageenan, *l*-carrageenan, and λ-carrageenan. κ-Carrageenan has 4-*O*-sulfato-β-D-galactopyranose and 3,6-anhydro-α-D-galactopyranose as the main repeating disaccharide unit. The structure of *l*-carrageenan is similar to that of κ-carrageenan except that the 3,6-anhydro-α-D-galactopyranose residue (the second residue of the repeating disaccharide unit) in κ-carrageenan is replaced by 3,6-anhydro-2-*O*-sulfato-α-D-galactopyranose in *l*-carrageenan. The structure of λ-carrageenan is similar to that of κ-carrageenan and *l*-carrageenan, except that the repeating disaccharide unit in λ-carrageenan consists of 2-*O*-sulfato-β-D-galactopyranose and 2,6-di-*O*-sulfato-α-D-galactopyranose (Robyt 1998). All carrageenans are soluble in hot water.

Hemicellulose

Algae and seaweed can produce hemicellulose, with its type differing among various species. For example, in red and green algae, hetero-β-mannans are common, abundant hemicelluloses in red and green algae, which can even replace cellulose as the dominant structural carbohydrate of the cell walls (Voiniciuc 2022). Minor hemicelluloses in these organisms include β-1,4-D-glucuronans, β-1,4-D-xyloglucans, and β-(1,3;1,4)-glucans containing a small amount of xylose and arabinose residues (Khan et al. 2018). In contrast, β-(1,3;1,4)-glucans are the major form of hemicellulose in brown algae (Fuertes-Rabanal et al. 2025). β-(1,3;1,4)-glucans in red algae and brown algae contain sulfated D-glucosyl residues. Note that mixed-linkage β-glucans that comprise mostly β-D-glucose residues but also small amounts of other types of sugars) are sometimes incorrectly called β-glucans (which are homopolysaccharides). The presence of hemicellulose in algae and seaweed provides evidence for their evolutionary relationship to land plants.

PHENOLIC POLYMERS IN PLANTS

Lignins

Lignins are cross-linked highly methoxylated ($–OCH_3$) phenolic polymers with a high molecular weight in plants (Boerjan et al. 2003). They are not soluble in H_2O and are not carbohydrates. These substances are present in plants (forming tough woody fibers) and some algae, but are absent from animals. Because of its association with cellulose and hemicellulose, lignin is usually discussed together with carbohydrates in nutrition. Lignin stiffens the cellulose fibers, providing strong structural support for the plant, and constitutes about 5–10% of the DM of annual plants. Lignin's composition, such as the numbers of 3-phenylpropanol units and methyl groups, and N content, varies with plant species and maturity. For example, the methoxyl content ($–OCH_3$) of wood lignin is ~15%, that of grass lignin is ~8%, and that of legume lignin is ~5% (Mottiar et al. 2016; Robyt 1998). Lignin is very stable due to its condensed structure and is rarely attacked by microbes. Because of its close association with cellulose and hemicellulose present in forages, lignin reduces their hydrolysis by microbes. However, the methoxyl content of lignin decreases progressively on passage through the ruminant GIT. The digestibility of

low-quality forages and straw can be improved by treatment with alkali, which partially dissolves the lignin and breaks the bonds between lignin and cellulose or hemicellulose.

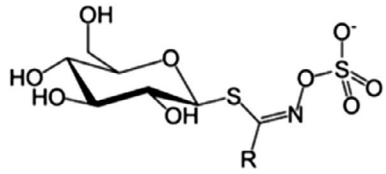
Tannins

Tannins (also known as tannic acids) are phenolic polymers with numerous hydroxyl groups (–OH) in plant cell walls and have a bitter taste (Sieniawska 2015). These substances are relatively abundant in trees, nuts, legumes, and fruits. Tannins are soluble in H_2O, but they are not carbohydrates. Thus, there are major differences in the chemical structures and properties between tannins and lignins. Tannins can be classified as hydrolyzable tannins and condensed tannins (proanthocyanidins). The hydrolyzable tannins include tara gallotannins (gallic acid and quinic acid) and caffetannins (e.g., caffeic acid and chlorogenic acid). Condensed tannins include cyanidin, delphinidin, malvidin, peonidin, pelargonidin, and petunidin. Tannins can bind proteins, irons and other metal ions, carbohydrates, and alkaloids. Thus, a high content of tannins in the diet can decrease feed intake, growth rate, feed efficiency, and protein digestibility in nonruminants but may protect high-quality dietary protein from ruminal degradation in ruminants (Patra and Saxena 2011). Because of their polyphenolic structures, tannins can scavenge oxygen free radicals and ameliorate oxidative stress in animals.

TOXIC CARBOHYDRATES IN PLANTS

Some glycosides in the Brassicaceae family of plants (including broccoli, cabbage, cauliflower, rapeseed, mustard, and horseradish), such as glucosinolates (glycosides of β-D-thioglucose), are antinutritional factors in the feeding of livestock, poultry, and fish due to adverse effects. The latter include reductions in feed intake and growth performance, GIT irritation, goiter, anemia, and the abnormalities of tissues (e.g., liver and kidneys; Bischoff 2016). Interestingly, appropriate levels of glucosinolates in diets may have beneficial effects on intestinal health in animals, such as increases in anti-inflammatory, antioxidative, and antistress responses and direct antimicrobial properties (Bischoff 2016). This is an example of the two sides of a substance.

Glucosinolates in cruciferous plants contribute to natural defense against pests and diseases. These sulfur- and N-containing substances have an aliphatic, indole, or aromatic group, depending on the amino acid precursor. The aliphatic group is derived from alanine, leucine, isoleucine, valine, and methionine; the indolic group from tryptophan; and the aromatic group from phenylalanine and tyrosine (Lietzow 2021). Glucosinolates themselves are not toxic, but are hydrolyzed by specific ß-thioglucosidases (myrosinases) that are present in intestinal bacteria to generate glucose and an unstable intermediate (thiohydroximate-*O*-sulfonate). The latter is nonenzymatically metabolized to toxic hydrolysis products, including isothiocyanates (R-N=C=S; contributing to 60–90% of the total glucosinolate breakdown products), nitriles (R-C≡N), thiocyanates (R-S-C≡N), thiocyanate ion (S-C≡N$^-$), oxazolidine-2-thiones, and epithionitriles (Lietzow 2021). When fed at high dosages to swine, poultry, ruminants, and fish, glucosinolates adversely affect these animals (e.g., reducing feed intake and growth performance, and causing GIT irritation, goiter, anemia, and the abnormalities of tissues such as the liver and kidneys; Bischoff 2016). Interestingly, despite acting as anti-nutritional factors, appropriate levels of glucosinolates in diets may have beneficial effects on intestinal health in animals, such as increases in anti-inflammatory, antioxidative, and antistress responses and direct anti-microbial properties (Bischoff 2016).



Glucosinolates (glycosides of β-D-thioglucose) in plants. The R group varies, depending on amino acid precursors and their modifications, and may be an alkyl, alkenyl, aryl, or indolyl.

Other common examples of toxic carbohydrates in food plants include (a) lectins (e.g., phytohemagglutinin) in legumes (e.g., green beans, red kidney beans, and white kidney beans); (b) cyanogenic glycosides (e.g., linamarin from cassava, amygdalin from the seeds of stone fruit and bitter apricot seed, taxiphyllin from bamboo shoots, and linustatin/neolinustatin and linamarin from flaxseeds); (c) glycoalkaloids (e.g., α-chaconine and α-solanine) in any green, damaged, or sprouting bits of potatoes; (d) 4'-methoxypyridoxine in ginkgo seeds; (e) colchicine in fresh lily flowers; and (f) muscarine in some wild mushrooms (Gleadow and Møller 2014; Gupta 1987). Certain weather conditions (e.g., drought or frosts) can increase the production of cyanogenic glycosides by some plants in the sorghum family (e.g., Johnsongrass). Of note, sorghum grain is the major source of carbohydrates in swine and poultry diets in the Southwestern United States and many other regions of the world (Cohen and Tanksley 1976; McCuistion et al. 2019; Wu and Knabe 1994). In animals (e.g., cattle and sheep), cyanogenic glycosides break down to yield hydrogen cyanide (H–C≡N), which is also known as prussic acid, hydrocyanic acid, or hydrogen cyanide. This cyanide strongly inhibits the mitochondrial electron transport system. The amounts of these toxins in foods may be reduced to safe levels through soaking in H_2O, cooking, fermenting, or roasting.

CHEMICAL REACTIONS OF CARBOHYDRATES

Knowledge about chemical reactions of carbohydrates is useful for their analysis and therefore in conducting nutritional research. Some of these reactions may occur in animals and may provide a biochemical basis for adverse effects of excess

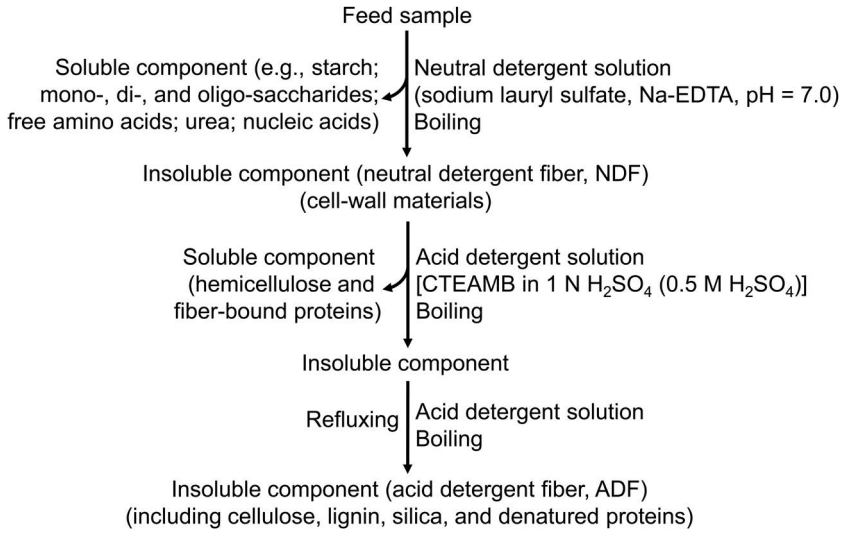


FIGURE 2.22 Proximate analysis of fiber in plants. This method, developed by P.J. Van Soest in the 1960s, is based on the concept that plant cells can be divided into less digestible cell walls (containing hemicellulose, cellulose, and lignin) and mostly digestible cell contents (e.g., starch; mono-, di-, and oligo-saccharides; urea, ammonia, free amino acids and peptides; and nucleic acids). These cell components are separated with the use of neutral detergent solution [6.81 g sodium tetraborate, 18.6 g disodium ethylenediaminetetraacetate (EDTA), 30 g sodium lauryl sulfate, 10 ml cellosolve (2-ethoxyethanol), and 4.56 g disodium hydrogen phosphate (Na_2HPO_4) in 1 L distilled water] and an acid detergent solution [20 g cetyltrimethylammonium bromide (CTEAMB, also known as hexadecyltrimethylammonium bromide) in 1 L of 0.5 M sulfuric acid (H_2SO_4)]. Neutral detergent fiber (NDF) is composed of acid detergent fiber (ADF) plus hemicellulose. ADF includes mainly cellulose and, to a much lesser extent, lignin, cutin, insoluble minerals (mainly silica), and acid- and heat-damaged proteins.

carbohydrates in the body. Overall, chemical reactions of carbohydrates are dependent upon their functional groups, such as ketones and aldehydes. Based on the chemistry of complex carbohydrates, P.J. Van Soest developed a useful method for the proximate analysis of plant fiber in the 1960s (Figure 2.22). Because a significant proportion of soluble NSPs are lost when they are extracted with Van Soest's neutral detergent, an enzymatic-chemical-gravimetric approach is useful to determine complex carbohydrates in feeds (Figure 2.23).

MONOSACCHARIDES

Epimerization

In alkaline solutions, a monosaccharide is converted into a complex mixture of polyhydroxy aldehydes and polyhydroxy ketones. This reaction forms a pair of epimers at C-2, and is therefore called epimerization.

$$\text{D-Glucose} + {}^{-}\text{OH} \rightarrow \text{D-Mannose (epimerization at C-2)}$$

Reduction

The carbonyl group of a monosaccharide can be reduced by $NaBH_4$ to form an alditol. For example, D-mannose is reduced to form D-mannitol, and D-fructose is reduced to yield both D-mannitol and D-sorbitol (also known as D-glucitol).

$$\text{D-Mannose} + \text{NaBH4} + H_3O^+ \rightarrow \text{D-Mannitol}$$

$$\text{D-Fructose} + \text{NaBH4} + H_3O^+ \rightarrow \text{D-Mannitol} + \text{D-Sorbitol}$$

Oxidation

a. Oxidation by Tollens' reagent (Ag^+, NH_3, and OH^-). A ketose (–C=O) is converted to an aldose (–CHO) under alkaline conditions. An aldose sugar can be oxidized by Tollens' reagent to form a carboxylate ion. Thus, Tollens' reagent cannot be used to distinguish glucose from fructose.

$$\text{Ketose (e.g., Fructose)} + OH^- \rightarrow \text{Aldose (e.g., Glucose)}$$

$$+ \text{Tollens' reagent} \rightarrow \text{Carboxylate ion}$$

b. Oxidation by Benedict's reagent [sodium carbonate, sodium citrate, and copper (II) sulfate] or Fehling's reagent [potassium sodium tartrate, NaOH, copper (II) sulfate]. Under alkaline conditions, an aldose sugar (e.g., glucose) is oxidized to an enediol (–CH=C-OH) by Benedict's reagent. The enediol then reduces the cupric ion (Cu^{2+}) to the cuprous ion (Cu^+), producing an insoluble brick-red copper (I) oxide (Cu_2O). Because a ketose (e.g., fructose) is converted into an aldose under alkaline conditions, Benedict's reagent can also be used to determine the presence of fructose.

$$\text{Ketose (e.g., Fructose)} + 2Cu^{2+} + OH^- \rightarrow \text{Aldose}$$

$$\text{(e.g., Glucose)} + \text{Benedict's reagent}$$

$$\rightarrow Cu_2O \downarrow \text{(brick-red)}$$

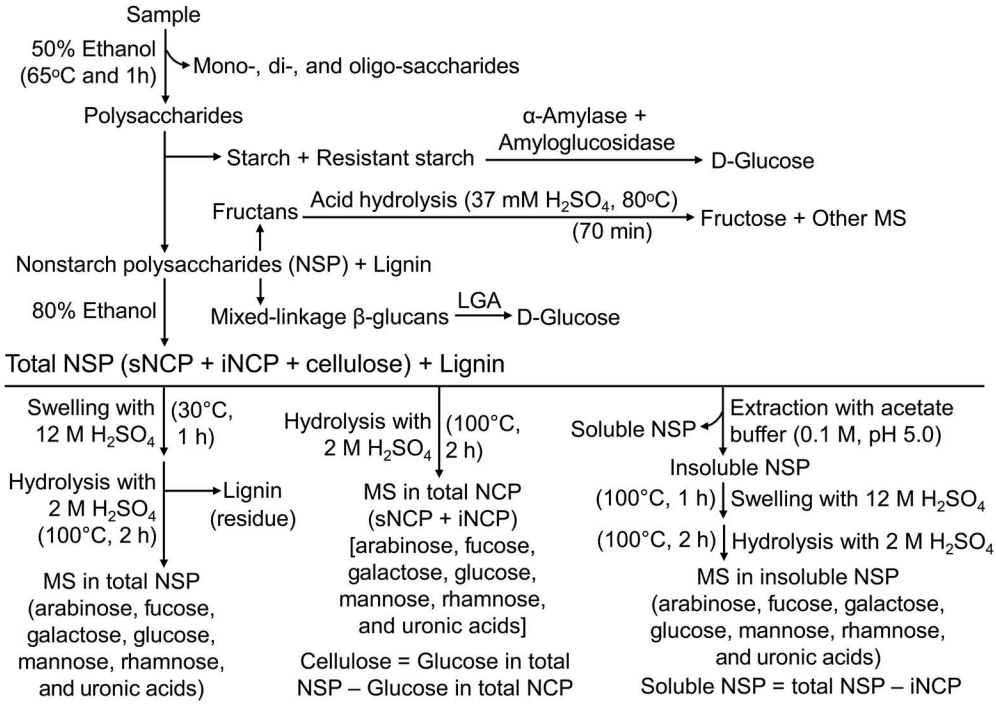


FIGURE 2.23 Analysis of carbohydrates in plants using combined chemical, enzymatic, and gravimetric procedures. These methods are based on the principles that (a) ethanol precipitates polysaccharides [including nonstarch polysaccharides (NSPs) and cellulose]; (b) cellulose is resistant to hydrolysis by 2 M H_2SO_4; (c) 0.1 M sodium acetate (pH 5.0) separates NSPs into water-soluble and water-insoluble fractions; and (d) NSPs are degraded after swelling with 12 M H_2SO_4 and hydrolysis by 2 M H_2SO_4 at 100°C to neutral and acidic carbohydrate monomers (Bach Knudsen 1997, 2014). Specifically, treatment with 50% (v/v) ethanol at 60°C for 1 h separates low-molecular-weight carbohydrates (mono-, di-, and oligo-saccharides) from polysaccharides (including starches, fructans, mixed linkage β-glucans, and NSPs). Starches (including resistant starch) are hydrolyzed by α-amylase and amyloglucosidase to D-glucose. Fructans are extracted with 0.1 M acetate buffer (pH 5.0) at 65°C and then hydrolyzed by 37 mM H_2SO_4 at 80°C to monosaccharides (MSs). Alternatively, fructans can be hydrolyzed by exo- and endo-inulinase and endo-levanase to D-fructose and D-glucose. Mixed linkage β-glucans are hydrolyzed by lichenase to β-gluco-oligosaccharides, which are further degraded by β-glucosidase to D-glucose; this series of reaction is indicated by LGA. NSPs devoid of starches, fructans, and mixed linkage β-glucans are precipitated by 80% ethanol to yield water-soluble NSPs [noncelluosic polysaccharides (NCPs)], water-insoluble NSPs (iNCP; cellulose), and lignin. Treatment with 0.1 M sodium acetate buffer (pH 5.0) separates water-insoluble NSPs from water-soluble NSPs. Total NSPs or water-soluble NSPs are swelled by 12 M H_2SO_4 and hydrolyzed by 2 M H_2SO_4 at 100°C for 2 h to constituent neutral and acidic MS. Low-molecular-weight carbohydrates are analyzed by enzymatic, chromatographic (gas or liquid), or colorimetric methods. Amounts of cellulose in NSPs are calculated as differences in D-glucose between samples that are swelled with 12 M H_2SO_4 and samples that are not treated with 12 M H_2SO_4. Lignin is determined as the insoluble residue after treatment with 12 M H_2SO_4 at 30°C for 1 h, followed by the addition of 28 volumes of water and heating at 125°C for 1 h (Theander and Westerlund 1986).

A similar reaction occurs for aldoses in the presence of Fehling's reagent, as follows:

$$\text{R-CHO (e.g., Fructose)} + 2Cu^{2+} + \text{NaOH} \rightarrow \text{R-COO}^{-}\text{Na}^{+} + Cu_2O \downarrow \text{(brick-red)}$$

c. Oxidation by Br_2. An aldehyde (e.g., glucose with –CHO) is oxidized by a Br_2 aqueous solution (red color) to gluconic acid (–COOH), with Br_2 being reduced to 2 Br^- (colorless). A ketose (e.g., fructose) does not react with Br_2. Therefore, the red color of a Br_2 aqueous solution does not disappear in the presence of a ketose. This assay is useful to distinguish a ketose from an aldehyde.

$$\text{Aldose (e.g., Glucose)} + Br_2 \text{ solution (red color)} \rightarrow \text{Gluconic acid} + 2\ Br^{-}\text{(colorless)}$$

Dehydration of Aldoses and Ketoses

A hexose can undergo dehydration under strong acidic conditions and at high temperatures to form a hydroxymethyl-furfural. The furfural derivative condenses with

two molecules of phenol (e.g., α-naphthol, resorcinol, or thymol) to generate a purple or cherry-red color product. Likewise, a pentose is dehydrated to yield furfural, which can react with anthrone to produce a blue-green color product.

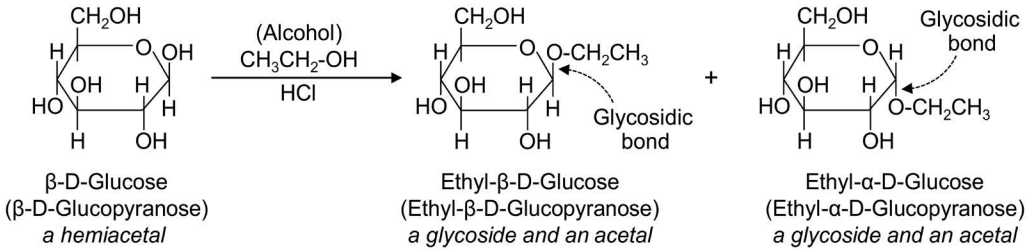
$$\text{Hexose} + \text{HCl (or } H_2SO_4) \rightarrow \text{Hydroxymethyl-furfural} + 3H_2O$$
$$\text{Pentose} + \text{HCl (or } H_2SO_4) \rightarrow \text{Furfural} + 3H_2O$$

Dehydration of a ketose is a chemical basis for the determination of fructose in biological samples. Specifically, fructose is dehydrated in the presence of 18% HCl at 80°C to form 5-hydroxymethyl-furfural. The furfural derivative then reacts with resorcinol (a benzenediol) in the presence of alcohol (20% of a solution consisting of 0.5 g resorcinol in 500 ml of 95% ethyl alcohol) to generate a product with a cherry-red color. Under these conditions, no color develops when glucose is present in the assay mixture. This is a satisfactory method for fructose analysis in deproteinized plasma, urine, semen, and fetal fluid.

$$\text{Fructose} + \text{HCl} \rightarrow \text{5-Hydroxymethyl-furfural} + 3H_2O$$

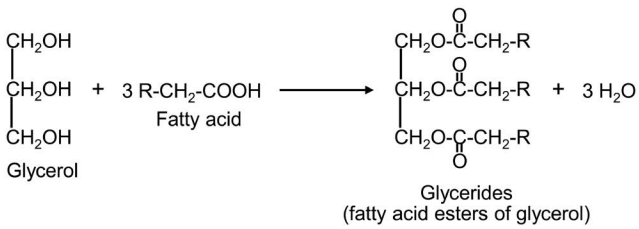
Formation of Glycosides

A hemiacetal (e.g., D-glucose) or hemiketal (e.g., D-fructose) reacts with an alcohol to form an acetal (e.g., β-D-glucoside and α-D-glucoside) or ketal (e.g., β-D-fructofuranoside and α-D-fructofuranoside). As noted previously, a hemiketal monosaccharide is a nonreducing sugar and cannot be oxidized by Ag^+ or Br_2. A glycoside of glucose, galactose or fructose is called a glucoside, galactoside, or fructofuranoside, respectively. An example of the formation of a glycoside from glucose and alcohol is given as follows:



Esterification

Esterification occurs chemically when an –OH group of a carbohydrate reacts with a –COOH group of a fatty acid in the presence of a catalyst (e.g., sulfuric acid) to form an ester. This reaction can be catalyzed by enzymes in cells. Glycerides, which are fatty acid esters of glycerol, are important esters in biology. Sulfate esters of galactose or glucosamine are present predominantly in proteoglycans of animals.



Nonenzymatic Glycation

In the open-chain form, an aldohexose (e.g., D-glucose) has the free aldehyde group that reacts with an amino acid (free or protein-bound) to form a Schiff base (a covalent bond) and then Amadori products. The amino acid involved in this Maillard reaction is mainly lysine or arginine but can also be histidine or glutamine. Heating accelerates the Maillard reaction and gives rise to melanoidin polymers (brown color), which are not digestible by animals. Thus, nonenzymatic glycation has very important implications in feed-pelleting and animal nutrition.

$$\text{D-Glucose (}-\text{CHO, open-chain)} + \varepsilon\text{-}NH_2 \text{ in Lysine}$$
$$\rightarrow \text{Schiff base (}-\text{CH = N-Lysine)} \rightarrow \text{Glycation}$$

DISACCHARIDES, OLIGOSACCHARIDES, AND POLYSACCHARIDES

Saccharification

Saccharification refers to the breaking of the glycosidic bond in carbohydrates, including di-, oligo-, and poly-saccharides. This reaction is weak under alkaline conditions (e.g., NaOH) but very strong under acidic conditions (e.g., H_2SO_4 or HCl). For example, the exposure of microalgae (*S. almeriensis*) to 1 and 5 M NaOH at 90°C for 30 min results in the release of <4

and 15 mg monosaccharides per g of dry weight, respectively (Hernández et al. 2015). However, the exposure of the microalgae to 7% H_2SO_4 at 121°C for 30 min results in the release of 93 mg monosaccharides per g of dry weight (Hernández et al. 2015). Alkaline hydrolysis generates large amounts of oligo- and poly-saccharides.

$$\text{Alkaline hydrolysis (weak): Sucrose} + \text{NaOH}\ (\text{e.g., 3 M}) \rightarrow \text{Glucose} + \text{Fructose}\ [\text{Elevated temperatures}]$$

$$\text{Alkaline hydrolysis (weak): Starch or cellulose} + \text{NaOH}\ (\text{e.g., 3 M}) \rightarrow \text{Glucose} + \text{OS} + \text{PS}\ [\text{Elevated temperatures}]$$

$$(\text{OS} = \text{oligosaccharides; PS} = \text{polysaccharides})$$

$$\text{Acidic hydrolysis (strong): Sucrose} + \text{H}_2\text{SO}_4\ (\text{e.g., 3 M}) \rightarrow \text{Glucose} + \text{Fructose}\ [\text{Elevated temperatures}]$$

$$\text{Acidic hydrolysis (strong): Starch or cellulose} + \text{H}_2\text{SO}_4\ (\text{e.g., 3 M}) \rightarrow \text{Glucose}\ [\text{Elevated temperatures}]$$

Carbohydrases degrade carbohydrates with various glycosidic linkages to mono- and/or oligo-saccharides, depending on substrate structures. Bacteria produce enzymes that can hydrolyze all types of carbohydrates. However, animal-sourced enzymes in the lumen of the digestive tract can break down only select carbohydrates such as sucrose (with the α-1,4 bond), lactose (with the β-1,4 bond), and starch (with α-1,4 and α-1,6 bonds), but cannot act on raffinose (with α-1,6 and α-1,2 bonds) or lactosucrose (with β-1,4 and α-1,2 bonds). Thus, it is incorrect to state that animal-sourced enzymes can only digest carbohydrates with α-type glycosidic bonds.

Oxidation

Benedict's reagent can react with reducing disaccharides (e.g., lactose and maltose) as indicated previously, but not with nonreducing disaccharides (e.g., sucrose) that do not have a hemiacetal (or hemiketal) group on both ends of the molecules. However, sucrose can be hydrolyzed to D-glucose and D-fructose if heated with dilute HCl. Subsequently, both glucose and fructose can be detected by Benedict's reagent. Starches and glycogen react very poorly with Benedict's reagent because they have only a limited number of reducing sugar moieties at the ends of their chains. Inositol (myo-inositol) is another carbohydrate that cannot be detected by this assay.

Reacting with Iodine

Starch or glycogen reacts with Lugol's reagent (I-KI; iodine dissolved in an aqueous solution of potassium iodide) to give rise to a blue-black or brown-blue color, respectively.

The straight chain component of starch (i.e., amylose) gives a blue color, while the branched component (e.g., amylopectin) yields a purple color. The intensity of the color decreases with increasing temperature and with the presence of water-miscible organic solvents (e.g., ethanol). The principle of this assay is that iodine binds to the helices of starch or glycogen to form a starch-iodine or glycogen-iodine complex, respectively. The helices of starch are longer than those of glycogen, therefore binding more iodine atoms and generating a more intense color as compared with glycogen. This test should not be performed at very low pH due to the hydrolysis of starch and glycogen. Other polysaccharides and monosaccharides do not react with Lugol's reagent.

The Molisch Test for Nearly All Carbohydrates

Except for sugar alcohols, 2-deoxy sugars, and 2-amino-2-deoxy sugars, all carbohydrates in an aqueous solution are determined by the Molisch test (Robyt 1998). In this relatively broad assay with good specificity for carbohydrates, concentrated sulfuric acid hydrolyzes the glycosidic bonds in oligosaccharides or polysaccharides to monosaccharides. In the presence of a strong acid (H_2SO_4), monosaccharides are dehydrated to generate furfural derivatives, which condense with α-naphthol (a phenolic compound) to produce a characteristic purple color. The detection limit of the Molisch test for carbohydrates is 10 mg/L. 2-Deoxy sugars and 2-amino-2-deoxy sugars can be analyzed by high-performance liquid chromatography and mass spectrometry.

SUMMARY

Carbohydrates are polyhydroxy aldehydes or ketones, as well as their amidated or sulfated derivatives (e.g., glucosamine, *N*-acetyl-glucosamine, and dermatan sulfate). They are important components of plants, animals, and microbes (Table 2.12). Carbohydrates can be classified into two types: simple or complex. Simple carbohydrates are (a) monosaccharides (single sugars), which cannot be broken down by hydrolysis reactions; and (b) disaccharides [e.g., maltose (in plants and from the digestion of starch and glycogen), lactose (in the milk of mammals), and sucrose (in plants)]. Carbohydrates that have anomeric forms (i.e., their anomeric carbons are not involved in a glycosidic linkage) can spontaneously exhibit α- and β-configuration in H_2O (e.g., α- or β-glucose).

D-Glucose (an aldose), which is synthesized from CO_2 and H_2O in green plants and from many glucogenic precursors in animals, is the most abundant single sugar in nature, followed by D-fructose (a ketose). All hexose monosaccharides (6-carbon sugars), either aldoses or ketoses, have an α- or β-anomer. Aldoses (e.g., D-glucose) exist

TABLE 2.12
Glycosidic Linkages in Polysaccharides

Polysaccharide	Source	Monomer	Main Chain	Branch Linkage
Homopolysaccharides		**Sole sugar**		
Arabinan	Plants	D-Arabinose	α-1,3; α-1,5; β-1,2	---
Amylose of starch	Green plants	D-Glucose	α-1,4	---
Amylopectin of starch	Green plants	D-Glucose	α-1,4	α-1,6
Callose	Higher plants	D-Glucose	β-1,3	β-1,6
Cellulose	Plants, others[a]	D-Glucose	β-1,4	---
Chitin	Animals, others[b]	AGlcN	β-1,4	---
Dextrans	Some bacteria	D-Glucose	α-1,6	α-1,3
Galactan	Plants	D-Galactose	β-1,4	---
Galactan	Microbes (LAB)	D-Galactose	α-1,6	---
β-D-Glucans	Fungi, algae	D-Glucose	β-1,3	β-1,6
β-D-Glucans	Cereals, bacteria	D-Glucose	β-1,3; β-1,4	---
β-D-Glucans	Lichens	D-Glucose	β-1,3; β-1,6	---
Glycogen	Animals, others[c]	D-Glucose	α-1,4	α-1,6
Levan	Plants, microbes	D-Fructose	β-2,6	β-2,1
Pure mannans	Certain bacteria	D-Mannose	α-1,3	α-1,2; β-1,6
Pure mannans	Green algae	D-Mannose	β-1,4	---
Pure mannans	Plants	D-Mannose	β-1,4	---
Pure mannans	Yeast, other fungi	D-Mannose	α-1,6	α-1,2; α-1,3
Pullutan	Yeast, other fungi	D-Glucose	α-1,4; α-1,6	---
Heteropolysaccharides		**Main sugars**		
Alginate	Seaweed	MANU + GU	β-1,4; α-1,4	---
Agar	Red seaweed	D-Galactose	β-1,4; α-1,3	---
Arabinogalactan	Plants	D-Arabinose	α-1,5	β-1,3; β-1,6
		+ D-Galactose	α-1,4 or β-1,4	
Arabinogalactan	Microbes	D-Arabinose	α-1,3; α-1,5; β-1,2	β-1,3; β-1,6
		+ D-Galactose	β-1,5; β-1,6	
Carageenan	Red seaweed	G4S + G2S	β-1,4; α-1,3	---
Chitosan	Animals, others[b]	GlcN + AGlcN	β-1,4	---
Chondroitin sulfate	Animals	GA + AGN	β-1,3; β-1,4	---
Dermatan sulfate	Animals	Idu + AGN	α-1,3; β-1,4	---
Galactomannan	Plants	D-mannose (B)	β-1,4	α-1,6
		+ D-galactose		
Galactoglucomanann	Plants	D-Mannose (B)	β-1,4	α-1,6
		+ D-galactose (S)		
		+ D-glucose (S)		
Glucomannan	Plants	D-mannose (B)	β-1,4	β-1,6; α-1,6
		+ D-glucose		
Hemicellulose	Plants	Hexoses[d]	β-1,4; α-1,2	---
Heparan sulfate	Animals	GS + NGAS	β-1,4; α-1,4	---
Heparin	Animals	Idu + NGAS	α-1,4	---
Hyaluronan	Animals	GA + AGlcN	β-1,4; α-1-3	---
Inulin	Plant tubers	D-Fructose	β-2,1	---
α-Mannans	Yeast	D-Mannose	α-1,6	α-1,2; α-1,3
β-Mannans	Plants	D-Mannose	β-1,4	β-1,6; α-1,6
Murein	Bacteria	AGlcN + AM	β-1,4	---
Pectin	Green plants	GAU + others	α-1,4 and others	---
Xanthan	Bacteria	Hexoses[e]	β-1,4; α-1-3	---
Xylans	Plants, others[f]	D-Xylose	β-1,3	---

[a] Some bacteria also contain cellulose.
[b] Fungi (including yeasts), green algae, as well as brown and red seaweed (types of algae) also contain chitin.
[c] Bacteria, fungi (including yeasts), protozoa, and the blue-green algae also contain glycogen.
[d] Including β-D-xylopyranose, β-D-xylose, β-D-mannose, β-D-arabinose, β-D-glucose, β-D-galactose, and uronic acid.
[e] Including D-glucose, D-mannose, and D-glucuronic acid.
[f] Brown seaweed also contains xylans.

Note: AGlcN, *N*-acetyl-D-glucosamine; AGN, *N*-acetyl-D-galactosamine; AM, *N*-acetyl-D-muramic acid; B, backbone chain; GA, glucuronic acid; GlcN, D-glucosamine; GAU, D-galacturonate; GS, D-glucuronate-2-*O*-sulfate; GU, D-guluronate; G4S, D-galactose-4-sulfate; G2S, 3,6-anhydro-D-galactose-2-sulfate; Idu, α-L-iduronate; LAB, lactic acid bacteria; MANU, D-mannuronate; NGAS, *N*-sulfo-D-glucosamine-6-phosphate; S, side chain; ---, no side chain.

predominantly as cyclic hemiacetals (pyranoses with a 6-membered ring) in equilibrium with their open-chain forms with free aldehyde groups. Likewise, ketoses (e.g., D-fructose) exist mainly as cyclic hemiketals (pyranoses or furanoses) in equilibrium with their open-chain forms with ketone structures. In an aqueous solution, pyranoses are thermodynamically more stable and more abundant than furanoses. Only monosaccharides are absorbed by the small and large intestines.

Complex carbohydrates include oligosaccharides (consisting of 3 or 10 monosaccharide units) and polysaccharides (e.g., starch, glycogen, cellulose, hemicellulose, and pectin). In the main chains, monosaccharides are bonded primarily through α-1,4 and/or α-1,6 glycosidic linkages (starch and glycogen) or β-1,3 and β-1,4 glycosidic linkages (other complex carbohydrates). Starch and glycogen (α-glucan polymers) are the main homopolysaccharides in plants and animals, respectively. There are two types of starch (amylose and amylopectin, present in a 1:3 ratio) that differ in the level of branching, with amylose being essentially linear and amylopectin being highly branched. These branches affect starch characteristics and functionality. Other homopolysaccharides include cellulose, β-D-glucans, levans, mannans in plants, and chitin in animals. Heteropolysaccharides include (a) exudate gums, hemicelluloses, inulins, hetero-mannans, mucilages, and pectins in plants; (b) hyaluronic acid, chondroitin sulfates, dermatan sulfate, heparan sulfate, heparin, keratan sulfate, and chitosan in animals; (c) LPSs, murein, and xanthan in microbes; and (d) agar, algin (alginic acid), and carrageenans in algae and seaweed. Notably, yeast cell walls contain high amounts of β-glucan- and α-MOS. Dietary fiber refers to the plant material composed of complex NSPs and lignin, and is abundant in select feedstuffs (e.g., beet pulp, citrus pulp, cottonseed hulls, hay, rice bran, sillages, soy hulls, and wheat middlings).

Lactose (the major sugar in mammalian milk) has a galactosyl β-1,4-linkage that is hydrolyzed by the small intestine-derived lactase. Complex carbohydrates with D-glucosyl- or D-fructosyl β-1,4-linkages or dietary fibers are generally not broken down by animal-sourced enzymes but are fermented by microbes to varying degrees in the large intestine. NSPs are important components of diets for land animals and play an important role in maintaining their gut health. Although lignins and tannins are not carbohydrates, they are usually associated with polysaccharides in plants to affect their digestion in the GIT.

Monosaccharides undergo chemical reactions, such as epimerization, reduction, oxidation, dehydration, formation of glycosides, esterification, and glycation, whereas starch or glycogen, but not other complex carbohydrates, can bind to and react with iodine to form a colored product. These reactions provide the basis for the determination of certain types of carbohydrates in feedstuffs and animals. Except for a few sugar alcohols, all carbohydrates in an aqueous solution can be analyzed by the Molisch test. Optimal animal feeding must be designed to ensure: (a) adequate supply of plant-sourced fibers and starch for post-weaning animals; (b) sufficient synthesis of glucose, glycogen, and heteropolysaccharides in all animals; and (c) maximum production of lactose in milk for mammals. Knowledge about the chemistry of carbohydrates provides a foundation for our understanding of their metabolism, nutrition, and health benefits in animals.

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3 Chemistry of Lipids

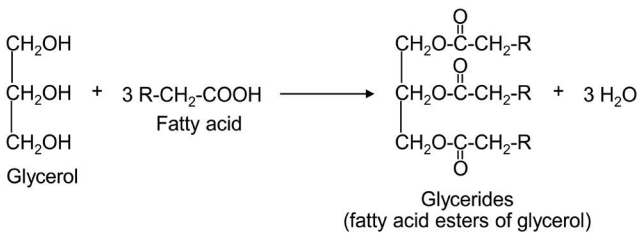
Lipids are defined as hydrocarbon compounds that are soluble in organic solvents (e.g., chloroform, ethanol, or ether) and, except for short- and medium-chain fatty acids, are insoluble in water (H_2O; Gunstone 2012). These substances have diverse chemical structures and biological functions. Of note, they are highly reduced molecules, with the highest proportions of hydrogen atoms among all dietary macronutrients. In nature, examples of lipids are fatty acids, triacylglycerols (TAGs; also known as triglycerides), glycerolipids, glycerophospholipids, sphingolipids, cholesterol, bile acid, cortisol, testosterone, estradiol, progesterone, and vitamin A (Mead et al. 1986). Lipids account for 1% to 50% of the body weight (BW) of animals, depending on their species, age, nutritional state, and disease (Cherian 2015; Pond et al. 200). Plants and algae are excellent sources of some fatty acids that normally are not synthesized by animals (NRC 2011, 2012). In proximate analysis, lipids are determined as ether extracts and are collectively called crude fats in animal nutrition.

Lipids are important structural and cellular components in plants, animals, and microbes. These substances are widely distributed in the cell membranes and the membranes of intracellular organelles to control the transport of gases, nutrients, ions, and metabolites into and out of cells (Ridgway and McLeod 2016). In animals, TAGs [e.g., those in white adipose tissue (WAT)] are the major form of energy storage, and fatty acids are the primary metabolic fuels for such key organs as the liver, skeletal muscle, and heart in both fed and postabsorptive states (Jobgen et al. 2006). Dietary fatty acids, along with liver-derived bile salts, also facilitate the digestion and absorption of lipid-soluble vitamins by the small intestine (Conde-Aguilera et al. 2013; Smith and Smith 1995). In neonates, subcutaneous WAT insulates the body from the loss of heat. In many mammals, brown adipose tissue (BAT) oxidizes fatty acids to produce large amounts of heat, thereby keeping the body warm during the neonatal period (Satterfield and Wu 2011; Smith and Carstens 2005). In all organisms, lipids serve as signaling molecules to regulate physiological processes. However, excessive fats also contribute to a variety of metabolic disorders and chronic diseases, including obesity, diabetes, and cardiovascular disease.

Our current knowledge of lipids has been built on the shoulders of the pioneers of the field (e.g., Block et al. 1946; Burr and Burr 1929; Mead et al. 1986). The 19th century witnessed great discoveries of lipids in nature. In 1813, M.E. Chevreul reported the composition of animal fats and proposed the concept of fatty acids. Ten years later, this same author published a landmark book on the chemistry of lipids, in which he described several straight-chain fatty acids (margaric, oleic, stearic, butyric and caproic acids) and a branched-chain fatty acid, isovaleric acid, which was isolated from the head oil of the dolphin and from porpoise oil. In 1884, J.-P. Couerbe introduced the method of using diethyl ether to extract lipids from animal tissues, which facilitated research in the field. In 1929, G.O. Burr discovered linoleic acid, a long-chain fatty acid (LCFA), as a nutritionally essential fatty acid for the growth and health of young rats. Subsequently, Wesson and Burr (1931) reported that animals do not synthesize α-linolenic acid or linoleic acid. In the 1940s, K.E. Bloch discovered the synthesis of cholesterol from acetate in the liver. These ground-breaking findings ushered in a new era of lipid nutrition research in the 20th century (Field et al. 2008; Kessler et al. 1970; Pedersen 2016; Spector and Kim 2015; Tso 1985; Wood and Harlow 1960). This chapter highlights the chemistry of lipids pertinent to animal nutrition.

CLASSIFICATION AND STRUCTURES OF LIPIDS

Lipids are classified in four groups: fatty acids, simple lipids, compound lipids, and derived lipids (Figure 3.1). Fatty acids, also known as precursor lipids, include those fatty acids with various numbers of carbons and unsaturated double bonds. Some of them have *cis, trans*, or both types of the chemical configuration (Ma et al. 1999), whereas others may have a branched methyl group. Along with glycerol (1,2,3-propanetriol), alcohols, serine, sphingosine, choline and ethanolamine, fatty acids are used to synthesize all other lipids (including TAGs) in animals (Ridgway and McLeod 2016). Oils and fats are defined as any greasy water-insoluble organic substances that are liquid and non-liquid (solid/soft), respectively, at room temperature (e.g., 20–25°C). In plants, animals, and foods, oils are composed primarily of polyunsaturated fatty acids (PUFAs) or their glycerol-based esters in a liquid state, whereas fats and waxes are the glycerol-based esters of fatty acids and fatty alcohols, respectively, in either a solid or a soft state (Mead et al. 1986).



Lipids have structural, storage, regulatory, protective, secretory, and transport functions in animals. For example, excess energy is stored as TAGs in all animals (Bergen and Mersmann 2005). Insulation of the body by TAGs is crucial

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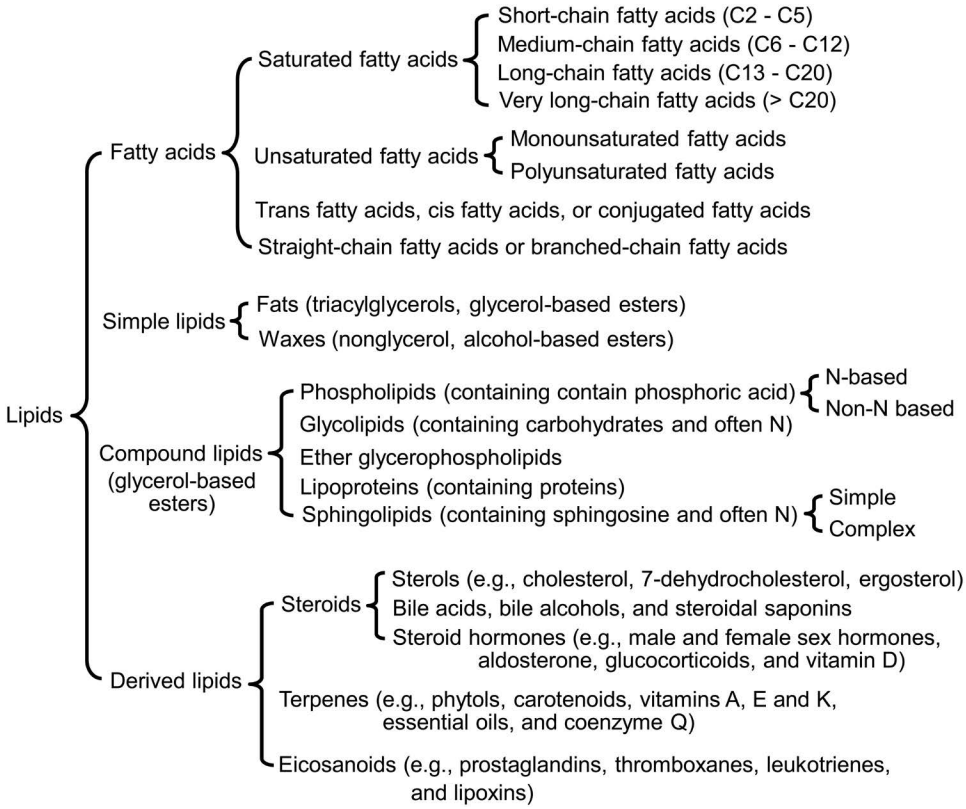


FIGURE 3.1 Classification of lipids in animal nutrition. Lipids are composed of fatty acids (precursor lipids) as well as simple, compound, and derived lipids. Serine, choline, and ethanolamine are often components of phospholipids and sphingolipids. N, nitrogen.

for keeping newborns warm and for protecting adult animals from the cold. In sperm whales, large amounts of lipids in the head may detect sound waves in H_2O. Lipids (including bile acids) are required for the digestion and absorption of dietary TAGs and lipid-soluble vitamins (Pond et al. 2005). Furthermore, phospholipids are signaling molecules that regulate nutrient metabolism, whereas lipoproteins play an important role in the interorgan transport of TAGs, cholesterol, and other lipids. Deficiencies of nutritionally essential fatty acids result in skin lesions, growth restriction, and diseases, but excess TAGs in the WAT as well as excess TAGs and cholesterol in blood are the major causes of obesity, insulin resistance, and cardiovascular disorders in animals (Fox et al. 2002; Hausman et al. 1982).

FATTY ACIDS

Definition of Fatty Acids

Fatty acids are hydrocarbon chains with 2 or more carbon atoms and a carboxyl group (Figure 3.2). The early isolation of these substances from animal fats led to the term "fatty acids." More than 300 fatty acids have been isolated from plants and animals (Gunstone 2012). According to the length of the carbon chain, fatty acids are categorized as: (a) short-chain fatty acids (SCFAs) with 2 to 5 carbon atoms; (b) medium-chain fatty acids (MCFAs) with 6 to 12 carbon atoms; (c) LCFAs with 13 to 20 carbon atoms; and (d) very long-chain fatty acids (VLCFAs) with >20 carbon atoms. Note that formic acid (an organic acid) is not a fatty acid.

Based on the number of double bonds in the carbon chain, fatty acids are classified as: (a) saturated fatty acids without a double bond; (b) monounsaturated fatty acids (MUFAs) with one double bond; and (c) PUFAs with two or more double bonds (Mead et al. 1986). An alkene (also known as olefin) is a hydrocarbon substance containing a carbon–carbon double bond (e.g., MUFAs and PUFAs). Because of the different structures of double bonds, fatty acids are grouped as (a) *cis*-fatty acids, with the hydrogen atoms being on the same side of the double bond; (b) *trans*-fatty acids, with the hydrogen atoms being on the opposite side of the double bond; and (c) conjugated fatty acids, with at least one pair of double bonds being separated by only one carbon atom (Azain 2003; Barnes et al. 2012; Brandebourg and Hu 2005).

Most naturally occurring fatty acids have an even number of carbons because of their synthesis from acetyl-CoA (a 2-carbon compound) and are unbranched (Table 3.1). However, some fatty acids have an odd number of carbons or a branched chain. Fatty acids with even- or odd-number carbons are called even- or odd-numbered fatty acids. Additionally, most fatty acids contain *cis* double bonds because fatty acid desaturases in plant and animal

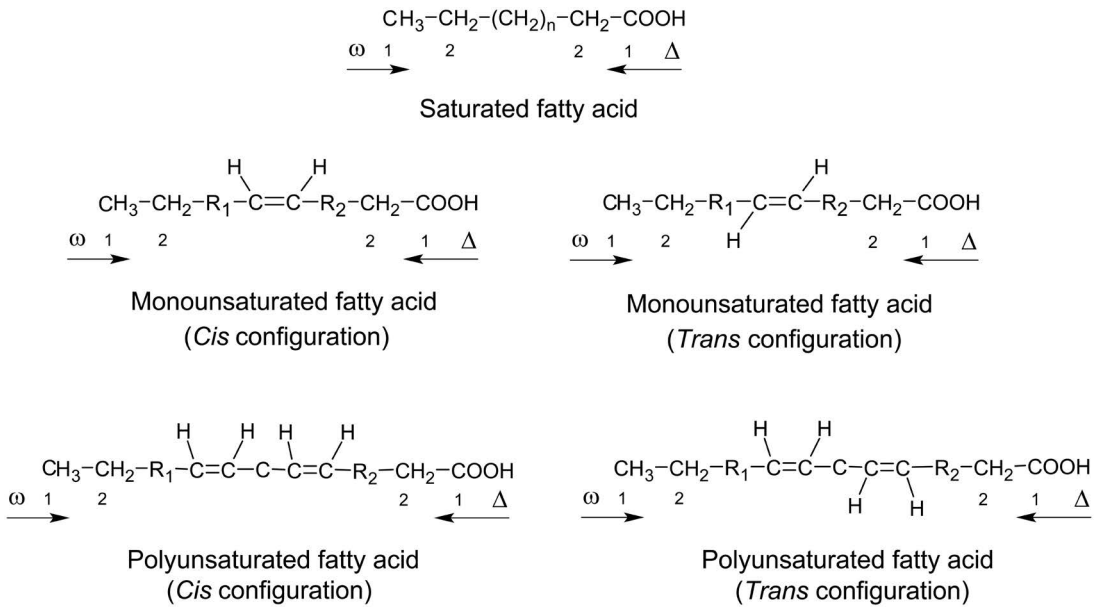


FIGURE 3.2 Nomenclature of fatty acids. Fatty acids are named according to delta (Δ), omega (ω), common (nonsystematic), and systematic (scientific) nomenclatures. In the Δ nomenclature, the carbon atom of the carboxyl group is counted as C-1. In the ω nomenclature, the carbon atom of the terminal methyl group is counted as C-1. Common names for fatty acids were often derived from the sources of their discoveries, whereas systematic names of fatty acids are given by following the nomenclature of organic chemistry. Saturated fatty acids have no double bond. Fatty acids with one or more double bonds are called mono- or poly-unsaturated fatty acids. Unsaturated fatty acids are grouped as: (a) *cis*-fatty acids, with the hydrogen atoms being on the same side of the double bond; (b) *trans* fatty acids, with the hydrogen atoms being on the opposite side of the double bond; and (c) conjugated fatty acids, with at least one pair of double bonds being separated by only one carbon atom. Most naturally occurring fatty acids have the *cis* configuration, although the prefix "*cis*" is often omitted.

TABLE 3.1
Fatty Acids Commonly Present in Feedstuffs and Animals

Common Name	Systematic Name (Δ Nomenclature for Unsaturated Fatty Acids; Carboxyl Group = C-1)	Number of Carbons	Number of Double Bonds	Symbols of Fatty Acids
Saturated fatty acid				
Acetic acid	Ethanoic acid	2	0	$C_{2:0}$
Propionic acid	Propanoic acid	3	0	$C_{3:0}$
Butyric acid	Butanoic acid	4	0	$C_{4:0}$
Isobutyric acid	2-Methylpropanoic acid	4	0	$C_{4:0}$[a]
Valeric acid	Pentanoic acid	5	0	$C_{5:0}$
Isovaleric acid	3-Methylbutanoic acid	5	0	$C_{5:0}$[b]
Caproic acid	Hexanoic acid	6	0	$C_{6:0}$
Caprylic acid	Octanoic acid	8	0	$C_{8:0}$
Capric acid	Decanoic acid	10	0	$C_{10:0}$
Lauric acid	Dodecanoic acid	12	0	$C_{12:0}$
Myristic acid	Tetradecanoic acid	14	0	$C_{14:0}$
Pentadecanoic acid[c]	1-Decanecarboxylic acid	15	0	$C_{15:0}$
Palmitic acid	Hexadecanoic acid	16	0	$C_{16:0}$
Margaric acid[c]	Heptadecanoic acid	17	0	$C_{17:0}$
Stearic acid	Octadecanoic acid	18	0	$C_{18:0}$
Nonadecanoic acid[c]	Nonadecanoic acid	19	0	$C_{19:0}$
Arachidic acid	Eicosanoic acid ($C_{20}H_{40}O_2$)	20	0	$C_{20:0}$
Behenic acid	Docosanoic acid	22	0	$C_{22:0}$
Lignoceric acid	Tetracosanoic acid ($C_{23}H_{47}COOH$)	24	0	$C_{24:0}$
Melissic acid	Triacontanoic acid ($C_{29}H_{59}COOH$)	30	0	$C_{30:0}$

(*Continued*)

TABLE 3.1 (*Continued*)
Fatty Acids Commonly Present in Feedstuffs and Animals

Common Name	Systematic Name (Δ Nomenclature for Unsaturated Fatty Acids; Carboxyl Group = C-1)	Number of Carbons	Number of Double Bonds	Symbols of Fatty Acids
Unsaturated fatty acid (all double bonds are *cis* unless indicated otherwise)				
Palmitoleic acid	9-Hexadecenoic acid	16	1	$C_{16:1}$ (ω7)
Vaccenic acid	*trans*-11-Octadecenoic acid (in dairy products)	18	1	*trans* $C_{18:1}$ (ω7)
Oleic acid	9-Octadecenoic acid	18	1	$C_{18:1}$ (ω9)
Elaidic acid*	*trans*-9-Octadecenoic acid	18	1	*trans* $C_{18:1}$ (ω9)
Linoleic acid	9,12-Octadecadienoic acid	18	2	$C_{18:2}$ (ω6)
Linolelaidic acid*	*trans*-9,12-Octadecadienoic acid	18	2	*trans* $C_{18:2}$ (ω6)
α-Linolenic acid	9,12,15-Octadecatrienoic acid	18	3	$C_{18:3}$ (ω3)
γ-Linolenic acid	6,9,12-Octadecatrienoic acid	18	3	$C_{18:3}$ (ω6)
Arachidonic acid	5,8,11,14-Eicosatetraenoic acid	20	4	$C_{20:4}$ (ω6)
Timnodonic acid	EPA	20	5	$C_{20:5}$ (ω3)
Erucic acid[d]	13-docosenoic acid	22	1	$C_{22:1}$ (ω9)
Brassidic acid[e]	*trans*-13-docosenoic acid	22	1	*trans* $C_{22:1}$ (ω9)
Cetoleic acid[f]	11-docosenoic acid	22	1	$C_{22:1}$ (ω11)
Clupanodonic acid	7,10,13,16,19-Docosapentaenoic acid (ω3 DPA)	22	5	$C_{22:5}$ (ω3)
Osbond acid	4,7,10,13,16-Docosapentaenoic acid (ω6 DPA)	22	5	$C_{22:5}$ (ω6)
Cervonic acid	DHA	22	6	$C_{22:6}$ (ω3)
Nervonic acid	15-Tetracosenoic acid	24	1	$C_{24:1}$ (ω9)

[a] $(CH_3)_2$-CH-COOH.
[b] $(CH_3)_2$-CH-CH_2-COOH.
[c] Present naturally in the skin of animals and in ruminant products (e.g., the milk of cows and goats).
[d] Toxic fatty acid highly abundant in the oil-rich seeds of the Brassicaceae (formerly Cruciferae) family of natural plants (e.g., wallflower, rapeseed, and mustard). This fatty acid is also present in some fish and seafoods at low concentrations.
[e] Present naturally along with bitter-tasting compounds in plants.
[f] Present naturally in certain plants (e.g., the Brassicaceae family) as well as some fish and seafoods at low concentrations.

Note: EPA, 5,8,11,14,17-eicosapentaenoic acid; DHA, 5,8,11,14,17,20-docosahexaenoic acid; * hydrogenated vegetable oil.

cells do not produce *trans*-double bonds. In contrast, bacteria can form both *cis*- and *trans*-LCFAs. The solubility and acidity (ionization of the carboxyl group) of fatty acids in H_2O decrease markedly with the length of the carbon chain (Table 3.2). The diversity of fatty acids fulfills their versatile nutritional and physiological functions in animals (Clandinin 1999; Swift et al. 1988).

Nomenclature of Fatty Acids

There are four naming systems used for fatty acids: delta (Δ) nomenclature, omega (ω) or n-nomenclature, common (nonsystematic) names, and systematic (scientific) names (Gunstone 2012). In the delta nomenclature, the carbon atom of the carboxyl (COOH) group is counted as C1, and the fatty-acid chain is numbered consecutively toward the methyl (CH_3) group; the positional number of the carbon (e.g., C9) in the first double bond is indicated by "Δ" plus the carbon number (e.g., Δ^9; Figure 3.3). In the ω or n-nomenclature, the carbon atom of the terminal methyl group is counted as C1, and the fatty-acid chain is numbered consecutively toward the carboxyl group; the positional number of the carbon (e.g., C3) in the first double bond is indicated by "ω" or "n-" plus the carbon number (e.g., "ω3" or "n-3"; Figure 3.3). There are ω3, ω6, ω7, and ω9 unsaturated fatty acids in microbes, plants, and animals (Smith and Smith 1995).

Common names for fatty acids were given before a systematic naming system was developed and were often derived from the sources of their discoveries (Mead et al. 1986; Ralston and Hoerr 1942). For example, lauric acid is a widely occurring fatty acid in *Lauraceae* seeds, palmitic acid is a major component of the oil from palm trees, oleic acid is abundant in olive oil, and melissic acid (a very long-chain saturated fatty acid) is abundant in the nectar of the flowers that attract bees (*melissa* in Greek). Stearic acid is derived from the Greek *stear* (meaning "tallow"). Systematic names of fatty acids are based on the nomenclature of organic chemistry. Greek prefixes (e.g., *di*, *tri*, *tetra*, *penta*, *hexa*, and *octa*) are used to designate the number of

TABLE 3.2
Melting Points of Fatty Acids and Their Solubility in Water

Fatty Acid	Chemical Formula	Molecular Weight (Da)	Melting Point (°C)	Solubility in Water (g/100 mL)	
				20°C	30°C
Saturated fatty acid					
Acetic acid (ethanoic acid; $C_{2:0}$)	$C_2H_4O_2$	60.05	16.5	miscible	miscible
Propionic acid (propanoic acid; $C_{3:0}$)	$C_3H_6O_2$	74.08	–20.5	miscible	miscible
Butyric acid (butanoic acid; $C_{4:0}$)	$C_4H_8O_2$	88.11	–5.1	miscible	miscible
Isobutyric acid (isobutanoic acid; $C_{4:0}$)	$C_4H_8O_2$	88.11	–47	miscible	miscible
Valeric acid (pentanoic acid; $C_{5:0}$)	$C_5H_{10}O_2$	102.13	–34.5	4.03	5.03
Isovaleric acid (3-methylbutanoic acid; $C_{5:0}$)	$C_5H_{10}O_2$	102.13	–29.3	4.07	5.27
Caproic acid (hexanoic acid; $C_{6:0}$)	$C_6H_{12}O_2$	116.16	–3.4	0.968	1.02
Caprylic acid (octanoic acid; $C_{8:0}$)	$C_8H_{16}O_2$	144.21	16.7	0.068	0.079
Capric acid (decanoic acid; $C_{10:0}$)	$C_{10}H_{20}O_2$	172.27	31.6	0.015	0.018
Lauric acid (dodecanoic acid; $C_{12:0}$)	$C_{12}H_{24}O_2$	200.32	44.2	0.0055	0.0063
Brassylic acid (dicarboxylic acid; $C_{13:0}$)	$C_{13}H_{24}O_4$	244.33	111	0.14	---
Myristic acid (tetradecanoic acid; $C_{14:0}$)	$C_{14}H_{28}O_2$	228.38	53.9	0.0020	0.0024
Pentadecanoic acid (pentadecylic acid; $C_{15:0}$)	$C_{15}H_{30}O_2$	242.40	52.0	0.0012	0.0014
Palmitic acid (hexadecenoic acid; $C_{16:0}$)	$C_{16}H_{32}O_2$	256.43	63.1	0.00072	0.00083
Margaric acid (heptadecanoic acid ($C_{17:0}$)	$C_{17}H_{34}O_2$	270.45	61.3	0.00042	0.00049
Stearic acid (octadecanoic acid; $C_{18:0}$)	$C_{18}H_{36}O_2$	284.48	69.6	0.00029	0.00034
Nonadecanoic acid (nonadecylic acid; $C_{19:0}$)	$C_{19}H_{38}O_2$	298.50	69.0	Insoluble	Insoluble
Arachidic acid (icosanoic acid; $C_{20:0}$)	$C_{20}H_{40}O_2$	312.54	75.5	Insoluble	Insoluble
Behenic acid (docosanoic acid; $C_{22:0}$)	$C_{22}H_{44}O_2$	340.59	81.0	Insoluble	Insoluble
Lignoceric acid (tetracosanoic acid; $C_{24:0}$)	$C_{24}H_{48}O_2$	368.63	86.0	Insoluble	Insoluble
Melissic acid (triacontanoic acid; $C_{30:0}$)	$C_{30}H_{60}O_2$	452.46	93.0	Insoluble	Insoluble
Unsaturated fatty acid (all double bonds are *cis* unless indicated otherwise)					
Palmitoleic acid ($C_{16:1}$, *cis*-9, ω7); Δ^9	$C_{16}H_{30}O_2$	254.41	–0.5	Insoluble	Insoluble
Sapienic acid ($C_{16:1}$, *cis*-6, ω10); Δ^6 (human sebum)	$C_{16}H_{30}O_2$	254.41	16.5	Insoluble	Insoluble
Oleic acid ($C_{18:1}$, *cis*-9, ω9); Δ^9	$C_{18}H_{34}O_2$	282.47	13.4	Insoluble	Insoluble
Ricinoleic acid (HO-$C_{18:1}$ *cis*-9, ω9); Δ^9	$C_{18}H_{34}O_3$	298.46	5.5	Insoluble	Insoluble
Elaidic acid ($C_{18:1}$, *trans*-9, ω9); Δ^9	$C_{18}H_{34}O_2$	282.47	45	Insoluble	Insoluble
Vaccenic acid ($C_{18:1}$, *trans*-11, ω7); Δ^{11}	$C_{18}H_{34}O_2$	282.47	44	Insoluble	Insoluble
Linoleic acid ($C_{18:2,}$ *cis*-9,12, ω6); $\Delta^{9,12}$	$C_{18}H_{32}O_2$	280.45	–5	Insoluble	Insoluble
Rumenic acid (a CLA; $C_{18:2,}$ *cis*-9, *trans*-11, ω7); $\Delta^{7,9}$	$C_{18}H_{32}O_2$	280.45	14.9	Insoluble	Insoluble
α-Linolenic acid ($C_{18:3}$, *all-cis*, ω3); $\Delta^{9,12,15}$	$C_{18}H_{30}O_2$	278.44	–11	Insoluble	Insoluble
γ-Linolenic acid ($C_{18:3}$, *all-cis*, ω6); $\Delta^{6,9,12}$	$C_{18}H_{30}O_2$	278.44	–11	Insoluble	Insoluble
Mead acid ($C_{20:3}$, *all-cis*, ω9); $\Delta^{5,8,11}$	$C_{20}H_{34}O_2$	306.48	–20	Insoluble	Insoluble
Sciadonic acid ($C_{20:3}$, *all-cis*, ω6); $\Delta^{5,11,14}$	$C_{20}H_{34}O_2$	306.48	---	Insoluble	Insoluble
Dihomo-γ-linolenic acid ($C_{20:3}$, *all-cis*, ω6); $\Delta^{8,11,14}$	$C_{20}H_{34}O_2$	306.48	---	Insoluble	Insoluble
Arachidonic acid ($C_{20:4}$, *all-cis*, ω6); $\Delta^{5,8,11,14}$	$C_{20}H_{32}O_2$	304.47	–49.5	Insoluble	Insoluble
Timnodonic acid (EPA; $C_{20:5}$, *all-cis*, ω3); $\Delta^{5,8,11,14,17}$	$C_{20}H_{30}O_2$	302.45	–53.5	Insoluble	Insoluble
Cervonic acid (DHA; *all-cis*, $C_{22:6}$ ω3); $\Delta^{4,7,10,13,16,19}$	$C_{22}H_{32}O_2$	328.49	–44.0	Insoluble	Insoluble
Erucic acid ($C_{22:1}$, *cis*-13, ω9); Δ^{13}	$C_{22}H_{42}O_2$	338.57	33.8	Insoluble	Insoluble
Brassidic acid ($C_{22:1}$, *trans*-13, ω9); Δ^{13}	$C_{22}H_{42}O_2$	338.57	61	Insoluble	Insoluble
Cetoleic acid ($C_{22:1}$, *cis*-11, ω11); Δ^{11}	$C_{22}H_{42}O_2$	338.57	33	Insoluble	Insoluble
Adrenic acid ($C_{22:4}$, *all-cis*, ω6); $\Delta^{7,10,13,16}$	$C_{22}H_{36}O_2$	332.52	---	Insoluble	Insoluble
Osbond acid ($C_{22:5}$, *all-cis*, ω6); $\Delta^{4,7,10,13,16}$	$C_{22}H_{34}O_2$	330.52	161.0	Insoluble	Insoluble
Nervonic acid ($C_{24:1}$, *cis*-15, ω9); Δ^{15}	$C_{24}H_{46}O_2$	366.62	42.5	Insoluble	Insoluble

Source: Bruice (2011), Lehninger et al. (1993), Mead et al. (1986), and Ralston and Hoerr (1942).

Note: Adrenic acid, 7,10,13,16-docosatetraenoic acid; EPA, 5,8,11,14,17-eicosapentaenoic acid; DHA, 5,8,11,14,17,20-docosahexaenoic acid; mead acid, 5,8,11-eicosatrienoic acid; osbond acid, 4,7,10,13,16 docosapentaenoic acid (ω6 DPA); ---, data are not available.

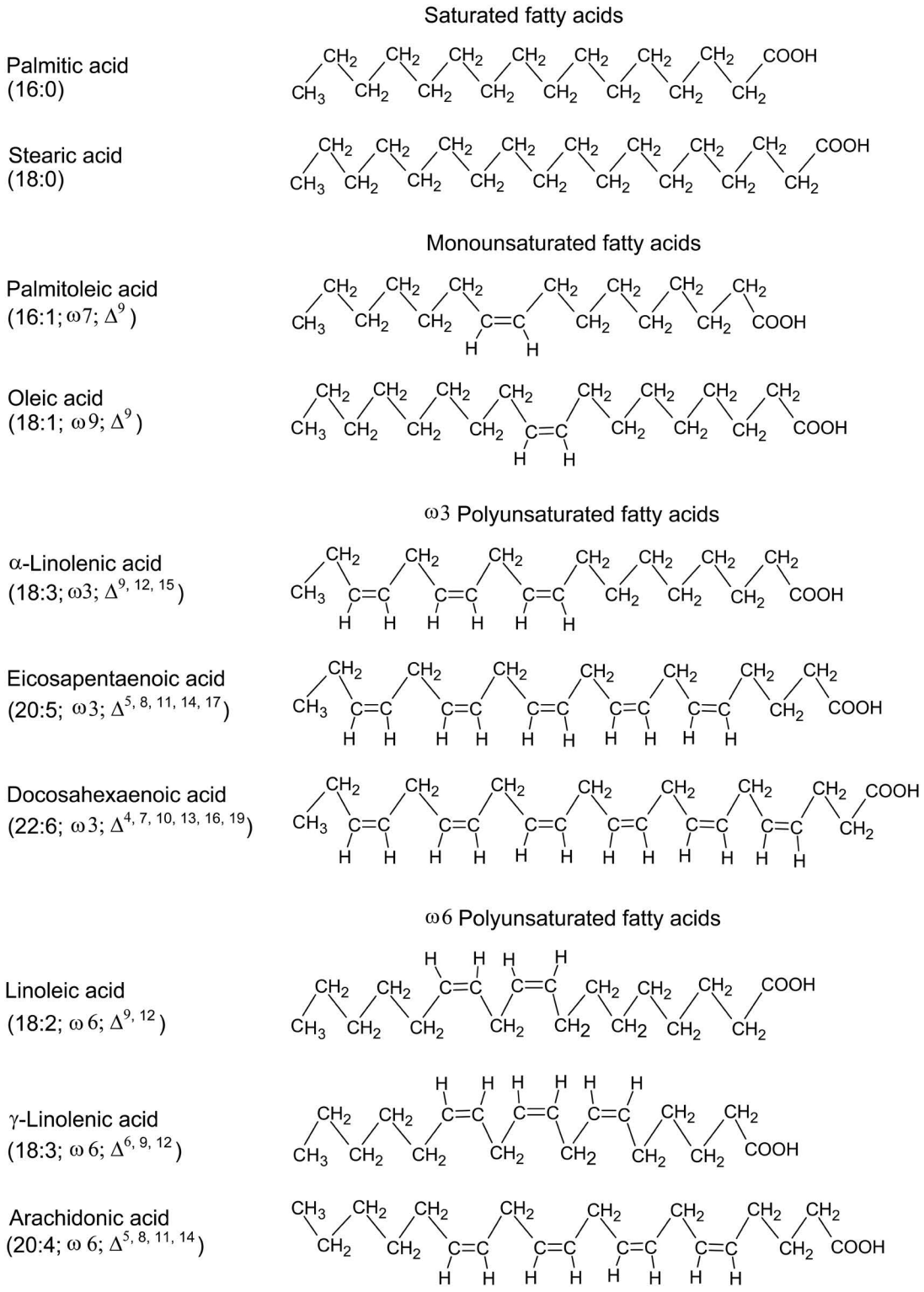


FIGURE 3.3 Saturated, monounsaturated (ω7 or ω9), ω3 polyunsaturated, and ω6 polyunsaturated fatty acids. In most animals, a double bond can be formed in a long-chain fatty acid between its Δ^1 and Δ^9 carbons, but not beyond the Δ^9 carbon. α-Linolenic acid (ω3) is the precursor of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in animals but is not synthesized by animals. Linoleic acid (ω6) is the precursor of γ-linolenic acid and arachidonic acid in animals but is not synthesized by animals. Thus, α-Linolenic acid and linoleic acid are nutritionally essential fatty acids in animals.

carbon atoms and double bonds in fatty acids (Bruice 2011). For example, octadecanoic acid (stearic acid) is a fatty acid with 18 carbon atoms and no double bond, the C_{18} fatty acid with one double bond is octadec*enoic* acid (oleic acid), the C_{18} fatty acid with two double bonds is octadeca*dienoic* acid (linoleic acid), and the C_{18} fatty acid with three double bonds is octadeca*trienoic* acid (linolenic acid).

Other aspects of the nomenclature deserve comments. A number preceding the name of a fatty acid indicates the locations of double bonds. For example, when the counting of carbon atoms begins from the carboxyl group, 9,12-octadeca*dienoic* acid is a fatty acid with 18 carbon atoms with two double bonds being located between carbons 9 and 10 and between carbons 12 and 13. In addition, the salt of a fatty acid (e.g., oleic acid) has a name ending with "-ate" (e.g., ole*ate*). The composition of fatty acids in common feedstuffs for animals and in livestock tissues is listed in Tables 3.3 and 3.4, respectively. Animal-derived foodstuffs are important sources of oleic acid (from livestock species), as well as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (from marine, cold-water fishes), but plant-derived foodstuffs contain little or no EPA or DHA.

TABLE 3.3
Composition of Individual Fatty Acids in Common Foodstuffs

		Fatty Acid, % of Crude Fat (Ether Extract)										
Foodstuff	Crude Fat[a] (%)	$C_{14:0}$ (SF)	$C_{16:0}$ (SF)	$C_{16:1}$ (ω7)	$C_{18:0}$ (SF)	$C_{18:1}$ (ω9)	$C_{18:2}$ (ω6)	$C_{18:3}$ (ω3)	$C_{18:4}$ (ω3)	$C_{20:0}$ (SF)	$C_{20:5}$ (ω3)	$C_{22:6}$ (ω3)
Alfalfa meal	1.70	0.95	12.8	0.70	1.90	2.20	9.65	18.5	0.02	0.4	0.00	0.00
Beef oil	100	5[b]	28	--	22.5	40	3	---	0.00	---	---	---
Beef tallow	100	2.7	24.9	4.2	18.9	36.0	3.1	0.6	0.00	1.1	0.06	0.16
Canola oil	100	0.10	3.99	0.38	1.71	55.1	19.5	9.31	0.00	0.64	0.00	0.00
Corn grain	3.48	0.00	12.0	0.08	1.58	26.3	44.2	1.37	0.00	0.52	0.00	0.00
Corn oil	100	0.00	10.9	0.00	1.8	24.2	59.0	0.7	0.00	0.41	0.00	0.00
Cottonseed meal	5.50	0.88	22.0	0.67	2.22	17.3	46.6	0.19	0.00	0.26	0.00	0.00
Feather meal	5.97	1.00	17.4	3.10	6.90	20.0	1.65	0.00	0.00	0.00	0.00	0.00
Fish oil (MHD)[c]	100	7.3	19.0	9.0	4.2	13.2	1.3	1.55	2.80	0.35	11.0	9.1
Flaxseed	33.8	0.02	5.14	0.06	3.15	17.5	14.0	54.1	0.01	0.50	0.00	0.00
MBM	9.21	1.89	19.3	2.59	13.4	28.5	2.52	0.63	0.00	1.05	0.00	0.00
Oats	5.42	0.22	15.0	0.19	0.94	31.4	35.1	1.61	0.00	0.1	0.00	0.00
Olive oil	100	0.0	11.0	0.8	2.2	72.5	7.9	0.6	0.00	0.4	0.00	0.00
Palm oil	100	1.0	43.5	0.3	4.3	36.6	9.1	0.2	0.00	0.39	0.00	0.00
PKO[d]	100	16.0	8.2	0.00	2.5	15.3	2.5	0.2	0.00	2.9	0.00	0.00
Peanut meal	6.50	0.00	8.73	0.00	1.82	39.8	26.0	0.0	0.00	1.2	0.00	0.00
Pork lard	100	1.3	23.8	2.7	13.5	41.2	10.2	1.0	0.00	1.3	0.12	0.20
Poultry fat	100	0.9	21.6	5.7	6.0	37.3	19.5	1.0	1.1	1.1	0.09	0.07
Rice grain	1.30	0.36	17.1	0.36	1.80	35.9	34.3	1.51	0.00	0.45	0.00	0.00
Safflower meal	2.24	0.08	6.03	0.08	2.18	11.3	65.9	0.25	0.00	0.54	0.00	0.00
SBM, dehulled	6.64	0.08	7.88	0.15	2.85	16.3	39.8	5.55	0.00	0.23	0.00	0.00
Skim milk	0.90	10.8	30.5	2.86	11.0	21.7	2.47	1.43	0.00	0.10	0.03	0.00
Sorghum	3.42	0.27	12.3	0.88	1.06	29.2	40.0	1.97	0.00	0.37	0.00	0.00
Soybean oil	100	0.1	10.3	0.2	3.8	22.8	51.0	6.8	0.00	0.41	0.00	0.00
SPC	1.05	0.22	8.26	0.22	2.83	17.0	38.5	5.22	0.00	0.3	0.00	0.00
SFM, dehulled	2.90	0.15	4.73	0.30	3.23	15.2	48.7	0.23	0.00	0.25	0.00	0.00
Wheat grain	1.82	0.06	15.2	0.52	0.84	12.5	39.0	1.75	0.00	0.10	0.00	0.00
White grease	100	1.9	21.5	5.7	14.9	41.1	11.6	0.4	0.00	0.22	0.05	0.10
Yellow grease	100	1.9	16.2	2.5	10.5	47.5	17.5	1.9	0.00	0.30	0.04	0.10

Source: Ackman (2023), INRAE (2023), Merriman et al. (2016), National Research Council (NRC) (2011), and National Research Council (NRC) (2012), Rohman et al. (2012), and and Wu et al. (2011).

[a] % of feedstuff (g/100 g) on a wet-weight basis.

[b] Including lauric acid.

[c] $C_{18:3}$ ω3 = 1.3%; $C_{18:3}$ ω6 = 0.25%. Total $C_{18:3}$ = 1.55%.

[d] Palm kernel oil contains the following fatty acids (% of the total crude fat): $C_{6:0}$, 0.3; $C_{8:0}$, 3.6; $C_{10:0}$, 3.5; and $C_{12:0}$, 48.3.

Note: CF, crude fat; MBM, meat and bone meal; MHD, menhaden; PKO, palm kernel oil; SBM, dehulled soybean meal (45% crude protein); SF, saturated fatty acid; SFM, sunflower meal (40% crude protein); SPC, soy protein concentrate (65% crude protein); ---, data are not available.

TABLE 3.4
Composition of Fatty Acids in White Adipose Tissue (WAT) and Skeletal Muscle of Steers and Swine

		Fatty Acids (%; g/g)								M/S Ratio
Animal	**Tissue**	**14:0**	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**18:3**	**TSFA**	**(g/g)**
Steer[a]	Perirenal WAT	2.9	24.3	1.1	28.4	38.3	4.4	---	55.6	0.71
	Subcutaneous WAT overlaying SM	4.3	25.8	2.2	17.2	43.4	5.3	---	47.3	0.96
	Subcutaneous WAT overlaying LD	4.1	26.7	2.8	14.9	44.7	5.6	0.28[c]	45.7	1.04
	Skeletal muscle (SM)	3.4	29.1	2.5	12.4	47.3	4.8	---	44.9	1.11
	Skeletal muscle (LD)	3.3	29.4	1.4	12.2	48.9	3.2	0.46[d]	44.9	1.12
Swine[b]	Perirenal WAT	1.0	19.4	0.5	9.4	49.0	17.4	4.3	28.8	1.72
	Subcutaneous WAT overlaying SM	0.9	18.0	0.8	6.8	53.5	16.4	3.6	25.7	2.11
	Subcutaneous WAT overlaying LD	0.8	16.7	0.8	9.2	53.2	16.2	4.1	26.7	2.02
	Skeletal muscle (SM)	1.7	26.2	1.9	6.3	52.2	10.6	0.59[e]	34.2	1.58
	Skeletal muscle (LD)	1.5	23.6	1.7	5.5	52.8	14.8	0.61[e]	30.6	1.78

Source: St John et al. (1987).

[a] Approximately 300- to 350-kg body weight steers (Angus × Hereford) in feedlots.

[b] Approximately 100-kg body weight barrows.

[c] 0.26% $C_{18:3}$ ω3 and 0.02% $C_{18:3}$ ω6 in subcutaneous WAT (Noci et al. 2007).

[d] 0.43% $C_{18:3}$ ω3 and 0.03% $C_{18:3}$ ω6 in subcutaneous WAT (Noci et al. 2007).

[e] Nowakowicz-Dębek et al. (2022).

Note: LD, longissimus dorsi muscle; M/S, monounsaturated fatty acids/saturated fatty acids; SM, Semimembranosus muscle; TSFA, total saturated fatty acids; ---, data are not available.

Short-Chain Fatty Acids

SCFAs are volatile organic substances, including acetic, propionic, butyric, isobutyric, valeric, and isovaleric acids. They are highly soluble in H_2O and are present in the rumen and blood of ruminants as products of carbohydrate and amino acid fermentation (Rook 1964). Because silages (e.g., corn, sorghum, and other cereals) are fermented during long-term storage at high moisture, they contain SCFAs. Valeric acid is also found naturally in the perennial flowering plant valerian. The production of SCFAs by ruminal bacteria is crucial for ruminant nutrition. Butyrate is a major metabolic fuel for colonocytes and also plays an important role in the health of the large intestine. Acetate is the primary substrate for *de novo* fatty acid biosynthesis in ruminant species.

Medium-Chain Fatty Acids

MCFAs contribute to approximately 58.7% and 56.4% of the total fatty acids in coconut oil and palm kernel oil, respectively (Table 3.5). Interestingly, rabbit milk contains MCFA-abundant TAGs that account for ~50% of its total TAGs, with the principal MCFAs being caprylic ($C_{8:0}$) and capric ($C_{10:0}$) acids (Lebas et al. 1996). MCFAs with 6 carbons have a good solubility in H_2O, whereas those with 8 to 12 carbons have a much lower solubility in H_2O. At physiological temperatures and concentrations, MCFAs are soluble in H_2O (Odle 1997). Approximately 10–20% of the fatty acids in the milk of horses, cows, sheep, and goats are MCFAs (Breckenridge and Kuksis 1967; Palmquist 2006; Park and Haenlein 2006). Because of their solubility in H_2O, these nutrients are absorbed from the small intestine into the portal vein. Of interest, MCFAs are highly toxic to bacteria, archaea, and protozoa, thereby possessing potent antimicrobial properties (Hassinen et al. 1951; Nieman 1954; Patra and Yu 2013).

Long-Chain Fatty Acids

LCFAs are insoluble in H_2O. They include saturated fatty acids (e.g., palmitic, stearic, and arachidic acids) and unsaturated fatty acids (e.g., palmitoleic, oleic, linoleic, α-linolenic, γ-linolenic, and arachidonic acids). Fatty acids with 20 carbons are collectively named as eicosanoic acids (e.g., arachidonic acid and EPA). In cells, myristic acid is covalently linked to some proteins, and nervonic acid is enriched in sphingolipids. In plants and animals, the most common saturated LCFA is palmitic acid, the most common monounsaturated LCFA is oleic acid, and the most common polyunsaturated LCFA is linoleic acid (Table 3.3).

TABLE 3.5
Types of Fatty Acids in Foodstuffs

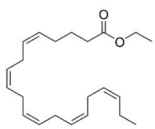
Type of Fatty Acid	% Saturated	% Monounsaturated	% Polyunsaturated
Mostly saturated			
Beef fat	52	40	8
Butter	66	30	4
Coconut oil[a]	92	6	2
Meat & bone meal	51	44	5
Palm oil	52	37	11
Palm kernel[b]	83	15	2
Skim milk powder	66	29	5
Mostly monounsaturated			
Canola (rapeseed) oil	7	62	31
Chicken fat	31	47	22
Lard (pork fat)	41	47	12
Margarine, hard	21	50	29
Olive oil	14	73	13
Peanut oil	18	46	36
Rice	21	40	39
Sesame seed oil	18	42	40
Vegetable shortening (hydrogenated)	27	50	33
Mostly polyunsaturated			
Alfalfa meal	40	6	54
Barley	25	14	61
Corn oil	13	24	63
Cottonseed oil	27	18	55
Fish oil, Menhaden	33	25	42
Flaxseed (linseed oil)	9	19	72
Margarine, soft	13	26	61
Margarine, tub	19	35	46
Oats	19	37	44
Safflower oil	10	12	78
Sorghum	16	35	49
Soybean oil	15	23	62
Soy protein concentrate	16	24	60
Sunflower oil	11	20	69
Wheat grain	23	19	58

Source: NRC 2011, 2012).

[a] Saturated fatty acids with ≤12 carbons account for 58.7% of the total fatty acids.

[b] Saturated fatty acids with ≤12 carbons account for 56.4% of the total fatty acids.

PUFAs are prone to oxidation during storage and processing, particularly at high temperatures because the $–CH_2–$ group between the two double bonds possesses reactive hydrogen atoms (Mead et al. 1986). The structure of ethyl EPA (also known as icosapent ethyl; Vascepa), a synthetic analog of EPA with a potent effect to reduce hepatic TAG production, is shown below.



Ethyl eicosapentaenoic acid [$C_{22}H_{34}O_2$; MW = 330.51; also known as icosapent ethyl; Vascepa; a synthetic analog of eicosapentaenoic acid]. Eicosapentaenoic acid is an ω3 polyunsaturated fatty acid (PUFA) that is highly abundant in fish oil and beneficial for health.

Arachidonic acid, DHA, and EPA are abundant in the plasma membranes of the brain, retina, erythrocytes, mononuclear cells, sperm, and spleen in a cell-specific manner. For example, approximate concentrations of DHA (% of the total fatty acids) in tissues of adult humans are as follows: retina, 23; cerebral cortex, 14; sperm, 14; erythrocyte, 4; spleen, 4; liver, 2.5; skeletal muscle, 2.5; heart, 2; rectal epithelium, 1.5; and WAT, 0.25 (Arterburn et al. 2006). Approximate concentrations of arachidonic acid (% of the total fatty acids) in tissues of adult humans are as follows: erythrocytes, 17; spleen, 17; liver, 12; skeletal muscle, 11; retina, 10; cerebral cortex, 10; heart, 9.5;

rectal epithelium, 7; sperm, 2.5; and WAT, 0.4 (Arterburn et al. 2006). In addition, arachidonic acid accounts for 16.0, 15.5, 9.6, 9.5, and 6.6% of the total fatty acids in mononuclear cells, erythrocytes, plasma phosphatidylcholine, platelets, and plasma cholesteryl esters, respectively, in the blood of adult humans (Walker et al. 2015). Furthermore, linoleic acid is an important component of skin ceramides in mammals, birds, and fish (Djuricic and Calder 2021). These fatty acids play important roles in cell integrity, cellular signaling, and health (including brain development, behavior, cardiovascular function, vision, reproduction, and immune responses). Whole milk is a good source of PUFAs (Table 3.6).

Vegetable oils usually contain more unsaturated LCFAs and ω6 PUFAs than animal-sourced fats (Table 3.5). Except for marine fish tissues, the content of ω3 PUFAs is much lower in animal than in plant products (Smith and Smith 1995). The composition of LCFAs in common feedstuffs is shown in Table 3.3. Linoleic acid ($C_{18:2}$ ω6) and α-linolenic acid ($C_{18:3}$ ω3) cannot be synthesized by animals and are nutritionally essential for them. Therefore, these two fatty acids must be provided to all animal diets (Spector and Kim 2015). In contrast, both linoleic acid and α-linolenic acid are synthesized by bacteria, marine algae, and plants. Safflower oil and corn oil are abundant sources of ω6 PUFAs, as are cod liver oil and salmon oil for ω3 PUFAs (Table 3.7). High amounts of both ω6 and ω3 PUFAs are available from certain plant products, such as canola oil and soybean oil (Adeola et al. 2013; Calder 2006). Most LCFAs (including arachidonic acid, oleic acid, and erucic acid) are esterified with glycerol as TAGs in microbes, plants, and animals (Ackman 2008; Gunstone 2012). Nonruminants (e.g., pigs) fed diets high in unsaturated fatty acids have thinner and

TABLE 3.7
Abundant Sources of ω6 and ω3 Polyunsaturated Fatty Acids (PUFAs) for Animal Diets

Foodstuff	ω6 PUFAs	ω3 PUFAs
Safflower oil	73% $C_{18:2}$	0.4% $C_{18:3}$
Corn oil	57% $C_{18:2}$	0.7% $C_{18:3}$
Cod liver oil	1.4% $C_{18:2}$; 1.6% $C_{20:4}$	11.2% $C_{20:5}$; 12.6% $C_{22:6}$
Salmon oil	1.2% $C_{18:2}$; 0.9% $C_{20:4}$	11% $C_{20:5}$; 13.8% $C_{22:6}$
Canola oil	22% $C_{18:2}$	9.5% $C_{18:3}$
Soybean oil	51% $C_{18:2}$	7% $C_{18:3}$

Source: National Research Council (NRC) (2011).

TABLE 3.6
Composition of Major Fatty Acids in the Milk of Mammals

Crude Fat or Fatty Acid	Cows	Sheep	Goats	Sows[a]	Humans	Cats	Dogs[b]
Crude fat (%)	3.76	6.09	4.27	6.88	3.4	12.7	9.94
			Fatty acid (% of total fatty acids, g/100 g)				
$C_{4:0}$	2.87	2.57	2.03	0.02	0.03	0.00	0.24
$C_{6:0}$	2.01	1.87	2.78	0.01	0.04	0.00	0.82
$C_{8:0}$	1.39	1.87	2.92	0.01	0.1–0.2	0.00	ND
$C_{10:0}$	3.03	6.63	9.59	0.07	1.0–1.7	0.042	0.17
$C_{12:0}$	3.64	3.99	4.52	0.27	3.0–6.1	0.12	0.09
$C_{14:0}$	10.9	10.2	9.83	3.42	4.0–6.8	1.77	3.23
$C_{14:1}$ *cis*-9	0.95	0.24	0.23	0.11	0.1–0.20	0.22	0.12
$C_{15:0}$	1.10	1.20	1.3	0.30	0.1–0.43	0.24	ND
$C_{16:0}$	28.7	25.1	24.6	31.0	21–22.2	23.2	32.4
$C_{16:1}$ *cis*-9	1.83	0.74	0.68	8.22	1.7–2.3	5.07	4.11
$C_{18:0}$	11.2	8.85	8.87	4.38	6–6.45	5.94	12.2
$C_{18:1}$ *cis*-9	22.4	20.2	18.7	30.3	33–36	37.9	16.9
$C_{18:1}$ *trans*-11	1.03	2.33	1.20	0.11	0.2–1.9	0.12	11.1
$C_{18:2}$ ω6	2.57	2.32	2.25	18.2	15–20	20.6	11.2
$C_{18:2}$ *cis*-9 *trans*-11	1.01	1.08	0.65	0.1–0.23	0.0–0.39	ND	ND
$C_{18:3}$ ω3	0.50	0.92	0.77	1.14	0.97–2.0	1.26	0.30
$C_{18:3}$ ω6	0.13	0.26	0.14	0.10	0.1–0.13	ND	ND
$C_{20:4}$ ω6	0.086	0.14	0.17	0.44	0.46–0.6	0.81	1.77

Source: Bai et al. (2017), Conte et al. (2022), Jacobsen et al. (2004), Jahreis et al. (1999), Lopez et al. (2019), Markiewicz-Kęszycka et al. (2013), Ni et al. (2021), Peterson et al. (2022), Ramiro-Cortijo et al. (2020), Ren et al. (2023), van Valenberg et al. (2013), Vieira et al. (2020), and Zhang et al. (2022).

[a] The diet of lactating sows contained 3% soybean oil.

[b] Labrador dogs.

Note: ND, not detected.

lower-quality bellies with a soft fat composition, as compared to diets with lower amounts of unsaturated fatty acids (St John et al., 1987). Importantly, dietary supplementation with saturated fat (e.g., 5% hydrogenated white grease) to growing-finishing pigs can improve pork quality (Gatlin et al. 2003).

Some unsaturated fatty acids, such as erucic acid [*cis* $C_{22:1}$ ω9; *cis*-13-docosenoic acid; $CH_3(CH_2)_7CH{=}CH(CH_2)_{11}COOH$] and cetoleic acid (*cis* $C_{22:1}$ ω11), have the same molecular weight but differ in their molecular structures, namely the position of a double bond and its hydrogen atoms in their carbon chains. The stereoisomer (*trans* $C_{22:1}$ ω9) of erucic acid is brassidic acid (*trans*-13-docosenoic acid), which occurs in nature along with bitter-tasting compounds in plants. The chemical hydrogenation of erucic acid yields behenic acid and, as a byproduct, brassidic acid. In contrast to cetoleic acid, erucic acid is an abundant fatty acid in oil-rich seeds of the *Brassicaceae* (formerly *Cruciferae*) family of natural plants (wallflower, rape, canola, and mustard), constituting up to 60% of the total fatty acids on a weight basis. For example, natural rapeseed and mustard species contain high levels of erucic acid, which usually accounts for >40% of the total fatty acids (EFSA 2016). However, the content of erucic acid in commercially bred cultivars of rapeseed developed since the 1970s is typically <0.5% of the total fatty acids. In addition to plants, erucic acid and cetoleic acid occur naturally in some fish and seafoods (e.g., salmon fillet, mackerel, and cod) at low concentrations (Ackman 2008; Batista et al. 2001). Unlike some other MUFAs that may reduce the risk of cardiovascular disease, a high intake of erucic acid (e.g., >1% of the total dietary fatty acids in infant formulae) may cause adverse health effects primarily in the heart, such as myocardial lipidosis (an accumulation of lipids in heart muscle fibers that may reduce their contractility; EFSA 2016). Interestingly, nervonic acid ($C_{24:1}$ ω9) occurs in the brain, kidneys, and liver of healthy animals (e.g., pigs and humans) at relatively high concentrations but is not toxic (Phung et al. 2024).

In bacteria, lipid metabolism results in the formation of conjugated fatty acids, which are present at relatively high concentrations in the milk and meat of ruminants (Bauman et al. 2011). Nutritionally significant conjugated fatty acids include conjugated linoleic acids (CLA) that contain both *cis*- and *trans*-double bonds in the same molecule (Figure 3.4), such as the *cis*-9, *trans*-11 configuration in ruminants (Azain 2003). *Trans*-fatty acids are rare in nature, with only small amounts occurring in the milk and fat of ruminants (e.g., cow, goat, and sheep) due to bacterial synthesis in the rumen. Most *trans*-fatty acids found in food can be produced during food processing (e.g., stir-frying, cooking at high temperatures, and the industrial hydrogenation of vegetable oils) and may adversely affect animal and human health (Nagpal et al. 2021). *cis*-fatty acids with 16 to 18 carbons comprise the bulk of the fatty acids in TAGs and compound lipids (Tan et al. 2011). Concentrations of *cis*- and *trans*-unsaturated fatty acids in cow's milk are shown in Table 3.6. *Trans*-unsaturated fatty acids are normally not found in the milk of nonruminants.

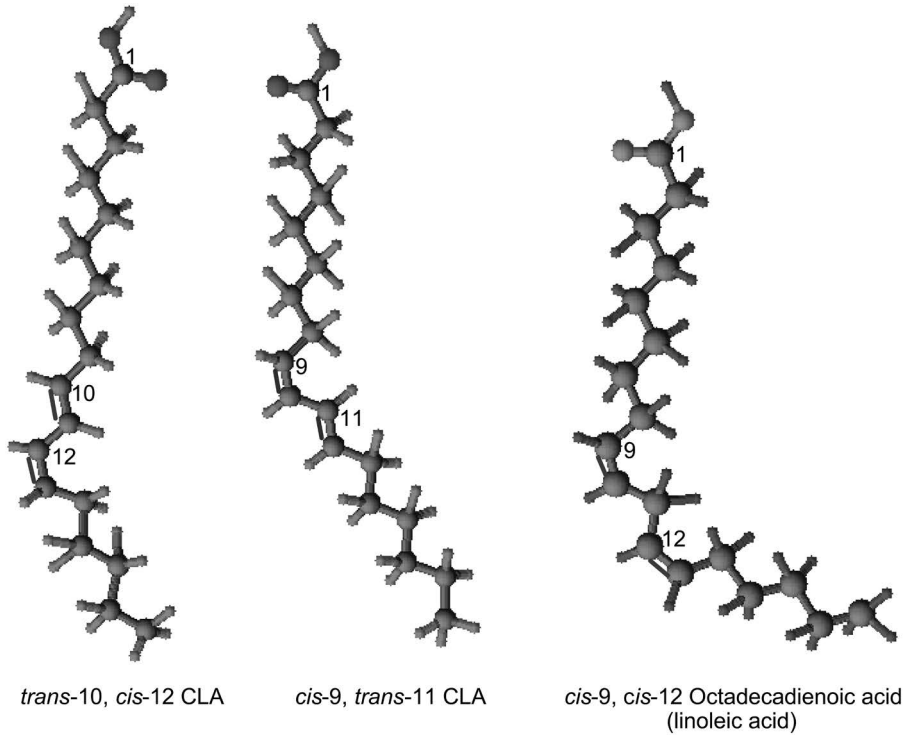


FIGURE 3.4 Conjugated fatty acids. These fatty acids are formed by bacteria (e.g., ruminal bacteria) and are present at relatively high concentrations in ruminant milk and tissues. CLA, conjugated linoleic acid. *Cis*-linoleic acid is shown for comparison.

Most naturally occurring unsaturated fatty acids have the *cis*-configuration, and the word "*cis*" is often omitted. They are commonly present in the fats and oils of animals, plants, microbes, and food. Dietary intake of LCFAs can affect the composition of *cis*-fatty acids in animal tissues (Field et al. 2008; St John et al. 1987) and reduce the microbial population in the gastrointestinal tract (GIT).

SIMPLE LIPIDS

Simple lipids consist of the glycerol-based esters of either fatty acids (e.g., in fats and oils) or fatty alcohols (e.g., in waxes). Fats differ from waxes in both chemical and biochemical properties (Rijpstra et al. 2007; Schreiber 2010). In animals, fats are generally TAGs in which all three hydroxyl groups of glycerol are esterified to either saturated or unsaturated fatty acids (Lehninger et al. 1993). TAGs are neutral molecules due to the lack of any acidic or basic groups. Solid and soft fats are made of primarily saturated and polyunsaturated LCFAs, respectively.

Fats

TAGs constitute the largest fraction (~98%) of lipids in most of concentrate foodstuffs and in animals (Ding et al. 2003; Pond et al. 2005; Rezaei et al. 2022a,b). The 1st, 2nd, and 3rd carbons of the glycerol molecule are denoted as the *sn*-1, *sn*-2 and *sn*-3 positions, respectively. TAGs are the most predominant lipids in nature and are used for the storage of LCFAs and the prevention of toxicity of LCFAs. About 95% of dry matter (DM) in adipocytes is TAGs. Small amounts of mono- and di-acylglycerols are present in bacteria, yeast, plants, and animals (Ridgway and McLeod 2016; Zinser et al. 1991). Within the same TAG molecule, the type of three fatty acids may be either the same (e.g., tristearin and triolein), which is rare, or different (e.g., 1-palmitoleoyl-2-oleoyl-3-stearoyl-glycerol; Figure 3.5; Smith and Tume 2008). At room temperature (e.g., 20–25°C), TAGs consisting of saturated fatty acids with ≥10 carbons are usually solid, whereas those with <10 carbons are liquid.

In plant oils, the percentage of unsaturated fatty acids greatly exceeds that of saturated fatty acids. In animals, fats are either solid or soft, depending on species and the composition of dietary fatty acids (Ramsay et al. 2001; Smith et al. 1998; Whitney and Smith 2015). At room temperature (e.g., 20–25°C), ruminant fats (e.g., butter and tallow) are solid, but the firmness of fats in nonruminants depends on the ratio of saturated fatty acids to PUFAs in diets. The fat or white adipose tissue content of the body is relatively

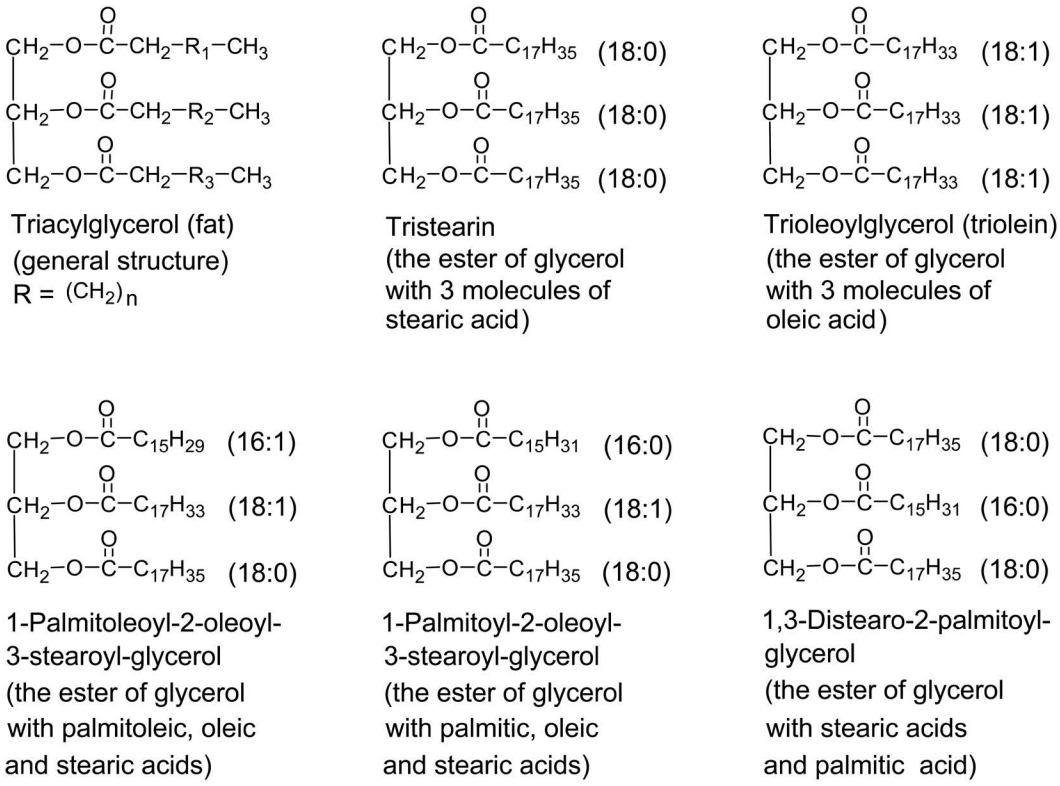


FIGURE 3.5 Triacylglycerols (triglycerides or fats) in plants, bacteria, and animals. Fats are the esters of saturated or unsaturated fatty acids with glycerol. Within the same fat molecule, the three fatty acids may be the same (e.g., tristerin and triolein) or different (e.g., 1-palmitoleoyl-2-oleoyl-3-stearoyl-glycerol). R_1, R_2, and R_3 represent alkyl groups [$-(CH_2)_n$], with either the same or different numbers of carbons. The first, second, and third carbons of the glycerol molecule are denoted as the sn1, sn2, and sn3 positions, respectively.

TABLE 3.8
Fat Content in the Whole Bodies of Animals at Birth and Older Ages[a]

	At Birth		Older Ages		
Animal Species	Body Weight (kg)	Fat Content (%, g/100 g)	Bogy Weight (kg)	Age (days)	Fat Content (%, g/100 g)
Broiler	0.038	1.5	2.6	42	13
Cat	0.12	1.8	4.5	360	15
Cattle	35	3.0	545	540	31
Dog[b]	0.1–0.6	1.5	2–80	730	15–25
Goat[b]	2.0–3.6	2–3	25–100	1095	8–16
Guinea pig	0.08	10.1	1.0	200	30
Horse	45	3.0	450	1800	18
Human	3.5	16.1	70	9125	13
Mouse	0.0016	2.1	0.03	60	15
Pig	1.4	1.1	110	180	36
Rabbit	0.054	2	2.3	63	8.0
Rat	0.0058	1.1	0.3	90	15
Sheep	4.0	3.0	52	180	20

Source: Cherian (2015), Conde-Aguilera et al. (2013), Fox et al. (2002), and Pond et al. (2005).

[a] In all animal species, the values for body weight and fat content were reported in the literature, and may vary with breeds, sex, feeding levels, and the ambient environment.

[b] Body weight and fat content at both birth and adults vary greatly among different breeds.

low at birth but increases with age (Table 3.8). As noted previously, TAGs are the main form of energy storage in animals and confer protective functions in the skin and subcutaneous tissues. Interestingly, in marine mammals (e.g., whales whose heads represent 30% of the BW), fats in their heads have an acoustic sensory role (Madsen and Surlykke 2013). Excess WAT results in overweight, obesity, insulin resistance, and numerous health problems (Bergen and Brandebourg 2016; Jobgen et al. 2006). Thus, the content of fats in animals must be precisely regulated.

Waxes

Waxes are esters of aliphatic or alicyclic fatty acids with long-chain monohydric alcohols (one hydroxyl group). Natural waxes are often mixtures of such esters and occur as malleable solids at room temperature (e.g., 20–25°C). The fatty acids are usually saturated LCFAs ($\geq C_{13}$), including stearic acid ($C_{18:0}$), lignoceric acid (also called carnaubic acid, $C_{24:0}$), and melissic acid ($C_{30:0}$), while fatty acids with $\leq C_{12}$ are rare (Butovich et al. 2009). Waxes are widely present in plants and animals (e.g., animal skins and bird feathers) to provide protective function and a waterproof layer (Buschhaus and Jetter 2011; Chung and Carroll 2015).

The most common alcohols in natural waxes are oleoyl [CH_3-$(CH_2)_7$-CH=CH-$(CH_2)_8$-OH], cetyl [CH_3-$(CH_2)_{15}$-OH], carnaubyl [CH_3-$(CH_2)_{23}$-OH], and melissyl [CH_3-$(CH_2)_{29}$-OH]. Of note, the uropygial glands of birds produce unique waxes, which are esters of hydroxy acids (type-I wax) or alkane-1,2-diols (type-II wax) with medium- to long-chain fatty acids (Kolattukudy et al. 1987; Rijpstra et al. 2007). Carnauba wax [$CH_3(CH_2)_{30}CO_2$-$(CH_2)_{33}CH_3$] is abundant in the leaves of the Carnauba palm, a plant native to the northeastern Brazilian states. Lanolin (also called wool wax or wool grease) is an animal wax secreted by the sebaceous glands of wool-bearing animals (e.g., sheep). Spermaceti [$CH_3(CH_2)_{14}CO_2$-$(CH_2)_{15}CH_3$] is another type of animal wax produced by some marine animals (e.g., sperm whale; Takagi and Itabashi 1977). Cetyl palmitate is a major component of beeswax [$CH_3(CH_2)_{24}CO_2$-$(CH_2)_{29}CH_3$]. Waxes are highly water-insoluble due to the weakly polar nature of the ester group (Figure 3.6). Unlike fats, waxes cannot be digested or utilized as a nutrient by animals.

COMPOUND LIPIDS

Compound lipids are the glycerol-based esters of fatty acids (e.g., usually LCFAs) that contain phosphoric acid (i.e., phospholipids and ether phospholipids), carbohydrates (i.e., glycolipids), sphingosine (i.e., simple and complex sphingolipids), and/or proteins. These lipids often possess nitrogen (N) in the form of choline, ethanolamine, or serine (Guidotti 1972; Ridgway and McLeod 2016). Like simple lipids, compound lipids are saponifiable in plants, bacteria, and animals. Note that the proteins in lipoproteins are not covalently bound to the phospholipids of compound lipids.

Glycolipids (Glycerol-Glycolipids)

Glycolipids are esters of glycerol. They are lipids with a carbohydrate (e.g., monosaccharide or oligosaccharide)

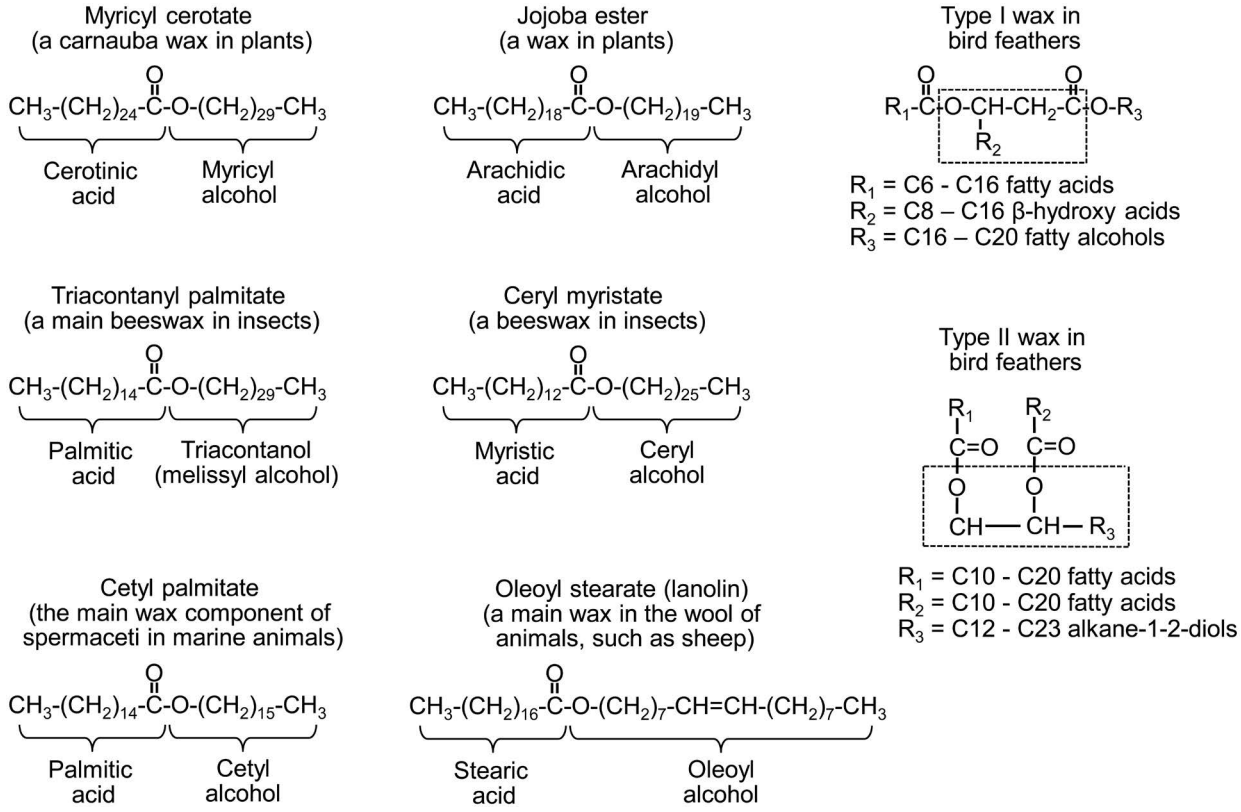


FIGURE 3.6 Waxes in plants and animals. Waxes are the esters of fatty acids with aliphatic or alicyclic higher molecular weight monohydric alcohols. This class of lipids provides plants and animals with a waterproof layer of protection. Unlike fats, waxes cannot be digested or utilized as a nutrient by animals.

joined together by a glycosidic bond (Figure 3.7) and are widely present in plants, bacteria, fungi, and animals (Mead et al. 1986). These substances are amphiphilic components of cell membranes, with a hydrophilic polar sugar backbone on the outer surface of all eukaryotic cell membranes and a hydrophobic nonpolar lipid moiety anchoring in the membrane.

Glycolipids are the major lipid component of forages (McDonald et al. 2021). In these compounds, two of the hydroxy groups of glycerol are esterified by fatty acids, mainly linoleic acid, and one or two moles of a sugar (e.g., galactose) are added to the *sn*-3 position of the glycerol. In the leaf tissue, lipid content (consisting of ~60% galactolipids) ranges from 3% to 10% of the DM. In myelin, which coats the axon of a neuron to confer protection and help speed nerve transmission, glycolipids are the most abundant compound lipids (Tanford 1980). In animals, glycolipids serve as markers for cell recognition, energy sources, and regulators of signal transduction and metabolism.

Phospholipids (Phosphatides; Phosphatidic Acid)

Phospholipids are esters of glycerol, in which two of its three hydroxyl groups are esterified by LCFAs and its third hydroxyl group is esterified to phosphoric acid (Figure 3.7). Phosphatidic acid (the glycerol-based phospholipid with fatty acids being esterified at the *sn*-1 and *sn*-2 positions, as well as a phosphate group in the *sn*-3 position) is the common precursor for the synthesis of other phospholipids (Colbeau et al. 1971). In most cases, a saturated fatty acid is at the *sn*-1 position and an unsaturated fatty acid at the *sn*-2 position (Table 3.9). The *sn*-3 position contains a N or non-N compound (Gunstone 2012).

Based on its chemical structure, the phospholipid molecule generally has two "tails" of hydrophobic fatty acids and one "head" of hydrophilic phosphate moiety, joined together by a glycerol molecule (Vance 2014). The replacement of the "X" group in the general glycerol-phospholipid structure (Figure 3.7) by ethanolamine, choline, serine, myo-inositol, glycerol, or phosphatidylglycerol generates a corresponding phospholipid derivative (Figure 3.8). For example, phosphatidylcholine (termed lecithin), which was the first phospholipid to be identified in the egg yolk of chickens in 1847, contains choline. Other common phospholipids are phosphatidylethanolamine (cephalins), phosphatidylserine, phosphatidylinositol, and diphosphatidylglycerol (cardiolipin). Phosphatidylcholine is the most abundant phospholipid in animal tissues and is also predominant in

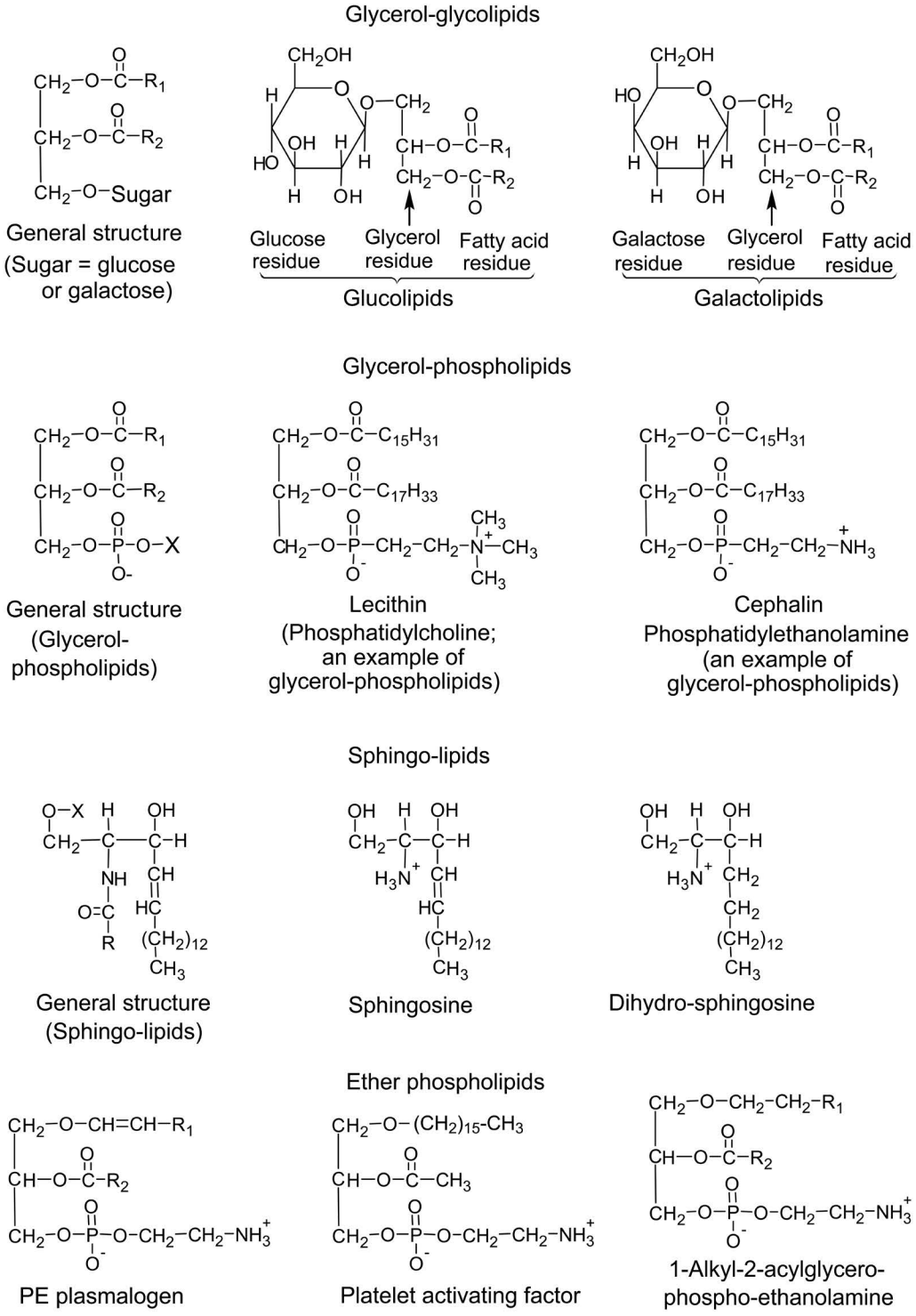


FIGURE 3.7 The general structures and examples of glycerol-based glycolipids, glycerol phospholipids, and ether phospholipids, as well as sphingosine-based sphingolipids in plants, bacteria, and animals. Glycolipids and phospholipids contain carbohydrates and phosphate groups, respectively. Sphingosine is a long-chain amino alcohol and is synthesized from serine and acetyl-CoA in tissues (e.g., the brain and liver). Ether glycerophospholipids have an ether linkage ($-CH_2-O-CH_2-$) in the *sn*-1 position, an ester linkage ($-O-CO-CH_2-$) at the *sn*-2 position, and often a phosphoethanolamine ($-PO_3-CH_2-CH_2-NH_3^+$) group at the *sn*-3 position. R_1 is commonly the alkyl group [$-(CH_2)_n-CH_3$] of a long-chain saturated fatty acid and R_2 is often the hydrocarbon of an unsaturated fatty acid (e.g., oleic acid). In sphingolipids, R is an alkyl group of a saturated fatty acid with different numbers of carbons. The symbol "X" represents a specific group. PE, phosphatidylethanolamine.

TABLE 3.9
Positional Distribution of Fatty Acids in Liver and Brain Phosphatidylserine

		Fatty Acid (% of Total Fatty Acids)					
Tissue	**Position**	**16:0**	**18:0**	**18:1**	**18:2**	**20:4**	**22:6**
Rat liver	*sn*-1	5	93	1	0	0	0
	sn-2	6	29	8	4	32	19
Bovine brain	*sn*-1	3	81	13	0	0	0
	sn-2	2	1	25	trace	1	60

Source: Wood and Harlow (1960) and Yabuuchi and O'Brien (1968).

Note: *sn*, a "stereospecific numbering" system for the positions of fatty acids in the glycerol backbone of a triglyceride molecule.

the membranes of animal cells and yeast (Table 3.10). In nutrition, soybean lecithin (98% acetone insolubles) is often used as a rich source of phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol. Other good sources of phospholipids are rapeseed, sunflower, chicken eggs, bovine milk, brain, liver, and fish. Phospholipids are (a) a major component of all cell membranes; (b) second messengers in cell signaling (in which they frequently contain arachidonic acid in the *sn*-2 position); (c) sources of choline, inositol, and physiologically important fatty acids; (d) mediators of the metabolism of nutrients (including lipids, amino acids, and glucose), intestinal absorption of lipids; and (e) facilitators for the interorgan transport of cholesterol and lipoproteins (Jacobs et al. 2005).

Sphingolipids

This class of lipids is based on sphingosine (a C_{18} amino alcohol) instead of glycerol (Figure 3.7). They were discovered in brain extracts in the 1870s and are now known

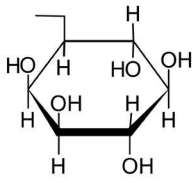
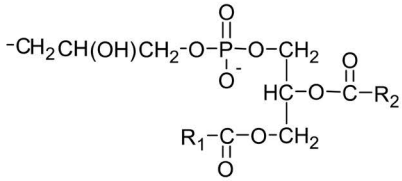
Name of X	Formula of X	Name of Phospholipid
-	-H	Phosphatidic acid
Ethanolamine	$-CH_2CH_2\overset{+}{N}H_3$	Phosphatidylethanolamine (cephalin)
Choline	$-CH_2CH_2\overset{+}{N}(CH_3)_3$	Phoshatidylcholine (lecithin)
Serine	$-CH_2CH(\overset{+}{N}H_3)COO^-$	Phosphatidylserine
myo-Inositol		Phosphatidylinositol
Glycerol	$-CH_2CH(OH)CH_2OH$	Phosphatidylglycerol
Phosphatidylglycerol		Diphosphatidylglycerol (cardiolipin)

FIGURE 3.8 The structures of abundant phospholipids in plants, bacteria, and animals. The symbol "X" in the general structure of phospholipids (Figure 3.7) is replaced by various groups (e.g., ethanolamine, choline, serine, or myo-inositol) to form different phospholipids (e.g., phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, or phosphatidylinositol). These compounds are all glycerol-based lipids.

TABLE 3.10
Composition of Lipids, Proteins, and Carbohydrates in Some Biological Membranes

	Cell Membrane				Outer MM		Inner MM		Bovine Heart		
Variable	Human RBCs	Rat Liver[b]	E. Coli	Yeast[c]	Rat Liver	Yeast	Rat Liver	Yeast	Mit	ER	Human Myelin
Protein, % (g/100 g)	49	52	68	53	52	52	76	84	74	55	18
Carbohydrates, % (g/100 g)	8	8	VL	VL	2–4	---	1–2	VL	VL	VL	3
Lipids, % (g/100 g)	43	40	32	47	48	48	24	16	24	45	79
Percent (%) of total lipids											
Phosphatidic acid[a]	1.5	2.4	0	1	1	4	1	1.7	0	1	0.5
Phosphatidylcholine[a]	19	20	0	4.4	42	46	40	32	39	60	10
Phosphatidylethanolamine[a]	18	11	65	5.1	27	33	34	20	27	23	20
Phosphatidylglycerol[a]	0	2.7	18	1.8	0.4	VL	VL	4	VL	VL	VL
Phosphatidylinositol[a]	1	4.0	2	4.7	11	10	2	13	7	10	1
Phosphatidylserine[a]	8.5	5.0	2	8.7	2	1	3	3.3	0.5	2	8.5
Cardiolipin[a]	0	0.3	12	0.3	5	6	18	13	22.5	1	0
Sphingomyelin[a]	17.5	10	0	0	2	0	2	0	0	3	8.5
Glycolipids[a]	10	5.6	0	70	0	0	0	0	0	VL	26
Cholesterol[a]	25	20.4	0	0[d]	10	0	1.5	0	3	VL	26

Source: Colbeau et al. (1971), Daum (1985), Guidotti (1972), Horvath and Daum (2013), Ray et al. (1969), Tanford (1980), and Zinser et al. (1991).

[a] Expressed as % of the total lipids by weight.

[b] In the liver cell membrane, phospholipids, glycolipids, and neutral lipids account for 55.4%, 5.6%, and 39% of the total lipids by weight. Neutral lipids (% of the total lipids) consist of the following: triacylglycerols, 3.9; diacylglycerols, 1.6; monoacylglycerols, 1.8; free cholesterol, 18.1; esterified cholesterol, 0.9; cholesterol esters, 1.4; free fatty acids, 7.9; hydrocarbons, 2.5; unidentified lipids, 0.9 (Ray et al. 1969).

[c] Yeast *Saccharomyces cerevisiae*.

[d] Yeast *Saccharomyces cerevisiae* contains no sterol or sphingomyelin, but its cell membrane has ergosterol and sphingolipids instead. The content of phospholipids, ergosterol and sphingolipids in the yeast cell membrane (mg/mg protein) is 0.23, 0.4 and 0.27, respectively.

Note: ER, endoplasmic reticulum; MM, mitochondrial membrane; RBCs, red blood cells; VL, very low.

to be synthesized from serine plus acetyl-CoA (Morell and Braun 1972). The replacement of the "X" group in the general sphingolipid structure (Figure 3.7) by phosphocholine, glucose, galactose, lactose, and complex oligosaccharides yields a corresponding new sphingolipid derivative (Figure 3.9). An example of sphingolipids is ceramide, which is composed of sphingosine and a fatty acid joined by an amide bond. Typically, the sphingosine backbone is linked to: (a) an acyl group (e.g., a fatty acid) via the amide linkage to form a ceramide; (b) a nitrogenous charged head group (e.g., ethanolamine, serine, or choline) to yield a sphingomyelin; or (c) various sugar monomers or dimers to generate glycosphingolipids (e.g., cerebrosides; Ridgway and McLeod 2016). Like phosphatidylcholine and cephalins, sphingomyelins are surface-active and serve as important components of cell membranes in animal cells (Daum 1985). Of note, *Escherichia coli* and yeasts do not have sphingomyelin (major phosphosphingolipids in mammals). Instead, the cell membrane of *E. coli* consists primarily of phospholipids (e.g., phosphatidylglycerol, phosphatidylethanolamine, and cardiolipin), and yeasts have inositol phosphosphingolipids. These phospholipids may function like the mammalian sphingomyelin (Horvath and Daum 2013).

Sphingolipids have a common structural feature: a backbone of sphingoid bases (aliphatic amino alcohols), including sphingosine. Based on their structures, sphingolipids are classified as simple or complex. Simple sphingolipids include sphingoid bases (the fundamental building blocks of all sphingolipids) and ceramides, whereas complex sphingolipids are sphingomyelins, glycosphingolipids, cerebrosides (including sulfated cerebrosides), gangliosides (having at least three sugars, with one being sialic acid), and inositol-containing ceramides (Figure 3.10). Sphingolipids are particularly abundant in tissues (e.g., the brain) of the nervous system, constituting, for example, up to 25% of the total lipids in the myelin sheaths of nerve cells (Maceyka and Spiegel 2014). In addition, sphingolipids serve as adhesion sites for extracellular proteins and play important roles in cell signal transmission and cell recognition. Meat, dairy products, eggs, and soybeans are good dietary sources of ceramides, sphingomyelins, cerebrosides, gangliosides, and sulfatides as sphingolipids.

Ether Glycerophospholipids

Ether glycerophospholipids consist of a glycerol molecule, two fatty acid molecules, a phosphate group, and an ethanolamine molecule. This class of lipids possesses an ether

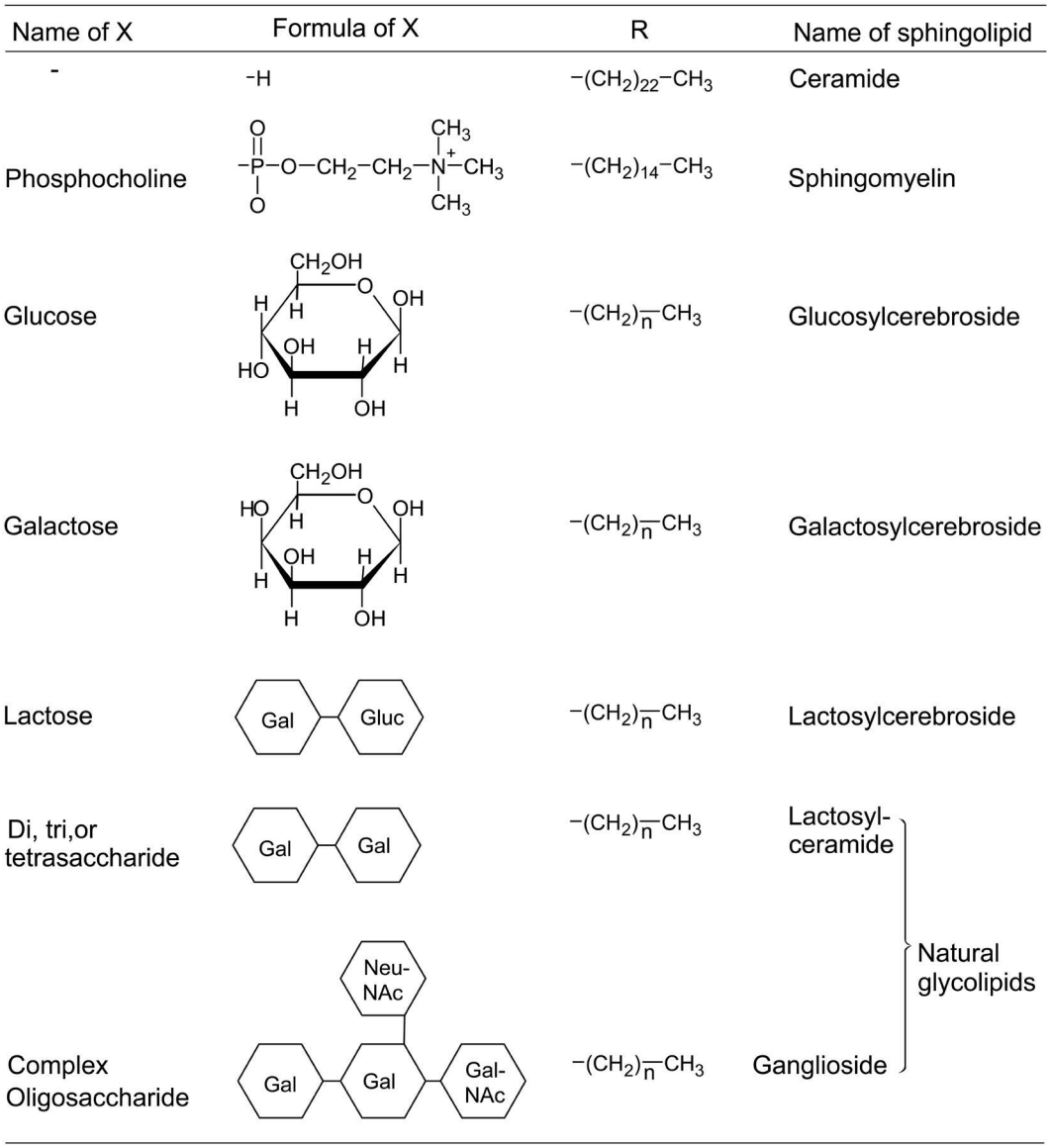


FIGURE 3.9 The structures of abundant sphingolipids in plants, bacteria, and animals. The symbols "X" and "R" in the general structure of sphingolipids (Figure 3.7) are replaced by various groups to form different sphingolipids. For example, when X = "H" and R = "-$(CH_2)_{22}$-CH_3)," the sphingolipid is called ceramide; when X = "phosphocholine" and R = "-$(CH_2)_{14}$-CH_3)," the sphingolipid is called sphingomyelin.

linkage (-CH_2-O-CH_2-) in the *sn*-1 position, an ester linkage (-O-CO-CH_2-) at the *sn*-2 position, and a phosphoryl nitrogenous head group up at the *sn*-3 position (Lehninger et al. 1993). Examples of ether glycerophospholipids are plasmalogens (alkenyl ether phospholipids) and the platelet activating factor (Figure 3.10).

Plasmalogens, which were first reported in 1924, are a major group of ether phospholipid. In these compounds, the *sn*-1 position is usually derived from a $C_{16:0}$, $C_{18:0}$, or $C_{18:1}$ fatty alcohol, the *sn*-2 position is most commonly linked to a PUFA, and the head group at the *sn*-3 position contains choline, ethanolamine or serine. In mammals and birds, the most common head groups present in plasmalogens are phosphoethanolamine [-PO_3-CH_2-CH_2-NH_3^+] or phosphocholine (Lehninger et al. 1993). Plasmalogens are abundant in many tissues, particularly those of the nervous, immune, and cardiovascular systems. Notably, in the heart, plasmalogens represent 30–0% of choline glycerophospholipids, 30% of the glycerophospholipids in the brain, and up to 70% of myelin sheath ethanolamine glycerophospholipids (Ridgway and McLeod 2016).

The platelet activating factor (acetyl-glyceryl-etherphosphorylcholine) was discovered in the 1970s and was the first phospholipid known to have intracellular messenger functions. Its *sn*-1 position is linked via an ether linkage to a saturated fatty acid (palmitate), its *sn*-2 position is connected to an acetate unit via an ester linkage (for increasing solubility in H_2O), and its *sn*-3 position is occupied by phosphocholine (Gunstone 2012). The platelet activating factor

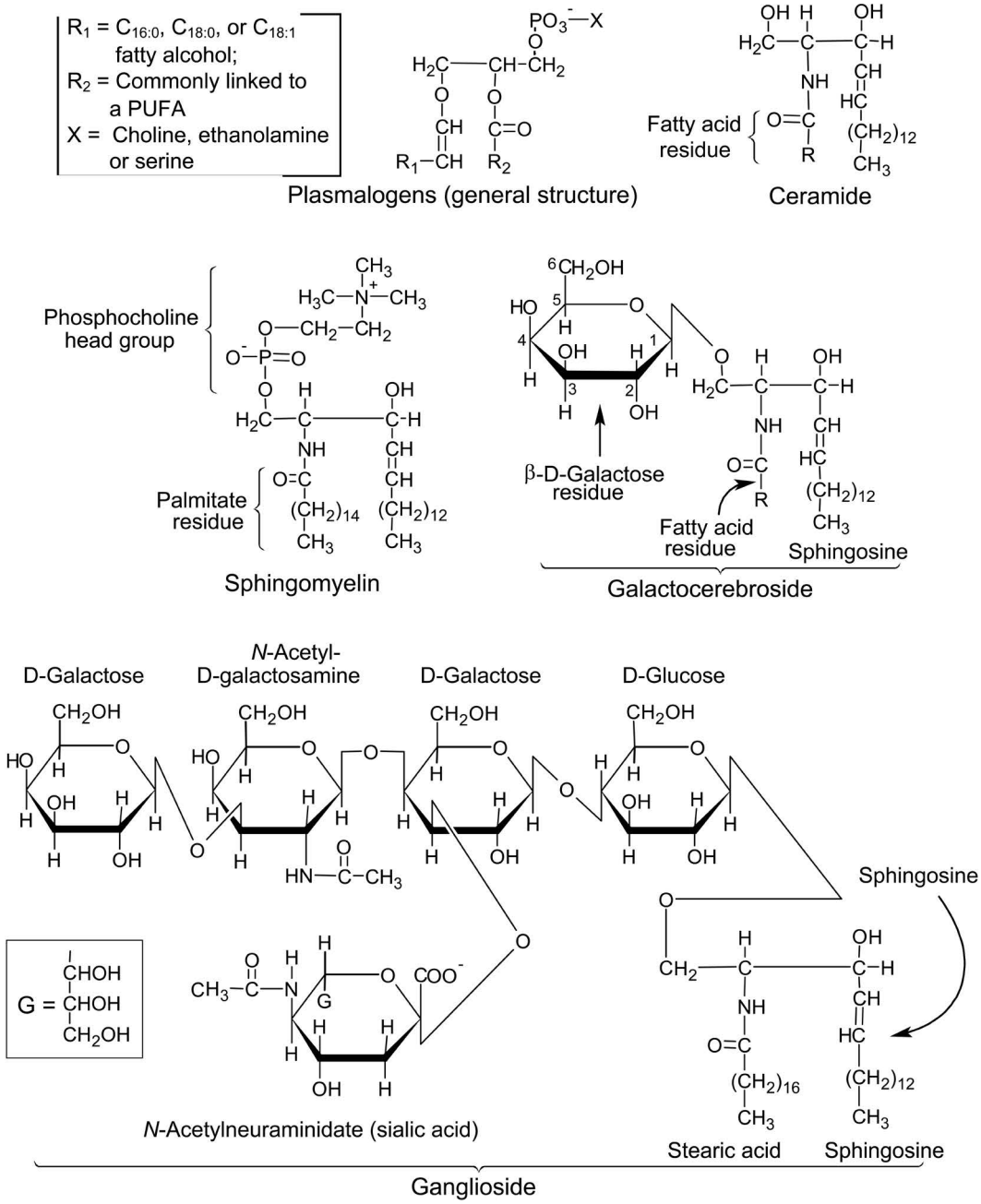


FIGURE 3.10 The general structure of plasmalogens, as well as specific structures of ceramide, phosphocholine sphingomyelin, galactocerebroside, and ganglioside. The sphingosine backbone is linked to: (a) an acyl group (e.g., a fatty acid) via the amide linkage to form a ceramide; (b) a nitrogenous charged head group (e.g., ethanolamine, serine, or choline) to yield a sphingomyelin; or (c) various sugar monomers or dimers to generate glycosphingolipids (e.g., cerebrosides).

plays an important role in mediating the chemotaxis and functions of leukocytes, platelet aggregation and degranulation, inflammation, anaphylaxis, vascular permeability, and the oxidative burst in animals.

Lipoproteins

Lipoproteins are lipids associated noncovalently with special proteins and are synthesized and released by the small intestine and the liver (Ridgway and McLeod 2016). The protein moiety serves to carry and emulsify the lipid molecules. Examples of lipoproteins are high-, intermediate-, low-, and very low-density lipoproteins, as well as chylomicrons (Table 3.11). They have different proportions of protein, cholesterol, cholesteryl ester, phospholipids, and TAGs, and, therefore, different density values. In essence, high-density lipoproteins and chylomicrons (the major small-intestinal lipoprotein) contain the highest proportions of protein and TAGs, respectively (Besnard et al. 1996).

TABLE 3.11
Composition of Small-Intestinal (SI) and Plasma Lipoproteins in Animals

		Class of Lipoprotein				
Physicochemical Property	**Source**	**Chylomicrons**	**Very-Low-Density Lipoproteins**	**Intermediate-Density Lipoproteins**	**Low-Density Lipoproteins**	**High-Density Lipoproteins**
Density, g/mL	SI	< 0.95	0.95–1.006	1.006–1.040	1.04	1.12
	Plasma	0.94	0.94–1.006	1.006–1.019	1.019–1.063	1.063–1.21
Size, nm in diameter	SI	70–600	28–70	40	22–26	5–15
	Plasma	80–500	30–80	25–30	16–25	7–13
Protein, %	SI	1.5–2	5–10	11	20–22	40–50
	Plasma	1.5–2.5	5–10	15–20	20–25	40–55
Major apoproteins	SI	B48,A1,A4	B48,A1,A4	B48,A1,A4	B48	A1
(Apo)	Plasma	B48,A1,A2 C1,C2,C3,E	B100,C1,C2 C3,E	B100,C3,E	B100	A1,A2,C1 C2,C3,D,E
Total lipids, %	SI	98–98.5	90–95	89	78–80	50–60
	Plasma	97–98.5	90–95	80–85	75–80	45–60
Triacylglycerols, %	SI	85–88	50–55	24–30	10–15	3–5
	Plasma	84–89	50–65	22	7–10	3–5
Cholesteryl ester, %	SI	3	12–15	25–30	37–45	15–20
	Plasma	3–5	10–15	30	35–42	12–20
Free cholesterol[a], %	SI	1	8–10	8–10	8–10	2–5
	Plasma	1–3	5–10	8	7–10	3–5
Phospholipids, %	SI	8	18–20	25–27	20–28	26–30
	Plasma[b]	7–9	15–20	22	15–22	25–30
Nonesterified fatty	SI	0	1	1	1	2
acids, %	Plasma[b]	0	1	1	1	2

Source: Besnard et al. (1996), Devlin (2006), Kessler et al. (1970), Mead et al. (1986), and Swift et al. (1988).

[a] Surface components.

[b] Mainly phosphatidylcholine.

Note: A1 and A2, apolipoproteins A-1 and A-2; B100, apolipoprotein B-100; C1, C2 and C3, apolipoproteins C1, C2 and C3; D, apolipoprotein D; E, apolipoprotein E; SI, small intestine.

Lipoproteins are present in the blood, transmembrane proteins, and intracellular fatty acid-binding proteins, as well as some antigens, adhesins, and toxins. Apolipoproteins function to (a) guide the formation of lipoproteins; (b) the transport LCFAs, TAGs, and cholesterol from the small intestine to lymphatic vessels (lacteals), from the liver to peripheral tissues, and in the circulation; (c) act as ligands for lipoprotein receptors; and (d) regulate the metabolism of lipoproteins (Gunstone 2012; Ridgway and McLeod 2016). Chylomicron remnants, very low-density lipoproteins (VLDLs), intermediate-density lipoproteins (IDLs), and low-density lipoproteins (LDLs) are pro-atherogenic, whereas high-density lipoproteins (HDLs) are antiatherogenic and antioxidative.

DERIVED LIPIDS

Definition

Derived lipids are lipids that are formed from cholesterol or isoprene compounds (Gunstone 2012). Their structures are complex and can be classified as steroids, eicosanoids, and terpenes. Both steroids and terpenes are nonsaponifiable. Steroids have the cyclopentanophenanthrene ring (Figure 3.11) and include sterols (e.g., cholesterol), steroid hormones (e.g., male and female reproductive hormones), bile acids, and bile alcohols. Of note, steroidal saponins are present only in plants (e.g., Yucca). Eicosanoids, which contain 20 carbons and one or more oxygen atoms in addition to a carboxyl group, are the oxygenated or hydroxylated derivatives of C_{20} PUFAs (dihomo-γ-linolenic acid, arachidonic acid, and EPA) in animal cells (Cannon 1984). Eicosanoids include prostaglandins (PGs), thromboxanes (TXs), leukotrienes, and lipoxins. Terpenes (e.g., carotenoids, coenzyme Q, vitamins E and K, essential oils, and phytols) are a diverse group of organic compounds that contain isoprene units (Zerbe and Bohlmann 2015). The name "terpene" is derived from the Greek word "turpentine," meaning a species of the terebinth tree. Terpenes are produced by a variety of plants, green algae, and some insects (e.g., termites and swallowtail butterflies) and frequently consumed by animals including (including pigs, humans, ruminants, poultry, and fish (Pichersky 2006).

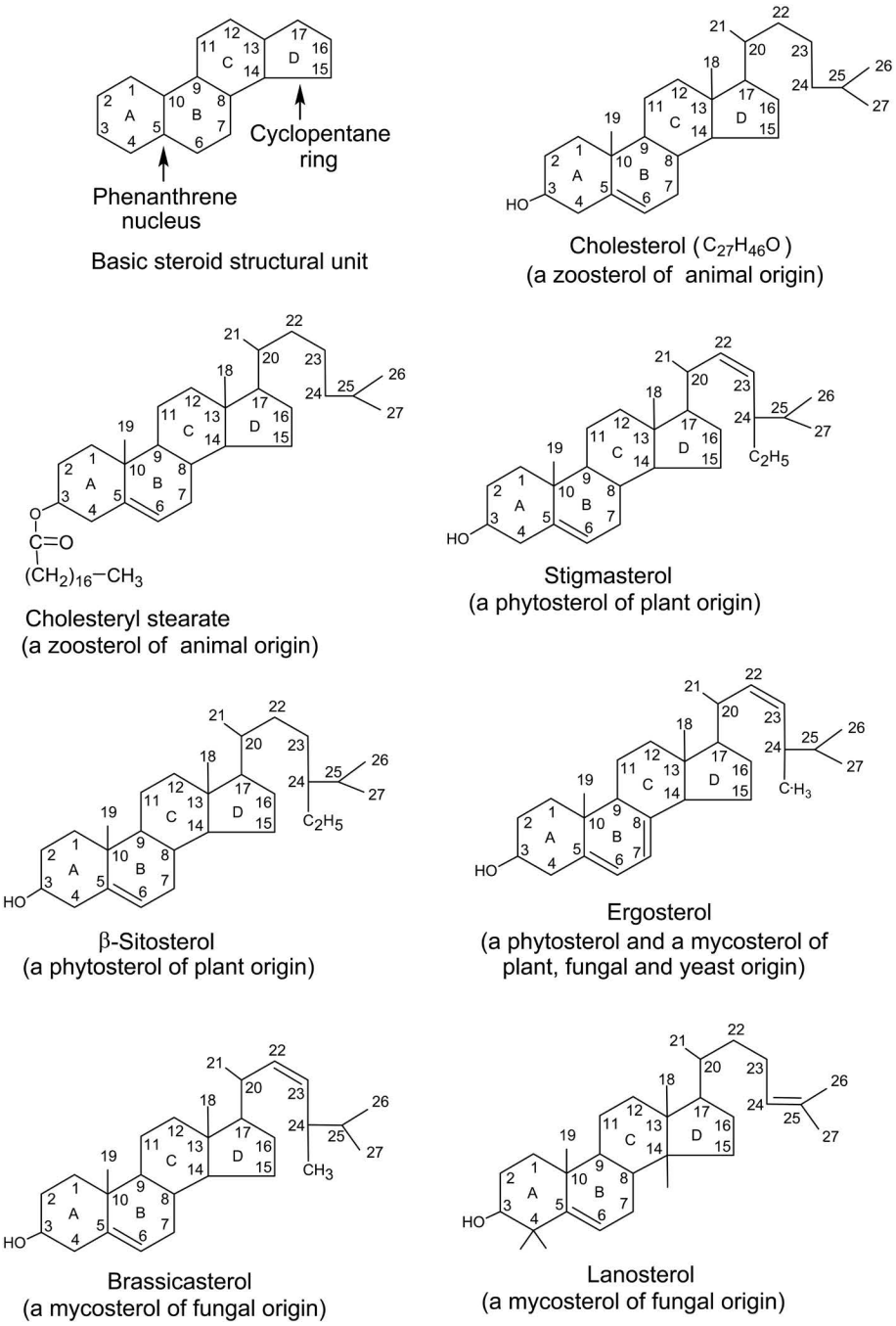


FIGURE 3.11 Structures of sterols in plants, animals, and microbes. Sterols are steroid alcohols, including zoosterols of animal origin [e.g., cholesterol (5-cholesten-3β-ol) and cholesteryl stearate], phytosterols of plant origin (e.g., stigmasterol and β-sitosterol), and mycosterols of fungal origin (e.g., brassicasterol and lanosterol). Ergosterol is present in plants, fungi, and yeasts. In cholesterol, the –OH group at C-3 faces up in the six-membered ring, constituting the β-anomer.

The difference between terpenes and terpenoids is that terpenoids contain additional functional groups beyond the terpene hydrocarbon chain.

Steroids

Sterols

Sterols are steroid alcohols, such as phytosterols of plant origin, mycosterols of fungal origin, and zoosterols of animal origin. Zoosterols include cholesterol, 7-dehydrocholesterol, and the sulfated conjugate of cholesterol, all of which are components of skin lipids (Gunstone 2012). The term cholesterol was originally coined from the Greek *chole-* (bile) and *stereos* (solid), followed by the chemical suffix *-ol* meaning an alcohol. Cholesterol is an essential component of cell membranes and plays an important role in the metabolism, growth, development (including neurological development), and reproduction of animals, but an excessive amount of this lipid in the blood increases the risk for

cardiovascular disorders. Note that animal-sourced, but not plant-sourced, foodstuffs contain cholesterol. The content of cholesterol in beef tallow, pork lard, and poultry fat is 0.11%, 0.10%, and 0.09%, respectively (USDA 2022).

Zoosterols, but not phytosterols or mycosterols, are absorbed by the small intestine of animals (Ridgway and McLeod 2016). However, ergosterol is a sterol widely distributed in brown algae, bacteria, yeast, and higher plants, but these organisms contain little or no cholesterol. Ergosterol is converted by sunlight into ergocalciferol (vitamin D_2), which is then biologically available to animals. In contrast, animals contain cholesterol but not ergosterol. In mammals, birds, and fish, cholesterol is the common precursor for the synthesis of 7-dehydrocholesterol (the precursor of vitamin D_3), steroid hormones, bile acids, and bile alcohols (Mead et al. 1986; Stamp and Jenkins 2008). In the liver, bile acids conjugate with either taurine or glycine to form bile salts, depending on animal species. Quantitatively, sterols are the most abundant steroids in nature, and cholesterol is the most predominant sterol in animals. As a component of cell membranes, cholesterol affects membrane fluidity and nutrient transport (including the absorption of dietary nutrients by the small intestine). As noted in the following paragraphs, physiological levels of cholesterol are the substrate for the synthesis of all steroid hormones and bile acids or bile alcohols and therefore are essential to animal nutrition and metabolism. However, excess cholesterol increases the risk for cardiovascular disease in animals.

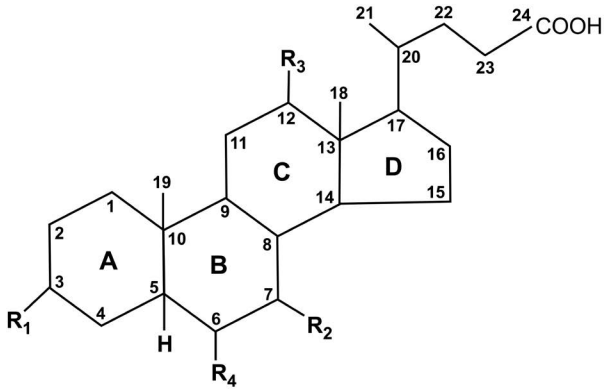
Steroid Hormones

Steroid hormones are steroids with hormone functions in biology (Gunstone 2012). They are synthesized from cholesterol in animals and can be classified as corticosteroids (synthesized in the adrenal cortex) and sex steroids (generated in the male and female gonads and in placentae). Corticosteroids are further grouped into glucocorticoids (e.g., cortisol in most mammals such as pigs and ruminants, or corticosterone in chickens and rats) and mineralocorticoids (e.g., aldosterone). Sex hormones include progesterone, estrogen, estradiol, and testosterone. Vitamin D is another steroid hormone that can be synthesized by animals. As a hydrophobic molecule, a steroid hormone binds to its specific receptor in the cytosol, forming a steroid-receptor ligand complex (Ridgway and McLeod 2016). This complex then enters the nucleus to bind with a specific DNA sequence, thereby inducing the transcription of its target genes. Steroid hormones play important roles in reproduction, immune functions, and anti-inflammatory reactions, as well as the metabolism of protein, lipids, carbohydrates, minerals, and H_2O in the body.

Bile Acids

Bile acids consist of primary bile acids and secondary bile acids. They are all C_{24} compounds in nearly all mammals, most birds, and some fish (e.g., teleost fish; Agellon 2008), but C_{27} bile acids are present in ancient mammals (e.g., elephants and manatees), reptiles, and certain species of aquatic animals (Hagey et al. 1993, 2010; Hofmann 1999; Kurogi et al. 2011; Lefebvre et al. 2009). The C_{27} bile acids contain the C_8 side chain of cholesterol, while the C_{24} bile acids have a truncated C_5 side chain. Primary bile acids are synthesized from cholesterol in the liver, and their molecular forms differ among species (Figure 3.12). Examples are cholic acid and chenodeoxycholic acid (major bile acids in most mammals), as well as hyocholic acid (pig), ursodeoxycholic acid (some species of bears; members of Ursidae), and β-muricholic acid (Table 3.12). Interestingly, β-muricholic acid is formed only in rats and mice among all the animals studied to date (Rudling 2016). In mammals, most C_{24} bile acids are 3α-OH,5β-H anomers; namely, there is the –OH group at C-3 in the α position, and the H at C-5 H is in the β position.

Before secretion from the liver, the terminal carboxyl group of 98% of bile acids is conjugated covalently with taurine or glycine to form water-soluble bile salts in the livers of mammals and birds (Stamp and Jenkins 2008). The



Structures of bile acids in animals

Bile acid	R_1	R_2	R_3	R_4
Lithocholic acid	αOH	H	H	H
Deoxycholic acid	αOH	H	αOH	H
Chenodeoxycholic acid	αOH	αOH	H	H
Ursodeoxycholic acid	αOH	βOH	H	H
Cholic acid	αOH	αOH	αOH	H
Ursocholic acid	αOH	βOH	αOH	H
Hyocholic acid	αOH	αOH	H	αOH
β-Muricholic acid	αOH	βOH	H	βOH
Hyodeoxycholic acid	αOH	H	H	αOH

FIGURE 3.12 Structures of primary and secondary bile acids in animals. The carboxyl group of bile acids can conjugate with taurine or glycine. Dehydroxylation of a primary bile acid at the C-6 position by liver and intestinal bacteria generates a secondary bile acid. The major forms of bile acid differ among animals. When the C6-position of chenodeoxycholic acid contains an α-hydroxyl group, the modified molecule is called hyocholic acid (the major bile acid synthesized by the liver of pigs). When the C6-position of ursodeoxycholic acid contains a β-hydroxyl group, the modified molecule is called β-muricholic acid (a major bile acid synthesized by the liver of mice and rats). In a bile acid, the –OH group at a carbon may face down or up in the six-membered ring, constituting the α- or β-anomer, respectively.

TABLE 3.12
Primary and Secondary Bile Acids (BAs) in Animals

Bile Acid	Animal
Primary bile acids	
Chenodeoxycholic acid[a]	Chicken, giant panda, guinea pig, hamster, horse, human, penguin, and sun bear
Cholic acid[b]	Brown bear, cat, cattle, chicken, dhole (Chinese), dog, ferret (European), fox, hamster, human, mouse, lion (Asian), penguin, Polar bear, rabbit, rat, red panda, raccoon, sheep, striped skunk, suricate, and tiger (Sumatran)
Hyocholic acid	Pig (~75% of total primary BAs in the bile; chenodeoxycholic acid, ~20%)
β-Muricholic acid[c]	Mouse and rat
Ursocholic acid	Black bear (a member of Ursidae) and nutria (an amphibious herbivore)
Secondary bile acids[d]	
Lithocholic acid[e]	Bear, hamster, human, and pig
Deoxycholic acid	Bear, cat, cattle, chicken, dog, hamster, human, mouse, rabbit, rat, and sheep
Ursodeoxycholic acid	Bear (all ages), and pig [21-day-old (weaning age) and 1 week after weaning]
Hyodeoxycholic acid	Pig (25–30% of total BAs in feces at 28 days of age; 60–65% of total BAs in feces in 25- to 50-kg pigs)

[a] Principal primary bile acid in hamsters, humans, and horses.
[b] Principal primary bile acid in ruminants, carnivores, poultry, omnivorous rodents, and some species of bears.
[c] Another principal primary bile acid in mice and rats. β-Muricholic acid is formed only in rats and mice among all the animals studied to date.
[d] The dehydroxylation of a primary bile acid at the C6 position results in the formation of the corresponding secondary bile acid.
[e] A toxic secondary bile acid produced by intestinal microbes to induce cholestatic liver damage.

bile salts in teleost fish are the same as those in mammals. There is little unconjugated bile acid in the bile. Of note, the amounts of taurine and glycine in the conjugated bile salts vary with animal species (Washizu et al. 1991). In general, bile acids are conjugated mainly or exclusively with taurine in carnivores, mainly or almost exclusively with glycine in herbivores, and with both taurine and glycine in substantial proportions in omnivores (Agellon 2008). The corresponding products are taurocholic acid (the derivative of cholic acid and taurine) and glycocholic acid (the derivative of cholic acid and glycine). The formation of bile salts helps to reduce the toxicity of bile acid, increase its solubility in H_2O, and improves its function as an emulsifier of dietary lipids. It should be borne in mind that both taurine- and glycine-conjugated forms of bile acids can be present in the same animal, but their concentrations may be markedly different as noted previously. For example, 70–75% and 25–30% of bile acids are conjugated with glycine and taurine, respectively, in humans, whereas all bile acids are conjugated with taurine in dogs. The liver releases bile salts into the gall bladder for storage. Of interest, the concentrations of bile salts in the gallbladder are extremely high (>300 mM) because of a constant removal of H_2O and electrolytes (Stamp and Jenkins 2008). In response to feeding, the gall bladder secretes the bile salts, via the bile duct, into the duodenum of the small intestine (Tso 1985).

The bile salts are actively secreted as bile into the lumen of the duodenum where their concentrations range from 0.2% to 2% (g/100 mL fluid; Agellon 2008). The typical mammalian bile (a yellow-green aqueous solution) comprises 82% H_2O, 12% bile salts, 4% phospholipids (mostly phosphatidylcholines), 1% unesterified cholesterol, and 1% other compounds [including proteins and pigments (biliverdin and bilirubin conjugated with the water-soluble glucuronides)] (Ridgway and McLeod 2016). In the small intestine, bile salts facilitate the digestion and absorption of lipids by forming micelles, while activating the farnesoid X receptor (FXR; a member of the superfamily of ligand-activated nuclear receptor transcription factors). There is only a very small proportion of endogenous bile acids (not conjugated with glycine or taurine) in the lumen of the ileum and large intestine, where they exist mainly in the water-soluble ionized form as sodium salts at a slightly alkaline pH.

Bile Alcohols

Bile alcohols are usually C_{27} compounds synthesized from cholesterol in the liver of fish and reptiles, with the terminal –OH group at the C_{27} position (Figure 3.13). These bile alcohols are either 5α- or 5β-anomer; namely, the –H atom at C-5 is in either the α- or β-position. In mammals, impaired mitochondrial 26-hydroxylation of intermediates in the pathway for the conversion of cholesterol into bile acids generates bile alcohols. Like the C_{27} bile acids, the C_{27} bile alcohols contain the C_8 side chain of cholesterol. Thus, the conversion of cholesterol into bile alcohols does not involve the removal of any carbon.

The molecular forms of bile alcohols differ among fish and reptile species. Typically, bile alcohols are secreted from the liver of these animals as sulfate conjugates, which usually form salts with minerals, such as sodium (Kurogi et al. 2011). For example, zebrafish produce cyprinol and cyprinol sulfate but no bile acids. In contrast, myxinol and myxinol sulfate are synthesized by hagfish, and scymnol and scymnol sulfate by marine elasmobranch vertebrates (Figure 3.13). Concentrations of bile alcohol sulfate salts in the lumen of the fish intestine are comparable to those of bile salts in mammals and birds (Lefebvre et al. 2009).

Steroidal Saponins

Steroidal saponins are plant metabolites. They are abundant (up to 10% of DM) in Yucca trees native to North America (e.g., Mexico, California, and Arizona). The name Yucca applies to as many as 40 species of trees and shrubs, including *Yucca aloifolia, Y. whipplei,* and *Y. schidigera*. Steroidal

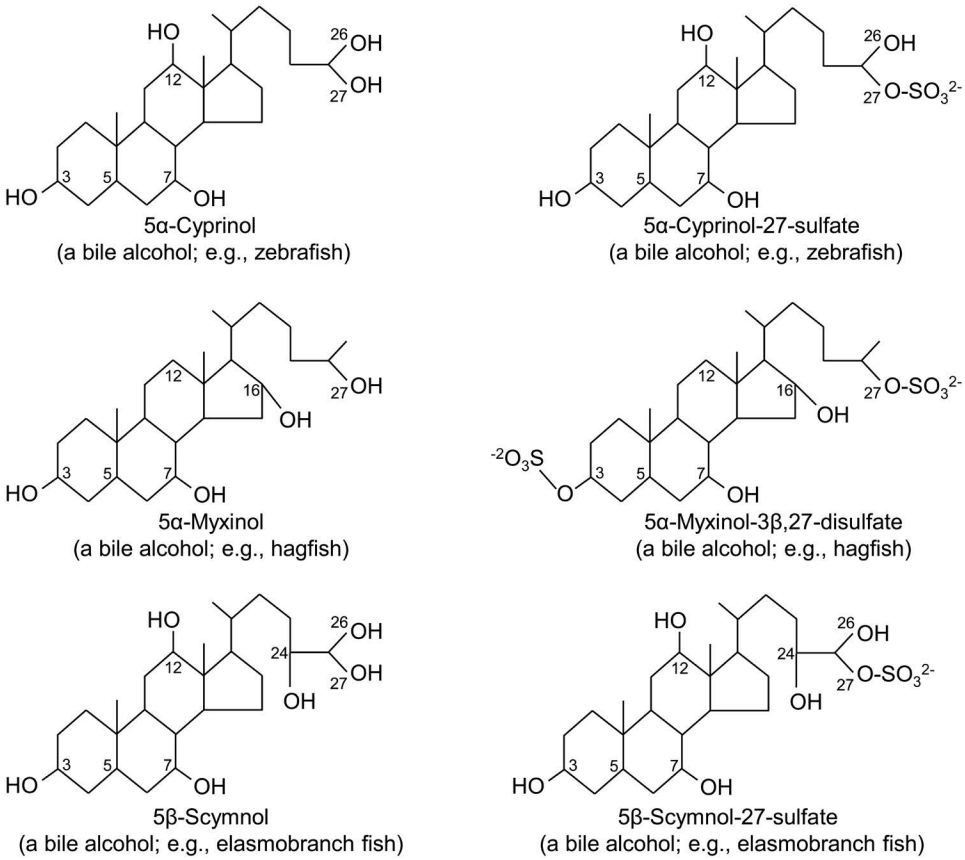


FIGURE 3.13 Structures of bile alcohols in fish. They are usually C_{27} compounds synthesized from cholesterol in the liver of aquatic animals and reptiles, with the terminal –OH group at the C_{27} position. The sulfate group forms a salt with sodium in the body.

saponins contain an ether linkage (–C–O–C–) and one or more monosaccharides joined via β-1,2 or β-1,3 bonds. Oleszek et al. (2001) reported the structures of 8 steroidal saponins in *Y. schidigera* Roezl ex Ortgies (Figure 3.14). They are similar in structure to glucocorticoids in animals.

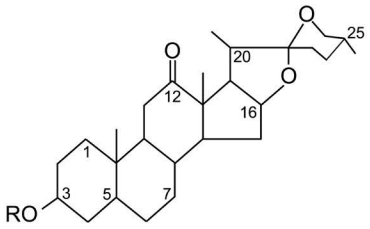
The *Y. schidigera* extract is generally recognized as a safe (GRAS) product and approved by the U.S. Food and Drug Administration (FDA) as a natural food adjuvant under Title 21CFR 172.510. This product is also widely used as a feed additive for livestock species (including swine) and poultry to improve air quality in the production barns, as well as the health, growth, and productivity of the animals (Cheeke 2000). It has been suggested that the Yucca extract binds ammonia and, therefore, reduces its emission from animals (Colina et al. 2001). As steroidal derivatives, Yucca saponins may also have potent anabolic and anti-oxidative effects on animals. For example, under high-temperature conditions, steroidal saponins may antagonize the action of glucocorticoids and oxidative stress in animals, thereby enhancing feed efficiency and production performance. This view represents a paradigm shift in our understanding of the mechanisms responsible for the beneficial effects of the *Y. schidigera* extracts on the growth and health of livestock species and poultry.

Eicosanoids

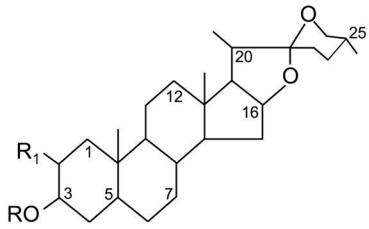
Eicosanoids are formed from ω3 and ω6 PUFAs with 20 carbon atoms in nearly all cell types, particularly platelets, vascular endothelial cells, vascular smooth muscle cells, immunocytes, brain, and reproductive organs (Ridgway and McLeod 2016). Prostaglandins (PGs) were originally found to be synthesized in the male prostate gland, thromboxanes in platelets (thrombocytes), and leukotrienes in leukocytes, hence their names. Like LCFAs, eicosanoids circulate in the blood mainly as albumin complexes.

Prostaglandins

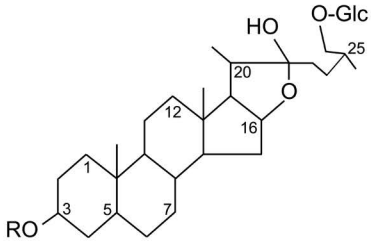
Each PG molecule contains 20 carbon atoms, including a 5-carbon ring (Figure 3.15). They act like hormones but differ from endocrine hormones because these eicosanoids are produced in many cell types (rather than at a specific site) throughout the body (Gunstone 2012). There are 3 series of PGs. Series 1 and 2 PGs (with 1 and 2 double bonds, respectively) are derived from linoleic acid and arachidonic acid (ω6 PUFAs), respectively. Series 1 PGs are PGH_1, PGE_1, $PGF_{1\alpha}$, PGI_1, and PGD_1, whereas series 2 PGs are PGH_2, PGE_2, $PGF_{2\alpha}$, PGI_2, and PGD_2. Series 3 PGs (with 3 double bonds) are derived from α-linolenic acid (an ω3 PUFA), and include PGH_3, PGE_3, $PGF_{3\alpha}$, PGI_3, and PGD_3.



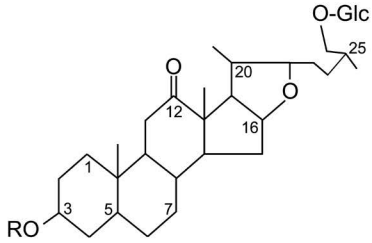
Steroidal saponins in Yucca extracts
Compound 1: R = Glc (1-2)[Xyl(1-3)] Glc
Compound 2: R = Glc (1-2)[Glc(1-3)] Glc



Steroidal saponins in Yucca extracts
Compound 3: R = Glc (1-2) Xyl; R_1 = H
Compound 4: R = Glc (1-2)[Xyl(1-3)] Glc; R_1 = H
Compound 5: R = Glc (1-2) Gal; R_1 = OH

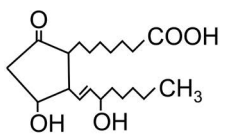


Steroidal saponins in Yucca extracts
Compound 6: R = Glc (1-2) Glc
Compound 7: R = Glc (1-2)[Xyl(1-3)] Glc

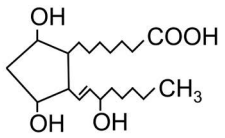


Steroidal saponins in Yucca extracts
Compound 8: R = Glc (1-2)[Xyl(1-3)] Glc

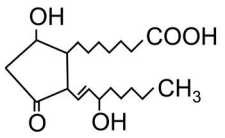
FIGURE 3.14 Structures of steroidal saponins in plants. Steroidal saponins are abundant (up to 10% of dry matter) in Yucca trees native to North America. These substances contain an ether linkage (C-O-C) and one or more monosaccharides joined via β-1,2 or β-1,3 bonds. The structures of 8 steroidal saponins present in *Yucca schidigera* Roezl ex Ortgies are illustrated to show their similarities and differences.



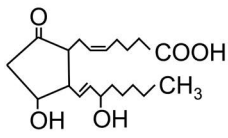
Prostaglandin E_1
($C_{20}H_{34}O_5$)



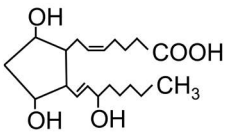
Prostaglandin $F_{1\alpha}$
($C_{20}H_{36}O_5$)



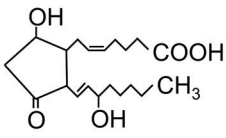
Prostaglandin D_1
($C_{20}H_{34}O_5$)



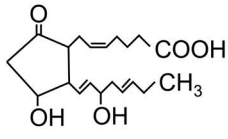
Prostaglandin E_2
($C_{20}H_{32}O_5$)



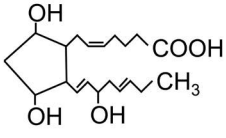
Prostaglandin $F_{2\alpha}$
($C_{20}H_{34}O_5$)



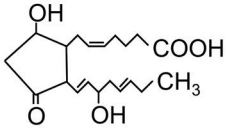
Prostaglandin D_2
($C_{20}H_{32}O_5$)



Prostaglandin E_3
($C_{20}H_{30}O_5$)



Prostaglandin $F_{3\alpha}$
($C_{20}H_{32}O_5$)



Prostaglandin D_3
($C_{20}H_{30}O_5$)

FIGURE 3.15 Structures of prostaglandins (PGs) E, F, and D in animals. These substances contain 20 carbon atoms, including a 5-carbon ring. There are 3 series of PGs. Series 1 and 2 of PGs (with 1 and 2 double bonds, respectively) are derived from linoleic acid and arachidonic acid, respectively. Series 3 PGs (with 3 double bonds) are derived from EPA (an elongation product of α-linolenic acid).

Series 2 PGs are proinflammatory mediators, but series 1 and 3 PGs are anti-inflammatory (Folco and Murphy 2006). Series 2 PGs are derived from linoleic acid, and an excessive intake of linoleic is now recognized as being proinflammatory.

Leukotrienes

Leukotrienes are derivatives of unsaturated LCFAs and contain three conjugated double bonds. Leukotriene A (LTA) is an epoxide, whereas leukotriene B (LTB) has two –OH groups (Figure 3.16). Series 3 leukotrienes (LTA_3 and LTB_3) are synthesized from eicosatrienoic acid ($C_{20:3}$ ω9, Mead's acid) and have three conjugated double bonds (Devlin 2006). LTC_3 (5-hydroxy-6-S-glutathionyl-7,9,ll-eicosatrienoic acid) is the conjugate of a hydroxylated LTA_3 intermediate with glutathione (Glu-Cys-Gly), LTD_3 is the conjugate of a hydroxylated LTA_3 intermediate with cysteine-glycine, and LTE_3 is the conjugate of a hydroxylated LTA_3 intermediate with one cysteine molecule.

Series 4 leukotrienes have four double bonds (including three conjugated double bonds) and were the first leukotrienes discovered. They are synthesized from arachidonic acid, including LTA_4 and LTB_4, as well as LTC_4, LTD_4, LTE_4, and LTF_4. LTC_4, LTD_4, LTE_4 and LTF_4 are derivatives of LTA_4, and they all contain a cysteine moiety. Specifically, LTC_4 is the conjugate of a hydroxylated LTA_4 intermediate with glutathione, LTD_4 is the conjugate of a hydroxylated LTA_4 intermediate with cysteine-glycine, LTE_4 is the conjugate of a hydroxylated LTA_4 intermediate with one cysteine molecule, and LTF_4 is the conjugate of a hydroxylated LTA_4 intermediate with cysteine and glutamate. Thus, LTC_4, LTD_4, LTE_4 and LTF_4 are called cysteinyl leukotrienes. Production of series 4 leukotrienes is enhanced in response to inflammatory mediators, thereby inducing the adhesion and activation of leukocytes on the endothelium of blood vessels (Folco and Murphy 2006).

Series 5 leukotrienes (e.g., LTA_5, LTB_5, LTC_5, LTD_5 and LTE_5) have five double bonds, including three conjugated double bonds. They are synthesized from EPA, which is abundant in marine fish oil. LTC_5 (5-hydroxy-6-S-glutathionyl-7,9,11,14,17-EPA) is the conjugate of a hydroxylated LTA_5 intermediate with glutathione, LTD_5 is the conjugate of a hydroxylated LTA_5 intermediate with cysteine-glycine, and LTE_5 is the conjugate of a hydroxylated LTA_5 intermediate with one cysteine molecule. Series 5 leukotrienes inhibit the synthesis of series 4 leukotrienes and exert anti-inflammatory effects in blood vessels. Series 5 leukotrienes also are converted to resolvins in vascular endothelial cells by cyclooxygenase and cytochrome P450 in the presence of aspirin (Serhan et al. 2019). Resolvins reduce cellular inflammation by inhibiting the production and transportation of inflammatory cells and chemicals to sites of inflammation.

Series 6 leukotrienes have six double bonds as they are derived from DHA and can be converted to resolvins. Unlike series 5 leukotrienes, series 6 leukotrienes are precursors for the formation of neuroprotectins that have similar effects as resolvins (Serhan et al. 2019). The production of resolvins and neuroprotectins underscores the importance of consuming cold-water fish, the primary sources of EPA and DHA.

Thromboxanes

Thromboxane is named for its role in constricting blood vessels and promoting clot formation (thrombosis). This class of lipids contains a 6-membered ether-containing ring. Three series of thromboxanes have been identified (Figure 3.17). Series 1 thromboxanes (TXA_1 and TXB_1, each with one double bond), series 2 thromboxane (TXA_2 and TXB_2, each with two double bonds), and series 3 thromboxanes (TXA_3 and TXB_3, each with three double bonds) are formed from dihomo-γ-linolenic acid (an ω6 PUFA), arachidonic acid, and EPA, respectively (Folco and Murphy 2006). TXA_2 and TXB_2 are quantitatively

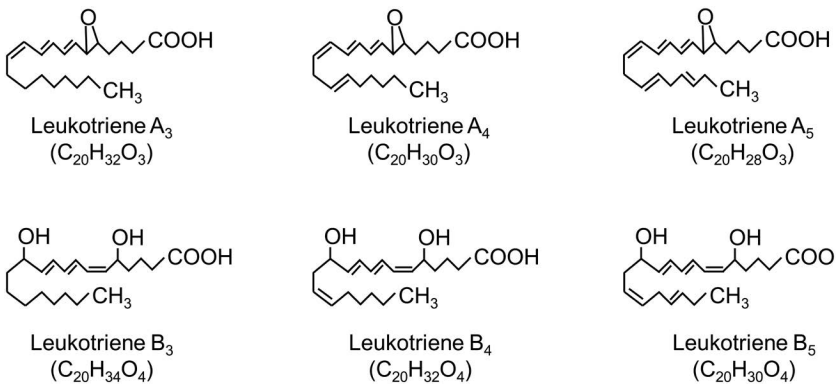


FIGURE 3.16 Structures of leukotrienes in animals. These substances are derivatives of unsaturated long-chain fatty acids and contain three conjugated double bonds. Leukotriene A (LTA) is an epoxide, whereas leukotriene B (LTB) has two –OH groups. Series 3, 4, and 5 leukotrienes (LTA_3 and LTB_3) are synthesized from eicosatrienoic acid (20:3 ω9), arachidonic acid (20:4 ω6), and eicosapentaenoic acid (C20:5 ω3), respectively.

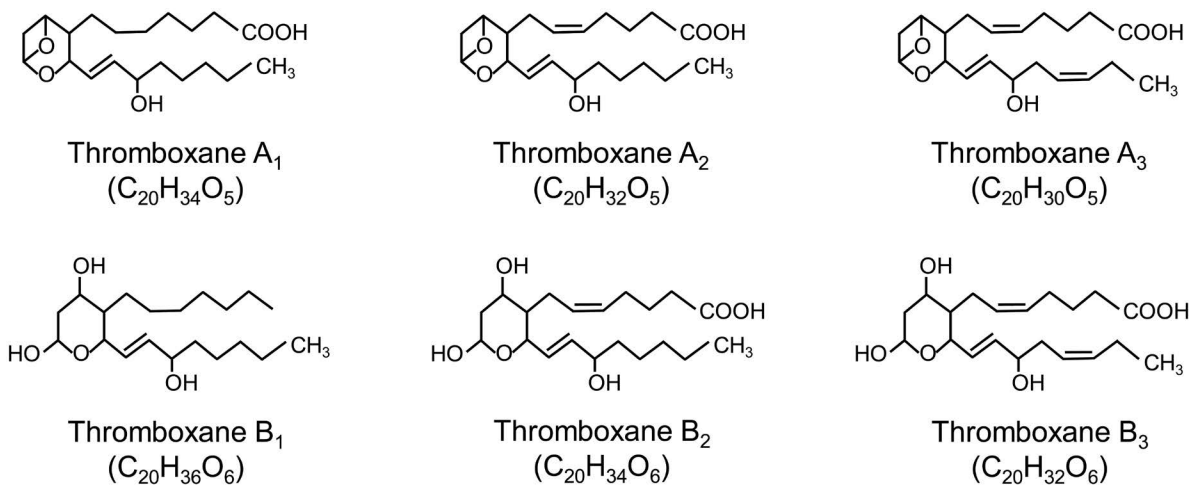


FIGURE 3.17 Structures of thromboxanes in animals. Thromboxanes are derivatives of unsaturated long-chain fatty acids and have a 6-membered ether-containing ring. Thromboxane A (TXA) is an epoxide, whereas TXB has two –OH groups. There are 3 series of thromboxanes. Series 1 thromboxanes (TXA_1 and TXB_1, each with one double bond), series 2 thromboxanes (TXA_2 and TXB_2; each with two double bonds), and series 3 thromboxanes (TXA_3 and TXB_3; each with three double bonds) are formed from dihomo-γ-linolenic acid (an ω6 PUFA), arachidonic acid (an ω6 PUFA), and eicosapentaenoic acid, respectively (an ω3 PUFA).

two major thromboxanes identified to date. All three series of thromboxanes act on specific receptors on the plasma membrane of platelets, thereby modulating the functions of these cells. PGI_2, a product of arachidonic acid in vascular endothelial cells, inhibits platelet aggregation and relaxes the smooth muscle cells of blood vessels.

Lipoxins

Lipoxins were first described in 1984 and are the newest type of eicosanoids to be discovered. They have four conjugated double bonds and three –OH groups (Figure 3.18). This class of lipids is synthesized from arachidonic acid via the formation of 15-hydroxyicosatetraenoic acid (15(*S*)-HETE) and its 15-hydroperoxy precursor. At present, four lipoxins have been identified in animal tissues: lipoxins A_4, A_5, B_4, and B_5. Lipoxins A_4 and B_4 have the same molecular weight. Lipoxins A_5 and B_5 also have the same molecular weight. These lipoxins stimulate superoxide anion generation and degranulation, as LTB_4 does. Like leukotrienes, lipoxins conjugate with cysteine to form cysteinyl-lipoxins. Emerging evidence shows that lipoxins have anti-inflammatory effects in animals (Romano et al. 2015).

Cannabinoids

Diverse tissues (particularly the brain) contain endocannabinoids (endogenous ligands of cannabinoid receptors) that serve as neurotransmitters (Piomelli 2003). These substances also occur in some plants, such as the cacao tree. The word "Ananda" is derived from Sanskrit, meaning joy, bliss, and delight. Two representative endocannabinoids are *N*-arachidonoylethanolamine (anandamide, an

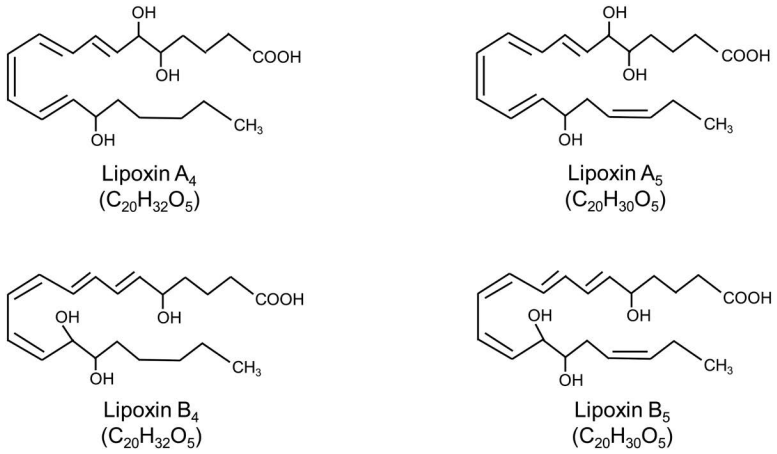
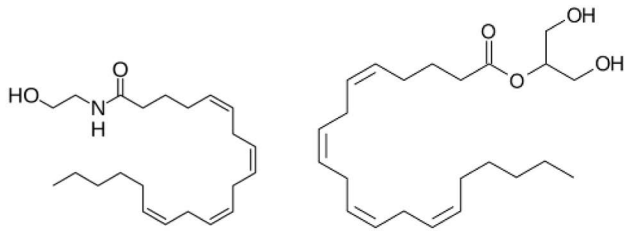


FIGURE 3.18 Structures of lipoxins in animals. Lipoxins contain four conjugated double bonds and three –OH groups. Thus, this class of lipids has a conjugated trihydroxytetraene structure. At present, lipoxins A4, A5, B4, and B4 are known to be synthesized from arachidonic acid.

N-acylethanolamine) and 2- arachidonoylglycerol (an ester of glycerol with arachidonic acid), which are neurotransmitters in the brain.



N-Arachidonoylethanolamine (anandamide) [$C_{22}H_{37}NO_2$; MW 347.53 Da]

2-Arachidonoylglycerol [$C_{23}H_{38}O_4$; MW 378.55 Da]

Terpenes

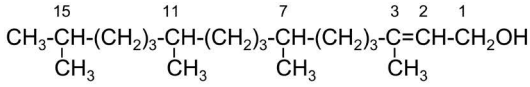
Phytols

Phytol is an acyclic diterpene alcohol. It is freely present in plants and algae as a constituent of chlorophyll and is also produced by bacteria (including those in the GIT of animals). An example of a phytol of plant and algae origin is 3,7,11,15-tetramethyl-2-hexadecen-1-ol ($C_{20}H_{40}O$) (Figure 3.19). Plants, algae, and bacteria can convert phytol into vitamin E and vitamin K_1, as well as phytanic acid and pristine (a saturated terpenoid alkane). Thus, these substances are found in common fruits, vegetables, and algae products (Chung et al. 1989). Likewise, the liver of certain animals (e.g., the shark) can metabolize phytol into pristane, which was originally coined from the Latin *pristis* "shark." As compounds with fragrant properties, phytols are used by plants to protect against predation. Similarly, some insects (e.g., the sumac flea beetle) depend on plant phytols for defense.

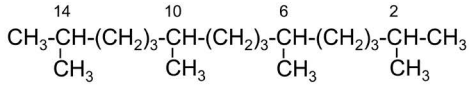
Carotenoids

Carotenoids are lipids consisting of isoprenoid units with conjugated double bonds, and, thus, are called isoprenoids (Mead et al. 1986). They are derived from the acyclic $C_{40}H_{56}$ structure (Figure 3.19) and are produced by bacteria, algae, fungi, phytoplankton, and higher plants. Carotenoids are plant or algae pigments responsible for bright red, yellow, and orange colors in fruits, vegetables, and other foods (e.g., sweet potatoes and papaya). There are more than 600 different types of carotenoids in nature, which can be classified as carotenes (e.g., α-carotene, β-carotene, γ-carotene, and lycopene) and xanthophylls (e.g., lutein, zeaxanthin, antheraxanthin, and β-cryptoxanthin). Both groups are hydrocarbons by definition and differ in their chemical structures. Specifically, xanthophylls contain oxygen, whereas carotenes do not. Animals do not synthesize carotenoids (Pond et al. 2005). However, these substances are important antioxidants in the diets of animals and are bound to proteins in the body for storage.

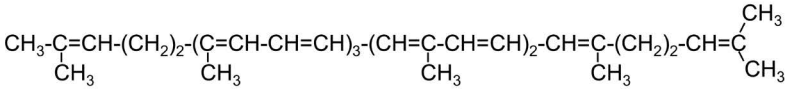
Astaxanthin is a keto-carotenoid. There is not *de novo* synthesis of astaxanthin in fish, crustaceans, and other aquatic animals. However, astaxanthin can be formed from β-carotene and zeaxanthin (Katayama et al. 1971, 1972). Thus, astaxanthin or its precursors must be available in either the native habitat or manufactured diet to meet the requirements of these animals. The dietary supplementation with pigmented preparations from



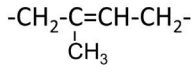
3,7,11,15-Tetramethyl-2-hexadecen-1-ol ($C_{20}H_{40}O$; a phytol in plants and algae)



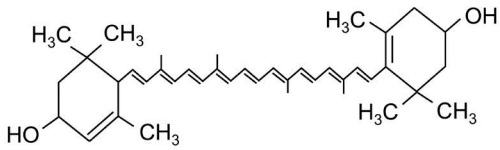
2,6,10,14-Tetramethylpentadecane (pristane) [$C_{19}H_{40}$; a terpenoid alkane in plants and certain animals (e.g., the shark); a metabolite of phytol]



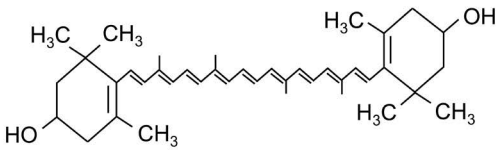
Carotenoid of bacterial origin (a general structure; $C_{40}H_{56}$)



Isoprene unit



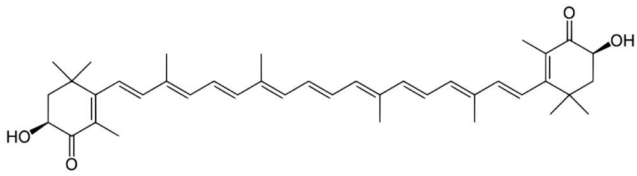
Lutein (a carotenoid in plants and green algae $C_{40}H_{56}O_2$)



Zeaxanthin (a carotenoid in plants and green algae $C_{40}H_{56}O_2$)

FIGURE 3.19 Structures of phytols and carotenoids. Phytols and carotenoids are classified as terpenes that have an isoprene structure. Phytol is an acyclic diterpene alcohol present in plants, algae, and bacteria. Carotenoids consist of isoprenoid units with conjugated double bonds and are derived from the acyclic $C_{40}H_{56}$ structure. More than 600 different types of carotenoids exist in nature, yielding pigments responsible for bright red, yellow, and orange colors in plants and certain organisms.

corn gluten, alfalfa, and Spirulina (blue-green alga) can increase the content of astaxanthin in crustaceans (e.g., the prawn; Tanaka et al. 1976). Astaxanthin causes the red color in cooked crustaceans (e.g., shrimp, crabs, and lobsters) and the pink-red color in some cooked fish (e.g., salmon) during heating through the release of astaxanthin from denatured caroteno-proteins. The intensity of the final color in these seafoods depends on the content of astaxanthin.



Astaxanthin ($C_{40}H_{52}O_4$; MW = 596.84 Da)

Lipid-Soluble Vitamins

Terpenes are lipid-soluble vitamins, including vitamins A, E, and K. Plants or algae contain vitamin-A precursors but not vitamin A. In animals, the small intestine converts β-carotene into vitamin A. In contrast, plants, algae, and bacteria synthesize vitamins E and K from shikimate (Bentley and Meganathan 1983; DellaPenna 2005), but these two vitamins are not made by animals (Pond et al. 2005).

Essential Oils

An essential oil is a concentrated hydrophobic liquid containing a phenolic ring. It is not an "oil" in the same way as the liquid state of the ester of a LCFA with glycerol. Steam distillation is the usual method for extracting essential oils from certain plants (Tongnuanchan and Benjakul 2014). The term "essential" does not imply that these substances are nutritionally essential for animals. Rather, the name was used because the *essence* of the plant has been extracted. The structures of various components of some essential oils are shown in Figure 3.20. Many of them have the same molecular weight but different structures, whereas some are isomeric (e.g., thymol and carvacrol). Essential oils are volatile aromatic compounds with characteristic fragrances and flavors.

Essential oils are unique to certain plants. For example, cinnamaldehyde is a pale yellow, viscous liquid present in the bark of cinnamon trees and other species of the genus *Cinnamomum*. Eugenol is an allyl chain-substituted guaiacol, which is a member of the phenylpropanoids class of phytochemicals (Figure 3.20). It is a pale yellow to colorless liquid extracted from certain plants, such as cloves, nutmeg, cinnamon, basil and bay leaves. Thymol (also known as 2-isopropyl-5-methylphenol) is a natural monoterpene phenol derivative of cymene and is isolated from *Thymus vulgaris* (common thyme) and various other kinds of plants. Thymol is a white crystalline substance with a pleasant aromatic odor.

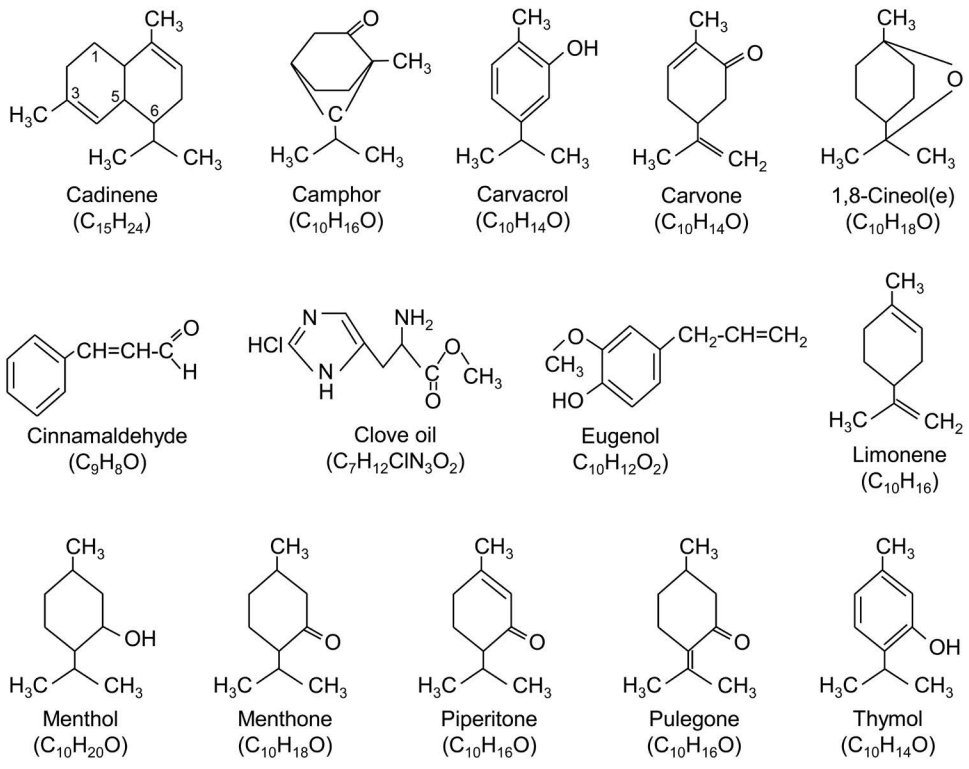


FIGURE 3.20 Structures of essential oils in plants. Essential oils are concentrated hydrophobic liquids containing a phenolic ring. They are present in certain plants as volatile aromatic compounds with characteristic fragrances and flavors. Many of them have the same molecular weight but different structures, whereas some are isomeric (e.g., thymol and carvacrol).

Essential oils have antimicrobial, antiparasitic, anti-inflammatory, antioxidative, and immunomodulatory functions (Benchaar et al. 2008; Zeng et al. 2015). They can be added to a diet as a single substance or a mixture of two or more compounds. For example, supplementing cinnamaldehyde to the diet of weanling piglets reduces the intestinal expression of inflammatory cytokines in response to lipopolysaccharide (LPS) challenge genes (Wang et al. 2015). In addition, supplementing cinnamaldehyde plus thymol to the diet of chickens can beneficially enhance feed intake, modulate the gut microbiota, and improve BW gain (Zeng et al. 2015). However, it should be borne in mind that because of their lipid-soluble properties and involvement in chemical reactions, excessive amounts of essential oils are toxic to animals.

Coenzyme Q

Coenzyme Q (CoQ), which was discovered in 1957, is chemically known as 2,3-dimethoxy-5-methyl-6-multiprenyl-1,4-benzoquinone. It is a hydrophobic compound present in the organelles (particularly the mitochondria) of all animals. The various forms of CoQ are distinguished by the number (usually between 6 and 10) of isoprenoid repeats in their side chains. In mammals and birds, the most common CoQ is either CoQ_{10} or CoQ_9, where the number refers to the number of isoprene repeats in the molecule (Turunen et al. 2004). For example, ~90% of ubiquinone is CoQ_9 in mice and rats. In contrast, in chickens, cows, goats, guinea pigs, horses, humans, rabbits, and pigs, ≥96% of ubiquinone is CoQ_{10}, with ≤4% of ubiquinone being CoQ_9 (Lass et al. 1997). Fish is also an excellent source of CoQ_{10}. CoQ plays an essential role in mitochondrial electron transport and ATP synthesis, and also acts as an antioxidant in cell membranes, lipoproteins, and mitochondria.

All animals can synthesize CoQ and do not require this fat-soluble ubiquinone from diets. This synthetic pathway occurs in many tissues, including the liver and brain. The biosynthesis of coenzyme Q_{10} involves three major steps: (a) the synthesis of the benzoquinone structure from tyrosine, with the first step being the vitamin B_6-dependent conversion of tyrosine to 4-hydroxyphenylpyruvic acid; (b) the synthesis of the isoprene side chain from acetyl-CoA via the mevalonate (cholesterol-synthetic) pathway; and (c) the condensation of the benzoquinone and mevalonate structures (Turunen et al. 2004). Hydroxymethylglutaryl (HMG)-CoA reductase plays a critical role in regulating the synthesis of both cholesterol and CoQ.

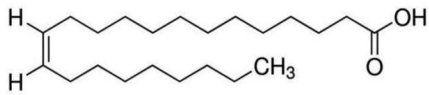
TOXIC LIPIDS IN PLANTS

Oxidized Fatty Acids

Unsaturated fatty acids, particularly PUFAs, are highly susceptible to chemical oxidation (e.g., peroxidation; see below). This induces oxidative stress as well as damage to proteins, amino acids, nucleic acids, and membranes in cells. Feedstuffs must be stored and transported under optimum conditions (including the use of antioxidants) to preserve unsaturated fatty acids.

Erucic Acid

Erucic acid, which is a very long-chain monounsaturated ω9 fatty acid ($C_{22:1}$ ω9), is a toxic docosenoic acid ($C_{22:1}$) to animals (including humans) when present in foods at high concentrations. It is particularly abundant in the oil- and protein-rich seeds of the *Brassicaceae* (formerly *Cruciferae*) family of natural plants [e.g., wallflower, rapeseed (a yellow flowered plant), and mustard] and is also present in some fish and seafoods at low concentrations. For example, on a DM basis, rapeseeds and rapeseed meal contain about 46% oil and 40% crude protein, respectively. Canola, which is a variety of rapeseed produced in Canada through traditional plant crossbreeding, contains much higher amounts of oleic acid and correspondingly much lower amounts of both erucic acid (e.g., ≤2% of the total lipids in canola seeds) and glucosinolates (e.g., ≤30 μg/g in canola meal) than the natural rapeseed. Through the formation of erucylcarnitine (Christophersen and Bremer 1972) and stimulating malonyl-CoA formation (Chen et al. 2020), erucic acid inhibits the mitochondrial oxidation of other fatty acids in animals, resulting in lipid deposits (lipidosis) in the heart, skeletal muscles, and adrenals; reductions in food intake and whole-body growth; and abnormal structures of tissues such as the liver and kidneys (Lietzow 2021).

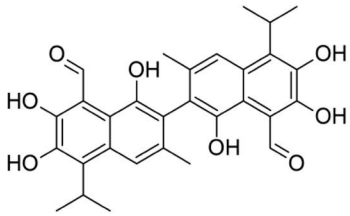


Erucic acid ($C_{22:1}$, ω9; $C_{22}H_{42}O_2$; molecular weight = 338.57 Da)

Gossypol

Gossypol is a lipid-soluble polyphenolic aldehyde present in cotton seeds as a yellow pigment, and its chemical name is 1,1′,6,6′,7,7′-hexahydroxy-3,3′-dimethyl-5,5′-di(propan-2-yl) [2,2′-binaphthalene]-8,8′-dicarbaldehyde. Gossypol has two optical enantiomers [(−)-gossypol and (+)-gossypol], as well as three different tautomers (aldehyde, ketone, and lactol; Gamboa et al. 2001; Liu et al. 2022). These substances can be degraded by GIT microbes, particularly in the rumen of ruminants. Thus, as a protein-rich feed, cottonseed meal (CSM) is commonly used as a partial substitute for soybean meal in the diets of ruminants in cotton-producing countries (Briggs and Hines 2021). By contrast, CSM is not used for feeding swine during all phases of their production due to concerns over the toxicity of gossypol (Briggs and Hines 2021). Gossypol inhibits the activities of some dehydrogenase (including lactate dehydrogenase, 5α-reductase 1, and 3α-hydroxysteroid dehydrogenase) and enzymes of the mitochondrial electron transport chain (e.g., succinic dehydrogenase and cytochrome oxidase), thereby reducing ATP

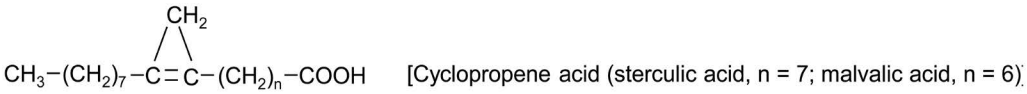
production from energy substrates and the formation of androgen from cholesterol in animals (Nicholson 2012). The tolerance of swine to gossypol depends upon age, with older animals being less sensitive than younger animals to its toxicity (Eisele 1986). Based on poor growth performance and severe skin disorders, the inclusion of 33.5% to 36.5% CSM (with 980 ppm free gossypol) in diets was highly toxic for postweaning pigs (Wallace et al. 1955). However, postweaning pigs could tolerate up to 35% CSM (with 240 ppm free gossypol) in diets for at least 10 weeks (Wallace et al. 1955). Likewise, postweaning pigs (28–63 days of age) could tolerate 12.6% CSM (with 380 ppm total gossypol) for at least 35 days in diets (LaRue et al. 1985), whereas nonpregnant sows could tolerate 29% CSM (with 225 ppm free gossypol) in diets for at least 15 days (Dong et al. 2020) without adverse effects. Furthermore, Wallace et al. (1955) reported that gestating and lactating swine could tolerate 17% CSM (with 240 ppm free gossypol) in diets for 114 and 56 days, respectively. A safe upper limit of free gossypol in CSM (at its 35% inclusion level in diets) appeared to be 400 ppm for swine (Wallace et al. 1955). Of note, such a high inclusion level of soybean meal (35%) is usually not used in the diets of gestating sows (e.g., ~10%; Herring et al. 2022) or lactating sows (e.g., 27%; Rezaei et al. 2022a,b). Thus, appropriate levels of low-gossypol CSM in swine diets should be reconsidered, particularly when the price of soybean meal is high and its supply is inadequate.



Gossypol ($C_{30}H_{30}O_8$; a lipid-soluble polyphenolic aldehyde

Cyclopropene Acids

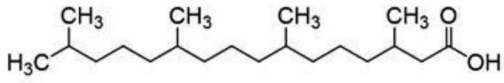
When laying hens are fed diets containing a relatively small amount of cyclopropene acids present in cottonseed oil or kapok seed oil, their eggs have a pink discoloration, and egg yields are markedly reduced. These unsaturated fatty acids include sterculic acid and malvalic acid. Feeding cyclopropene acids (representing 40% of dietary energy) to rats can kill most of the animals within a few weeks. Female rats become infertile when their diets contain as low as 2–3% cyclopropene acids. Pigs and cows are also sensitive to the toxic effects of these fatty acids in diets. In addition, cyclopropene acids can cause liver tumors in animals, including trout. Industrial processing can help to eliminate most or all cyclopropene acids in cottonseed and kapok seed. Some but probably not all the toxic effects of sterculic acid are attributed to its potent inhibition stearoyl-CoA desaturase, the enzyme responsible for converting saturated fatty acids to monounsaturated fatty acids.



[Cyclopropene acid (sterculic acid, n = 7; malvalic acid, n = 6)

Phytanic Acid

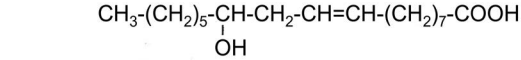
Phytanic acid (3,7,11,15-tetramethylhexadecanoic acid) is a branched chain fatty acid present in some plants. This saturated has 20 carbons ($C_{20}H_{40}O_2$). In plants, chlorophyll contains phytol (3,7,11,15-tetramethylhexadec-2-ene-1-ol). Unconjugated phytol can be readily absorbed by the small intestine of animals (including humans) and then converted into phytanic acid in the body. However, the phytol-chlorophyll ester is poorly absorbed by the intestine of animals (including humans) and is not a significant source of dietary phytanic acid. Under normal conditions, Phytanic acid can be oxidized by α-oxidation in peroxisomes and does not have any adverse effects on animals. However, in humans with an inborn defect in the α-oxidation, the dietary intake of phytanic acid results in Refsum's disease (a disorder of peripheral neuropathy).



Phytanic acid (3,7,11,15-tetramethylhexadecanoic acid)

Ricinoleic Acid

Ricinoleic acid (12-hydroxy-9-*cis*-octadecenoic acid; $C_{18}H_{34}O_3$) is a monounsaturated (ω9) hydroxyl fatty acid that is highly abundant in Castor seeds and in ergots. Note that this fatty acid differs from oleic acid in that the latter does not contain a hydroxyl group. Ricinoleic acid can stimulate the secretion of intestinal fluid, therefore exerting laxative effects in animals. Nonetheless, this unusual fatty acid is widely used in cosmetic, pharmaceutical, oleochemical, and agricultural industries (Nitbani et al. 2022).



Ricinoleic acid (12-hydroxy-9-cis-octadecenoic acid; HO-$C_{18:1}$ ω9)

TOXIC LIPIDS IN MARINE OILS

Oxidized Fatty Acids

Marine unsaturated fatty acids (particularly PUFAs) are more prone to peroxidation than plant unsaturated fatty acids due to their greater number of *cis*-double bonds,

resulting in unpleasant appearance, smell, and side effects. Thus, antioxidants such as vitamins C and E are required to stabilize marine fatty acids (e.g., EPA and DHA) during storage even at room temperature (e.g., 20–25°C).

Cetoleic Acid

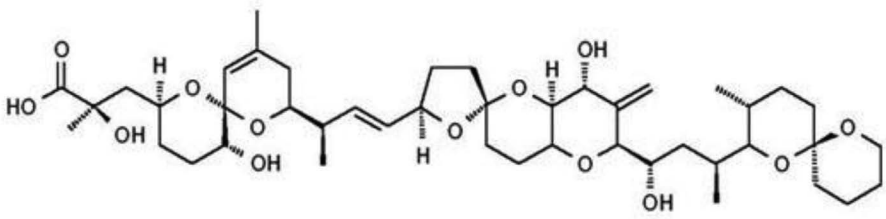
Anchoveta and menhaden oils contain a very small amount of docosenoic acid. However, herring and capelin oils have 12–20% docosenoic acid, with cetoleic acid (*cis*-11-docosenoic acid; $C_{22:1}$ ω11, *cis*; an isomer of erucic acid) being the major species (Dhopeshwarkar 1980). Acute lipidoses can occur in animals (e.g., rats and primates) consuming a significant amount of cetoleic acid.

CH_3-$(CH_2)_9$-CH=CH-$(CH_2)_9$-COOH

Cetoleic acid (cis-11-docosenoic acid; $C_{22:1}$ ω11)

Okadaic Acid

Okadaic acid ($C_{44}H_{68}O_{13}$) is produced by *Prorocentrum lima*, which is a marine, freshwater or terrestrial dinoflagellate (Encinas-Yánez et al. 2024). This unsaturated fatty acid readily accumulates in shellfish (e.g., bivalve mollusks and fish) and then may be consumed by humans. As a toxin (also a polycyclic ether), okadaic acid penetrates the plasma membrane into the cell, inhibits the activities of several types of serine/threonine protein phosphatases (1, 2A, and 2B), and acts as a carcinogen (possibly via inducing DNA mutations) in animals (Abal et al. 2018). In addition, okadaic acid negatively affects gastrointestinal, immune, and nervous systems, as well as embryonic development (Abal et al. 2018).



Okadaic acid ($C_{44}H_{68}O_{13}$; MW = 805.02)

CHEMICAL REACTIONS

Hydrolysis of Triacylglycerols and Saponification of Fatty Acids

TAGs can be hydrolyzed by boiling in the presence of an alkali compound (e.g., NaOH) to form glycerol and fatty acids. The fatty acids then react with the base to produce soaps (i.e., alkali salts of fatty acids). This reaction of salt formation is called saponification, a process of manufacturing soaps in the chemical industry (Ridgway and McLeod 2016). Similarly, fatty acids react with calcium hydroxide or calcium oxide to form salts. Such a reaction has been used to manufacture rumen-protected fatty-acid calcium salts as feed additives.

Triacylglycerols + 3 H_2O —(Boiling with alkali)→ 3 R-COOH + Glycerol

R-COOH + NaOH → R-COONa + H_2O

2 R-COOH + $Ca(OH)_2$ → $(R\text{-}COO)_2Ca$ + 2 H_2O

2 R-COOH + CaO + H_2O → $(R\text{-}COO)_2Ca$ + 2 H_2O

In animals, fat hydrolysis, which takes place in the lumen of the small intestine, is catalyzed by lipases in the presence of bile salts to yield *sn*-2-mono- or di-acylglycerols, fatty acids, and/or glycerol (Lefebvre et al. 2009). These digestion products, together with the bile salts, form water-miscible micelles, with the hydrophilic "head" regions in contact with the surrounding H_2O and the hydrophobic single-tail regions being sequestered in the micelle center.

Feed analysis of lipids often calls for the determination of "the acid value" of fatty acids, namely the quantity of potassium hydroxide (KOH) that is required to neutralize the free organic acids in a gram of fat. The acid value is a measure of the amount of free fatty acids present in the sample. This method provides a very rapid way to quantify fatty acids, but its accuracy should be verified by high-performance liquid chromatography (HPLC) or gas chromatography.

Esterification with Alcohols

A fatty acid forms an ester with glycerol (a sugar alcohol with three –OH groups) as noted previously. This condensation reaction is important for fat storage in the animal body (Tso 1985; Yabuuchi and O'Brien 1968). For comparison, phosphoric acid reacts with a hydroxyl group in glycerol to yield a phosphate ester (e.g., phospholipids). Furthermore, a fatty acid forms an ester with a straight-chain alcohol (e.g., ethanol), as shown in the following reaction, which is the chemical basis for the industrial production of polyesters (Gunstone 2012). Of note, ethyl acetate is the most common ester in wine that is formed enzymatically from acetic acid and ethanol during the fermentation process.

CH_3-C(=O)-OH + CH_3CH_2-OH —(H_2SO_4)→ CH_3-C(=O)-O-CH_2CH_3 + H_2O

Acetic acid Ethanol Ethyl acetate

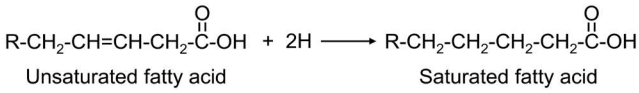
Substitution of the Hydroxyl Hydrogen

An electrophile (an electron-seeking reagent) extracts the hydrogen atom from the hydroxyl group of a long-chain fatty acid (Gunstone 2012). Electrophiles have vacant orbitals attracted to an electron-rich center and participate in chemical reactions by accepting a pair of electrons. Examples of this class of substances are alkyl halides, acyl halides, carbonyl compounds, hydrogen sulfide anion, alcohols and hydrogen, and oxidizing agents. Substitution of the hydroxyl hydrogen in fatty acids renders both saturated and unsaturated fatty acids prone to oxidation, leading to reductions in their chemical stability and biological activity.

$$\text{R-COOH} + \text{E}^+ \rightarrow \text{R-COO-E} + \text{H}^+ \ [\text{E}^+ = \text{an electrophile}]$$

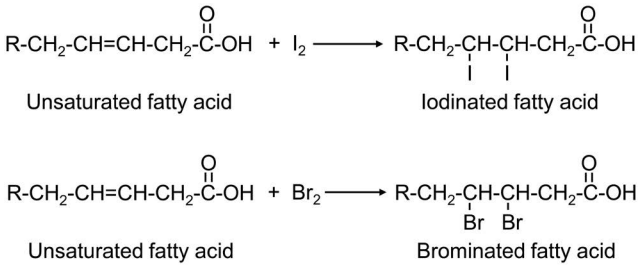
Hydrogenation of Unsaturated Fatty Acids

An unsaturated fatty acid, which is liquid at 37°C, can be converted into a saturated fatty acid by the hydrogenation of its double bonds (Mead et al. 1986). This process is used to convert oil into solid margarine. Ruminal microorganisms enzymatically hydrogenate unsaturated fatty acids, which is called biohydrogenation. This explains why the percentage of saturated fatty acids among the total lipids in the WAT and skeletal muscle is higher in ruminants than in nonruminants (Smith and Smith 1995; Tan et al. 2011) and why the body fat is "harder" in ruminants than in nonruminants.



Iodination and Bromination of the Double Bonds of Unsaturated Fatty Acids

A double bond in an unsaturated fatty acid is highly reactive with iodine (I_2), forming an iodinated fatty acid. This reaction has been widely used as a measure of the degree of unsaturation of a lipid (e.g., an oil, fat, or wax), and quantified as iodine value (or iodine number), which is defined as the amount of iodine (in g) that is taken up by 100 g of a lipid. Saturated oils, fats, and waxes do not react with iodine and, therefore, have an iodine value of zero. The more double bonds a fatty acid has, the higher its iodine value will be. This test is often performed with a known excess of iodine, usually in the form of iodine monochloride (ICl), and the amount of iodine remaining unreacted is then determined by titration. Note that a double bond in an unsaturated fatty acid also reacts with bromine (Br_2) to yield a brominated fatty acid.



Peroxidation of Unsaturated Fatty Acids

The double bonds in unsaturated fatty acids are chemically reactive (Mead et al. 1986). Thus, unsaturated fatty acids, particularly PUFAs, undergo peroxidation (the attack of lipids with one or more double bonds by oxidants) by reactive oxygen species (ROS, such as •OH and HOO•) to form lipid peroxides or lipid oxidation products. This reaction occurs *in vitro* and *in vivo*, and is accelerated at high ambient temperatures and increased oxygen exposure (Rosero et al. 2015). Lipid peroxidation involves three major steps: initiation, propagation, and termination (Figure 3.21). During initiation, a free radical extracts a proton and an electron from the carbon adjacent to the double bond, yielding an unstable fatty acid radical. The fatty acid radical then reacts with molecular oxygen to produce an unstable peroxyl-fatty acid radical, which reacts with another molecule of unsaturated fatty acid to form a new fatty acid radical and a lipid peroxide (Gunstone 2012). This ROS-initiated chain reaction results in the propagation of fatty acid radicals.

A radical reaction stops after two radicals react to produce a nonradical species. In animals, termination of the free radical reaction can be facilitated by antioxidants (e.g.,

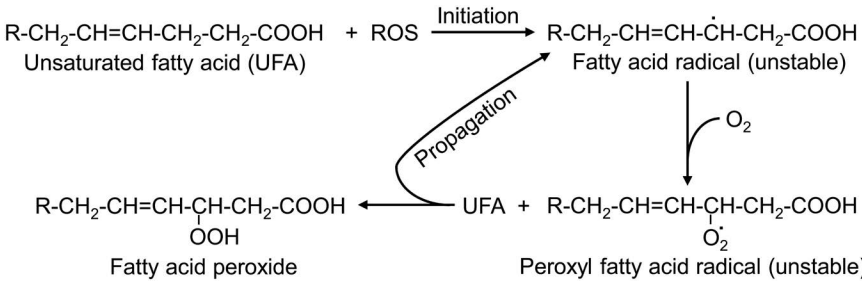


FIGURE 3.21 Peroxidation of unsaturated fatty acids. The double bonds in unsaturated fatty acids undergo peroxidation by reactive oxygen species to form lipid peroxides or lipid oxidation products. Lipid peroxidation involves three major steps: initiation, propagation, and termination. An unstable fatty acid radical is formed during the initiation phase, followed by the propagation of fatty acid radicals. The radical reaction stops when two radicals react and produce a nonradical species.

vitamins E and C, and phenolic compounds) and antioxidant enzymes (e.g., superoxide dismutase, catalase, and peroxidase; Fang et al. 2002). The antioxidants react with fatty acid radicals to form nonreactive products, thereby breaking the propagation chain of the peroxidation reaction (Mao et al. 2010). In fact, these compounds are able to donate a hydrogen atom to the fatty acid radical formed during the propagation phase of lipid acid oxidation.

Malondialdehyde (MDA; OCH-CH_2-CHO; pKa, 4.45) is generated by enzymatic and nonenzymatic peroxidation of PUFAs. MDA occurs in biological systems in its free form and in a conjugated form adducted to free amino acids and protein-bound amino acid residues, particularly lysine residue. MDA in plasma, mammalian colostrum, and milk, as well as animal cells and tissues, is widely used as a biomarker of lipid peroxidation (Rezaei and Wu 2022; Tsikas and Mikuteit 2022).

The knowledge of fatty acid peroxidation is useful for the storage (shelf life) and analysis of unsaturated fatty acids and for protecting animals from oxidative damage, a contributor to chronic diseases in mammals and birds. The Rancimat method for detecting peroxides provides useful information about the oxidative stability of fats and oils, as well as unsaturated fatty acids in feedstuffs (Aparicio et al. 1999). Noninvasive detection of products of PUFA peroxidation in plasma and urine has implications for preventing ROS-induced oxidative stress and diseases in humans and other animals.

Epoxidation of Unsaturated Fatty Acids

Epoxidation occurs via the Prileschajew reaction, in which the olefinic double bonds of unsaturated fatty acids are converted to epoxy (oxirane; the functional group of epoxides) by a peroxyacid (an acid containing a –OOH group; i.e., R-CO-OOH). The epoxy group (a highly reactive three-membered ring) contains one oxygen atom and two carbon atoms. A peroxyacid (e.g., performic acid or peroleic acid) is formed via the reaction of H_2O_2 with an acid (e.g., formic acid or oleic acid) in the presence of a strong mineral acid (e.g., sulfuric acid) as a catalyst. Vegetable oil-based epoxides are chemically produced from soy oil on an industrial scale.

$$\text{HCOOH (Formic acid) or Oleic acid} + H_2O_2 \longrightarrow \text{HCOOOH (Performic acid, PFA) or Peroleic acid} + H_2O$$

$$\underset{\text{Unsaturated fatty acid (alkene)}}{CH_3\text{-}(CH_2)_n\text{-}CH_2\text{-}CH{=}CH\text{-}(CH_2)_n\text{-}COOH} \xrightarrow[\text{(e.g., PFA)}]{\text{Peroxyacid}} \underset{\text{Epoxide of unsaturated fatty acid}}{CH_3\text{-}(CH_2)_n\text{-}CH_2\text{-}\overset{\overset{O}{\diagup\diagdown}}{CH}\text{-}CH\text{-}(CH_2)_n\text{-}COOH}$$

In animals, the epoxidation of unsaturated fatty acids (e.g., oleic, linoleic, α-linolenic, and γ-linolenic acids) at one or more double bonds can be catalyzed by the following enzymes. The main cytochrome P450 (CYP) isoforms are CYP2CAA, CYP2C2, CYP2C and CYP3A. Thus, fatty acid epoxides [e.g., *cis*-9,10-epoxystearic acid (*cis*-9,10-epoxyoctadecanoic acid, epoxidized oleic acid)] can be produced from oleic acid by both enzymic and nonenzymic epoxidation.

$$\text{Unsaturated fatty acid} + H_2O_2 \rightarrow \text{Peroxyacid (lipase)}$$

$$\text{Unsaturated fatty acids} + O_2 \rightarrow \text{Hydroxy and epoxy fatty acids (cytochrome P450)}$$

Nitration of Unsaturated Fatty Acids

An unsaturated fatty acid (e.g., oleic acid and linoleic acid) can spontaneously react with nitrogen dioxide ($NO_2^{\bullet}$) to form a nitroalkene fatty acid (an unsaturated fatty acid that contains a nitro group adjacent to a double bond) under both in vitro and in vivo conditions. $NO_2^{\bullet}$ is generated from several chemical reactions: (a) nitrite (NO_2^-) under acidic conditions, from nitric oxide (NO) autooxidation, from peroxynitrite ($ONOO^-$) decay or from peroxidase-mediated nitrite oxidation. The nitration of unsaturated fatty acids is favored in the processes of food digestion by the GIT and of inflammation. There is evidence that NO_2-oleic acid is formed from oleic acid and peroxynitrite ($ONOO^-$) at a very low rate (Tsikas 2023). Thus, the concentrations of NO_2-oleic acids (i.e., the sum of 9- and 10-NO_2-oleic acid) in the plasma of healthy, obese and type 2 diabetes subjects range from 1100 to 1300 pM (Tsikas 2023). The concentrations of *cis*-9,10-epoxy-oleic acid plus 9-NO_2-oleic acid plus 10-NO_2-oleic acid in the plasma of healthy subjects and the patients with end-stage liver disease are 1041–1218 pM and 746–782 pM, respectively (Tsikas 2023).

$$\text{Unsaturated fatty acid} + \text{Nitrogen dioxide radical } (NO_2^{\bullet}) \rightarrow \text{Nitroalkene fatty acid}$$

Reaction of the Hydrogen Atom in Methylene and Carboxyl Groups with Halogens

A hydrogen atom in a $-CH_2$ group of a fatty acid can be replaced by a halogen (fluorine, chlorine, bromine, or iodine) to yield a halogenated fatty acid (Dembitsky and Srebnik 2002). More than 200 different halogenated fatty acids are present in microorganisms, algae, marine invertebrates, higher plants, and some animals (Dembitsky and Srebnik 2002). In addition, the hydrogen atom of the carboxyl group in a fatty acid can be substituted by the halogen from a halogenated compound. For example, short-, medium-, or long-chain fatty acids (including unsaturated fatty acids) react with 9-chloromethyl-anthracene (Xie et al. 2012) or 2-bromomethyl-anthraquinone (Tapia 2014) in the presence

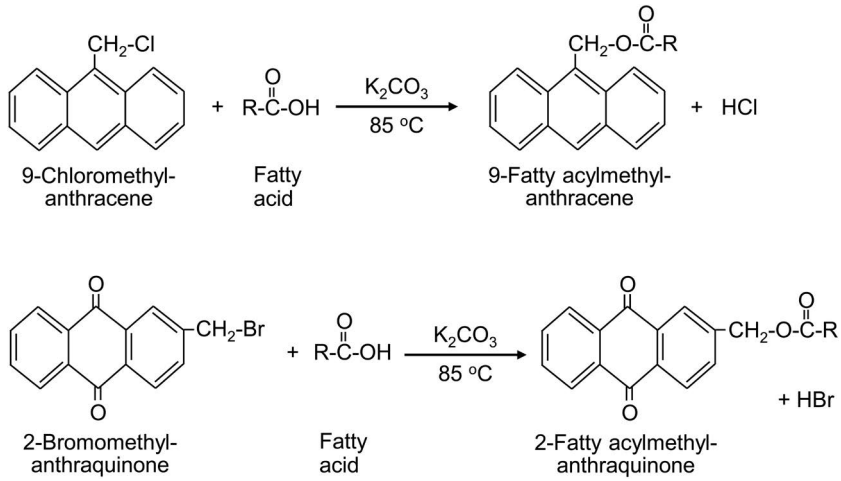


FIGURE 3.22 Reaction of fatty acids with halogens. The hydrogen atom of the carboxyl group in a fatty acid can be substituted by the halogen of a halogenated compound, such as 9-chloromethyl-anthracene or 2-bromomethyl-anthraquinone in the presence of a base (e.g., K_2CO_3) to yield a fluorescent, stable fatty acyl derivative. These reactions provide a basis for the analysis of fatty acids by chromatographic methods, such as high-performance liquid chromatography.

of a base (e.g., K_2CO_3) to yield fluorescent, stable fatty acyl derivatives (Figure 3.22). The derivatives are separated on a reversed-phase HPLC column and detected by fluorescence. These chemical reactions provide a useful tool for sensitive analysis of fatty acids in biological samples.

SUMMARY

Lipids are a diverse group of hydrocarbons that are defined according to their common hydrophobic property. This class of organic substances consists of fatty acids, as well as simple, compound, and derived lipids, with essential nutritional and physiological functions. Fatty acids have 2 to 30 carbon atoms and a carboxyl group, including short ($C_{2\text{-}5}$)-, medium ($C_{6\text{-}12}$)-, long ($C_{13\text{-}20}$)-, and very long (>C_{20})-chain fatty acids. LCFAs are much more abundant than SCFAs and MCFAs in the tissues of animals and possess 0 to 6 double bonds. In unsaturated fatty acids, the hydrogen atoms are usually on the same (*cis*) side of the double bond, but may also be on its opposite (*trans*) side. Most of the naturally occurring fatty acids have an even number of carbons, but some have an odd number of carbons or a branched chain. Because elevated concentrations of LCFAs are toxic to cells, they are stored in animals primarily as glycerol-based esters (e.g., TAGs) and, to a much lesser extent, as alcohol-based waxes. Oils contain mainly PUFAs and their esters. Fats and waxes are collectively known as simple lipids, but they differ in composition and functions. For example, fats are digestible as the major source of energy, whereas waxes are indigestible but provide a waterproof layer of protection.

Compound lipids are composed of (a) glycolipids containing a carbohydrate moiety; (b) phospholipids containing a phosphate group and an ethanolamine, choline, serine, myo-inositol, glycerol, or phosphatidylglycerol group; (c) sphingosine-based sphingolipids containing an acyl group to form a ceramide, a nitrogenous group (e.g., ethanolamine, serine, or choline) to yield a sphingomyelin, or sugars to generate glycosphingolipids; (d) ether glycerophospholipids containing an ether linkage (-CH_2-O-CH_2-) in the phospholipids (e.g., PAF and plasmalogens); and (e) lipoproteins formed and released by the small intestine and liver, with the protein moiety transporting LCFAs and cholesterol. Phosphatidylcholine, cephalins, and sphingomyelins are important components of cell membranes (Table 3.11) and highly abundant in the nervous system. Plasma chylomicrons and HDLs have the highest content of TAGs and proteins, respectively. The proportions of cholesterols in lipoproteins have important implications for cardiovascular health.

Derived lipids include steroids, eicosanoids, and terpenes. Steroids include sterols (e.g., cholesterol) and their derivatives, such as steroid hormones (e.g., male and female reproductive hormones), bile acids, and bile alcohols that are synthesized in animals, as well as steroidal saponins that are present in plants. Bile acids that are conjugated with taurine or glycine are known as bile salts, which facilitate the digestion and absorption of dietary lipid-soluble nutrients. Eicosanoids are composed of series 1, 2, and 3 prostaglandins and thromboxanes with 1, 2, or 3 double bonds, as well as series 4 and 5 leukotrienes and lipoxins with 4 or 5 double bonds. Terpenes, which have one or more isoprene units, include carotenoids, coenzyme Q, vitamins A, E, and K, essential oils, and phytols.

Lipids undergo many reactions, such as the hydrolysis of TAGs, the saponification of fatty acids, esterification with alcohols, the substitution of the hydroxyl hydrogen, the hydrogenation of unsaturated fatty acids, the peroxidation of unsaturated fatty acids (particularly at high temperatures), and reactions with chlorine or bromine. Although the diverse structures of lipids present challenges in their laboratory analysis, recent advances in chemistry have laid

down a solid foundation for nutritional research on precursor fatty acids and their metabolites. Knowledge about the chemistry of lipids provides a foundation for our understanding of their metabolism and nutrition in animals.

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4 Chemistry of Protein and Amino Acids

The word "protein" originated from the Greek word *proteios,* meaning prime or primary (Meister 1965). A protein is a large polymer of amino acids linked via the peptide bond (–CO–NH–). Different proteins have different chemical properties (e.g., amino acid sequences, molecular weights, ionic charges, three-dimensional structures, hydrophobicity, and function). The general structure of an amino acid is shown in Figure 4.1. There may be one or more polypeptide chains in a protein, which contains its constituents [nitrogen (N), carbon (C), oxygen (O), hydrogen (H), and sulfur (S) atoms]. A protein may be covalently bonded to other atoms and molecules (e.g., phosphates) and noncovalently attached to minerals (e.g., calcium, iron, copper, zinc, magnesium, and manganese), certain vitamins (e.g., vitamin B_6, vitamin B_{12}, and lipid-soluble vitamins), and/or lipids. Protein is the major nitrogenous macronutrient in foods and the fundamental component of animal tissues (Wu 2016). It has structural, signaling, and physiological functions in animals (Table 4.1).

Amino acids are classified according to their various properties. The classification schemes are based on molecular weights (small vs. large), physiological functions (e.g., proteinogenic vs. nonproteinogenic), and chemical properties. The latter include hydrophobic or hydrophilic; net ionic charges (neutral, acidic, or basic); straight- or branched-chain; structure (the primary amino group vs. the imino group); and the composition of N (one to four N atoms) and S (zero to two S atoms). The twenty amino acids that are the precursors for protein synthesis are called proteinogenic amino acids (Figure 4.2), whereas the amino acids that are not substrates for protein synthesis are referred to as nonproteinogenic amino acids (Wu et al. 2016). Post-translationally modified amino acids (e.g., 4-hydroxyproline and 3-methylhistidine) that are constituents, but not precursors, of proteins are loosely considered as proteinogenic amino acids. Examples of nonproteinogenic amino acids with important physiological functions in animals are shown in Figure 4.3. Both proteinogenic and nonproteinogenic amino acids have diverse roles in animals, and all of them are needed to maintain the homeostasis of organisms (Wu 2022). Animal- and plant-sourced proteins in feed ingredients have different compositions of amino acids (Li et al. 2011; Wu et al. 2024), as do various proteins in the body.

There is a rich history of amino acid chemistry (Greenstein and Winitz 1961; Vickery and Schmidt 1931). Research on natural and synthetic amino acids was pioneered in Europe and has spanned over 200 years. In 1806, asparagine was the first amino acid to be discovered

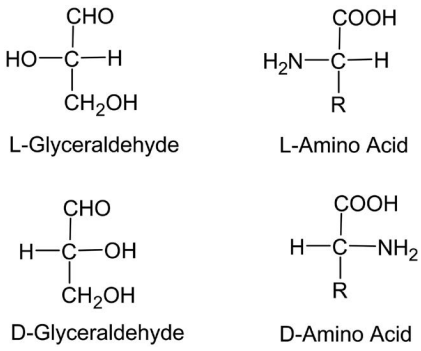


FIGURE 4.1 Fisher projections for configurations of amino acids relative to L-glyceraldehyde and D-glyceraldehyde. The general structure of an amino acid in the nonionized form is shown. For amino acids, L- or D-isomers refer only to the chemical configuration of their α-carbon.

TABLE 4.1
Structural and Physiological Roles of Proteins in Animals

Roles	Examples of Proteins
Buffering	Hemoglobin, myoglobin, and hemocyanins (crustaceans)
Cell and tissue structures	Collagen, elastin, filaggrin (skin), keratin, mucins, proteoglycans, and vimentin
Colloidal properties	Proteins in plasma (albumin and globulins) and gelatin
Enzyme-catalyzed reactions	Decarboxylase, dehydrogenase, kinase, lipases, proteases, and transaminase
Gene expression	DNA-binding proteins, DNA polymerase, histones, and repressor proteins
Hormone-mediated effects	FSH, glucocorticoids, insulin, LH, placental lactogen, somatotropin, and TSH
Muscle contraction	Actin, ATPase, myosin, nebulin, titin, tropomyosin, troponin, and tubulin
Protection	Blood clotting factors, immunoglobulins, interferon-γ, and interferon-tau
Regulation of cell signaling	AMPK, calmodulin, integrins, GPCRs, leptin, MTOR, osteopontin, and PKG
Storage of nutrients and O_2	FABs, ferritin, metallothionein, myoglobin, and perilipins
Transport of nutrients and O_2	Albumin, hemoglobin, plasma lipoproteins, and amino acid and glucose transporters

Note: AMPK, AMP-activated protein kinase; FABs, fatty acid-binding proteins; FSH, follicle-stimulating hormone; GPCRs, G protein-coupled receptors; LH, luteinizing hormone; MTOR, mechanistic target of rapamycin; PKG, cGMP-dependent protein kinase; TSH, thyroid-stimulating hormone

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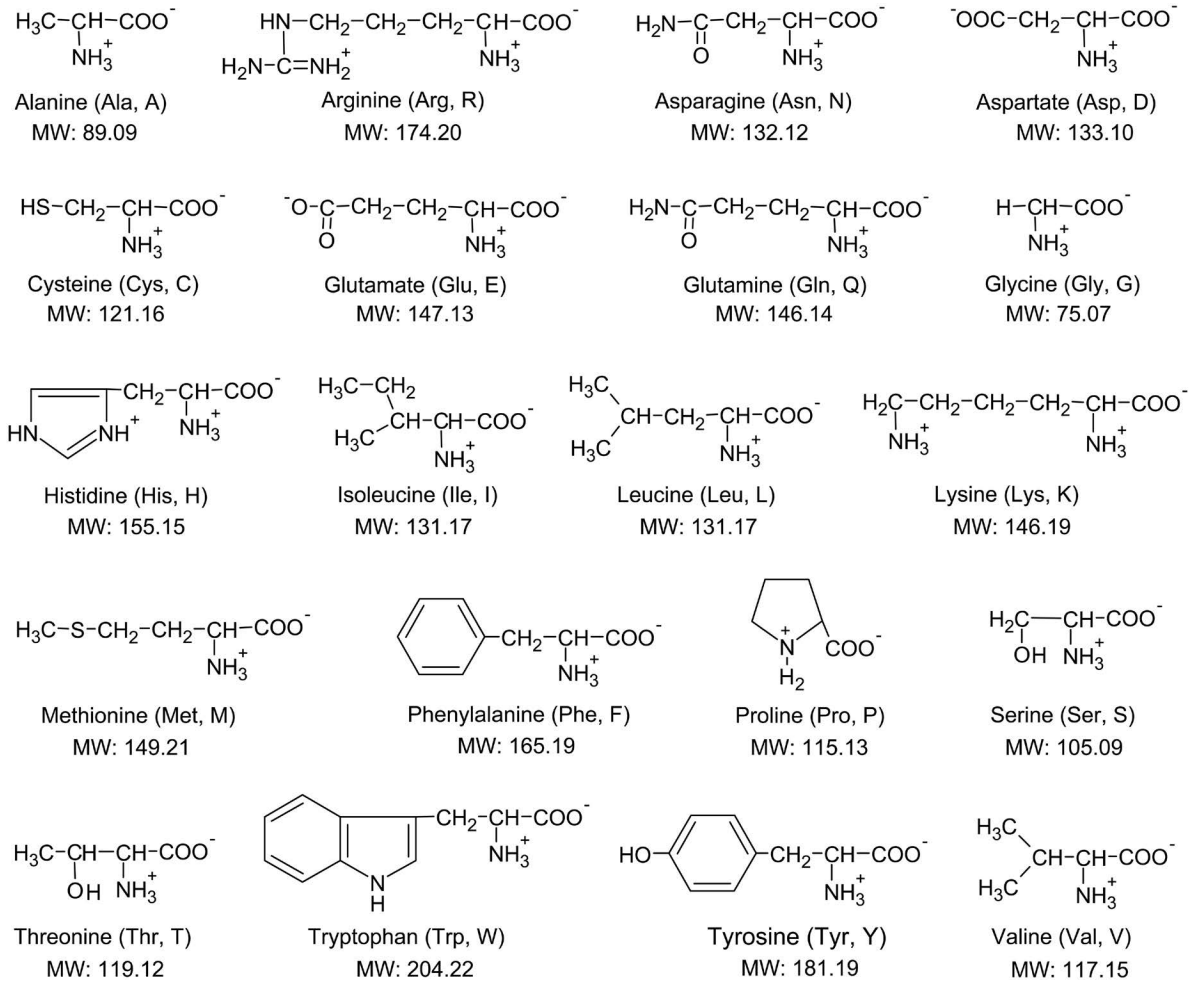


FIGURE 4.2 Chemical structures of amino acids that are substrates for the synthesis of proteins in animals at a neutral pH. These proteinogenic amino acids also occur in microbes and plants. Except for glycine, proteinogenic amino acids exist as L-isomers.

in nature by French chemists L.N. Vauquelin and P.J. Robiquet. This was followed by the first isolation of glycine from a protein (i.e., gelatin) in 1820 by another French chemist, H. Braconnot. In 1925, threonine was independently discovered in oat protein (by S.B. Schryver and H.W. Buston) and teozein (by R.A. Gortner and W.F. Hoffmann) and was the last addition to the long list of 20 proteinogenic amino acids in organisms. Ten years later, W.C. Rose identified the presence of threonine in casein. This work led to the formulation of purified diets with which to determine dietary requirements of individual amino acids by animals. The analysis of peptide-bound amino acids in feedstuffs and animal proteins is now feasible based on their acid, alkaline and enzymatic hydrolysis (Dai et al., amino acids 2014; Li et al. 2011). Protein ingredients (the primary sources of dietary amino acids) are the most expensive components of animal diets. Thus, maximum economic returns and sustainability of animal production require adequate knowledge of protein and amino acid chemistry (Wu et al. 2014a,b). The knowledge of protein and amino acid chemistry pertinent to animal nutrition is highlighted in this chapter.

DEFINITION, CHEMICAL CLASSIFICATION, AND PROPERIES OF AMINO ACIDS

Definition of Amino Acids: α-, ß-, γ-, δ-, or ϵ-Amino Acids

An amino acid is defined as an organic substance that contains both amino and acid groups. The acid group is the carboxyl group (–COOH) in proteinogenic amino acids but can be a sulfonic acid group (-SO_2-OH; its ionized form is called sulfonate) in nonproteinogenic amino acids (Wu 2022). The number of carbons in an amino acid is ≥2, with the carbon adjacent to the primary acid group being called the α-carbon. The other carbon atoms of the amino acid beyond the α-carbon are named in

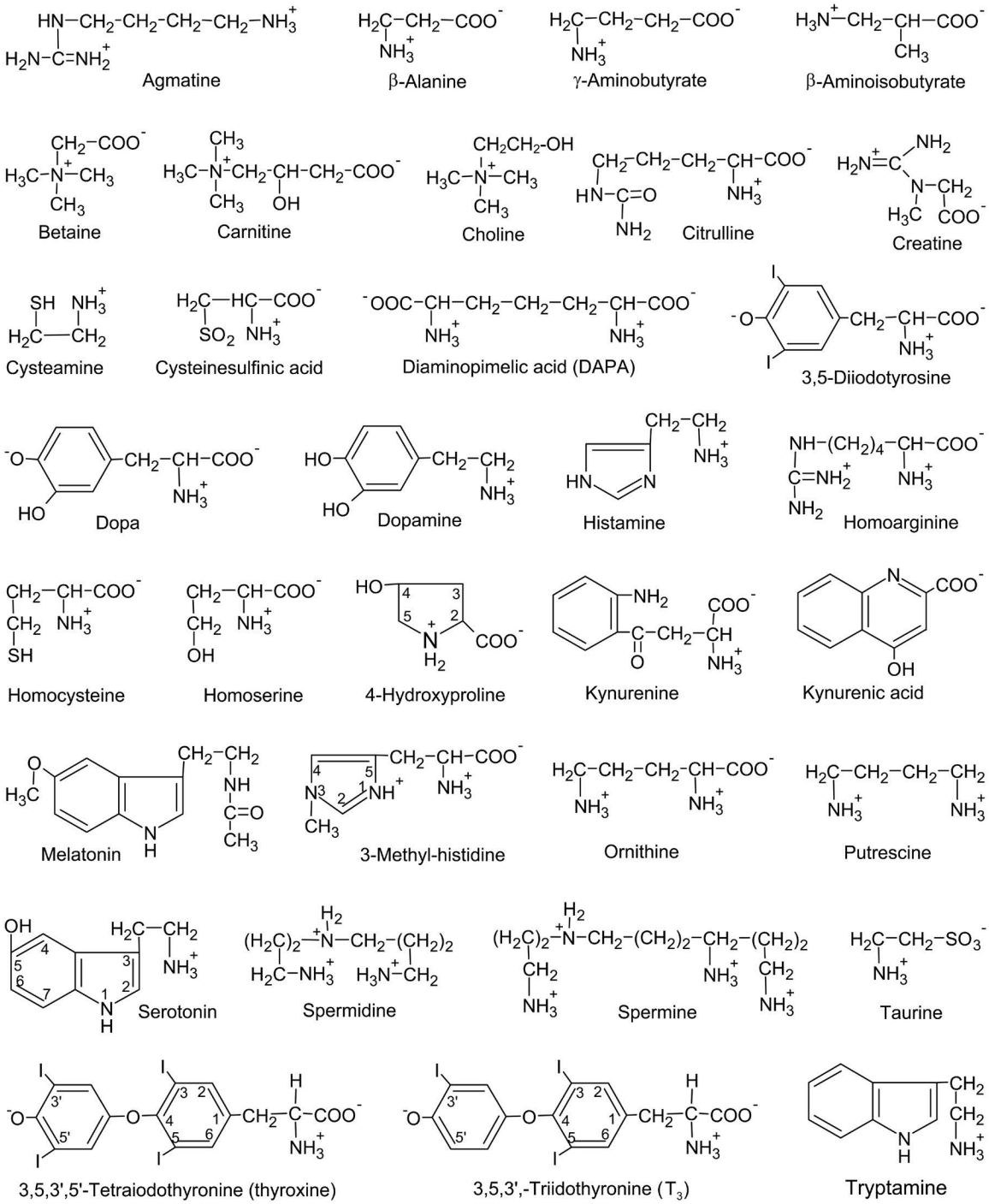
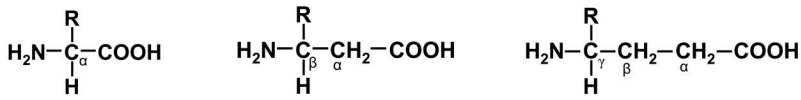


FIGURE 4.3 Chemical structures of nonproteinogenic amino acids and related metabolites in animals at a neutral pH. These substances occur in physiological fluids of animals, and some of them are also present in microbes and plants. Taurine is present only in animals as a free amino acid.

sequence according to the Greek alphabet, i.e., ß-, γ-, δ-, or ε-carbon. If the amino group ($-NH_2$) is linked to the α-carbon, the amino acid is called an α-amino acid. Likewise, if the amino group is linked to the ß-, γ-, δ-, or ε-carbon, the amino acid is known as a ß-, γ-, δ-, or ε- amino acid, respectively.



Imino Acids

Proline and hydroxyproline are special nitrogenous compounds with a pyrrolidine ring. They contain a secondary α-amino (α-imino; –NH) group and, therefore, are α-imino acids (Phang et al. 2008). The chemical reactivity of the imino group differs remarkably from that of the amino group. Because proline is a common substrate for protein synthesis like α-amino acids and hydroxyproline is the post-translational derivative of proline, both proline and hydroxyproline are loosely called α-amino acids in biochemistry and nutrition (Hu et al. 2022). 4-Hydroxyproline occurs as both *trans*-L-4-hydroxyproline (the major form) and *trans*-L-3-hydroxyproline (the minor form) in animals, but is rare in plants and microbes.

Differences in the Structures of Amino Acids

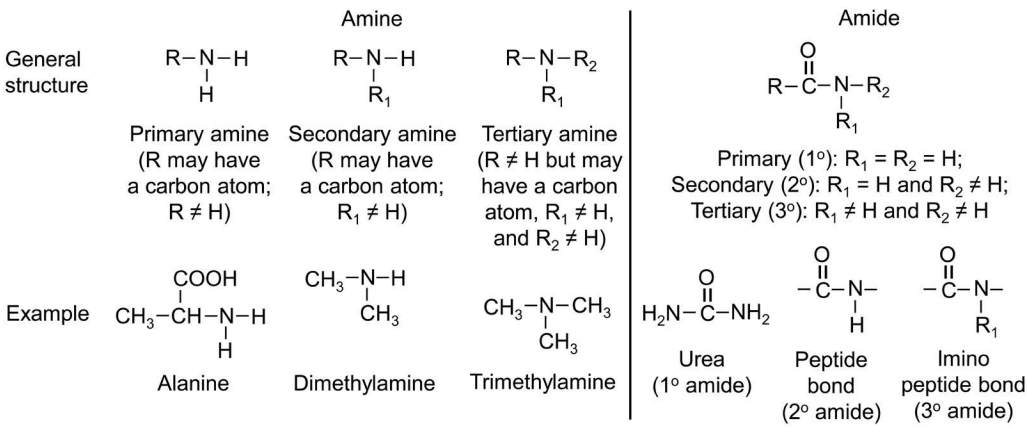
The numbers of amino and acid groups, as well as side-chain groups, vary with amino acids (Greenstein and Winitz 1961). For example, glutamate and aspartate have two carboxyl groups and one α-amino group, whereas lysine and ornithine have two amino groups and one carboxyl group. Both arginine and some of its metabolites (e.g., methylarginines and homoarginine) possess a guanidino group, while other members of the arginine family of amino acids, citrulline and homocitrulline, contain a ureido group. Although most amino acids have a straight carbon chain (e.g., alanine, asparagine, glutamine, and glycine), some amino acids (e.g., leucine, isoleucine, and valine) are branched in structure. The side chain of asparagine or glutamine that contains a [–(C=O)-NH_2] group is an amide [R-(C=O)-N(R_1)-R_2], where R_1 may be the same as R_2. The differences in the side chains of amino acids greatly affect their chemical properties.

All amino acids are composed of N, C, O, and H atoms. The abundance of the N atom in amino acids can be very different. For example, the number of N atoms per molecule is 4 in arginine, homoarginine and argininosuccinate; 3 in citrulline and histidine; 2 in glutamine, lysine, ornithine, tryptophan, and cystine; and 1 in alanine, glutamate, glycine, and leucine. The mean content of N in skeletal muscle and most other tissues is 16%. In fact, the reciprocal of this percentage (6.25) is used to convert the N content of a sample into the crude protein (CP) content.

Some amino acids (e.g., methionine, cysteine, homocysteine, taurine, and selenocysteine) also contain one S atom, whereas there are two S atoms in cysteine and Djenkolic acid. They are all called S-containing amino acids. Selenocysteine is a rare amino acid only present in selenoproteins (Stadtman 1996) and is derived, at the translation step, from serine and selenium (in the form of selenophosphate synthesized from selenide and ATP by selenophosphate synthetase 2; Leibundgut et al. 2005). Free selenocysteine is not a substrate for protein synthesis. The presence of the S atom greatly influences the reactivity of the α-amino group in the S-containing amino acids and proteins.

Amines and Amides

Amines are derivatives of ammonia (NH_3), wherein one or more hydrogen atoms are substituted with one or more organic groups. Amines can be classified as primary, secondary, or tertiary, depending on the replacement of one, two, or three of the hydrogen atoms of ammonia, respectively. For example, amino acids, imino acids, and trimethylamine are primary, secondary, and tertiary amines, respectively. Some amino acid metabolites [e.g., polyamines, dimethylamine, and betaine (trimethylglycine)] also belong in these groups of substances. Amides are substances that contain a carbonyl group (C=O) linked to an N atom. Amines can be classified as primary, secondary, or tertiary, depending on whether two, one, or no hydrogen is bonded to the N atom, respectively. Examples of primary, secondary, or tertiary amides are urea, peptide bonds, and imino acid-based bonds in peptides, respectively. Some amino acids (e.g., asparagine and glutamine) are both amines and amides.



Naming and Chemical Expression of Amino Acids

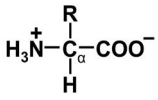
The common or trivial names of amino acids were derived from (a) the history of their discoveries; (b) their characteristics, including appearance (e.g., arginine and leucine), taste (e.g., glycine), and chemical structure (hydroxyproline, isoleucine, lysine, methionine, proline, and threonine); (c) their sources of isolation (e.g., asparagine,

citrulline, cysteine, glutamate, serine, tryptophan, tyrosine, and valine); or (d) the precursors of their chemical syntheses (e.g., alanine and phenylalanine). Because of variations in their side chains and associated characteristics, amino acids have remarkably different chemical properties and physiological functions (Greenstein and Winitz 1961).

According to the International Union of Biochemistry and Molecular Biology, a 3-letter abbreviation can be used to designate a protein amino acid, with one capital letter followed by two lower-case letters (e.g., Gln for glutamine; Figure 4.2). A one-letter abbreviation is used to represent an amino acid in protein or polypeptide sequences (e.g., E, Q, and R for glutamate, glutamine, and arginine, respectively). This annotation is very useful to indicate the primary structure of a protein.

The Zwitterionic (Ionized) Form of Amino Acids

The amino (or imino) and acid groups have opposite electrical charges. Therefore, an amino acid can act as a base or an acid by accepting or donating a hydrogen ion (H^+, also called a proton), respectively. All amino acids form intramolecular salts both in the crystalline state and in an aqueous solution. This structure, in which a molecule has both positive and negative electrical charges, is known as a zwitterion. All amino acids are ionized in physiological fluids and cellular proteins.



The zwitterionic (ionized) form of an amino acid

The strength of an acid (HA), such as an amino acid, is indicated by its dissociation constant (Ka). The stronger the acid, the more protons that will dissociate from it, and the lower the pKa value. The pKa for the acid, amino, and side-chain groups in amino acids are termed pKa_1, pKa_2, and pKa_3, respectively (Table 4.2).

$$HA \rightleftharpoons H^+ + A^-;\ Ka = [H^+] \times [A^-]/[HA];$$
$$pKa = -\log Ka.$$

Henderson-Hasselbalch equation:

$$pH = pKa + \log([A^-]/[HA])$$

Although all amino acids are ionized, the charges of the amino (imino) and acid groups can counterbalance each other. Here, the pH of an aqueous solution at which an amino acid has no net electrical charge is called the isoelectric point (pI). When an amino acid is dissolved in pure water until the solution is saturated, the pH of this solution will approach the pI value of the amino acid. For an amino acid without an ionizable side chain (e.g., glutamine and glycine), $pI = (pKa_1 + pKa_2)/2$.

Examples: for glutamine, $pI = (2.17 + 9.13)/2 = 5.65$;
for glycine, $pI = (2.35 + 9.78)/2 = 6.07$.

For an amino acid with an ionizable side chain (e.g., glutamate and arginine), pI = the average of the pKa values of the two most similar acid groups.

Examples: for glutamate, $pI = (2.19 + 4.25)/2 = 3.22$;
for arginine, $pI = (9.04 + 12.48)/2 = 10.76$.

Because the pKa values of the α-carboxylic acid groups of α-amino acids are approximately 2.0–2.4 (Table 4.2), these groups are almost entirely in their carboxylate forms at pH > 3.5. Similarly, because the pKa values of the α-amino groups of α-amino acids are approximately 9.0–10.0, these groups are almost entirely in their ammonium ion forms at pH < 8.0. Thus, at a physiological pH (e.g., pH 7.4 in the blood and pH 7.0 in the cytoplasm), the α-carboxylic acid and α-amino groups of α-amino acids are completely ionized to take the zwitterion form. In their ionized form, glutamic acid and aspartic acid are called glutamate and aspartate, respectively.

Based on their net charges at a neutral pH, amino acids are classified as neutral (net charge = 0), basic (net charge ≥ +1), or acidic (net charge ≤ −1). Thus, the addition of an acidic or basic amino acid to a solution with a weak buffering capacity will substantially decrease or increase its pH, respectively. Similarly, intravenous infusion of large amounts of an acidic amino acid (e.g., aspartic acid) or basic amino acid (e.g., arginine) into animals (including humans) will adversely disturb the acid-base balance of the body. Thus, acidic or basic amino acids should be neutralized (e.g., in the form of the aspartate-sodium salt or the arginine-HCl salt) before their use for intravenous administration. Interestingly, supplementing an appropriate amount of an acidic amino acid (e.g., 1% glutamic acid) or basic amino acid (e.g., 1% arginine) to a corn- and soybean meal (SBM)-based diet does not affect the pH in the lumen of the swine gastrointestinal tract. This is likely due to the highly acidic environment of the stomach (pH 2.0–2.5) and bicarbonate-containing pancreatic secretions in the lumen of the small intestine.

D- or L-Configurations of Amino Acids

Definition of L-Amino Acids and D-Amino Acids

The absolute configuration of amino acids (L- or D-isomers as introduced by Emil Fischer in 1908) was arbitrarily defined with reference to glyceraldehyde (Figure 4.1) and is now mostly used in amino acid chemistry and nutrition. For amino acids, L- or D-isomers refer only to the chemical configuration of their α-carbon. An asymmetric carbon is also called a chirality center. Because each asymmetric carbon can have two possible configurations, an amino acid with n asymmetric carbons has 2^n different possible stereoisomers. Except for glycine (the simplest amino acid in nature) which has no chirality center and, therefore, no L- or D-configuration, all proteinogenic amino acids (Figure 4.2) exist only as L-amino acids in animal cells. Most nonproteinogenic amino acids can exist as L-amino acids, D-amino

TABLE 4.2
Molecular Weights and Chemical Properties of Amino Acids

Amino Acid	MW (Da)	MP (°C)	Solubility[a]	pK_1[b]	pK_2[c]	pK_3[d]	pI
Neutral Amino Acids							
L-Alanine	89.09	297	16.5	2.35	9.87	–	6.11
β-Alanine	89.09	197	82.8	3.55	10.24	–	6.90
γ-Aminobutyrate	103.12	202	107.3	4.03	10.56	–	7.30
L-Asparagine	132.12	236	2.20	2.02	8.80	–	5.41
L-Citrulline	175.19	222	15.2	2.43	9.41	–	5.92
L-Cysteine	121.16	178	17.4	1.96[e]	8.18[e]	10.28[e]	5.07[e]
L-Cystine	240.30	261	0.011	<1.0[f]	8.02[f]	–	5.06[f]
				2.10[f]	8.71[f]	–	
L-Glutamine	146.14	185	4.81[e]	2.17	9.13	–	5.65
Glycine	75.07	290	25.0	2.35	9.78	–	6.07
L-Hydroxyproline	131.13	270	36.1	1.92	9.73	–	5.83
L-Isoleucine	131.17	284	4.12	2.36	9.68	–	6.02
L-Leucine	131.17	337	2.19	2.33	9.75	–	6.04
L-Methionine	149.21	283	5.06	2.28	9.21	–	5.74
L-Phenylalanine	165.19	284	2.96	2.20	9.31	–	5.76
L-Proline	115.13	222	162.3	1.99	10.6	–	6.30
L-Serine	105.09	228	41.3	2.21	9.15	–	5.68
Taurine	125.15	328	10.5	1.50	8.74	–	5.12
L-Threonine	119.12	253	9.54	2.15	9.12	–	5.64
L-Tryptophan	204.22	282	1.14	2.38	9.39	–	5.89
L-Tyrosine	181.19	344	0.045	2.20	9.11	10.07	5.66
L-Valine	117.15	315	5.82	2.29	9.72	–	6.01
Basic Amino Acids							
L-Arginine	174.20	238	18.6	2.17	9.04	12.48	10.76
L-Histidine	155.15	277	4.19	1.80	9.33	6.04	7.69
L-Lysine	146.19	224	78.2[g]	2.18	8.95	10.53	9.74
L-Ornithine	132.16	231[h]	54.5[h]	1.94	8.65	10.76	9.71
Acidic Amino Acids							
L-Aspartic acid	133.10	270	0.45	1.88	9.60	3.65	2.77
L-Glutamic acid	147.13	249	0.86	2.19	9.67	4.25	3.22

Source: Wu (2022).

[a] Solubility in water (g/100 mL at 25°C unless otherwise indicated).

[b] pK for α-COOH (SO_3H for taurine) at 25°C unless otherwise indicated.

[c] pK for α-NH_3^+ (α-NH_2^+ for proline) at 25°C unless otherwise indicated.

[d] pK for the ionized group in the side chain at 25°C unless otherwise indicated.

[e] Determined at 30°C.

[f] Determined at 35°C.

[g] L-Lysine-H_2O.

[h] L-Ornithine-HCl.

Note: MP, melting point; MW, molecular weight.

acids, or both. However, some nonproteinogenic amino acids (e.g., taurine, β-alanine, and γ-aminobutyric acid) do not have a chirality center and, therefore, have no D- or L-isomers. Chemical synthesis may yield both D- and L-isoforms of the proteinogenic amino acids (except for glycine).

Optical Activity of L-Amino Acids and D-Amino Acids

An optical isomer (e.g., L- or D-amino acid) exhibits optical activity or rotatory polarization. That is, when a beam of plane-polarized light is passed through a solution of the optical isomer, the light will be rotated either to the right or to the left. Optical rotations of amino acids can be determined with a photoelectric polarimeter at 20–28°C and their concentrations of 0.5–2% (Greenstein and Winitz 1961). When equal amounts of D- and L-amino acids (e.g., synthetic DL-methionine) are present in a solution, the resulting mixture has no optical activity.

Optical isomerism of organic molecules has traditionally been described as dextrorotatory (right, "+" or *d*) or levorotatory (left, "–" or *l*), depending on the direction of optical

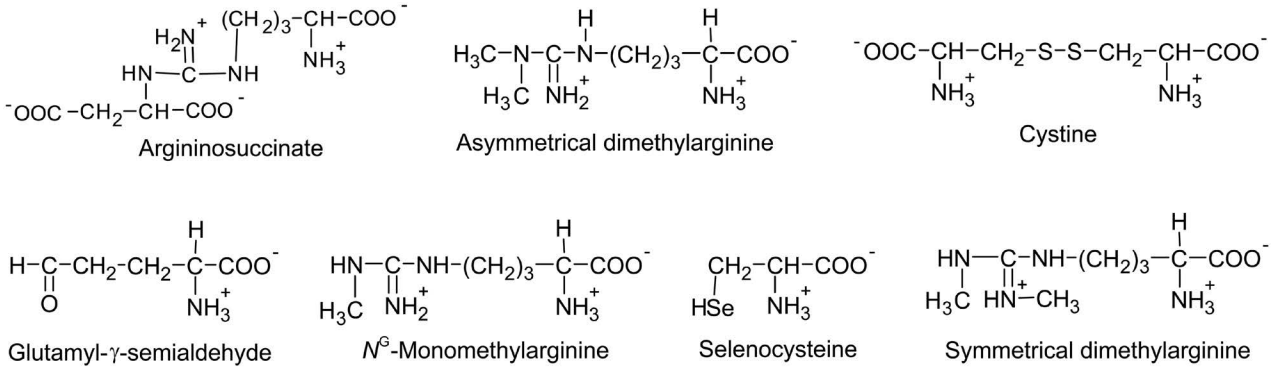


FIGURE 4.4 Chemical structures of methylarginines and some special amino acids in animals at a neutral pH. These substances also occur in microbes and plants.

rotation. Because measurement conditions can affect the angle of optical rotation, the terms "dextrorotatory" and "levorotatory" have been abandoned. It should be noted that the D and L-configurations for an organic substance may not necessarily determine the rotation of plane-polarized light. For example, the naturally occurring form of fructose is the D(–) isomer (D-configuration) with a levorotatory optical activity.

L-Amino Acids and D-Amino Acids in Nature

Approximately 700 natural L- and D-amino acids had been reported to be present in animals, plants, and microbes by the 1980s (Wagner and Musso 1983). Interestingly, only 20 of them are the building blocks of cellular proteins. Thus, >97% of the naturally occurring amino acids are nonproteinogenic. Some of them are present in animals (Figure 4.4), as well as feedstuffs, microbes, and plants (Figure 4.5). Besides proteinogenic amino acids, many nonproteinogenic amino acids are commonly present in all living organisms. However, some nonproteinogenic amino acids are specific to only certain kingdoms of organisms. For example, taurine is synthesized only by the liver of animals and is not formed in either plants or microbes (Wu 2022). In contrast, theanine

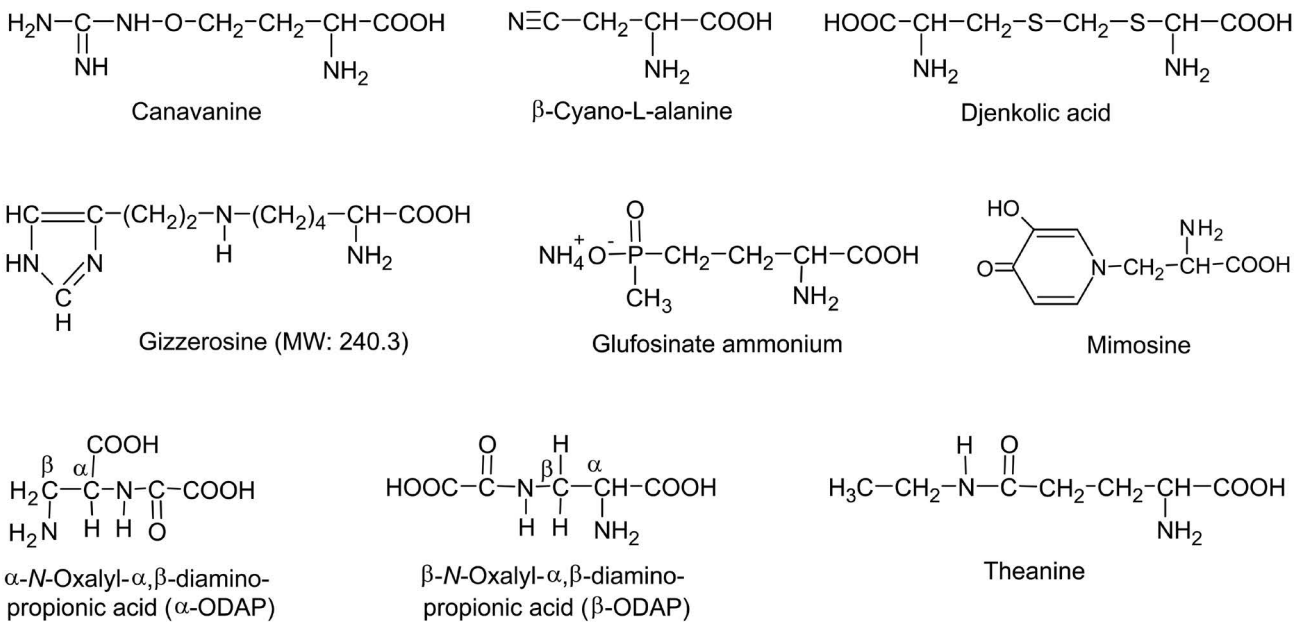
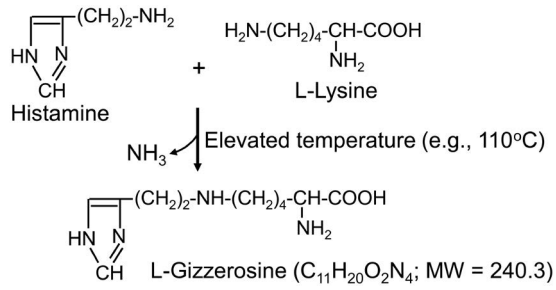


FIGURE 4.5 Chemical structures of gizzerosine, glufosinate ammonium, and some plant amino acids in the nonionized form. Gizzerosine may be formed from histamine and lysine in fishmeal during processing and storage at high temperature. Glufosinate ammonium is produced by microbes, whereas β-cyano-L-alanine is synthesized by both plants and microbes. All the other amino acids presented in this figure occur in plants. Canavanine, Djenkolic acid, mimosine, and theanine are structurally similar to arginine, cystine, tyrosine, and glutamine, respectively. Except for theanine, all the amino acids shown herein are highly toxic to animals.

(*N*-ethyl-L-glutamine) is found only in plants and is not produced in either animals or microbes.

Some nonproteinogenic amino acids in plants (e.g., L-theanine in teas and citrulline in watermelon) are beneficial for animal health, whereas others [e.g., β-cyanoalanine, djenkolic acid (two cysteine radicals connected by a methylene group), mimosine (chemically similar to tyrosine), gizzerosine, and glufosinate ammonium] can be highly toxic to animals (Nunn et al. 2010). Gizzerosine, which is formed from histamine and lysine in fish meal (and possibly other protein feedstuffs) at high temperature (e.g., 110°C), causes severe gizzard erosion (known as black vomit) in chickens even at a very low dose of 0.2 ppm in the diet (Sugahara 1995). It is L-gizzerosine, but not D-gizzerosine, that exerts the toxic effect in animals. Derivatives of some naturally occurring amino acids possess activities against weeds, fungi, and insects (Lamberth 2016). For example, glufosinate ammonium, a naturally occurring broad-spectrum herbicide from several species of *Streptomyces* (soil bacteria), can inhibit glutamine synthesis in animals (Hack et al. 1994).



Some D-amino acids also exist naturally in microbes, animals, and plants (Table 4.3) due to the racemization of free or protein-bound L-amino acids. The presence of D-amino acids in nature was first recognized in 1935 when W.A. Jacobs and L.C. Craig reported the presence of D-proline in ergotinine (a tripeptide alkaloid isolated from ergot). Examples of other D-amino acids are numerous: D-aspartate, D-glutamate, and D-alanine in bacterial cell-wall peptidoglycans; D-alanine in the rat pancreas, mouse brain, and peripheral tissues, as well as fishes; D-aspartate in rat endocrine glands (e.g., pancreas, pineal, adrenal, and pituitary), reproductive organs (e.g., testis, ovary, and placenta), the immune system (e.g., spleen and thymus), heart, and physiological fluids (e.g., plasma and saliva); and D-serine in the mouse brain and peripheral tissues (e.g., blood, heart, pancreas, spleen, liver, kidney, testis, epididymis, lung, skeletal muscle, and retina), as well as funnel web spiders (Nishikawa 2011). Furthermore, processing food at high temperatures can result in the formation of both free and peptide-bound D-amino acids in a time- and pH-dependent manner (0.2–2%). During cooking, rates of racemization of different L-amino acid residues to their respective D-isomers in a food protein vary, but the relative rates of formation through racemization for the same D-amino acid in different proteins are similar under the same conditions (Friedman 1999). Thus, the intrinsic chemical properties of amino acids, but not protein structures, are the predominant factor affecting their racemization rates during food processing. Compared to L-amino acids, D-amino acids

TABLE 4.3
Presence of Naturally Occurring Peptides Containing D-Amino Acids

Common Name	Structure and Origin
Ergotinine (ergocristinine)	Phe-D-Pro-Val-Lysergic acid (a tripeptide, with the NH_2 of Val linking to lysergic acid) Produced by ergot (fungus)
Tyrocidine A[a]	D-Phe-Pro-Phe-D-Phe-Asn-Gln-Tyr-Val-Orn-Leu (a cyclic decapeptide) Produced by Gram-positive bacteria *Bacillus brevis* found in soil
Gramicidin[a]	Formyl-Val-Gly-Ala-D-Leu-Ala-D-Val-Val-D-Val-Trp-D-Leu-Trp-D-Leu-Trp-D-Leu-Trp-Ethanolamine (a cyclodecapeptide) Produced by Gram-positive bacteria *Bacillus brevis* found in soil
Lactocin S[a]	A peptide containing 37 amino acid residues, including lanthionine and D-Ala
Enkephalins	Tyr-Gly-Gly-Phe-Met and Tyr-Gly-Gly-Phe-Leu (pentapeptides) Produced by the brain (pentapeptide); bind opioid receptors
Dermorphin[b]	Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2; Tyr-D-Ala-Phe-Gly-Tyr-Pro-Lys-NH_2; Tyr-D-Ala-Phe-Trp-Tyr-Pro-Asn-NH_2 (a hepta-peptide) Produced by South American frogs *Phyllomedusa,* bacteria, amphibians and molluscs
Deltorphins	Tyr-D-Met-Phe-His-Leu-Met-Asp-NH_2; Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH_2; Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH_2 Produced by opioid peptides from the amphibian skin
Achatin I[c]	Gly-D-Phe-Ala-Asp-NH_2 (a tetrapeptide) Produced by the ganglia and atrium of the African snail *Achatina fulica*
Fulicin	Phe-D-Asn-Glu-Phe-NH_2 (a tetrapeptide) Produced by the ganglia of the African snail *Achatina fulica*
ω-Agatoxins	A peptide containing 48 amino acid residues, including lanthionine and D-Ser Produced by the funnel web spider *Agelenopsis aperta* and present in its venom
Peptidoglycans	Polypeptides containing D-Ala, D-Asp and D-Glu Produced by bacteria and present on the bacterial cell walls

Source: Kreil (1997) and Li et al. (2016). Except for Gly, amino acids not indicated as the D-isoform are present in the L-isoform.

[a] Antibiotic.

[b] Act through binding to opiate receptors. Dermorphin is more potent than morphine in inducing deep long-lasting analgesia. This peptide has no biological activity if D-Ala is replaced by L-Ala.

[c] Act as a neuropeptide for the control of muscle contraction. This peptide has no excitatory activity on the heart or other muscles of the snail if D-Phe is replaced by L-Phe.

are quantitatively minor amino acids in nature (Li et al. 2016; Wu 2022). The ratio of total L-amino acids to total D-amino acids is likely greater than 100:0.02 (mol/mol) in organisms. The physiological functions of D-amino acids in animals remain to be defined (Brosnan 2001).

The configuration of L-amino acid and D-amino acid in water or buffered solutions does not change at room temperature (e.g., 25°C). Standard conditions for hydrolysis under acid (6 M HCl at 110°C for 24 h under N gas), base (4.2 M NaOH plus 1% thiodiglycol, antioxidant, at 110°C for 20 h), or enzymes do not affect the preexisting isomer of an amino acid in peptides. However, L- or D-amino acids may lose their optical activity (known as racemization) in higher concentrations of acid or alkaline solutions at high temperatures (e.g., >105°C). Heating feed protein above 105°C may generate a small amount of D-amino acid (Friedman 1999). To date, D-amino acids are often analyzed by high-performance liquid chromatography on a chiral or ligand column or involving precolumn derivatization with reagents that convert the enantiomers to diastereomers to improve chromatographic resolution.

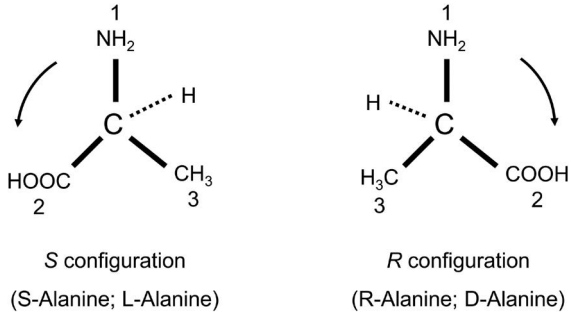


FIGURE 4.6 *R* and *S* configurations of organic substances. This nomenclature system does not involve a reference molecule such as glyceraldehyde. 1 = the group with the highest atomic number; 2 = the group with the second-highest atomic number; 3 = the group with the third-highest atomic number; 4 = the group with the lowest atomic number. To determine whether the chirality center is *R* or *S* configuration, one needs to first prioritize all four of the groups connected to the chirality center based on their atomic numbers (i.e., a higher atomic number takes precedence over a lower atomic number). Then, rotate the molecule so that the fourth (lowest) priority group points away from the viewer (a dash line). If the sequence for the first three priority groups 1-2-3 is clockwise, the designation is *R*. If the sequence for the first three priority groups 1-2-3 is counterclockwise, the designation is *S*.

R/S Configurations of Amino Acids

To better distinguish some naturally occurring and synthetic amino acids (e.g., threonine, isoleucine, and hydroxyproline) with more than one chirality center, the *R/S* nomenclature is used to name their absolute configurations, where *R* is *rectus* (right) and *S* is *sinister* (left) in Latin (Figure 4.6). This nomenclature system does not involve a reference molecule such as glyceraldehyde, but is based on the prioritized spatial arrangement of the four different groups which are attached to the chirality center. The *R/S* system has no fixed relation to the (+; dextrorotatory form)/(−; levorotary form) system. An *R* isomer can be either dextrorotatory or levorotatory, depending on its exact substituents. In the *R/S* system, naturally occurring amino acids are mostly in the *S* configuration for the first chiral center, just like carbohydrates. The *R/S* configurations of L-threonine, D-threonine, L-isoleucine, and D-isoleucine are shown in Figure 4.7.

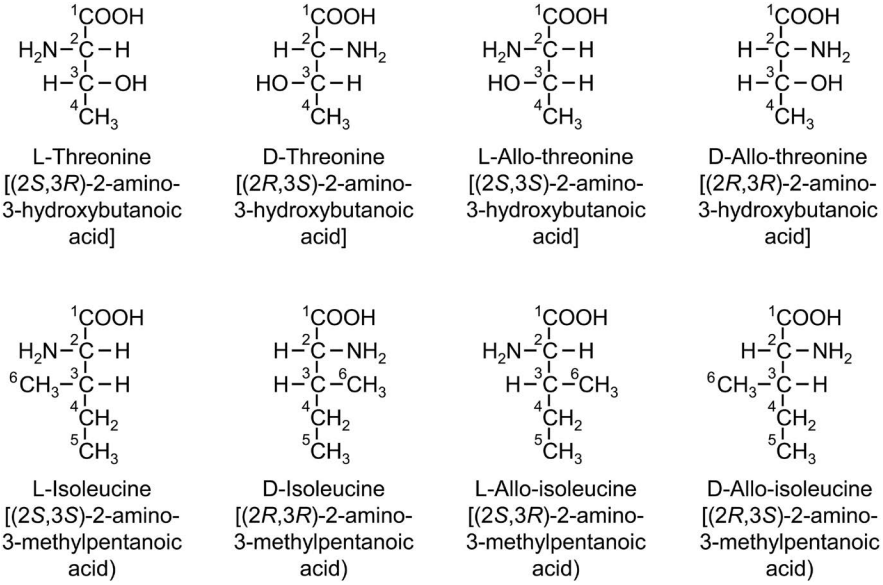


FIGURE 4.7 The *R/S* configurations of L-threonine, D-threonine, L-Ile, and D-Ile. Namely, if two diastereomers have a different configuration in the β- or γ-carbon, they are designated with the prefix "allo." Note that diastereomers are stereoisomers with two or more stereocenters that are not mirror images of one another and are nonsuperimposable on one another.

The collagen and plasma of animals contain 4-hydroxy-L-proline (4-hydroxy-L-pyrrolidine-2-carboxylic acid) and 3-hydroxy-L-proline (3-hydroxy-L-pyrrolidine-2-carboxylic acid), with their ratio being approximately 100:1. According to the *R/S* nomenclature system, the physiological isoform of 4-hydroxy-L-proline in collagen and its enzymatic hydrolysates is (2*S*,4*R*)-4-hydroxyproline (Figure 4.8), and that of 3-hydroxy-L-proline is (2*S*,3*S*)-3-hydroxyproline; both L-4-hydroxyproline and L-3-hydroxyproline are present only in the *trans* isoform. However, the acid hydrolysis of collagen at high temperatures (e.g., 110°C) yields a small amount of 4-hydroxyproline and 3-hydroxyproline in the *cis* isoform. *cis*-4-Hydroxy-L-proline [*cis*-(2*S*,4*S*)-4-hydroxyproline], a diastereomer of L-4-hydroxyproline, is present in the toxic cyclic peptides from *Amanita* mushrooms. Also, marine sponges and cyclopeptide antibiotics (e.g., telomycin) have both (2*S*,3*S*) and (2*S*,3*R*) 3-hydroxyproline.

Allo-Forms of Amino Acids

The nomenclature of an amino acid with more than one asymmetric carbon includes an allo-form. Namely, if two diastereomers have a different configuration in the β- or γ-carbon, they are designated with the prefix "allo." For example, a synthetic L-threonine (2*S*,3*S*) whose β-carbon has a configuration opposite to that of its naturally occurring diastereomer [L-(2*S*,3*R*)-threonine] is called L-allo-threonine (Figure 4.7). L-Threonine and L-allo-threonine are called diastereomers, and the relationship between the two amino acids is known as diastereomerism. This also applies to D-threonine and D-allo-threonine. The allo-forms of isoleucine are illustrated in Figure 4.7.

Free Amino Acids

Free amino acids are defined as those amino acids that are not covalently bound in peptides or proteins. Except for selenocysteine, all proteinogenic amino acids are present in the free pool. All nonproteinogenic amino acids in nature occur as free amino acids (e.g., β-alanine, γ-aminobutyrate, citrulline, and ornithine), but some of them (e.g., β-alanine and γ-aminobutyrate) are abundant in certain small peptides.

In most food ingredients of plant and animal origin, only small amounts of amino acids (< 2% of total amino acids) occur in a free form (Li et al. 2011). However, some free amino acids represent significant amounts of total amino acids in certain animal- and plant-sourced foods. For example, free glutamate and glutamine (1 and 4 mM, respectively) account for approximately 2.5% and 10% of total glutamate and glutamine (free plus peptide-bound), respectively, in sow's milk on day 28 of lactation (Table 4.5). Similar results have been reported for human milk (Wu 2022). Also, the concentration of free taurine in human and porcine milk can be as high as 1 mM. In addition, free amino acids represent 34.4% and 28.5% of total amino acids in potatoes and sweet potatoes, respectively (Hou et al. 2019). Notably, asparagine accounts for 32.3%, 17.5%, and 19.4% of total free amino acids in potatoes, sweet potatoes, and white rice, respectively (Hou et al. 2019). Free amino acids, particularly milk-borne glutamine and glutamate, play an important role in the growth and development of the neonatal small intestine.

Concentrations of individual free amino acids can differ markedly among cells, tissues, and species. (Li et al. 2021a,b,c). For example, concentrations of free glutamine are 0.5 and 1 mM in the plasma of healthy humans and chickens, respectively, and are 20–25 and 1.5–15 mM in the skeletal muscle of the same species, respectively, depending on muscle type (Hou et al. 2016; Watford and Wu 2005). Also, concentrations of free glycine are 0.25 and 1 mM in the plasma of healthy humans and pigs, respectively (Table 4.4). Interestingly, glycine, rather than glutamine, is the most abundant free amino acid in the plasma of postnatal pigs (Flynn et al. 2000); this may be due, in part, to high requirements of these rapidly growing animals for glycine to deposit proteins (particularly collagen) in the body

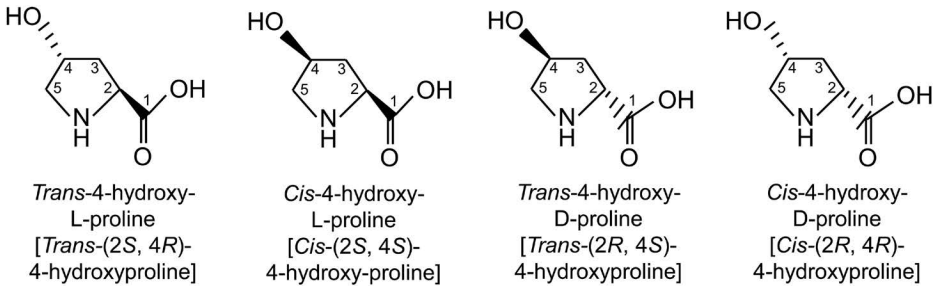


FIGURE 4.8 The *R/S* configurations of 4-hydroxy-L-proline and 4-hydroxy-D-proline in *trans* and *cis* forms. Proline is also known as pyrrolidine-2-carboxylic acid. The physiological isoform of 4-hydroxyproline in animal collagen and its enzymatic hydrolysates is *trans* 4-hydroxy-L-proline. In general, when an organic compound contains double bonds that cannot rotate, or when an organic compound (e.g., hydroxyproline) with two or more functional groups has a ring structure where the rotation of bonds is restricted, *cis* and *trans* isomers occur. A dashed line represents a bond pointing away from the viewer. A solid line represents a bond pointing toward the viewer. When two functional groups of a diastereomer are oriented in the same direction, the diastereomer (also called diastereoisomer) is referred to as *cis*. In contrast, when two functional groups of a diastereomer are oriented in opposite directions, the diastereomer is referred to as *trans*. Note that the *cis*/*trans* nomenclature is not the same as the *R*/*S* nomenclature.

TABLE 4.4
Concentrations of Amino Acids (AAs) in the Plasma of Animals (μmol/L)[a]

AA	Cat[b]	Cattle[c]	Chicken[d]	Dog[e]	Goat[f]	Horse[g]	HSB[h]	LMB[i]	Mouse[j]	Pig[k]	RBT[l]	Rat[m]	Sheep[n]	Shrimp[o]
Ala	570	192	665	312	186	121	778	1377	673	763	865	492	270	958
Arg	111	55	297	187	145	70	114	27	235	126	144	279	191	576
Asn	96	25	121	26	67	28	72	146	50	101	145	120	38	189
Asp	8	10	87	5.7	21	9.7	29	37	39	16	8	46	17	80
Cit	19	47	1.0	39	111	61	31	102	70	65	20	70	181	0.24
Cys*	14	156	162	33	92	147	152	217	124	158	9	157	188	152
Gln	816	200	912	967	256	323	180	224	754	521	281	559	248	562
Glu	43	56	290	48	131	22	48	58	308	151	35	137	137	95
Gly	299	243	391	257	912	405	229	673	234	912	505	384	566	1024
His	141	45	89	73	67	59	198	168	75	103	107	120	70	101
Ile	84	62	96	65	136	39	194	360	149	119	225	144	66	116
Leu	157	131	219	179	158	64	294	612	279	180	419	216	111	162
Lys	124	57	162	234	146	100	73	302	246	237	437	259	119	257
Met	54	27	70	62	18	19	145	210	120	80	96	107	22	33
Orn	16	72	19	19	84	32	7.5	149	54	75	57	68	98	148
Phe	89	47	137	59	42	42	139	224	67	95	95	106	33	71
Pro	149	102	315	160	225	128	224	579	195	580	115	286	115	308
Ser	138	72	446	145	134	210	78	463	472	252	160	353	80	254
Tau	136	31	170	69	52	23	2001	1733	632	127	417	670	54	717
Thr	156	41	220	207	81	61	100	539	195	254	278	381	98	162
Trp	68	44	58	83	38	62	69	34	45	43	21	115	30	28
Tyr	65	48	158	55	52	45	116	175	126	164	87	125	43	31
Val	210	54	156	250	238	115	308	761	485	294	468	249	171	224

[a] Values refer to the plasma of all animal species, except for largemouth bass (serum) and shrimp (hemolymph).
[b] The plasma was prepared from blood samples of adult cats in the fed state (Sabatino et al. 2013).
[c] Growing Angus steers fed a ground corn-, ground milo-, and cottonseed hulls-based diet (Choi et al. 2014).
[d] Forty two-day-old broilers fed a corn- and soybean meal-based diet containing 18.4% crude protein (CP); plasma was prepared from the wing vein blood at 2 h after the feeding. AAs were analyzed as described by Watford and Wu (2005).
[e] The plasma was prepared from blood samples of adult dogs at 3 h after feeding for the analysis of most AAs, except for Asn, Cit, Gln and Orn (Ikada et al. 2002). The values for these 4 AAs were reported by Outerbridge et al. (2002).
[f] Lactating goats; the plasma was prepared from the arterial blood samples for the analysis of most AAs, except for Cys, Taurine and Trp (Mepham and Linzell 1966). Values of these three AAs were obtained by Wu (2022).
[g] Twelve-month-old horse housed on pasture with free access to grass and hay and received twice daily supplementation with a pellet diet containing 15% CP; the plasma was prepared from blood samples at 14 h after the last feeding (Manso Filho et al. 2009).
[h] Plasma was obtained, at 4 h post-feeding, from juvenile hybrid striped bass [34 g body weight (BW)] fed a fishmeal (58%)- and fishmeal (15%)-based diet containing 44.2% CP (on a dry matter basis; Li et al. 2023b).
[i] Serum was obtained, at 4 h post-feeding, from juvenile largemouth bass (56 g BW) fed a fishmeal (65.3%)-based diet containing 45.5% CP (on a dry matter basis; Li et al. 2021a).
[j] The plasma was prepared from the blood samples of adult mice in the fed state (Nagasawa et al. 2012; G. Wu, unpublished work).
[k] Twenty one-day-old piglets nursed by lactating sows; the plasma was prepared from blood samples at 1.5 h after suckling (Flynn et al. 2000).
[l] Rainbow trout with a BW of 695–1483 g. The plasma was prepared from arterial blood samples at 3 h after the last feeding (Karlsson et al. 2006).
[m] Thirteen-week-old male rats fed a semi-purified diet containing 20% casein; the plasma was prepared from blood samples at 5 h after feeding (Assaad et al. 2014).
[n] Sheep on day 30 of gestation fed an alfalfa-based diet containing 15.8% CP; the plasma was prepared from blood samples at 24 h after feeding (Kwon et al. 2003).
[o] Hemolymph was obtained, at 24 h post-feeding, from white-leg shrimp (15 g BW) fed a fishmeal (20%)- and plant-based diet containing 43% CP (on a dry matter basis; Li et al. 2021b).
[*] Total cysteine (1/2 cystine + free cysteine). In the pig plasma, free cysteine = 84 μmol/L and cysteine = 4.6 μmol/L. The values of total cysteine for cats and rainbow trout as well as possibly dogs might have been underestimated in the previous studies due to technical problems.

Note: Cit, citrulline; HSB, hybrid striped bass; LMB, largemouth bass; Orn, ornithine; RBT, rainbow trout; Tau, taurine.

(Wu 2022). Concentrations of free arginine, glutamine, citrulline, and serine can be as high as 1, 25, 10, and 20 mM, respectively, in ovine allantoic fluid during gestation, whereas values for the corresponding free amino acids in porcine allantoic fluid are 6, 2.5, 0.1, and 0.2 mM, respectively (Kwon et al. 2003; Wu et al. 1996). These differences in concentrations of free amino acids reflect tissue- and species-differences in their metabolism.

Chemically Modified Free Amino Acids

Some free amino acids are phosphorylated by ATP-dependent kinases to generate new derivatives [e.g., phospho-arginine from arginine in various invertebrates (e.g., crustaceans), phospho-glutamate from glutamate in certain plants, and phospho-homoserine from homoserine in microbes]. In addition, some free amino acids (e.g., glutamate, aspartate, and ornithine) are acetylated enzymatically to form *N*-acetyl-amino acids (e.g., *N*-acetylglutamate, *N*-acetylaspartate, and *N*-acetylornithine, respectively). Furthermore, some free amino acids undergo spontaneous carbonylation by carbonyl species at very low rates. For example, at 25°C or 37°C, the ε-amine group of free lysine reacts with glyoxal (OCH-CHO) or methylglyoxal [OCH-C(=O)-CH_3] (metabolites of endogenous substances including D-glucose) to produce carboxymethyllysine (Lys-N^{ε}H-CH_2-COOH) and carboxyethyllysine [Lys-N^{ε}H-C(CH_3)H-COOH] in very low yields, respectively (Baskal and Tsikas 2022). Chemically modified free amino acids may serve as allosteric activators or inhibitors of enzymes and may modify the functions of proteins. Note that a few amino acid residues in proteins may undergo post-translational modifications, such as hydroxylation, methylation, and phosphorylation (Figure 4.9).

Physical Appearance, Melting Points, and Tastes of Amino Acids

The crystals of amino acids are generally white (Ajinomoto 2003). All crystalline α-amino acids, except for glutamine and cysteine, have a high melting point of over 200°C. Glutamine and cysteine have melting points of 185°C and 178°C, respectively. At or above their melting points, amino acids decompose spontaneously. The hydrochlorides of L-arginine (L-arginine-HCl), L-lysine (L-lysine-HCl), and L-ornithine (L-ornithine-HCl) have melting points of 235°C, 236°C, and 231°C, respectively (Table 4.2). Interestingly, the melting points of L-arginine and L-arginine-HCl are nearly identical.

The tastes of amino acids result from their interactions with specific receptors [guanine nucleotide-binding protein

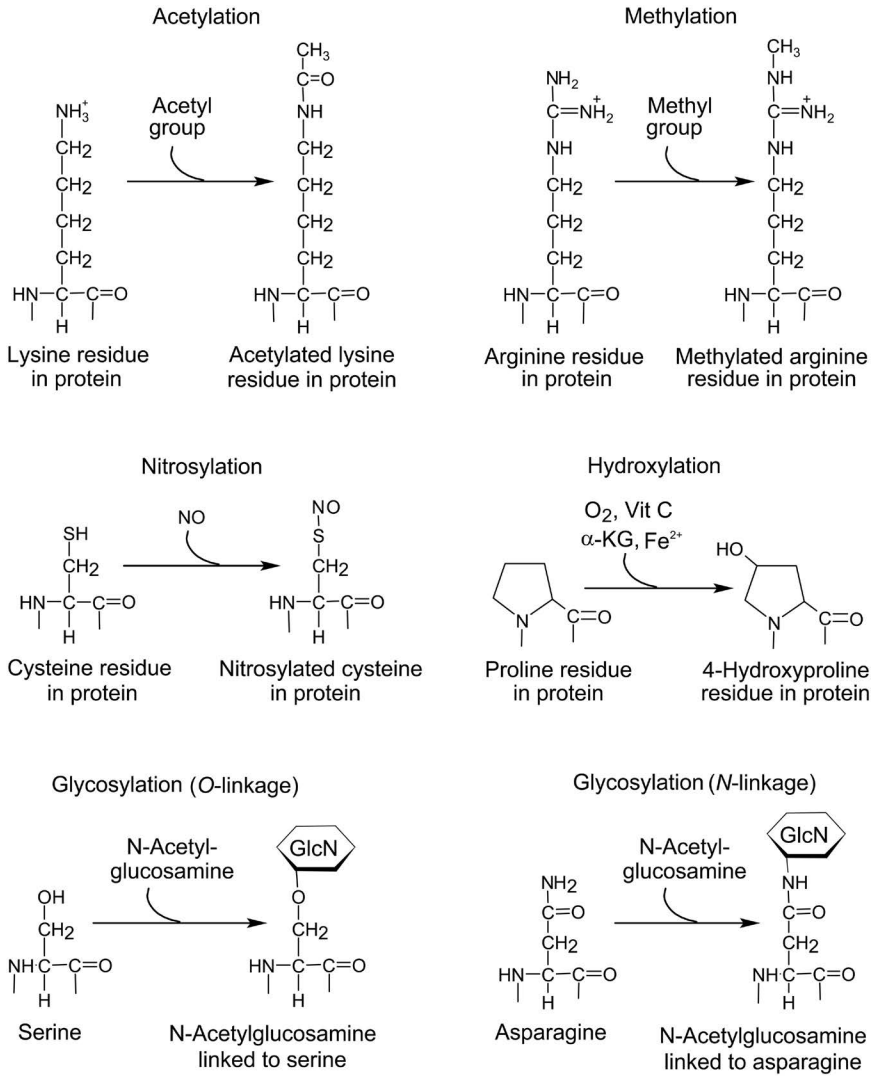


FIGURE 4.9 Modifications of certain amino acid residues in proteins through acetylation, methylation, nitrosylation, hydroxylation, and glycosylation. These reactions occur at the post-translational step in animals, microbes, and plants.

(G protein)-coupled receptors] on the tongue (Fernstrom et al. 2012). L-Glutamate has a "meaty" taste. L-alanine and glycine have a sweet taste, L-serine and L-threonine have a faintly sweet taste, and L-citrulline has a slightly sweet taste. L-Arginine base has a bitter and unpleasant taste by itself in humans, but in a mixture with citric acid in drinking water, it is palatable. L-Isoleucine has a bitter taste, whereas L-lysine, L-aspartate, and L-phenylalanine have a slightly bitter taste. L-Glutamine, β-alanine, and taurine are flat (lacking tastiness). L-Asparagine, L-cysteine, L-cystine, L-methionine, L-tryptophan, L-proline, L-ornithine, L-histidine, L-leucine, L-tyrosine, and L-valine have a flat-to-bitter taste. D-Glutamate is almost tasteless, whereas D-aspartate is flat. D-alanine, D-leucine, D-serine, D-tryptophan, and D-valine are very sweet, whereas D-glutamine, D-histidine, D-isoleucine, D-methionine, D-phenylalanine, D-threonine, and D-tyrosine are sweet (Kawai et al. 2012). The taste of a basic amino acid is altered by its hydrochloride salt (San Gabriel et al. 2009).

Solubility of Amino Acids in Water and Solutions

All amino acids are soluble in water at room temperature. Leucine, isoleucine, valine, phenylalanine, tryptophan, methionine, tyrosine, and cysteine are among the most hydrophobic amino acids. All amino acids (except for cystine) are readily soluble in Krebs bicarbonate buffer (pH 7.4 at, e.g., 25–40°C) at concentrations that are at least 10 times greater than those in animal plasma. The solubility of α-amino acids in water varies with their side chains, with proline and cystine being the most and least soluble, respectively (Table 4.2). The solubility of β-alanine and γ-aminobutyrate in water is higher than that of lysine. Salts affect the solubility of amino acids in water, and such an effect depends on their structures (Wu 2022). The hydrochlorides of amino acids (both neutral and basic), such as cystine, arginine, histidine, and lysine, are generally more soluble in water than the corresponding free amino acids. Most of the amino acid-hydrochloride salts are highly soluble in absolute ethanol. The hydrochloride salts of basic amino acids (e.g., arginine and lysine) are often used for their neutralization in water and in physiological solutions. The sodium salts of most amino acids dissolve more readily in water and are more ethanol-soluble than the corresponding free amino acids.

The solubility of amino acids generally increases in acidic or alkaline solutions and with elevated temperatures. Except for proline and hydroxyproline, amino acids are generally insoluble in organic solvents (e.g., absolute ethanol). Because of their pyrrole ring structures, proline and hydroxyproline are fairly soluble in absolute ethanol (approximately 1.6 g/100 ml at 20°C). Thus, proteins with a high content of proline are soluble in 70–80% ethanol.

Chemical Stability of Amino Acids

Stability of Crystalline Amino Acids

The crystalline forms of all amino acids are stable at room temperature (i.e., 25°C) for at least 30 years without any detectable loss. This applies to L-amino acids and D-amino acids, as well as proteinogenic and nonproteinogenic amino acids. However, like all substances, amino acids should be protected from light and should not be exposed to a high-humidity or high-temperature environment during storage (Ajinomoto 2003).

Stability of Amino Acids in Water and Buffers

Except for cysteine, all amino acids in water, in buffered solutions (e.g., Krebs bicarbonate buffer), or in deproteinized and neutralized biological samples, are stable at –80°C for 6 months without any detectable loss. Likewise, amino acids are generally stable in an aqueous solution at a physiological pH and 37–40°C, except for (a) cysteine, which undergoes rapid oxidation to cystine particularly in the presence of metal ions and the absence of reducing agents; and (b) glutamine, whose side-chain amide group and the α-amino group spontaneously and slowly interact to form the cyclic product, the ammonium salt of pyrrolidone carboxylate (pyroglutamate, a potential neurotoxin). This reaction occurs at a rate of <1%/day for 1 mM glutamine (sterile) at 37°C.

The ratio of cystine to cysteine is approximately 10:1 in the plasma or serum of healthy subjects. Cystine is readily converted to cysteine inside the cell under physiological reducing conditions. *N*-Acetylcysteine (a water-soluble synthetic substance) is a stable precursor of cysteine for cultured cells and for intravenous or oral administration into humans and other animals (Hou et al. 2015a). In chemical analysis, the thiol (–SH) group of cysteine can be protected by iodoacetic acid, whereas cystine can be readily reduced to cysteine by 2-mercaptoethanol. In contrast to cysteine, few means are available to protect glutamine from spontaneous cyclization in an aqueous solution.

Stability of Amino Acids in Acid and Alkaline Solutions

At concentrations between 0.01 and 5 mM, all amino acids, except for cysteine and glutamine, are stable in a 5% TCA or 0.75 M $HClO_4$ solution, or an alkaline solution (pH 8.4), at room temperature (e.g., 25°C) for at least 12 h or at –80°C for 2 months, without any detectable loss. However, under the standard conditions of acid hydrolysis at high temperatures (i.e., 6 M HCl, 110°C, and 24 h under N gas), changes in the following amino acids occur: (a) all glutamine and asparagine are converted to glutamate and aspartate, respectively; (b) tryptophan is completely destroyed; and (c) 20% of methionine undergoes oxidation to generate methionine sulfoxide. Notably, under these conditions, other amino acids are either completely stable (i.e., no detectable loss for alanine, arginine, cystine, glutamate, glycine, histidine, leucine, lysine, phenylalanine, and valine) or relatively stable (3% loss for aspartate and threonine; 5% loss for tyrosine and proline; and 10% loss for serine). Under the conditions of alkaline hydrolysis at a high temperature (e.g., 4.2 M NaOH and 105°C), most amino acids (including glycine, histidine, serine, and threonine) are almost

completely destroyed, many amino acids (e.g., cysteine and methionine) undergo degradation to a great extent, and some amino acids are hydrolyzed (e.g., glutamine to glutamate, asparagine to aspartate, and arginine to ornithine). In contrast, tryptophan is stable (100% recovery) in alkaline solution for 20 h even at 105°C. Thus, the analysis of tryptophan in protein can be successfully accomplished by alkaline hydrolysis in the presence of 4.2 M NaOH and 1% thiodiglycol (an antioxidant) at 105°C for 20 h. It must be recognized that the determination of proline, cysteine, methionine, and tryptophan in feed and animal-tissue proteins is technically challenging, and, therefore, values for these amino acids from any studies without verifying the accuracy of their analysis should be considered with caution (Dai et al. 2014).

With three exceptions, free amino acids in water or a neutral solution are stable under high pressure and high temperature conditions (e.g., in an autoclave); glutamine and asparagine are almost completely destroyed, whereas cysteine is oxidized to cystine. Cystine, however, is stable under these same conditions. In a dipeptide form (e.g., L-alanyl-glutamine and glycyl-glutamine, as well as L-leucyl-asparagine and glycyl-asparagine), glutamine and asparagine are stable under these conditions. To prevent the loss of glutamine and asparagine under autoclaving conditions, a solution containing free glutamine and asparagine can be sterilized through a 0.2-μm filter before use for cell or tissue culture. Note that, under autoclaving conditions, cysteine (2%, w/v) in deoxygenated water is stable at pH 4.9 but undergoes 8% and 17% losses at pH 7 and 8, respectively.

DEFINITION, CHEMICAL CLASSIFICATION, AND PROPERTIES OF PEPTIDES AND PROTEINS

Definitions of Peptides and Proteins

A peptide is defined as an organic molecule consisting of 2 or more amino acid residues linked by peptide bonds. In most peptides, the typical peptide bonds are formed from the α-amino and α-carboxyl groups of adjacent amino acids. A peptide bond [–(C=O)–NH–] is an amide type of covalent chemical bonding. Peptides can be chemically and biochemically synthesized from amino acids (Fridkin and Patchornik 1974). The formation of one peptide bond results in the removal of one water molecule (a H atom from an amino acid and a –OH group from the other amino acid) with a molecular weight of 18 Da (Figure 4.10). An amino acid that loses the H atom and the –OH group is called a residue. The smaller the amino acid, the greater the difference between its intact and residue molecular weights. The molecular weights of intact glycine, glutamate, and tryptophan are 32%, 14%, and 10% greater than those for their residues, respectively.

Peptides can be classified according to the number of their amino acid residues. An oligopeptide is composed of 2–20 amino acid residues (Hughes 2012). An oligopeptide containing ≤10 amino acid residues is called a small oligopeptide (or small peptide); an example is oxytocin with 9 amino acid residues, which stimulates lactation. An oligopeptide containing 11–20 amino acid residues is called a large oligopeptide (or large peptide); an example is somatostatin with 14 amino acid residues, which is an inhibitor of growth hormone release. A peptide that contains ≥21 amino acid residues and does not have a 3-dimensional structure is termed a polypeptide; an example is endothelin with 21 amino acid residues, which is a potent vasoconstrictor. A protein consists of one or more polypeptides and has a well-defined 3-dimensional structure; an example is mature bovine serum albumin with a molecular weight of 66,463 Da (583 amino acid residues). All peptides and proteins are polymers.

The dividing line between proteins and polypeptides is usually their molecular weights and the number of their amino acid residues (Kyte 2006). Generally speaking, a protein has a molecular weight of ≥8,000 Da (i.e., ≥72 amino acid residues), because such a molecule will have a well-defined 3-dimensional structure. For example, growth hormone, which has 191 amino acids with a molecular weight of ~22,000 Da, is a protein. Ubiquitin (a single chain of 72 amino acid residues) and casein α-S1 (200 amino acid residues) are proteins as well. Polypeptides with a molecular weight less than 8000 Da and 72 amino acid residues are generally not considered to be proteins unless they have a well-defined 3-dimensional structure. For example, glucagon (29 amino acid residues with a molecular weight of ~3500 Da) is a polypeptide. Likewise, PEC-60 (a single chain of 60 amino acid residues; Agerberth et al. 1989) and dopuin (a single chain of 62 amino acid residues; Chen et al. 1997), which are isolated from the pig small-intestinal mucosae, are called polypeptides. However, the classification of proteins or polypeptides simply based on their molecular weights or their number of amino acid residues is certainly not absolute. For example, insulin [51 amino acid residues (20 in

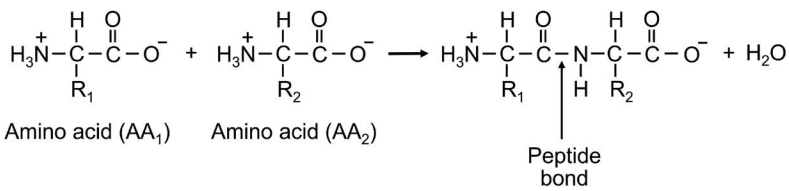


FIGURE 4.10 Synthesis of a dipeptide from two amino acids. R_1 and R_2 represent the side chains of the two amino acids. With the loss of one H_2O molecule, a peptide bond (–CONH–) is formed.

chain A and 31 in chain B)] is well recognized as a protein because it has the defined 3-dimensional structure exhibited by proteins. To date, the number of different proteins with various amino acid compositions has been estimated to be ~100,000 in domestic animals and up to 50,000 in plants (Sterck et al. 2007). Note that 100 g of protein is equal to 100 g of amino acid residues, but is not equivalent to 100 g of amino acids (Wu 2022).

Some substances with nontypical peptide bonds [e.g., glutathione (GSH) and *N*-pteroyl-L-glutamate (folate)] are beneficial for nutrition and physiology, whereas others [e.g., ergovaline (present in endophyte-infected Tall fescue, a cool-season grass) and phalloidine] are toxic to animals, including humans. GSH, which consists of L-glutamate, L-cysteine and glycine, is the most abundant low-molecular-weight antioxidant tripeptide in animal cells and fluids (up to 10 mM). GSH is a very special small peptide, as it is the γ-carboxyl group (Figure 4.11), rather than the α-carboxyl group, of the L-glutamate molecule that reacts with the α-amino group of L-cysteine to form a peptidic γ-linkage, which protects GSH from hydrolysis by extracellular or intracellular peptidases.

Peptide (Protein)-Bound Amino Acids

Peptide (protein)-bound amino acids are those amino acids that are present in peptides or proteins. The composition of peptide-bound amino acids in animal products or tissues can vary greatly among different proteins (Davis et al. 1994; Wu et al. 2016). For example, porcine milk protein contains ~10% glutamate and 10% glutamine (Table 4.5), but muscle protein contains ~5% glutamate and ~5% glutamine (Wu 2022). The overall composition of amino acids in whole body proteins is largely similar among different species, such as chickens, fishes, humans, cattle, rats, pigs, and sheep. In most food ingredients of plant and animal origin, more than 98% of total amino acids are present in proteins and polypeptides (Li et al. 2011; Li and Wu 2020).

In animal tissues and in foods, the total amount of a proteinogenic amino acid is its sum in the peptide

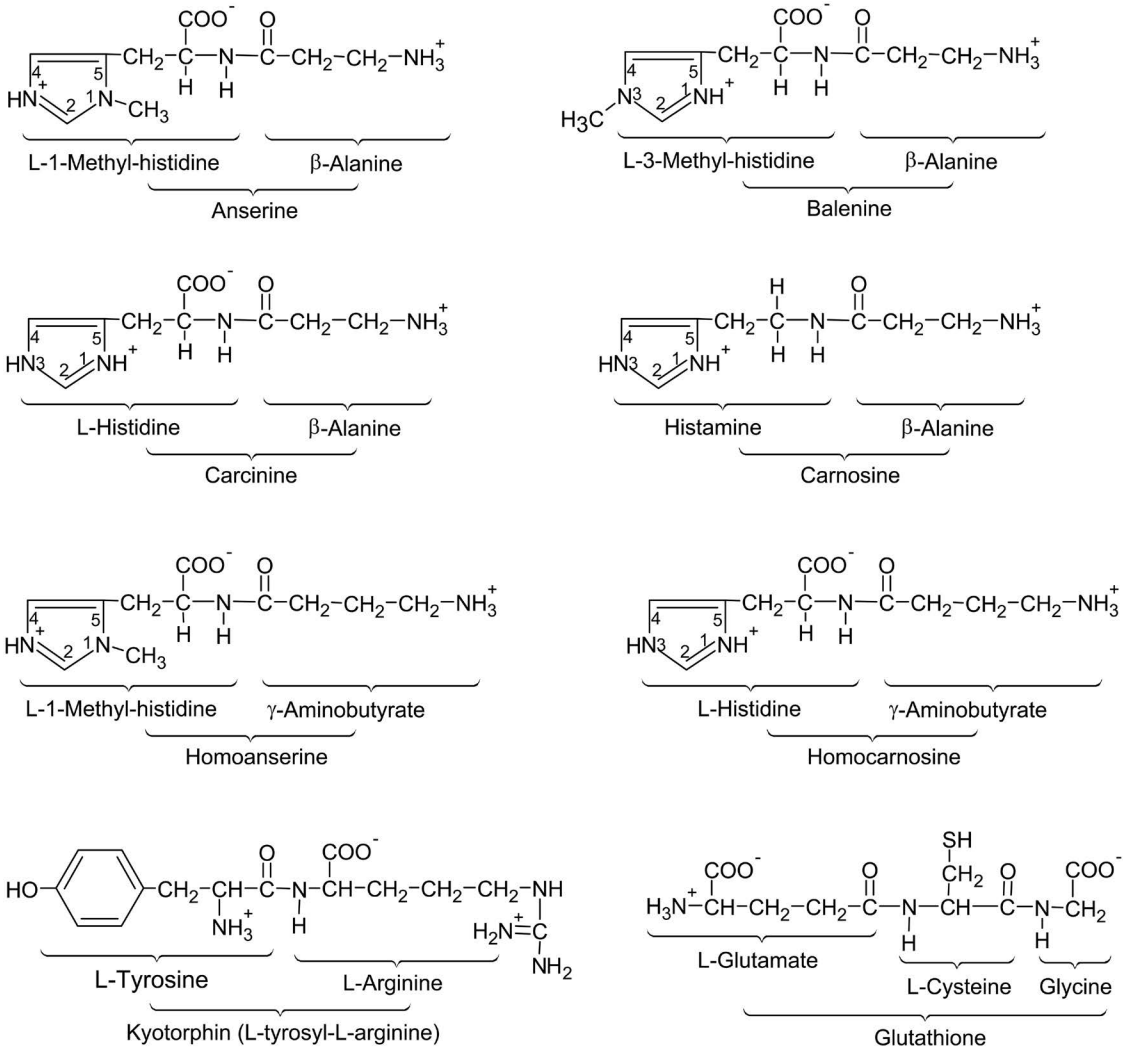


FIGURE 4.11 Physiologically important dipeptides and glutathione in animal tissues. These small peptides play important roles in antioxidative reactions, cellular pH homeostasis, and cell signaling.

TABLE 4.5
Composition of Free, Peptide-Bound, and Total Amino Acids (AAs) in Sow's Colostrum and Milk

	Day 2 of Lactation			Day 14 of Lactation			Day 28 of Lactation		
AA	Total AA	Free AA	Peptide-Bound AA	Total AA	Free AA	Peptide-Bound AA	Total AA	Free AA	Peptide-Bound AA
	g/L of whole milk								
Ala	3.74	0.025	3.72	1.99	0.056	1.93	1.84	0.058	1.78
Arg	2.89	0.008	2.89	1.43	0.010	1.42	1.35	0.011	1.34
Asn	4.31	0.004	4.31	2.51	0.017	2.49	2.17	0.032	2.14
Asp	4.58	0.027	4.55	2.77	0.060	2.71	2.35	0.066	2.28
Cys*	1.72	0.028	1.69	0.72	0.048	0.67	0.75	0.095	0.65
Gln	7.65	0.054	7.41	4.85	0.226	4.62	4.71	0.546	4.16
Glu	7.72	0.062	7.59	4.97	0.187	4.78	4.40	0.176	4.22
Gly	2.20	0.024	2.18	1.12	0.068	1.05	1.03	0.104	0.93
His	1.54	0.136	1.40	0.94	0.116	0.82	0.83	0.079	0.75
Ile	3.89	0.001	3.89	2.29	0.002	2.29	2.11	0.003	2.11
Leu	8.04	0.003	8.04	4.58	0.005	4.57	4.23	0.006	4.22
Lys	4.23	0.005	4.22	4.18	0.008	4.17	3.77	0.009	3.76
Met	1.81	0.001	1.81	1.05	0.003	1.05	0.93	0.003	0.93
Phe	3.35	0.003	3.35	2.03	0.006	2.02	1.90	0.006	1.89
Pro	7.49	0.004	7.49	5.61	0.009	5.60	5.28	0.014	5.27
OH-Pro	1.37	0.015	1.35†	1.04	0.009	1.03†	0.69	0.007	0.68†
Ser	4.48	0.005	4.47	2.35	0.031	2.32	2.20	0.048	2.15
Thr	4.36	0.013	4.35	2.28	0.019	2.26	2.07	0.054	2.02
Trp	1.28	0.001	1.28	0.66	0.003	0.66	0.62	0.004	0.62
Tyr	3.71	0.007	3.70	2.32	0.012	2.31	1.95	0.014	1.94
Val	4.70	0.006	4.69	2.53	0.013	2.52	2.26	0.017	2.24
β-Ala	0.001	0.001	0.000	0.003	0.003	0.000	0.004	0.004	0.000
Cit	0.001	0.001	0.000	0.007	0.007	0.000	0.009	0.009	0.000
Orn	0.005	0.005	0.000	0.008	0.008	0.000	0.008	0.008	0.000
Taurine	0.134	0.134	0.000	0.176	0.176	0.000	0.186	0.186	0.000
All AAs	81.2	0.57	80.7	50.1	1.09	49.0	45.7	1.55	44.1

Milk samples were obtained from lactating sows fed an 18.5% crude protein (Mateo et al. 2008) and analyzed for free and peptide-bound AAs (Wu et al. 2016). Values are the means for 10 sows. Day 0 of lactation is defined as the day of farrowing. The molecular weights of intact AAs were used for the calculation of concentrations of peptide-bound AAs in the milk.

* Total cysteine (1/2 cystine + free cysteine); † Present in small peptides.
Note: Cit, citrulline; OH-Pro, 4-Hydroxyproline; Orn, ornithine.

(protein)-bound plus free pools. The ratio of total free amino acids to total peptide-bound amino acids in the whole body of animals and most tissues (e.g., skeletal muscle, liver, and small intestine) is approximately 1:30 (g:g), meaning that total free amino acids represent ~3% of total amino acids (free plus peptide-bound) in humans and other animals (Wu 2022). However, a ratio of some individual free amino acids to the same peptide-bound amino acids can be greater than 1:10 or lower than 0.1:100 (g:g). For example, in human skeletal muscle, the ratio of free glutamine to peptide-bound glutamine is approximately 2:10 (g:g), whereas the ratio of free tryptophan to peptide-bound tryptophan is only 0.06:100 (g:g). Depending on age and nutritional status, protein content in animals ranges from 12% to 16%. Selenocysteine is rare in proteins as noted previously, but there is virtually no free selenocysteine in animal cells or plasma (Wu 2022).

Major Proteins in Animals

Actin and Myosin

Actin and myosin are major intracellular proteins in most animal cells (e.g., muscles). For example, actin and myosin comprise 65% and 2–10% of total cellular protein in skeletal muscle and nonmuscle cells, respectively (Rennie and Tipton 2000). Actin has three α-isoforms (e.g., in skeletal, cardiac, and smooth muscles), as well as β- and γ-isoforms (e.g., in both muscle and nonmuscle cells; Dominguez and Holmes 2011). The actin isoforms differ by only a few amino acid residues and undergo post-translational modifications

(e.g., the methylation of histidine-73 of skeletal muscle α-actin to form 3-methyl-histidine). Of interest, actin can exist in monomeric globular (G-actin) and filamentous (F-actin) states. Myosin consists of six polypeptides: two heavy chains, as well as two pairs of different light chains (essential light chains and regulatory light chains; Sweeney and Houdusse 2010). Both myosin and F-actin possess ATPase activity. In the resting state, actin and myosin filaments partially overlap each other in an inter-digitating manner. When muscle contracts, the actin and the myosin slide past each other, with the required energy being provided through ATP hydrolysis by the myosin globular head. Besides contraction, actin and myosin also have other biological activities, such as cell motility, division, signaling, and organelle movement, as well as the maintenance of cell cytoskeleton and shape (Wu 2022).

Proteins in Connective Tissues

The four major types of proteins in connective tissue (e.g., the skin) are collagen, elastin, glycosaminoglycans, and proteoglycans. They are extracellular proteins, which are synthesized and released by fibroblasts. Collagen, which is unusually rich in proline, hydroxyproline, and glycine in the repeated form of tripeptides (Gly-Pro-Y and Gly-X-Hyp, where X and Y can be any amino acid), has a structural role to confer form and strength to tissues and organisms. In addition to 4-hydroxyproline (9%) and 3-hydroxyproline (0.1%), collagen also contains 5-hydroxylysine (0.6%). Collagen protein (~30–33% of the total protein in the body) is the most abundant protein in animals. There are approximately 20 different types of collagen in the animal kingdom. Each mature collagen contains three polypeptide chains, which may be the same or different. In type I collagen (e.g., mainly in the skin, tendons, bones, teeth, ligaments, and between organs), there are two α1(I) chains and one α2(I) chain, with 1000 amino acid residues per polypeptide chain. In type II collagen (e.g., mainly in the cartilage and eyes), there are three identical α1(II) chains. Of note, both type I and type II collagens contain no cysteine or cystine. In type III collagen (e.g., mainly in blood vessels, muscle, intestinal and uterine walls, newborn skin), there are three identical α1(III) chains. In type IV collagen (mainly in the basement membrane, eye lens, and the capillaries and glomeruli of the kidneys), there are three identical α1(IV) or α2(IV) chains. Interestingly, both type III and type IV collagens contain cysteine residues. The heart and skeletal muscle contain type I, type III, and type IV collagens.

Elastin, which contains two special derivatives of lysine residues (i.e., desmosine and isodesmonsine), is more stretchable than collagen, thereby helping to maintain tissue resilience and elasticity. Glycosaminoglycans (GAGs) and proteoglycans hold water within a tissue and also provide mechanical support. GAGs are composed of *N*-acetyl glucosamine, *N*-acetylgalactosamine, and glucosamine sulfate. These units form various types of GAGs, such as hyaluronic acid, keratin sulfate, heparin, heparin sulfate, dermatin sulfate, and chondroitin sulfate. Proteoglycans, which are larger than GAGs, are formed when GAGs are attached to a protein backbone.

Chemically Modified Amino Acid Residues in Proteins and Peptides

Certain amino acid residues in proteins undergo modifications, such as phosphorylation (Bischoff and Schlüter 2012), as well as acetylation, methylation, nitrosylation, hydroxylation, glycosylation (*N*- or *O*-linkage), and carbonylation (Figure 4.9). The post-translational phosphorylation of proteins results in the covalent linkage of an amino acid to the phosphate group, yielding phospho-serine, phospho-tyrosine, and phospho-threonine. The methylation of amino acid residues in proteins yields methylated amino acids (e.g., 1-methylhistidine and 3-methylhistidine from histidine; asymmetric dimethylarginine, symmetric dimethylarginine, and N^G-monomethyl-arginine from arginine; Wu and Morris 1998). Furthermore, the hydroxylation of amino acid residues in proteins yields hydroxylated amino acids (e.g., 4-hydroxyproline and 3-hydroxyproline from proline; 5-hydroxylysine from lysine; hydroxyserine from serine; hydroxythreonine from threonine; and hydroxytyrosine from tyrosine). Other examples of the formation of new amino acid residues in polypeptides due to post-translational modifications include citrulline from arginine; nitrosylated tyrosine from tyrosine; and acetylated lysine, hydroxylysine, methylated lysine, and hypusine from lysine. Of note, hypusine is present in eukaryotic translation initiation factor 5A (eIF5A) and is formed by two reactions. First, deoxyhypusine synthase catalyzes the cleavage of the polyamine spermidine and the transfer of its 4-aminobutyl moiety to the ε-amino group of one specific lysine residue of the eIF-5A precursor to form deoxyhypusine and 1,3-diaminopropane. Second, deoxyhypusine hydroxylase mediates the formation of hypusine through the addition of a hydroxyl group to the deoxyhypusine residue. The terminal ε-amine group of some lysine residues in proteins can be carbonylated spontaneously by carbonyl species to form carbonylated proteins (e.g., glyoxal and methylglyoxal; Tsikas 2022). Proteins with carboxymethyllysine and carboxyethyllysine residues contribute to advanced glycation end-products in animals and are elevated under some conditions (e.g., obesity and diabetes) in the plasma and red blood cells (Tsikas 2022).

Finally, glutamine residues in certain proteins (e.g., blood coagulation protein factor XIII, as well as skin and hair proteins) can be modified by primary amines or protein-bound lysine through transglutaminases 1 and 2 to incorporate amines into proteins or cross-linked proteins, respectively. Specifically, transglutaminase 2 catalyzes protein polymerization via an intermolecular cross linkage (isopeptide bond) between the side-chain amine ($-NH_2$) group of a glutamine residue of a protein and the ε-amine (ε-NH_2) group of a lysine residue of another protein. Bonds formed by transglutaminases exhibit high resistance to proteolytic degradation. Transglutaminases also catalyze the removal of the $-NH_2$ group in the side chain of glutamine residues in

proteins (deamidation) to yield glutamate residues and NH_3. Examples of these reactions are provided below:

$$\text{Protein-Gln-NH}_2 + \text{Primary amine (H}_2\text{N-R)} \rightarrow \text{Protein-Gln-NH-R} + \text{NH}_3 \text{ (Transglutaminases 1)}$$

$$\text{Protein-Gln-NH}_2 + \text{H}_2\text{N-Lys-Protein} \rightarrow \text{Protein-Gln-(C = O)-NH-Lys-Protein} + \text{NH}_3 \text{ (Transglutaminases 2)}$$

$$\text{Protein-Gln-NH}_2 + \text{H}_2\text{O} \rightarrow \text{Protein-Glu} + \text{NH}_3 \text{ (Transglutaminase, glutamine deamidation)}$$

Microbial transglutaminase 2 has been used as a protein glue in multiple processed food industries for over 30 years to improve the texture (e.g., hardness and gelation) of foods (Miwa 2020). The latter include fishery products such as kamaboko (boiled fish paste), meat products such as sausages, milk products such as yogurt, processed-soybean products such as tofu, and wheat products such as bread and noodles. Of note, transglutaminase 2 is known to play a key role in the pathogenesis of celiac disease (a chronic immune disorder in the small intestine in humans triggered by gluten ingestion), resulting in damage to the intestinal epithelium, diarrhea, fatigue, weight loss, bloating, and anemia (Iversen et al. 2020). Thus, there is concern that the microbial transglutaminase 2 used for food processing may increase risk for celiac disease by serving as a target of autoantibodies and the generation of immunogenic cross-linked gliadin complexes (Lerner and Matthias 2020).

Separation of Peptides from Proteins

Proteins can be denatured by heat, acids, bases, alcohols, urea, and salts of heavy metals. Trichloroacetic acid (TCA; at a final concentration of 5%) or perchloric acid ($HClO_4$, PCA; at a final concentration of 0.2 M) can fully precipitate proteins, but not peptides, from animal tissues, cells, plasma, and other physiological fluids (e.g., rumen, allantoic, amniotic, intestinal-lumen fluids, and digesta) (Moughan et al. 1990; Rajalingam et al. 2009). Tungstic acid (1%) can precipitate small peptides consisting of ≥4 amino acid residues. Thus, PCA or TCA can be used along with tungstic acid to distinguish small and large peptides. Ethanol (80%, vol/vol) can effectively precipitate both nonprolamin proteins and nucleic acids from aqueous solutions (Wilcockson 1975). This method may be useful to remove water-soluble inorganic compounds (e.g., aluminum) from protein hydrolysates. Note that certain plant proteins with high proline content (e.g., zein, glidin, and hordein) are soluble in 70–80% ethanol but insoluble in water or 100% ethanol; these proteins are called prolamines. Finally, ammonium sulfate (e.g., at a final concentration of 35%) can precipitate proteins from a solution without altering their biological structure.

Protein Structures

Proteins are the most abundant macromolecules in cells. They have four orders of structures: (a) a primary structure (the sequence of amino acids along the polypeptide chain); (b) a secondary structure (the conformation of the polypeptide backbone); (c) a tertiary structure (the three-dimensional arrangement of the protein molecule); and (d) a quaternary structure (the spatial arrangement of polypeptide subunits), if applicable (Figure 4.12). The primary sequence of amino acids in protein determines its secondary, tertiary, and quaternary structures, as well as its biological functions. The forces stabilizing polypeptide aggregates are hydrogen and electrostatic bonds between amino acid residues. Peptides and proteins are usually stable in a sterile aqueous solution at room temperature (Lubec and Rosenthal 1990).

The secondary structure of protein consists of several repeating patterns (Jones 2012). The most commonly observed types of secondary structure are the α-helix and the β-pleated sheet. The α-helix is a rigid, rod-like structure that arises when a polypeptide chain twists into a right-handed helical conformation, where hydrogen bonds form between the NH group of each amino acid and the carbonyl group of the amino acid four residues away. Some amino acids foster or inhibit the formation of specific secondary structures. For example, glycine's R group (a hydrogen atom) is so small that the polypeptide chain may be too flexible, whereas proline contains a rigid ring that prevents the N-C bond from rotating. In addition, proline has no NH group available to form the intra-chain hydrogen bonds that are

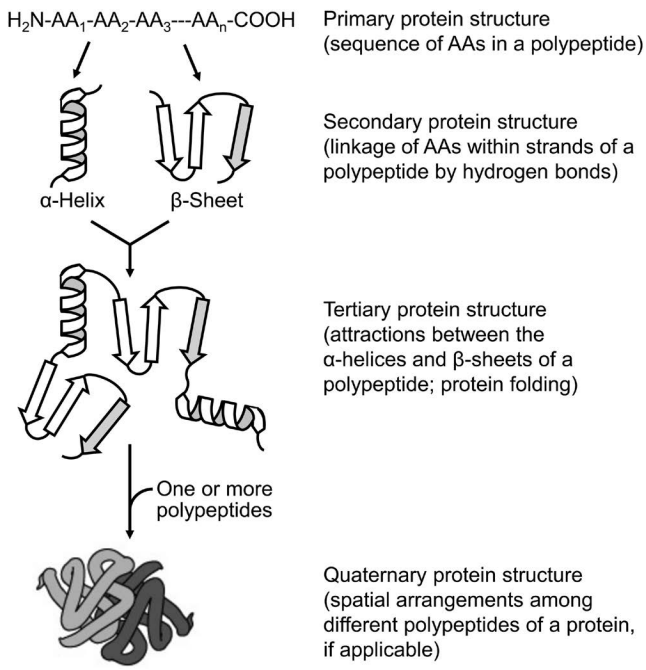


FIGURE 4.12 The four orders of protein structures. A protein has (a) a primary structure (the sequence of amino acids along the polypeptide chain), (b) a secondary structure (the conformation of the polypeptide backbone), (c) a tertiary structure (the three-dimensional arrangement of protein), and (d) a quaternary structure (the spatial arrangement of polypeptide subunits, if applicable). The primary sequence of amino acids in protein determines its secondary, tertiary, and quaternary structures, as well as its biological functions. [Reproduced from a figure freely available in the public domain (Hou et al. 2017), an open-access article].

crucial in α-helix structure. Likewise, amino acid sequences with large numbers of charged amino acids (e.g., glutamate and aspartate) and bulky R groups (e.g., tryptophan) are also incompatible with α-helix structures. On the other hand, β-pleated sheets form when two or more polypeptide chain segments line up side by side, with each individual segment being called a β-strand. Instead of being coiled, each β-strand is fully extended. The β-pleated sheets are either parallel or antiparallel. In the parallel β-pleated sheet structures, the hydrogen bonds in the polypeptide chains are arranged in the same direction; in the antiparallel chains, these bonds are arranged in opposite directions. In the secondary structure, both α-helix and β-pleated sheet patterns are stabilized by localized hydrogen bonding between the carbonyl and NH groups in the protein's backbone. Proline plays a key role in forming the β-turns of β-sheets.

Proteins can be classified according to their overall shape (globular or fibrous), solubility in water (hydrophobic or hydrophilic), three-dimensional structure, or biological functions (Table 4.1). Based on the combined chemical properties, proteins can be classified as simple proteins, scleroproteins, and complex proteins (e.g., flavoproteins, glycoproteins, hemoproteins, lipoproteins, metalloproteins, mucoproteins, nucleoproteins, and phosphoproteins). Albumin (e.g., in rice and wheat), globin (e.g., phytoglobin), and G-actin are globular proteins, which are common to all feedstuffs of plant origin. Fibrous proteins include F-actin, myosin, collagens, elastin, α-keratins, and β-keratins, which are limited to the feedstuffs of animal and marine origin. Collagens are rich in proline and glycine (~1/3 each) and constitute ~30% of total proteins in animals. Keratins are rich in cysteine, with wool protein containing ~4% S. Mature collagens are insoluble in water. Feedstuffs with high percentages of water-insoluble protein are fish meal, meat and bone meal, beet pulp, dried brewers grains, dried distillers grains, forages, sorghum, and soy hulls (NRC 2001). Because of their large size, proteins have colloidal properties to maintain stability in physiological fluid. A colloid is a solution that has evenly distributed particles ranging between 1 nm and 1 μm in diameter.

Proteins interact among themselves and the surrounding molecules. Depending on concentration and type, proteins differ in their solubility in water, with the membrane-bound proteins being hydrophobic. Like amino acids, proteins have characteristic isoelectric points and buffering capacities. All proteins can be denatured or modified from their natural state by heat, acids, alkalis, alcohols, urea, and the salts of heavy metals. The susceptibility of foodstuff proteins to heat damage during processing is increased in the presence of carbohydrates, owing to the Maillard reaction, which involves a condensation between the carbonyl group of a reducing sugar with the free amino group of an amino acid residue (e.g., lysine).

The structures of proteins in diets affect their susceptibility to animal-sourced proteases. For example, digestive proteases of mammals, birds, and fishes do not hydrolyze natural α-keratins or β-keratins (Wu 2022). α-Keratins primarily have the α-helical structure and are the major proteins of the hair, wool, hooves, horns, and nails in mammals. β-Keratins have stacked β-sheets and are the major proteins of nails, scales, and claws in birds and reptiles, and of beaks and feathers in birds. In contrast, digestive proteases readily degrade: (a) casein, which has limited α-helix and β-sheet secondary structure, as well as no disulfide bridges and no tertiary structure; and (b) actin and myosin, which are the main globular proteins in skeletal, smooth, and heart muscles.

The Concept of Crude Protein and True Protein

The content of mixed proteins in a feed or biological sample is often obtained by multiplying their N content by a factor of 6.25 based on the average N content (16%) in protein. The value obtained from the analysis is referred to as CP content. Such calculation, however, is not very precise for determining true protein content (Mariotti et al. 2008) because (a) some proteins contain more or less N due to their amino acid composition, and (b) different feedstuffs contain various percentages of nonprotein N (NPN), including peptides, amino acids, ammonia, urea, amides, amines, choline, betaine, purines, pyrimidines, nitrite, and nitrate. The NPN substances are abundant in grasses and legume forages (NRC 2001). The content of NPN is generally higher in hays and silages than the same feedstuffs when fresh because proteolysis occurs during wilting or fermentation. Some N-rich substances (e.g., melamine, which contains 66% N) lead to high CP content and are highly toxic to animals. With the availability of advanced analytical methods, the composition of amino acids in dietary or tissue proteins can be precisely determined and used to calculate the content of true protein based on the molecular weights of constituent amino acid residues (Dai et al. 2014).

Nitrogen in animal tissues and feedstuffs is commonly determined using the Kjeldahl method, which was developed in 1883 by a Danish chemist, Johan Kjeldahl (1849–1900). This method involves the heating of a sample in the presence of sulfuric acid (H_2SO_4) to generate ammonium sulfate, which is then converted by sodium hydroxide into ammonia. The ammonia is captured by boric acid (H_3BO_3) to form ammonium borate ($NH_4HB_4O_7$). The latter is then titrated with H_2SO_4 or hydrochloric acid (HCl) to regenerate boric acid (H_3BO_3). The main chemical reactions are summarized as follows:

1. Digestion: Sample + H_2SO_4 → $(NH_4)_2SO_4$ + SO_2 + H_2O + CO_2 ↑
   [This step requires heating and a catalyst, such as potassium sulfate (K_2SO_4), mercuric oxide (HgO), copper sulfate ($CuSO_4$), or sodium sulfate (Na_2SO_4)].
2. Distillation: $(NH_4)_2SO_4$ + 2NaOH → Na_2SO_4 + $2H_2O$ + $2NH_3$ ↑
3. Trapping of ammonia: $4H_3BO_3$ (red color) + NH_3 → $NH_4HB_4O_7$ (green color) + $5H_2O$
4. Titration: $NH_4HB_4O_7$ (green color) + H_2SO_4 → $(NH_4)_2SO_4$ + $2H_3BO_3$ (red color)
   or $NH_4HB_4O_7$ (green color) + HCl + $5H_2O$ → NH_4Cl + $4H_3BO_3$ (red color)

The Kjeldahl procedure requires an hour or longer to complete. Without proper modifications, there is an incomplete reduction of nitrite and nitrate into NH_3. As a result, total N is underestimated, but protein and amino acids can be determined more accurately. When a sample is pretreated with salicylic acid and sodium thiosulfate ($Na_2S_2O_3$) before acid digestion, N in nitrite and nitrate can be detected.

Nitrogen in animal tissues and feedstuffs can also be analyzed using the Dumas method, which was developed in 1826 by a French chemist, Jean-Baptiste Dumas (1800–1884). This method involves the combusting of a sample at 800–900°C in the presence of O_2 to generate the N_2 gas. The latter is determined with the use of a thermal conductivity detector (also known as a katharometer), which senses changes in the thermal conductivity (i.e., the property of a material to conduct heat) of the column effluent, compared with the flow of a reference carrier gas. This procedure is easy and rapid but requires an expensive instrument. On average, the Dumas method yields N content in samples that is about 1.4% higher than that for the Kjeldahl method, and the differences between the methods depend on the type of foodstuff: cereal: 1.5%; fish, 2.2%; meat, 0.63%; and milk, 1.5% (Thompson et al. 2002).

Animal-sourced ingredients [casein, gelatin, blood meal, hydrolyzed feather meal, meat and bone meal (MBM), poultry byproduct meal (PBM), and Menhaden fish meal] generally contain a higher content of CP and true protein than plant-sourced ingredients [cookie meal, hulled SBM, dehulled SBM, cottonseed meal, peanut meal, corn grain, sorghum grain, and white rice] (Table 4.6). Note that the

TABLE 4.6
Composition of Amino Acids (AAs) in Feed Ingredients

AA	Blood Meal	Casein	Cookie Meal	Corn Grain	CSM	Feather Meal	Fish Meal	Gelatin	MBM	Peanut Meal	PBM	SBM	SBM (PR)	SGH Grain	White Rice
General nutrients (% of foodstuff, as-fed basis)															
DM	91.8	91.7	90.8	89.0	90.0	95.1	91.8	88.9	96.1	91.8	96.5	89.0	96.4	89.1	90.7
CP	89.6	88.0	12.3	9.3	40.3	82.1	63.4	100.1	52.0	43.9	64.3	43.6	51.8	10.1	7.48
TP	88.0	86.2	10.5	8.2	32.3	81.0	63.7	97.4	50.7	35.1	60.4	38.2	41.6	8.8	6.62
EA	41.9	37.2	3.3	3.0	10.7	24.9	24.9	14.4	15.4	10.7	18.4	14.5	15.5	3.2	2.84
SA	46.1	49.0	7.2	5.2	21.6	56.1	38.8	83.0	35.3	24.3	42.0	23.7	26.1	5.6	4.86
Individual AA (% of foodstuff, as-fed basis)															
Ala	7.82	2.77	0.52	0.71	1.42	4.18	5.07	9.01	4.78	1.86	4.91	1.95	2.08	0.96	0.42
Arg	4.91	3.40	0.58	0.38	4.32	5.74	4.85	7.68	3.67	5.68	4.63	3.18	3.12	0.41	0.66
Asn	4.67	2.56	0.40	0.35	1.57	1.67	2.92	1.42	2.21	1.80	2.73	2.10	2.41	0.31	0.42
Asp	6.20	3.88	0.45	0.43	1.94	2.92	4.34	2.86	3.08	2.52	4.10	3.14	3.40	0.36	0.39
Cys	1.92	0.43	0.18	0.20	0.70	4.16	0.67	0.05	0.49	0.65	1.05	0.70	0.69	0.19	0.15
Gln	4.32	11.2	1.44	1.02	3.60	2.86	3.94	3.03	2.81	2.66	3.54	3.80	4.11	0.85	0.72
Glu	6.38	9.38	1.92	0.64	4.59	4.81	6.01	5.26	4.05	4.18	4.89	4.17	4.53	1.18	0.67
Gly	3.86	1.86	0.78	0.40	2.12	8.95	6.58	33.6	8.67	3.17	9.42	2.30	2.72	0.39	0.36
His	5.57	2.78	0.22	0.23	1.08	0.88	1.51	0.74	1.19	0.95	1.30	1.13	1.15	0.23	0.20
Hyp	0.20	0.14	0.00	0.00	0.05	4.95	1.86	12.8	2.88	0.07	3.31	0.09	0.07	0.00	0.00
Ile	2.54	4.91	0.51	0.34	1.19	3.79	3.26	1.17	1.92	1.41	2.32	2.03	2.10	0.38	0.34
Leu	11.4	8.82	0.88	1.13	2.26	6.75	5.24	2.61	3.56	2.48	4.21	3.44	3.70	1.21	0.66
Lys	8.25	7.49	0.41	0.25	1.66	2.16	5.29	3.75	3.13	1.37	3.44	2.80	2.87	0.21	0.21
Met	1.16	2.64	0.19	0.21	0.66	0.75	2.02	1.03	1.10	0.47	1.39	0.60	0.64	0.20	0.18
Phe	5.83	4.87	0.50	0.46	2.02	3.95	2.76	1.67	1.85	1.93	2.36	2.21	2.44	0.51	0.40
Pro	6.29	10.8	0.98	1.06	1.89	11.7	4.25	20.6	5.86	2.29	6.72	2.40	3.18	0.96	0.49
Ser	4.49	5.08	0.56	0.45	1.72	8.80	2.80	3.44	2.08	2.03	2.67	2.12	2.35	0.46	0.37
Thr	3.95	4.10	0.42	0.31	1.25	3.97	4.11	3.45	2.42	1.67	2.85	1.76	2.03	0.32	0.27
Trp	1.30	1.19	0.15	0.07	0.44	0.79	0.70	0.22	0.39	0.38	0.49	0.62	0.63	0.10	0.10
Tyr	2.86	5.06	0.55	0.43	1.10	2.04	2.36	0.93	1.45	1.39	1.84	1.66	1.72	0.45	0.22
Val	8.21	6.03	0.53	0.44	1.69	5.76	3.80	1.96	2.23	1.69	2.89	2.09	2.25	0.50	0.47

Source: Li et al. (2011) for all feedstuffs except for white rice, Hou et al. (2019) for white rice.
The molecular weights of intact AA were used to calculate the content of peptide-bound AA in feed ingredients.

Note: CP, crude protein (N % × 6.25); CSM, cottonseed meal; DM, dry matter; EA, total amounts of traditionally classified essential AA (His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val); Hyp, 4-hydroxyproline; MBM, meat and bone meal; PR, processed; PBM, poultry byproduct meal; SA, total amounts of amino acids synthesizable in animal cells (Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, Pro, Ser, and Tyr); SBM, soybean meal; SGH, sorghum; TP, true protein, which was calculated on the basis of the molecular weights of AA residues in protein.

TABLE 4.7
Content (%) of Dry Matter (DM) and Crude Protein (CP) in Forages and Roughages for Ruminant Diets (As-Fed Basis)

Feedstuff	DM	CP	Feedstuff	DM	CP
Bermudagrass hay	87.1	10.4	Legumes, forage		
Corn (yellow)			Pasture (intensively managed)	21.4	5.7
Cobs	90.8	3.0	Hay (immature)	84.2	22.8
Silage (immature)	23.5	9.7	Hay (mid mature)	83.9	20.8
Silage (normal)	35.1	8.8	Hay (mature)	83.8	17.8
Silage (mature)	44.2	8.5	Silage (immature)	41.2	23.2
Hulls	89.0	6.2	Silage (mid mature)	42.9	21.9
Grasses			Silage (mature)	42.6	20.3
Pasture (intensively managed)	20.1	5.3	Soybean hulls	90.9	13.9
Hay (immature)	84.0	18.0	Wheat		
Hay (mid mature)	83.8	13.3	Bran	89.1	17.3
Hay (mature)	84.4	10.8	Hay, headed	86.8	9.4
Silage (immature)	36.2	16.8	Middlings	89.5	18.5
Silage (mid mature)	42.0	16.8	Straw	92.7	4.8
Silage (mature)	38.7	12.7	Flour	95.1	13.4

Source: NRC (2001) for all feedstuffs except wheat flour; and Hou et al. (2019) for wheat flour.

term "byproduct" for a feedstock or foodstuff manufactured by the rendering industry is now replaced by "coproduct" in the United States and many regions of the world. The percentages of CP in these ingredients vary from 9 to 100%, depending on type, and are greater than those in forages and roughages (as low as 3% CP on the fed-basis) for ruminants (Table 4.7). The content of true protein is approximately 80–100% CP in ingredients, depending on type. Differences between CP and true protein are smaller for blood meal, casein, hydrolyzed feather meal, fish meal, gelatin, MBM, PBM, and SBM, compared with cookie meal, cottonseed meal, peanut meal, and dehulled SBM (Li et al. 2011; 2021d), as well as white rice (Hou et al. 2019). In all these ingredients, the total content of the amino acids that are not synthesizable in animal cells is lower than that of the amino acids which are synthesizable in animal cells.

CRYSTALLINE AMINO ACIDS, PROTEIN INGREDIENTS, AND PEPTIDE ADDITIVES FOR ANIMAL DIETS

Crystalline Amino Acids

A dietary supplement is a substance taken by mouth to provide a nutrient that animals may not obtain adequately from their regular diet relative to their optimal growth, development, and health. In the United States, most of the proteinogenic amino acids are permitted as feed additives for animals. Over the past 60 years, animal production has greatly benefited from dietary supplementation with some amino acids. Farm animals (e.g., chickens, pigs, cows, and sheep) are usually fed plant-based diets that generally contain low levels of lysine, methionine, threonine, and tryptophan. Deficiencies of these amino acids limit the maximum growth and production performance of these animals and impair their immunity (Li et al. 2007). To partially resolve this problem, DL-methionine was first used in the late 1950s as a supplement in feeds for broiler chickens. In the 1960s, L-lysine-HCl became commercially available for piglet diets. The 1980s witnessed the beginning of the use of L-threonine and L-tryptophan as supplements for swine and poultry feeds to enhance growth, improve immune function, and reduce glucocorticoid-induced stress. In the 1990s and 2000s, there was interest in the use of isoleucine and valine to improve milk production by lactating sows, but inconsistent results were reported in the literature, likely due to different experimental conditions (Wu et al. 2014b). In the past decade, rumen-protected lysine came into use for ruminants (e.g., cows and beef cattle) to increase milk production of lactating cows and growth performance of postweaning calves.

Although traditional research logically focused on dietary supplementation with amino acids that are not synthesized in animal cells, recent advances in the physiological roles of synthesizable amino acids have resulted in their use in swine and poultry production. For example, in 2005, a mixture of glutamate and glutamine was first produced for feeding postweaning pigs and chickens in some countries (including Brazil and Mexico) to prevent intestinal atrophy and improve feed efficiency. The nutrition of glutamate and glutamine in animals had long been ignored for historical reasons, including (a) the absence of data on glutamine content in feedstuffs due to analytical problems, (b) the complete lack of glutamine research in livestock production prior to the 1990s, and (c) the failure to describe glutamine as a component of proteins in classic animal nutrition textbooks. To date, many states in the United States have

approved the use of these two amino acids as supplements for animal feeds. Finally, based on discoveries driven by basic research, arginine (Progenos™) was first marketed in 2006 to enhance embryonic survival and litter size in gilts and sows. Feed-grade arginine is now available from Ajinomoto Inc. (Tokyo, Japan) as a feed additive for swine, poultry, and fishes. Aside from its use to promote lean tissue growth, arginine can also reduce whole-body white fat and enhance immunity in livestock and avian species.

Crystalline amino acids in the diet are directly available for absorption by the small intestine. Therefore, they are absorbed into enterocytes and appear in the portal vein more rapidly than peptide-bound amino acids released from protein digestion (Yen et al. 2004). This may result in a transient imbalance among amino acids in the systemic circulation, the extent of which likely depends on both the quality and quantity of dietary protein. Such a phenomenon raises a question about the bioequivalence of supplemental amino acids relative to amino acids in dietary proteins and peptides. However, experimental evidence from studies with humans, pigs, chickens, and rats consistently indicates that crystalline amino acids have high nutritional values when they are added to a diet deficient in those amino acids. In addition, some species of fishes, such as hybrid striped bass, can effectively use dietary free amino acids and can even grow faster when fed a purified diet based on free amino acids as the sole amino acid sources in comparison with a conventional diet containing 60% fish meal (He et al. 2023c). Advantages of dietary amino acid supplementation include (a) balancing amino acid composition in the diet, (b) reducing total protein content in the diet without compromising maximal growth or production performance, (c) minimizing the impact of animal production on environmental pollution, (d) improving health status and reducing infectious diseases and the associated costs of treatment, (e) enhancing feed efficiency and economic returns, and (f) mitigating the global shortage of protein resources. Extensive research has also shown that supplementing appropriate amounts of an amino acid (usually 0.2–2.5% of the diet on a dry matter (DM) basis, depending on individual amino acids, age, and species) is generally safe for animals. For example, supplementing up to 2% alanine, 1% arginine, 0.2% cysteine, 4% glutamate, 1% glutamine, 1% isoleucine, 2% leucine, 2% glycine, 0.2% methionine, 2% proline, 0.5% threonine, 0.2% tryptophan, or 1% valine in diets (on an as-fed basis) is safe for lactating sows and postweaning pigs fed a corn- and SBM-based diet containing 16–20% CP (Zhang et al. 2021). Pregnant gilts and sows fed a corn- and SBM-based diet containing 12% CP can also tolerate dietary supplementation with (a) 1% arginine between days 14 and 25 or between days 14 and 114 of gestation, or (b) 1% glutamine between days 90 and 114 of gestation, as can lactating sows between days 1 and 21 post-partum. However, dietary supplementation with 0.83% arginine immediately after breeding until day 25 of gestation (i.e., between days 0 and 25 of gestation) reduces progesterone production and embryonic survival in gilts.

Protein Ingredients from Plants, Animal Coproducts, and Single Cells

Both plant- and animal-sourced ingredients are regular sources of protein in animal diets. Different proteins in feedstuffs and animal tissues have different compositions of amino acids. On a DM basis, plant-sourced feedstuffs generally contain a lower content of protein and amino acids than animal-sourced feedstuffs (Table 4.6). For example, although corn grain and sorghum are excellent sources of energy for swine and poultry, they contain only 9–10% CP, 0.21–0.25% lysine, and 0.07–0.1% tryptophan, as well as imbalanced proportions of amino acids. For comparison, fish meal contains 63.4% CP, 5.28% lysine, and 0.7% tryptophan (Li et al. 2011). Also, poultry byproduct meal contains 64.3% CP, 3.44% lysine, and 0.49% tryptophan. The quality of a protein feedstuff depends upon many factors, including (a) its ability to supply sufficient amounts of all proteinogenic amino acids, (b) its protein digestibility, and (c) the presence of anti-nutritional or toxic substances. A combination of plant and animal products is desirable for the healthy diets of humans and omnivorous animals, and some of them can be replaced by single-cell protein foodstuffs (e.g., algae meal) to maximize environmental sustainability. In addition, animal-sourced feedstuffs can be used to cost-effectively replace a proportion or all of the fishmeal in the diets of fish and crustaceans. Furthermore, animal-sourced ingredients can be used in the diets of carnivores, such as cats, dogs, and tigers, to reduce their dependence on edible foods for the growing human population.

Plant-Sourced Protein Foodstuffs

Omnivores and herbivores consume plants for energy, proteins, and other nutrients. Plant-sourced protein foodstuffs include SBM, soybean protein concentrates, soybean protein isolates, canola meal, peanut meal, rice protein concentrates, and rice protein concentrates. Cereal grains generally contain low amounts of lysine, tryptophan, methionine, cysteine, threonine, and histidine. Barley and wheat provide a greater amount of tryptophan than corn and sorghum grains. SBM is a good source of most amino acids, including arginine, lysine, methionine, cysteine, threonine, and tryptophan, which are typically present in relatively low concentrations in cereal grains. However, it has recently been recognized that SBM is deficient in glycine when fed to growing-finishing pigs (particularly those with intrauterine growth restriction due to impaired glycine synthesis; He et al. 2023a) or fish such as juvenile hybrid striped bass (He et al. 2023b; Li et al. 2023b) and largemouth bass (Rossi et al. 2021). All plant-sourced foods do not contain creatine, taurine, anserine, or carnosine, which are all abundant antioxidants in animals (Wu 2022).

Globally, SBM, which contains 40–48% CP, depending on the processing method (e.g., the removal of hulls and oil extraction), is the preferred protein source for nonruminant feeds because of its wide availability, high protein digestibility, high protein content with a well-balanced profile

for most amino acids, and reasonably low cost. Various processed soybean products that have been used in animal (including fishes) feeding include soybean protein concentrates, soybean protein isolates, extruded SBM, and protein hydrolysates (McCalla et al. 2010). Other commonly used plant protein sources include wheat, canola meal, cottonseed meal, peanut meal, sunflower meal, peas, dried brewers grains, and dried distillers grains with solubles (DDGS). Of note, these products contain many anti-nutritional factors (e.g., gossypol, oxalates, goitrogens, tannins, cyanogenic glycosides, chlorogenic acids, and toxic amino acids), in addition to those found in SBM. The plant-sourced anti-nutritional factors can be divided into heat-labile (e.g., trypsin inhibitors, hemagglutinins, phytate, goitrogens, and anti-vitamin factors) and heat-stable (e.g., saponins, estrogens, flatulence factors, and lysinoalanine) factors. For example, canola meal should be analyzed for the presence of such anti-nutritional factors as glucosinolates and erucic acid.

Corn grain contains a variety of proteins, including albumins, globulins, glutelins, and prolamines (zein A and zein B). DDGS (a major co-product from the corn-based production of ethanol) as new sources of feed protein deserves much attention from animal nutritionists. With the rapid growth of the ethanol industry, amounts of low-cost DDGS for ruminant and nonruminant rations are increasing worldwide. Corn- and wheat-source DDGS contain 26–30% and 40–45% CP (DM basis), respectively (Cromwell et al. 2011; Widyaratne and Zijlstra 2007). DDGS contains a higher content of CP than its parent grain because of microbial fermentation (carbohydrate catabolism and protein/amino acid synthesis) during ethanol production. DDGS is an excellent source of fiber and phosphorus for animals. A concern over the use of DDGS to feed animals is its high and variable levels of S (0.4–1.5% of DM). This is primarily because (a) various amounts of sulfuric acid are employed to maintain fermenter pH levels in the biofuels industry with rapidly changing technologies and (b) grains and possibly water also contain some S. Inclusion rates of DDGS in rations depend on animal species and production phases, such as 15, 20, 30, and 40% for pigs during grower, finisher, lactation, and gestation periods, respectively (Cromwell, et al. 2011; Song et al. 2010), as well as 10, 20, and 40% for poultry, dairy cattle, and beef cattle, respectively (Shurson 2017).

Plants, particularly legumes, contain anti-nutritional factors, such as protease (e.g., trypsin) inhibitors, and heat processing can alleviate most or nearly all of these adverse effects on animals. However, methods and heating conditions for soybean processing [e.g., crushing (e.g., 135°C and 30 s), extrusion (e.g., 155°C and 15 to 20 s), and solvent extraction (e.g., hexane, ethanol, or their mixture) can substantially affect the nutritional values of the feedstuffs (e.g., the true digestibilities of both energy and protein, as well as animal growth performance). For example, excessive heating increases Maillard reactions between the side-chain NH_2 group of amino acids (e.g., lysine, arginine, and glutamine) and reducing sugars (e.g., glucose, fructose, and galactose), resulting in reduced energy and amino acid digestibility. On the other hand, under-processing may not effectively remove antinutritional factors from soybean products. Because SBM is the major source of dietary protein for swine, poultry, and many species of aquatic animals in most regions of the world, chemical and biological methods [including enzymatic (urease), protein solubility (e.g., in 0.2% or 35.6 mM KOH at 22°C for 20 min), and animal (e.g., young chick and postweaning pig) growth assays] have been developed to assess the degree of heating raw soybeans for manufacturing their meals. Increasing the temperature and period of soybean processing generally decreases protein solubility. Inclusion of SBM with a solubility of less than ~60% and ~65% can reduce, respectively, the feed efficiency of young chicks and postweaning pigs fed diets containing adequate amino acids (Parsons et al. 1991).

Animal-Sourced Protein Foodstuffs

As noted previously, plant protein ingredients (including SBM) contain a lower content of many amino acids (e.g., cysteine, glycine, lysine, methionine, tryptophan, and threonine) than animal-sourced proteins. There is a limitation to the inclusion of plant ingredients in the diet (particularly for young animals) due to their relatively high content of antinutritional factors (Wu 2022). Thus, animal nutritionists have used animal-sourced proteins to formulate amino acid-balanced diets. To date, animal protein supplements are manufactured from poultry, pork, beef, and fish byproducts at poultry, meat, rendering operation, fishes, and dairy processing plants (Wilkinson and Meeker 2021). These foodstuffs are manufactured by rendering, which involves cooking raw animal tissues at high temperatures to destroy all microbes, while separating fat, protein, and water into usable nutritional components or chemical products by using a variety of equipment and control processes (Swisher 2023). In the United States, approximately 49%, 44%, 37%, and 57% of the live weight of cattle, pigs, broilers, and most fish species, respectively, are not consumed by humans (Meeker and Hamilton 2006). Without rendering, the protein component of the inedible raw material would have been either discarded or used as fertilizer. Many regions of the world (including the United States, Canada, and Europe) have established quality assurance programs to produce animal-sourced feedstuffs for feeding farm and companion animals. Therefore, the rendering industry plays an important role in promoting circular bio-economies, while improving animal nutrition and health, reducing environmental pollution, and contributing to the sustainability of livestock, poultry, aquaculture, and pet industries.

Cost-effective feedstuffs for livestock, poultry, fishes, and crustaceans (including shrimp and crabs) include bone meal, meat and bone meal, poultry byproduct meal, chicken viscera digest, hydrolyzed feather meal, intestine-mucosa products, insect meal, black soldier fly larvae meal, blood meal, plasma meal (including spray-dried poultry

plasma), and spray-dried egg product (Li and Wu 2022; Li et al. 2021d, 2023b). Important nutritional features of these foodstuffs are noteworthy (Tables 4.8 and 4.9). First, animal-sourced foodstuffs are generally palatable due to their high content of glutamate and glycine, and can induce feed intake by animals. Second, blood meal, feather meal, meat and bone meal, and poultry byproduct provide large amounts of biosynthesizable amino acids. Third, blood meal is particularly rich in aspartate, asparagine, cysteine (a major amino acid in hemoglobin), glutamate, glutamine, histidine (another major amino acid in hemoglobin), leucine, lysine, methionine, tryptophan, and valine. The content of aspartate, asparagine, glutamate, glutamine, histidine, leucine, lysine, tryptophan, and valine in blood meal is the highest among all animal-sourced foodstuffs (Li et al. 2021d). Likewise, cysteine and histidine are highly abundant in spray-dried poultry plasma. By contrast, meat and bone meal contains less cysteine and tryptophan than other animal-sourced feedstuffs. This is because meat and bone meal contains a large proportion of bone, cartilage, tendons, and other connective tissue in which collagen (containing no tryptophan and little cysteine) is the major protein. Fourth, feather meal contains the highest content of arginine, cysteine, 4-hydroxyproline, isoleucine, proline, and serine among all common animal protein foodstuffs. Similarly, both glycine and serine are highly abundant in chicken viscera digest. Specifically, serine plus glycine plus 4-hydroxyproline accounts for ~25% of total protein

TABLE 4.8
Content of Nutrients and Total Amino Acids (Peptide-Bound Plus Free Amino Acids) in Feed Ingredients[a]

DM or AA	BSFM	CBPM	CVD	FM-M	FM-P	FM-SE	SDPM	PBM (PFG)	SDPP (PFG)	SDEP	Algae SM	Bermuda Grass Hay	DDGS	Sweet Potato
	% of feedstuff (as-fed basis)													
DM	95.3	98.0	93.2	92.8	91.6	92.1	88.7	96.8	95.4	95.1	96.5	87.2	90.3	22.9
CP	42.0	68.1	67.2	62.4	68.8	64.7	55.2	70.2	69.8	48.9	68.8	12.5	30.1	1.58
CF	33.5	13.4	8.57	11.1	7.32	5.07	4.63	11.5	4.02	35.0	0.25	2.01	4.2	0.21
Carb	13.6	4.20	5.44	0.38	0.37	0.39	9.14	3.91	3.35	6.34	20.3	67.8	48.0	20.2
GE[b]	5975	5106	4656	4431	4417	3986	3790	5032	4283	6191	4571	3644	3988	933
Ala	3.14	4.54	4.12	3.63	4.74	4.59	3.51	3.98	4.98	3.43	5.85	0.89	1.90	0.110
Arg	2.64	4.75	4.02	3.67	3.58	4.15	3.59	4.15	4.94	3.82	5.18	0.58	1.19	0.070
Asn	1.93	2.61	2.50	2.26	2.28	2.60	1.52	2.56	2.57	2.69	3.34	0.52	1.11	0.274
Asp	3.04	3.93	3.66	3.33	3.41	3.87	3.72	3.86	5.17	3.60	4.29	0.73	1.49	0.076
Cys	0.69	1.07	1.22	0.67	0.72	0.69	0.99	1.04	2.65	1.37	0.88	0.17	0.52	0.037
Gln	2.51	3.88	3.73	3.60	3.39	3.96	2.78	3.41	4.49	2.90	4.25	0.63	2.33	0.050
Glu	3.44	5.34	6.34	5.32	5.13	5.97	5.77	4.75	6.77	5.48	6.81	1.18	3.04	0.125
Gly	2.91	5.94	8.25	4.26	5.09	5.87	4.95	6.86	3.40	2.45	4.19	0.55	1.08	0.081
His	1.71	1.36	0.75	1.41	1.43	1.46	1.29	1.31	3.66	1.45	1.29	0.24	0.78	0.027
Hyp	1.27	1.85	1.73	1.57	1.82	1.98	0.79	2.25	0.021	0.023	0.014	0.010	0.035	0.001
Ile	2.02	2.72	3.84	2.42	2.75	2.79	2.44	2.38	2.77	2.97	4.09	0.50	1.01	0.076
Leu	3.25	5.28	6.10	4.50	5.27	4.59	4.30	4.33	7.56	4.61	6.32	0.92	3.14	0.112
Lys	3.21	4.52	5.02	4.46	5.25	4.57	4.07	3.54	6.85	4.09	4.18	0.52	0.92	0.083
Met	1.20	1.43	1.58	1.97	2.16	2.03	1.20	1.38	1.97	2.01	2.17	0.19	0.63	0.028
Phe	2.18	2.70	3.70	2.31	2.31	2.65	2.33	2.32	4.74	3.04	3.88	0.59	1.36	0.111
Pro	2.92	4.30	5.53	3.72	4.27	4.80	3.16	5.07	4.32	3.39	3.12	0.52	2.01	0.061
Ser	2.34	3.05	6.45	2.52	2.91	3.28	3.37	2.62	4.99	5.39	4.35	0.50	1.33	0.104
Thr	2.55	2.77	1.99	2.50	2.85	3.14	2.85	2.56	4.45	3.10	4.13	0.53	1.08	0.086
Trp	0.71	0.76	1.03	0.70	0.68	0.70	0.63	0.63	1.36	0.97	1.02	0.18	0.24	0.011
Tyr	3.85	2.29	2.56	1.89	2.01	2.36	2.24	1.88	3.51	2.61	3.79	0.38	0.95	0.069
Val	2.87	3.32	5.57	3.02	3.65	3.25	3.05	2.91	4.96	3.57	4.36	0.66	1.45	0.102
TAAs	50.4	68.4	79.7	59.7	65.7	69.3	58.6	63.8	86.1	63.0	77.5	11.0	27.6	1.69

Source: Li and Wu (2020, 2023), Gilbreath et al. (2021), and Hou et al. (2019).

[a] Molecular weights of intact amino acids (AAs) were used to calculate the content of AAs in feed ingredients.

[b] Expressed as kcal/kg of feedstuff (on an as-fed basis).

Note: AA, amino acid; BSFM, black soldier fly larvae meal; Carb, carbohydrates; CBPM, chicken byproduct meal; CP, crude fat; CVD, chicken visceral digest; DDGS, dried distillers grains with solubles; DM, dry matter; FM-M, fishmeal (United States Menhaden); FM-P, fishmeal (Peruvian anchovy); FM-SE, fishmeal (Southeast Asian miscellaneous marine fishes); Hyp, 4-hydroxyproline; PBM (PFG), poultry byproduct meal (pet-food grade); SDEP, spray-dried egg product; SDPM, spray-dried peptone from enzymes-treated porcine mucosal tissues; SDPP, spray-dried poultry plasma; SM, spirulina meal; TAAs, total amino acids.

TABLE 4.9
Composition of Amino Acids (AAs) in a Spray-Dried Porcine Peptone Product[a,b]

AA	Total AA	Free AA	AA in Proteins + Peptides	AA in Proteins	AA in Peptides
			g/kg of feedstuff (as-fed basis)		
Alanine	35.09	16.96	18.13	9.32	8.81
Arginine	35.92	12.82	23.10	14.56	8.54
Asparagine	15.18	3.36	11.82	2.08	9.74
Aspartate	37.17	15.21	21.96	8.00	13.96
Cysteine	9.85	4.14	5.71	4.96	0.75
Glutamine	27.84	0.0114	27.83	15.26	12.57
Glutamate	57.68	14.58	43.11	9.81	33.3
Glycine	49.5	7.12	42.37	21.80	20.57
Histidine	12.85	5.80	7.05	4.90	2.15
4-Hydroxyproline	7.89	2.54	5.35	1.99	3.36
Isoleucine	24.43	12.42	12.01	5.51	6.50
Leucine	43.01	22.16	20.86	15.52	5.34
Lysine	40.67	19.50	21.17	15.59	5.58
Methionine	12.01	5.45	6.56	6.12	0.44
Phenylalanine	23.31	12.79	10.52	5.28	5.24
Proline	31.63	14.42	17.22	6.54	10.68
Serine	33.72	12.06	21.65	10.09	11.56
Threonine	28.52	12.76	15.75	6.00	9.75
Tryptophan	6.28	4.19	2.09	1.62	0.47
Tyrosine	22.37	12.47	9.90	6.11	3.79
Valine	30.48	16.82	13.65	6.65	7.00

Source: Li and Wu (2022).

[a] Enzymes-treated porcine mucosal tissues. The content of dry matter in this product was 88.74%. Proteins and peptides are perchloric acid-insoluble and soluble molecules, respectively. Except for glycine, all amino acids are L-isomers.

[b] Containing the following functional molecules [g/kg product (as-fed basis)]: citrulline, 1.40; ornithine, 1.54; taurine, 1.64; β-alanine, 0.081; carnosine, 0.955; anserine, 0.067; creatine, 0.042; creatinine, 2.72; creatine phosphate, 1.43; putrescine, 0.53; spermidine, 1.36; spermine, 1.43, and total glutathione, 0.175.

in feather meal. Fifth, both blood meal and feather meal provide the highest content of threonine among all common animal protein foodstuffs. Thus, feather meal is a highly abundant source of all functional amino acids for animal feeding. Sixth, poultry byproduct meal contains the highest content of glycine among all common animal protein foodstuffs and is also rich in proline and 4-hydroxyproline. Likewise, meat and bone meal contains large amounts of glycine, Pro, and 4-hydroxyproline. Finally, tyrosine is the most abundant amino acid in black soldier fly larvae meal (Li and Wu 2020). Thus, animal-sourced feedstuffs are excellent sources of all amino acids in these foodstuffs to balance amino acid profiles in diets for livestock, poultry, fishes, and crustaceans.

In addition to their high amino acid content, animal-sourced feeds provide high levels of available phosphorus, calcium, and other minerals, as well as moderate levels of fat-soluble vitamins and energy (Li et al. 2023a). Importantly, animal-sourced ingredients contain little or no antinutritional factors. Although all animal protein products have a high content of amino acids, blood meal (a byproduct of slaughterhouses after protein coagulation by heating) has relatively low levels of isoleucine (only 2.54%) and glycine (3.86%) in comparison with other branched-chain amino acids and biosynthesizable amino acids, respectively (Li et al. 2011). Although some nutritionists believe that blood meal has an undesirable amino acid profile for animal feeding, this should not be a concern when this foodstuff is used as a protein supplement to a compounded diet that generally contains sufficient isoleucine. Of note, dried blood products (e.g., spray-dried plasma protein and spray-dried blood cells obtained by the separation of whole blood into plasma and cell fractions) have been used in the feed industry as high-quality protein sources. The quality of these products, which contain immunoglobulins, growth factors, polyamines, and bioactive peptides, is greatly influenced by the method of drying (e.g., heating vs. spray-drying).

Animal-sourced foodstuffs (e.g., intestinal mucosal products, intestinal peptones, and plasma meal) provide not only high-quality animal protein but also functional substances (Table 4.10). The latter include growth factors, enzymes, hormones, large peptides, small peptides, antimicrobials, cytokines, bioamines, regulators of nutrient metabolism, unique amino acids (e.g., taurine and

TABLE 4.10
Presence of Functional Molecules in the Small-Intestinal Mucosa of Animals

Molecule	Classification	Source	Major Functions
N-Acetylserotonin	Trp metabolite	Enteroendocrine cells	Inhibit production of inflammatory Cytokines and serve as an antioxidant
S-Adenosylmethionine	Met metabolite	Multiple mucosal cells	Stimulate growth of epithelial cells
β-Alanine	Nutrient	All cell types	Synthesis of carnosine, anserine, and related dipeptides; a component of pantothenic acid and coenzyme A
α-Amino acids	Nutrients	All cell types	Antioxidants, DNA and protein synthesis, cell signaling and growth; nutrient metabolism and transport; immunity
γ-Aminobutyrate	Glu metabolite	Enteroendocrine cells	Increase food intake by animals
Anserine	β-Ala-1MeHis	All cell types of most animals	Antioxidant and buffering
Anthranilic acid	Trp metabolite	IEL and mucosal T-cells	Inhibit production of inflammatory cytokines and enhance immunity
Antibodies (e.g., IgA)	Proteins	Plasma cells (B-cell)	Cell-mediated immunity
Azurocidin	Protein	Neutrophils	Antimicrobial and innate immunity
Belanine	β-Ala-3MeHis	All cell types of most animals	Antioxidant and buffering
Carnosine	β-Ala-Histidine	All cell types of most animals	Antioxidant and buffering
Catalase	Protein	Multiple mucosal cells	Kill pathogenic organisms and support innate immunity
Cathelicidins	Small peptides	Paneth cells	Antimicrobials and innate immunity
Cathepsin G	Protease	Neutrophils	Antimicrobial and innate immunity
Cathepsin K	Protease	Goblet cells & enterocytes	Antimicrobial and innate immunity
Cholecystokinin (CCK)	Polypeptide	Enteroendocrine cells	Stimulate pancreatic secretions
Chymase	Protease	Mast cells	Antimicrobial and regulation of intestinal mucosal homeostasis
Creatine and creatine-P	Arg, Gly and Met metabolite	All cell types	Energy metabolism, antioxidant and buffering
α-Defensins (cryptdins)	Small peptides	Paneth cells	Antimicrobials and innate immunity
β-Defensins	Small peptides	Enterocytes, plasma cells and granulocytes	Antimicrobials and innate immunity
Diamine oxidases	Enzymes	Multiple mucosal cells	Antimicrobial and innate immunity
Digestive enzymes[a]	Proteins	Enterocytes	Enhance digestion of protein, carbohydrates, and fat in diets
Dopamine	Bioamine	Mucosal neurons	Increase intestinal motility and food intake
Dopuin	62-AA peptide	Mucosa of pig small intestine	Antimicrobial
EGF	Protein	Mucosal Brunner's glands	Promote growth of epithelial cells and restitution of the epithelium
Fibroblast growth factors	Proteins	Fibroblasts	Stimulate growth of epithelial cells
Gastrin	Small peptide	Enteroendocrine cells in the duodenum	Stimulate gastric acid secretion to enhance digestion and kill bacteria, while promoting gut maturation
Ghrelin	Polypeptide	Enteroendocrine cells	Stimulate food intake by animals
Glucagon-like peptide-1	Polypeptide	Enteroendocrine cells	Regulate glucose metabolism
Glucagon-like peptide-2	Polypeptide	Enteroendocrine cells	Promote intestinal mucosal integrity
Glucosamine-6-phosphate	Aminosugar	Multiple mucosal cells	Enhance synthesis of glycoproteins
Glutathione	γ-Glu-Cys-Gly	Multiple mucosal cells	A major antioxidant
Glutathione reductase	Protein	Multiple mucosal cells	An antioxidant enzyme
Histadins[b]	Large peptides	Paneth cells	Antimicrobials and innate immunity
Histamine	His metabolite	Mast cells	Stimulate gastric acid secretion to enhance digestion and kill bacteria
IGF-I	Large peptide	Enterocytes and goblet cells	Stimulate growth of epithelial cells
Interferon-γ	Cytokine	IEL and mucosal T-cells	Regulates epithelial homeostasis
Interleukin-2	Cytokine	IEL	Protect the intestinal epithelium
Interleukin-4	Cytokine	IEL	Protect the intestinal epithelium
Interleukin-10	Protein	Fibroblasts and IEL	Increase survival of mucosal T-cells
Lysozymes	Glycoside hydrolases	Paneth cells, enterocytes and neutrophils	Antimicrobials, innate immunity and maintain extracellular matrix
Melatonin	Bioamine	Enteroendocrine cells	Regulate food intake and digestion
Minerals	Nutrients	All cell types	Antioxidants, DNA and protein synthesis, cell growth; coenzymes; nutrient metabolism and transport; immunity
Mucins	Glycoproteins	Goblet cells	Maintain intestinal mucosal integrity
Myeloperoxidase	Protein	Macrophages and lymphocytes	Kill pathogenic organisms and support innate immunity

(*Continued*)

TABLE 4.10 (*Continued*)
Presence of Functional Molecules in the Small-Intestinal Mucosa of Animals

Molecule	Classification	Source	Major Functions
Nucleotides	Metabolites	Multiple mucosal cells	Stimulate growth of epithelial cells
Oleoylethanolamide	Lipid derivative	Enterocytes	Stimulate uptake of fatty acids
Osteopontin	Glycoprotein	Enterocytes	Increase ion and nutrient transport
PEC-60	60-AA peptide	Mucosa of pig small intestine	Antimicrobial
Peroxidase	Enzyme	Multiple mucosal cells	Kill pathogenic organisms and support innate immunity
Phospholipase A2	Enzyme	Multiple mucosal cells	Antimicrobial and innate immunity
Polyamines[c]	Bioamines	Multiple mucosal cells	Stimulate DNA and protein synthesis; ion transport; intestinal cell proliferation, migration, maturation, and repair
Peptide YY	Small peptide	Enteroendocrine cells	Regulate gut development and motility
PR-39 peptide[d]	Small peptide	Paneth cells	Antibacterial and innate immunity
Serotonin	Bioamine	Enteroendocrine cells	Regulate luminal sensing of nutrients, mucosal secretions and inflammation, nutrient absorption, gut motility, food intake, and energy balance
Superoxide dismutase	Enzyme	Multiple mucosal cells	Kill pathogenic organisms and support innate immunity
Taurine	Cys metabolite	All cell types	Antioxidant and osmoregulation
TGF-α	Protein	Enterocytes and macrophages	Enhance growth of epithelial cells and restitution of the epithelium
TGF-β	Protein	T-cells and goblet cells	Enhance growth of epithelial cells and restitution of the epithelium
VEGF	Protein	Endothelial cells	Maintain microvasculature structure to support nutrient transport
Vitamins	Nutrients	All cell types	Antioxidants, DNA and protein synthesis, cell growth; coenzymes; nutrient metabolism and transport; immunity

Source: Li and Wu (2022).
[a] Including disaccharidases (lactase-phlorizin hydrolase and sucrase-isomaltase) and peptidases.
[b] Histine-rich peptides.
[c] Putrescine + spermidine + spermine.
[d] A *proline*-arginine-*rich peptide.*
Note: AA, amino acid; β-Ala, β-alanine; Arg, L-arginine; Cys, cysteine; EGF, epidermal growth factors; Glu, glutamate; Gly, glycine; IEL, intraepithelial lymphocytes; IGF-I, insulin-like growth factor-I; 1MeHis, L-1-methylhistidine; 3MeHis, L-1-methylhistidine; Met, L-methionine; TGF, transforming growth factor; and VEGF, vascular endothelial growth factor.

4-hydroxyproline), and other bioactive substances (e.g., creatine, creatine phosphate, glutathione, carnosine, and anserine; Li and Wu 2022; Li et al. 2021d). Note that both creatine and creatine phosphate undergo cyclization to creatinine at various rates under acidic (e.g., in the urine), neutral (e.g., in the blood), or heating (e.g., ~100°C) conditions, whereas strong alkaline conditions convert creatinine into creatine. Heating and strong acid or alkaline conditions do not generate creatine from creatine phosphate. Knowledge of these chemical properties facilitates the analysis of creatine and its related substances in foodstuffs and animal samples.

In nutrition, only a relatively small amount of animal-sourced foodstuffs is needed in animal diets to improve their amino acid profiles, and inclusion rates of these ingredients in diets vary with ingredient and animal species. For example, the content of animal-sourced foodstuffs ranges from 1.5–2% feather meal and 5–10% meat and bone meal in the diets of broilers and turkey to 20–30% poultry byproduct meal in the diets of cats and dogs as well as many species of fish, and to 33–66% feather meal in the diets of shrimp (Bureau 2006; Li et al. 2021d). Of note, appropriate amounts of blood meal, poultry byproduct, and feather meal can replace up to 80% fishmeal in the diets of fish and shrimp. Animal-sourced foodstuffs can be used in combination to bring about complementary effects in nutrition [such as improving feed intake, immunity, health (the intestine and the whole body), well-being, wound healing, growth performance, and feed efficiency in livestock, poultry, fish, and crustaceans]. Additionally, these can be used to optimize the health of skin, hair, bone, and joints in companion animals (Connolly and Wu 2024; Lopez et al. 2024).

Single-Cell Protein Foodstuffs

The term "single-cell protein" (SCP) was coined by Carroll Wilson at the Massachusetts Institute of Technology in 1966. SCPs are proteins extracted from edible unicellular microbes, including pure or mixed cultures of algae, yeasts, fungi, or bacteria. The SCPs have relatively balanced profiles of proteinogenic amino acids (e.g., algae, Li and Wu 2020). The composition of amino

acids in bacteria is well defined (Dai et al. 2010; Gilbreath et al. 2021). In addition, SCP products also provide some carbohydrates, lipids, vitamins, and minerals needed by animals and, therefore, are of high nutritive value as feed additives. Of note, *Saccharomyces cerevisiae* (commonly known as brewer's yeast or baker's yeast) was first cultured in Europe during World War I for human consumption as a food protein supplement. The industrial production of SCPs requires five processes: (a) preparing an adequate medium containing suitable C, N, and S sources for cultivation of microbes; (b) preventing contamination in the chosen fermentation medium and bioreactors (c) selecting and growing the desired microbes; (d) extracting microbial biomass with the use of combined mechanical, enzymatic, and engineering methods; and (e) eliminating the undesired substances, as well as drying and packaging the products (Bajić et al. 2023). Because the microbial mass can be grown from agro-industrial wastes or byproducts (e.g., ammonia, S, and indigestible carbohydrates), this renewable and environmentally sustainable technology is promising to provide future foods for livestock, poultry, fish, and crustaceans.

Peptides Used as Feed Additives

Industrially produced peptides are now widely used as feed supplements in animal nutrition (McCalla et al. 2010). Chemical, enzymatic, or microbial hydrolysis of proteins in animal byproducts or plant-sourced feedstuffs before feeding is an attractive means of generating high-quality small or large peptides that have both nutritional and physiological or regulatory functions in livestock, poultry, and fishes (Zhang et al. 2015). Antioxidative peptides generated from the hydrolysis of animal proteins are shown in Table 4.11.

Acid hydrolysis of protein (gelatin) at high temperatures was first reported by the French chemist H. Braconnot in 1920. A much shorter period of time (e.g., 2–6 h) can be used to produce peptides as flavor enhancers in animal diets (e.g., hydrolyzed vegetable protein; Pasupuleki and Braun 2010).

TABLE 4.11
Antioxidative Peptides Generated from the Hydrolysis of Animal Proteins

Source	Protease(s)	Amino Acid Sequence
Pig muscle actin	Papain + Actinase E	Asp-Ser-Gly-Val-Thr
Pig muscle	Papain + Actinase E	Ile-Glu-Ala-Glu-Gly-Glu
Pig muscle tropomyosin	Papain + Actinase E	Asp-Ala-Gln-Glu-Lys-Leu-Glu
Pig muscle tropomyosin	Papain + Actinase E	Glu-Glu-Leu-Asp-Asn-Ala-Leu-Asn
Pig muscle myosin	Papain + Actinase E	Val-Pro-Ser-Ile-Asp-Asp-Gln-Glu-Glu-Leu-Met
Pig collagen	Pepsin + Papain + Others[a]	Gln-Gly-Ala-Arg
Pig blood plasma	Alcalase	His-Asn-Gly-Asn
Chicken muscle	Thermolysin	His-Val-Thr-Glu-Glu
Chicken muscle	Thermolysin	Pro-Val-Pro-Val-Glu-Gly-Val
Deer muscle	Papain	Met-Gln-Ile-Phe-Val-Lys-Thr-Leu-Thr-Gly
Deer muscle	Papain	Asp-Leu-Ser-Asp-Gly-Glu-Gln-Gly-Val-Leu
Bovine milk casein	Pepsin, pH 2, 24 h	Tyr-Phe-Tyr-Pro-Glu-Leu
Bovine milk casein	Pepsin, pH 2, 24 h	Phe-Tyr-Pro-Glu-Leu
Bovine milk casein	Pepsin, pH 2, 24 h	Tyr-Pro-Glu-Leu
Bovine milk casein	Pepsin, pH 2, 24 h	Pro-Glu-Leu
Bovine milk casein	Pepsin, pH 2, 24 h	Glu-Leu
Bovine milk casein	Trypsin, pH 7.8, 24–28 h	Val-Lys-Glu-Ala-Met-Pro-Lys
Bovine milk casein	Trypsin, pH 7.8, 24–28 h	Ala-Val-Pro-Tyr-Pro-Gln-Arg
Bovine milk casein	Trypsin, pH 7.8, 24–28 h	Lys-Val-Leu-Pro-Val-Pro-Glu-Lys
Bovine milk casein	Trypsin, pH 7.8, 24–28 h	Val-Leu-Pro-Val-Pro-Glu-Lys
Bovine whey protein	Thermolysin, 80°C, 8 h	Leu-Gln-Lys-Trp
Bovine whey protein	Thermolysin, 80°C, 8 h	Leu-Asp-Thr-Asp-Tyr-Lys-Lys
Bovine β-lactoglobulin	Corolase PP, 37°C, 24 h	Trp-Tyr-Ser-Leu-Ala-Met-Ala-Ala-Ser-Asp-Ile
Bovine β-lactoglobulin	Corolase PP, 37°C, 24 h	Met-His-Ile-Arg-Leu
Bovine β-lactoglobulin	Corolase PP, 37°C, 24 h	Try-Val-Glu-Glu-Leu
Egg yolk	Pepsin	Tyr-Ile-Glu-Ala-Val-Asn-Lys-Val-Ser-Pro-Arg-Ala-Gly-Gln-Phe
Egg yolk	Pepsin	Tyr-Ile-Asn-Gln-Met-Pro-Gln-Lys-Ser-Arg-Glu

Source: Hou et al. (2017).

[a] Bovine pancreatic proteases plus bacterial proteases from *Streptomyces bacillus*.

Acid hydrolysis of protein offers the advantage of low cost but results in the complete destruction of tryptophan, a partial loss of methionine, and the conversion of glutamine into glutamate and of asparagine into aspartate. Alkaline hydrolysis of protein (e.g., 1 M NaOH) is performed at either 27–55°C for 4–8 h for the production of foaming agents (e.g., substitutes for egg proteins; Pasupuleki et al. 2010) or 105°C for 20 h for Try analysis (Dai et al. 2014). This method results in the complete destruction of most amino acids and the partial loss of many amino acids. Most recently, a rapid and noninvasive method based on Raman spectroscopy has been developed to estimate the amino acid composition and secondary structures of proteins (Altangerel et al. 2024).

Most enzymes for producing peptides are obtained from animal, plant and microbial sources, such as pancreatin, trypsin, pepsin, carboxypeptidases and aminopeptidases from pigs, papain and bromelain from plants, and proteases of bacterial and fungal sources with a broad spectrum of optimal temperatures, pH, and ion concentrations. Either a single enzyme (e.g., trypsin) or multiple enzymes (e.g., a mixture of proteases known as Pronase, pepsin, and prolidase) can be used for protein hydrolysis, depending on the raw materials and the desired degree of hydrolysis. Hydrophobic peptides and amino acids with bitterness can be degraded by treatment with porcine kidney cortex homogenate or activated carbon (Pasupuleki and Braun 2010). The enzymatic hydrolysis of proteins offers the following advantages: (a) the hydrolysis conditions (e.g., like temperature and pH) are mild and do not result in any loss of amino acids, (b) proteases are more specific and precise to control the degree of peptide-bond hydrolysis, (c) the small amounts of enzymes can be easily deactivated after the hydrolysis (e.g., 85°C for 3 min), and (d) the resulting products have antioxidative (Table 4.10) and antimicrobial activities (Table 4.12).

Microbial hydrolysis of protein generates large and small peptides using liquid (high-moisture conditions) or solid-state (high-moisture conditions) fermentation. The suitable microbes may be *Aspergillus oryzae, A. sojae*, and *A. tamari* (fungi); *Saccharomyces cerevisiae* (yeasts); and bacteria such as *Bacillus* and *Lactobacillus* species. Compared with the conventional SBM, fermented hydrolyzed soy products contain much lower levels of hyper-allergenic or anti-nutritional factors [e.g., protease (e.g., trypsin) inhibitors, amylase inhibitors, glycinin, β-conglycinin, phytate, lectins (carbohydrate-binding proteins), oligosaccharides (raffinose and stachyose), and saponins in soybeans], and can be included in diets at higher levels (McCalla et al. 2010). Note that horse beans (also known as fava beans) contain a toxic lectin called phytohemagglutinin, which can be denatured and deactivated through proper cooking.

TABLE 4.12
Antimicrobial Peptides Generated from the Hydrolysis of Animal Proteins

Source	Amino Acid Sequence	Against Gram-Positive Bacteria	Against Gram-Negative Bacteria
Bovine meat	Gly-Leu-Ser-Asp-Gly-Glu-Trp-Gln	*Bacillus cereus* *Listeria monocytogenes*	*Salmonella typhimurium* *Escherichia coli*
	Gly-Phe-His-Ile	No effect	*Pseudomonas aeruginosa*
	Phe-His-Gly	No effect	*Pseudomonas aeruginosa*
Bovine collagen	Peptides < 2 kDa (by collagenase)[a]	*Staphylococcus aureus*	*Escherichia coli*
Goat whey	GWH (730 Da) and SEC-F3 (1183 Da) (hydrolysis by Alcalase)	*Bacillus cereus* *Staphylococcus aureus*	*Salmonella typhimurium* *Escherichia coli*
Red blood cells	Various peptides (24-h hydrolysis by fugal proteases)	*Staphylococcus aureus*	*Escherichia coli* *Pseudomonas aeruginosa*
Hen egg white lysozyme	Asn-Thr-Asp-Gly-Ser-Thr-Asp-Tyr-Gly-Ile-Leu-Gln-Ile-Asn-Ser-Arg (hydrolysis by papain and trypsin)[b]	*Leuconostoc-mesenteroides*	*Escherichia coli*
Trout byproducts	Various peptides (20-30% of hydrolysis) (hydrolysis by trout pepsin)	*Renibacterium-salmoninarum*	*Flavobacterium psychrophilum*
Small intestine (Paneth cells)	α-Defensins, lysozyme C, angiogenin-4 and cryptdin-related sequence peptides	Gram-positive bacteria (broad-spectrum)	Gram-negative bacteria (broad-spectrum)
	Phospholipid-*sn*-2 esterase and C-type lectin	Gram-positive bacteria (broad-spectrum)	No effect

Source: Hou et al. (2017).
[a] Minimal inhibition concentrations = 0.6–5 mg/ml.
[b] Minimal inhibition concentrations = 0.36–0.44 μg/ml.

Toxic Amino Acids in Nature

Plant-Sourced Toxic Amino Acids

Plants contain nonproteinogenic amino acids that are toxic to animals, including mammals, birds, and fish (Table 4.13). These compounds include canavanine, hypoglycin A (the causative agent of Jamaican vomiting sickness), indospicine (an arginine analogue found in *Indigofera* species), lathyrogenic amino acids, mimosine, β-cyano-L-alanine, Djenkolic acid, and L-methylcysteine sulfoxide (Hegarty 1978). They act by inhibiting enzymes, interfering with transmembrane amino acid transport, and blocking neurotransmission. Dogs and horses are very sensitive to indospicine. Cattle consuming a large amount of marrow-stem kale, which contains L-methylcysteine sulfoxide, develops hemolytic anemia, because this compound is converted by ruminal microbes into dimethylsulfide (a hemolytic factor). In contrast, L-methylcysteine

TABLE 4.13
Toxic Nonproteinogenic Amino Acids in Plants

Amino Acid	Primary Source	Toxicity
Canaline (L-2-amino-4-aminoxybutanoic acid)	Legumes with canavanine	A product of canavanine by arginase; an analogue of ornithine; inhibits OAT
Canavanine (L-2-amino-4-guanidinooxy-butanoic acid)	Papilionoideae	An analogue of Arg; inhibits NOS and arginase
β-Cyano-L-alanine (L-2-amino-3-cyanopropanoic acid)	*L. sativus* seeds	Inhibits cystathionase and Asp decarboxylase
L-Djenkolic acid (methylene-bis-cysteine)	Mimosoideae species	Low solubility under acid conditions, leading to the formation of crystals; kidney dysfunction
Domoic acid (CMCMD-L-proline)	*Nitzschia pungens* (marine plant)	An analogue of Glu/proline; beurotoxin; inhibits adenylate cyclase; stimulates Glu vesicle release
L-Homoarginine (L-2-amino-6-guanidinohexanoic acid) (only at high doses)	*Lathyrus* species	Neurotoxin[a]; hypersensitivity and mortality in rats at 10 mmol/kg BW (not at 5 mmol/kg BW)
Hypoglycin A (L-α-amino-β-MCP-propionic acid)	Fruits of *Blighia Sapida* (Achee)	Metabolized to MCP acetate (an inhibitor of butyryl-CoA dehydrogenase and thus the β-oxidation of long-chain fatty acids) in the liver, reducing gluconeogenesis and blood [glucose]
Indospicine (L-6-amidino-2-aminohexanoic acid)	*Indigofera* species	Inhibits arginase; not a substrate for arginase
L-Methionine sulfoximine (MSO)	Seeds of the Connaraceae	Inhibits Gln synthetase and γ-glutamyl-cysteine synthetase; fatal in dogs (347 mg/kg BW)
L-Methylcysteine sulfoxide (methiin)	Marrowstem kale (leaf cabbage)	Metabolized by ruminal microbes to dimethyl disulfide, which causes hemolytic anemia in ruminants; not toxic to nonruminants
Mimosine (β-[N-(3-hydroxy-4-pyridone)])-L-2-APA	Leaves and seeds of leucaena	Metabolized by microbes to 3,4-DHP and 2,3- DHP (goitrogens); inhibits many enzymes
α-*N*-oxalyl-L-α,β-diaminopropionic acid (α-ODAP; α-oxalylamino-L-alanine)	*L. sativus* seeds	An analogue of glutamate; neurotoxin; less toxic than β-ODAP
β-*N*-oxalyl-L-α,β-diaminopropionic acid (β-ODAP; β-oxalylamino-L-alanine)	*L. sativus* seeds	An analogue of glutamate; activates neural protein kinase C; neurotoxin
γ-*N*-oxalyl-L-α,γ-diaminobutyric acid (γ-ODAB)	Lathyrus latifolius seeds	An analogue of glutamate; antagonizes NMDA; neurotoxin
L-α,γ-Diaminobutyric acid (DAB)[b]	Some legumes[c]	A homologue of ornithine; inhibitor of OAT
L-α-Amino-γ-(isoxazolin-5-on-2-yl)-butyric acid	*Lathyrus odoratus*	Metabolized to DAB in animals (e.g., chicks)

Source: Hegarty (1978) and Nunn et al. (2010).

[a] An uncompetitive inhibitor of human liver and bone alkaline phosphatases at elevated concentrations.

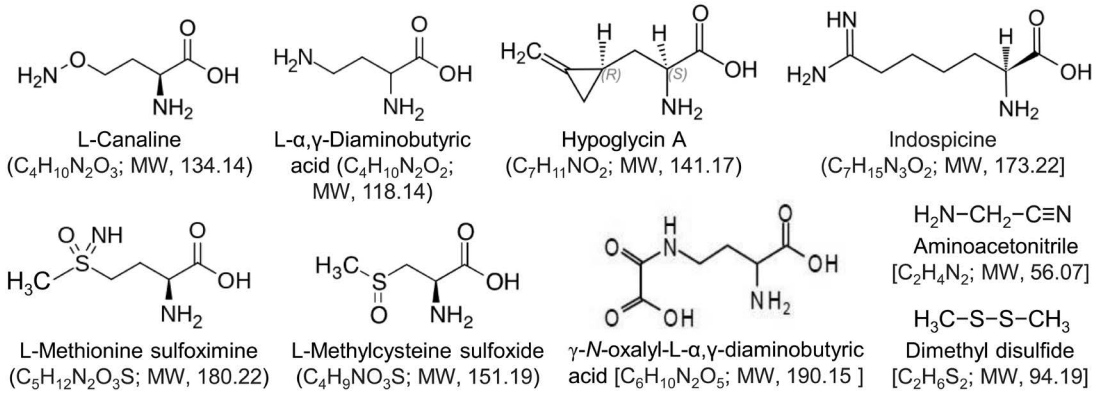
[b] Also known as L-2,4-diaminobutanoic acid.

[c] Lathyrus sylvestris (flat pea) and Phaseolus vulgaris (common bean).

Note: APA, aminopropanoic acid; BW, body weight; CMCMD; 3-carboxymethyl-4-(5-carboxy-1-methylhexa-1,3-dienyl; 3,4-DHP, 3-hydroxy-4-(1H)-pyridone; 2,3-DHP, 2,3-dihydroxy-pyridine; MCP, methylenecyclopropyl; NMDA, N-methyl-D-aspartate; NOS, nitric-oxide synthase; OAT, ornithine aminotransferase.

sulfoxide is not toxic to nonruminants including humans and instead may reduce the concentration of cholesterol in rats (D'Mello 1989). As seen in cattle and goats that graze on leucaena in Australia, mimosine is highly toxic to ruminants because it is metabolized by ruminal microbes to 3-hydroxy-4-(1H)-pyridone (3,4-DHP) and 2,3-dihydroxy-pyridine (2,3-DHP), which are potent goitrogens. Interestingly, cattle in Hawaii and Indonesia can tolerate mimosine because their ruminal microbes can not only convert mimosine to 3,4-DHP and 2,3-DHP but also further degrade the DHP to nontoxic products (Nunn et al. 2010). Note that L-homoarginine is beneficial for cardiovascular and neurological function in animals when present at physiological concentrations (Tsikas 2023), and this amino acid is not toxic even at a high dose of 5 mmol/kg body weight in rats (Nunn et al. 2010).

Lathyrogenic amino acids (found in legumes) are the metabolic antagonists of glutamate or aspartate (neurotransmitters) and include 4-*N*-acetyl-2,4-diaminobutanoic acid, α,γ-diamino-butyrate, α-*N*-oxalyl-L-α,β-diaminopropionic acid (α-ODAP; α-oxalylamino-L-alanine), β-*N*-oxalyl-L-α,β-diaminopropionic acid (β-ODAP; β-oxalylamino-L-alanine), exceedingly high doses of L-homoarginine (e.g., 10 mmol/kg body weight), acetyldiaminobutanoic acid. α-ODAP is less toxic than β-ODAP to animals. Under drought conditions, concentrations of some toxic amino acids (e.g., α-ODAP and β-ODAP) and their metabolites are markedly increased in plants.



Toxic nonamino acid nitrogenous metabolites include aminoacetonitrile (a liquid at room temperatures, e.g., 25°C). It is present in Astragalus plants such as locoweed. Aminoacetonitrile is an inhibitor of glycine decarboxylase of the glycine cleavage system, leading to birth defects and skeletal deformities in grazing animals such as sheep and cattle.

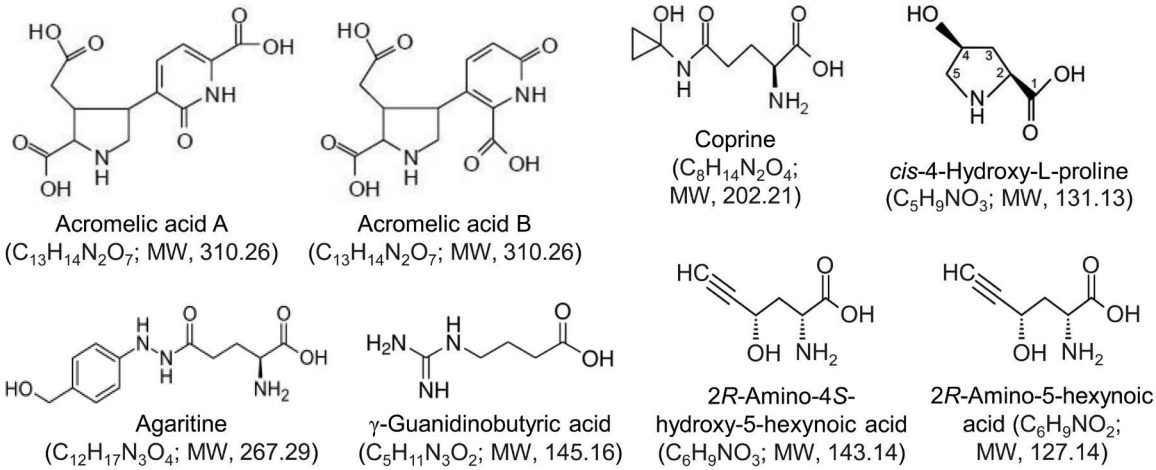
Animal-Sourced Toxic Amino Acids

In land animals, N^G-monomethylarginine (NMMA), asymmetric dimethylarginine (ADMA), and symmetric dimethylarginine (SDMA) are post-translational methylation products of arginine residues in proteins (Wu 2022). The degradation of these proteins releases the methylarginines. Both NMMA and ADMA are competitive inhibitors of NO synthase, whereas elevated levels of SDMA may inhibit arginine transport by cells. Under normal conditions, the concentrations of NMMA, ADMA, and SDMA in the plasma are very low (<1 μM) and have little or no effect on NO synthesis by endothelial cells and other cell types. However, the concentrations of these methylarginines in the plasma are elevated when their urinary excretion is limited due to renal dysfunction, causing cardiovascular disorders. Likewise, such diseases occur when elevated concentrations of homocysteine (a metabolite of methionine) oxidize NO and reduce the bioavailability of NO in the vasculature. Under heating conditions (e.g., 105°C), animal-sourced feedstuffs (e.g., fishmeal) that are relatively rich in lysine and histamine can generate gizzerosine, which is extremely toxic to animals (particularly chickens). All these metabolites can be analyzed by high-performance liquid chromatography (HPLC) methods (Wu and Meininger 2008; Li and Wu 2024).

Fungus-Sourced Toxic Amino Acids

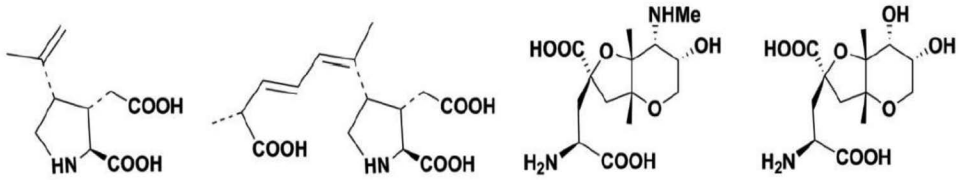
Some mushroom species have nonproteinogenic amino acids that are toxic to animals, including humans (Nieminen and Mustonen 2020). For example, the mushroom *Clitocybe acromelalga* contains acromelic acid A ([(3S,4S,5S)-5-carboxy-4-(carboxymethyl)pyrrolidin-3-yl]-6-oxo-1H-pyridine-2-carboxylic acid) and acromelic acid B, which are imino acids. They are neurotoxins by serving as neuroexcitatory agonists at kainite and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, which are ionotropic transmembrane receptors for glutamate. Acromelic acid A is also present in the mushroom *C. amoenolens*. Furthermore, the mushroom *Coprinus atramentarius* possesses coprine [N5-(1-hydroxycyclopropyl)-L-glutamine], which is hydrolyzed in the liver to glutamate and 1-aminocyclopropanol. The latter inhibits acetaldehyde dehydrogenase (an enzyme responsible for alcohol degradation) and possibly interferes with glutamine and glutamate metabolism. 2*R*-amino-4*S*-hydroxy-5-hexynoic acid, 2*R*-amino-5-hexynoic acid, and γ-guanidinobutyric acid in the fruiting bodies of the mushroom *Trogia venenata*,

as well as (2S,3R)-2-amino-3-hydroxypent-4-ynoic acid in the fungus *Sclerotium rolfsii* and 2-amino-4,5-hexadienoic acid in the amanita mushrooms, and agaritine (2-[4-(Hydroxymethyl)phenyl]-glutamohydrazide) in agaricus mushrooms, are highly toxic to cause death in humans. Interestingly, *cis*-4-hydroxy-L-proline, which is not a physiological metabolite in animals but occurs in some species of bacteria, fungi, and plants, is found in toxic cyclic peptides (e.g., alpha-amanitin and phalloidin) from Amanita mushrooms.



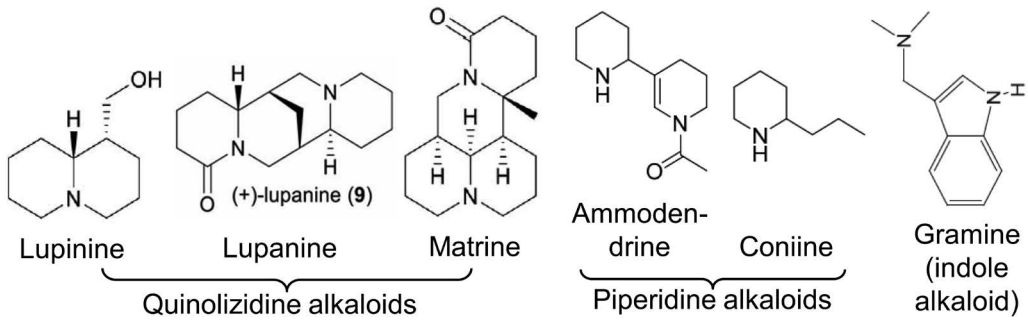
Marine-Sourced Toxic Amino Acids

Excitatory amino acids have been isolated from marine organisms including echinoderms, sponges, ascidians, algae, fungi, and bacteria (Stonik and Stonik 2020). Three groups of marine-derived excitatory amino acids are (a) kainic acid and related compounds, (b) domoic acid and some of its derivatives, and (c) sponge-derived dysiherbaine and neodysiherbaine A. They are synthesized by lower plants (including microalgae) or sponges and then accumulate in marine microbes, as well as invertebrates and vertebrates; therefore, they can be considered as marine plant-derived substances. All of these compounds are glutamate analogues, which bind and activate the kainate-type of glutamate receptors in the brain. Thus, such amino acids are potent excitotoxicants that are harmful to marine mammals and terrestrial animals (e.g., humans and birds) after ingestion.



Plant-Sourced Toxic Alkaloids

Alkaloids are a variety of complex heterocyclic nitrogenous compounds synthesized from amino acids (e.g., lysine and tryptophan) by plants (Qasem et al. 2022). Many alkaloids are toxic to animals. Notably, legumes contain many alkaloids, including quinolizidine, piperidine, and indole alkaloids (see examples of their chemical structures below; Mancinotti et al. 2022). Among them, quinolizidine alkaloids are the most toxic and abundant alkaloids in lupins. Interestingly, appropriate doses of some alkaloids (e.g., berberine, matrine, piperine, fritillarine, and rhynchophylline) have potential health benefits, including pain relief, anti-inflammatory properties, and cancer prevention (Qiu et al. 2014).



CHEMICAL REACTIONS OF FREE AMINO ACIDS

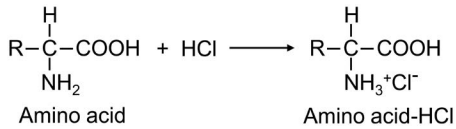
Knowledge regarding the chemical reactions of free amino acids is useful for their analysis and therefore research on amino acid nutrition and metabolism. Some of these reactions may occur in animals and may be of biochemical importance for understanding the safety of amino acids in animals. Many amino acid metabolites (including newly derived amino acids) are commonly present in animals, plants, and microbes (Wu 2022). Of particular interest are the biologically active amines, which are a class of compounds synthesized in living organisms, including polyamines and other amines (agmatine, serotonin, tyramine, histamine, phenylethylamine, tryptamine, and catecholamines). Overall, chemical reactions of amino acids are dependent upon the amino group, the carboxyl group, the side chain, and the intact molecule.

Chemical Reactions of the Amino Group in α-Amino Acids

The amino group of α-amino acids is chemically active and participates in reactions with a variety of substances. These chemical reactions include acetylation, benzoylation, carbobenzoxylation, condensation, deamination, dinitrophenylation, group protection, methylation, oxymethylation, and transamination (Wu 2022). Four of these reactions (conjugation, deamination, transamination, and oxymethylation) are briefly discussed herein because of their biological relevance and use in the analysis of amino acids and their metabolites.

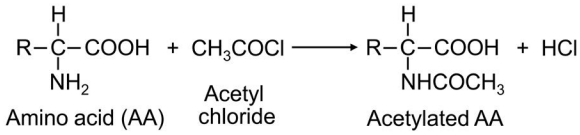
Reaction of the α-Amino Group of Amino Acids with a Strong Acid

Basic amino acids, such as arginine and lysine, are often prepared as their hydrochloride salts for intravenous infusion or dietary additives. This involves the reaction of the α-amino group of the amino acids with HCl at room temperature (e.g., 25°C). The resulting products have no bitter or unpleasant tastes.



Acetylation of the α-Amino Group of Amino Acids

The amino group of amino acids readily undergoes acetylation (i.e., the addition of -$COCH_3$ to an amino acid) by acetylating reagents such as acetyl chloride and acetic anhydride. In cells, the acetylation of amino acid residues in protein plays an important role in its biological activity. In the chemical synthesis of peptides, acetylation of the amino group by agents, such as benzyloxycarbonyl chloride and tert-butoxycarbonyl chloride, is required to generate a desired sequence of amino acids.



Conjugation of the α-Amino Group in Amino Acids with Benzene Dicarbaldehyde

o-Phthaldialdehyde (OPA, a nonfluorescent substance) is a benzene dicarbaldehyde reagent that reacts with primary amino acids, β-amino acids, and γ-amino acids, as well as small peptides (e.g., alanyl-glutamine and glutathione; Wu and Meininger 2008). OPA was first chemically synthesized from α,α,α',α'-tetrachloro-ortho-xylene by A. Colson and H. Gautier in 1887. OPA reacts rapidly with a molecule containing a primary amino group at room temperature in the presence of 2-mercaptoethanol or 3-mercaptopropionic acid to form a highly fluorescent adduct (Figure 4.13). Proline does not react with OPA (Wu 1993), and the reaction of cysteine or cystine with OPA is very limited (Dai et al. 2014). However, OPA also readily reacts with (a) 4-amino-1-butanol, which is produced from the oxidation of proline in the presence of chloramine-T and sodium borohydride at 60°C; and (b) *S*-carboxymethyl-cysteine, which is formed from cysteine in the presence of iodoacetic acid.

The OPA method is most widely used for amino acid analysis by HPLC because of the following advantages: simple procedures for the preparation of samples, reagents, and mobile phase solutions; rapid formation of OPA derivatives and their efficient separation at room temperature; high sensitivity of detection at picomolar concentration levels; easy automation of the instrument; few interfering side reactions; a stable chromatography baseline, as well as the accurate integration of peak areas; and rapid regeneration of guard and analytical columns. This method is suitable for the analysis

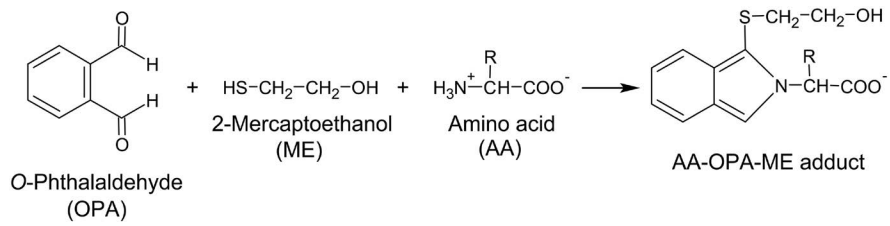
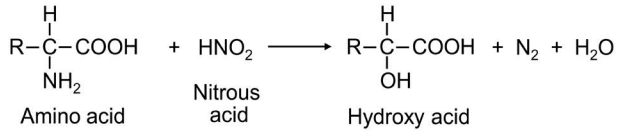


FIGURE 4.13 Analysis of primary amino acids as their *o*-phthaldialdehyde (OPA) derivatives. The concentration of each amino acid standard used for the reaction with OPA is 10 μM. The resultant products are separated by high-performance liquid chromatography, followed by detection at an excitation wavelength of 340 nm and an emission wavelength of 455 nm.

of amino acids and related metabolites in both tissues and protein hydrolysates (Dai et al. 2014; Hou et al. 2015b).

Deamination of Amino Acids

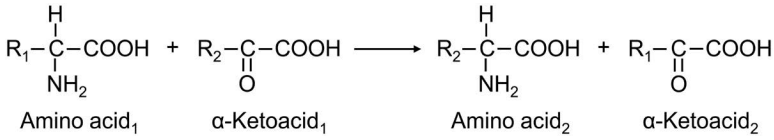
Removal of the α-amino group from an amino acid after treatment with nitrous acid to yield the corresponding hydroxy acid and N gas was recognized in the early 1910s. This reaction, known as the Van Slyke assay, was first used in 1911 by D.D. Van Slyke to determine amino acids. The N gas is measured by volumetric or manometric methods, and its production is directly proportional to the amount of the amino acid. Note that secondary amino acids (e.g., proline and hydroxyproline) do not react with nitrous acid.



An amino acid can undergo deamination to yield ammonia and the corresponding α-ketoacid in the presence of reactive carbonyls (e.g., alloxan, isatin, and quinones) or α-dicarbonyls (e.g., methylglyoxal and phenylglyoxal). In biology, deamination is catalyzed by D-amino acid deaminase (oxidase) and L-amino acid deaminase (oxidase). In 1909, O. Neubauer reported the deamination of α-amino acids in the mammalian body. Enzyme-catalyzed deamination of amino acids in animal tissues was discovered by H.A. Krebs in 1935. This biochemical reaction has important functions in both neurological and immunological systems.

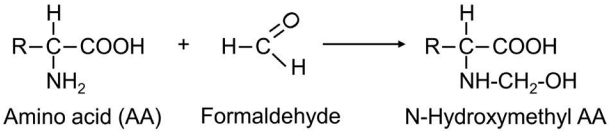
Transamination of Amino Acids with α-Ketoacids

The nonenzymatic transamination of amino acids with an α-ketoacid occurs in response to heating. For example, α-aminophenyl-acetic acid [$C_6H_5CH(NH_2)COOH$] reacts with pyruvic acid in an aqueous solution to yield alanine, benzaldehyde (C_6H_5CHO), and CO_2. This chemical reaction was discovered in 1934 by R.M. Herbst and L.L. Engel, and is used to synthesize dipeptides, such as alanyl-alanine. The enzyme-catalyzed transamination of amino acids is widespread in animals, plants, and microbes and plays an essential role in the synthesis and catabolism of many amino acids (Brosnan 2001).



Oxymethylation of Amino Acids

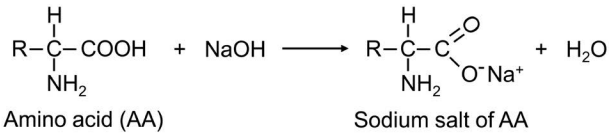
In oxymethylation, the α-amino group of an amino acid reacts with formaldehyde to form an *N*-hydroxymethyl amino acid. This reaction was proposed by H. Schiff in 1899 and developed in 1907 by S.P.L. Sörensen for amino acid analysis. In this method, excess formaldehyde is added to an amino acid solution, followed by titration with standard alkali to a strong red color with phenolphthalein as the indicator. It should be noted that: (a) the amino acid must be dissolved in a colorless solution to prevent any interference of the assay; (b) cysteine, but not cystine, reacts with formaldehyde or 1,2-naphthoquinone-4-sodium sulfonate; and (c) many nitrogenous substances, including ammonia, peptides, primary amines (substances in which one hydrogen atom in ammonia is replaced by an alkyl group). Nitrite and nitrate also react with formaldehyde. Thus, this method greatly overestimates concentrations of free amino acids in animal products or tissue enzymatic hydrolysates.



Chemical Reactions of the Carboxyl Group in α-Amino Acids

Reaction of the carboxyl group of amino acids with an alkaline

The carboxyl group of most amino acids reacts with strong bases (e.g., NaOH; pH > 12) to form sodium carboxylate salts at room temperature (e.g., 25°C). This explains the instability of these amino acids under strong alkaline conditions. An exception is tryptophan, which is very stable in strong bases (e.g., 4.2 M NaOH) for at least 20 h even at 105°C.



Decarboxylation of Amino Acids

The α-carboxyl group of amino acids is involved in several chemical reactions. These reactions include the following: decarboxylation, esterification, and reduction (Wu 2022). Examples of the decarboxylation of amino acids are the conversion of histidine into histamine and of glutamate into γ-aminobutyrate. These two reactions are catalyzed by enzymes in animal cells.

$$\text{Glutamate} \rightarrow \gamma\text{-Aminobutyrate} + CO_2 \quad \text{(Glutamate decarboxylase)}$$
$$\text{Histidine} \rightarrow \text{Histamine} + CO_2 \quad \text{(Histidine decarboxylase)}$$

Amino acids can be esterified by a strong base (e.g., NaOH). In addition, methyl, ethyl, and benzyl esters of many amino acids can be chemically prepared using 2,2-dimethoxypropane, absolute ethanol, and p-toluenesulfonic acid plus benzyl alcohol, respectively. Esterification of an amino acid serves to block its α-carboxyl group. Finally, α-amino acids can be chemically converted into 1,2-amino alcohols. In these methods, the modification

of unprotected or *N*-protected α-amino acids to the corresponding amino alcohols involves the activation of the acid group to become an anhydride, acid fluoride, or active ester, followed by reduction with sodium borohydride. Alternatively, amino acids can be reduced to form the corresponding alcohols directly using sodium borohydride and iodine in tetrahydrofuran.

Chemical Reactions of the Side Chains in α-Amino Acids

The amino or carboxyl group of the side chain of α-amino acids can take part in certain chemical reactions. For example, the ε-amino group of lysine and the guanidino amino group of arginine can participate in hydrogen bonding, methylation, and reactions involving carbohydrates. In addition, the guanidino group of arginine, not its amino group, can react specifically with diketones (Dinsmore and Beshore 2002). This reaction is used to determine a putative role of arginine residues in the stabilization of the tertiary and quaternary structure of proteins and in the allosteric and active sites of enzymes. Furthermore, the γ-amino group of asparagine can react with reducing carbohydrates, thereby providing key sites for N-linked glycosylation of proteins. Asparagine can also react with reactive carbonyls, i.e., carbohydrates, at high temperatures to generate acrylamide (a potential carcinogen), which is present in baked foods.

Amidation

An example of the amidation of an amino acid is the formation of glutamine from glutamate and NH_4^+. Another example is the synthesis of asparagine from aspartate and NH_4^+. These are important reactions in animals, plants and microbes.

$$\text{Glutamate} + NH_3 + \text{ATP} \rightarrow \text{Glutamine} + \text{ADP} + \text{Pi} \quad \text{(Glutamine synthetase)}$$

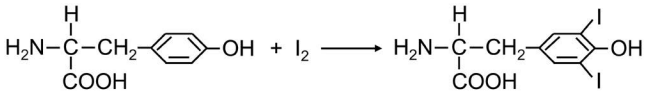
$$\text{Aspartate} + NH_3 + \text{ATP} \rightarrow \text{Asparagine} + \text{AMP} + \text{PPi} \quad \text{(Asparagine synthetase in microbes)}$$

Deamidation

As noted previously, glutamine and asparagine undergo deamidination in the presence of a strong acid (e.g., 6 M HCl) at a relatively high temperature (i.e., 110°C) to produce glutamate and aspartate, respectively. These reactions also yield NH_4^+. Hydrolysis of glutamine or asparagine is not detectable in the presence of 0.75 M HClO4 or 5% TCA at room temperature (e.g., 25°C).

Iodination of the Phenol Ring in Tyrosine

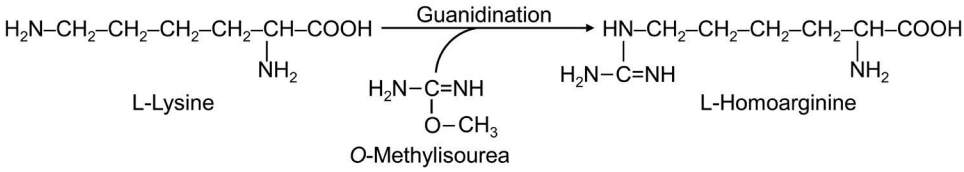
The phenol ring of tyrosine is iodinated under alkaline conditions. The synthesis of thyroid hormones from tyrosine in animals also involves tyrosine iodination, which includes a series of H_2O_2-dependent peroxidative reactions, such as the peroxidase-catalyzed oxidation of iodide, the iodination of tyrosyl residues, and the coupling of iodotyrosyl in thyroglobulin.



Tyrosine Iodinated tyrosine

Chemical Reactions Involving the ε-NH_2 Group of Lysine

As noted previously, the formation of L-gizzerosine in feedstuffs involves the reaction of the ε-NH_2 group of L-lysine with the amino acid of histamine. Likewise, a guanidination reaction of either free lysine or lysine residue in feed protein with methylisourea generates homoarginine. Specifically, a test material (containing 200 g CP) is thoroughly mixed with 1 L of 0.5 M methylisourea (adjusted to pH 10.5 using 1 M NaOH), and the mixture is then incubated at 4°C for 4–6 days. The protein with homoarginine residues is fed to animals (e.g., pigs and poultry) to determine the true digestibility of amino acids (Yin et al. 2015). This method was based on the assumption that homoarginine was not synthesized by the test animals. This assumption is now known to be invalid because many tissues of animals (e.g., pigs and rats) can synthesize homoarginine from arginine and glycine (Hou et al. 2016).



L-Lysine *O*-Methylisourea L-Homoarginine

Condensation of Two Amino Acids

At high temperatures (e.g., feed heating), two amino acids can be condensed to form a new amino acid through chemical reactions of their side chains. Such condensation does not involve the formation of a peptide bond. Examples are the generation of lanthionine and lysinoalanine from cystine plus cysteine and cysteine (or serine) plus lysine, respectively; as well as the formation of 3-methyl-lanthionine and 3-methyl-lysinoalanine from threonine plus cysteine and threonine plus lysine, respectively (Figure 4.14). Such reactions, which also occur in peptide-bound lysine, threonine, serine, and cysteine, can reduce protein quality because these amino acid derivatives are not hydrolyzed by any enzymes and have no nutritional value to animals.

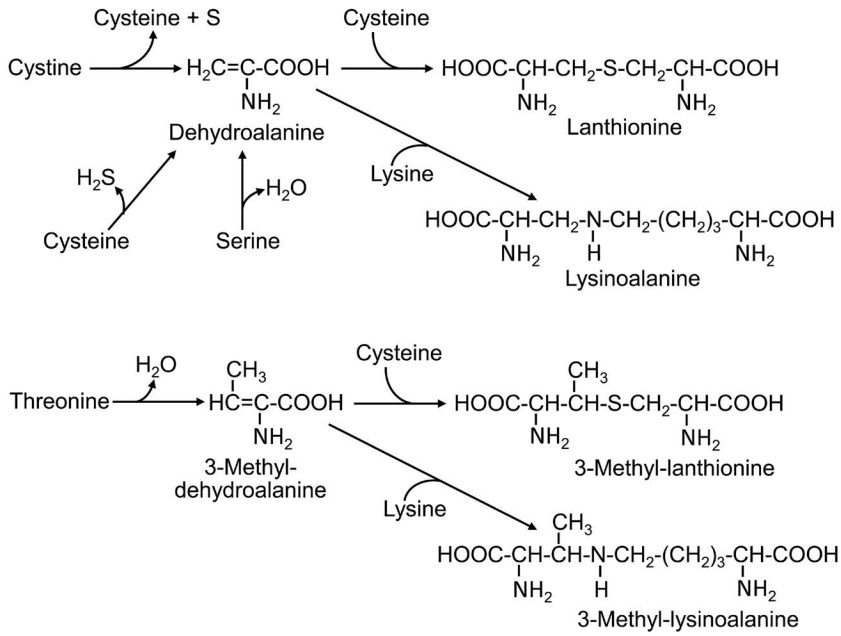
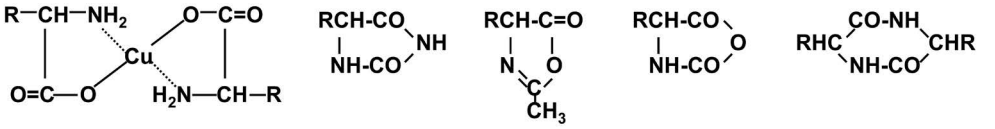


FIGURE 4.14 Formation of lanthionine, lysinoalanine, 3-methyl-lanthionine, 3-methyl-lysinoalanine after the heating of free amino acids. These reactions can also occur in peptide-bound amino acids.

Chemical Reactions Involving Both the Amino and Carboxyl Groups of the Same α-Amino Acid

Because α-amino acids contain both amino and carboxyl groups that are chemically active, they participate in some unique reactions, including chelation, cyclization, racemization, formation of N-carboxy anhydride, and oxidative deamination (decarboxylation) (Wu 2022). These reactions yield amino acid chelates, azlactone, diketopiperazine, N-carboxy amino acid anhydride (NCA), peptide bonds, and aldehyde. The structures of some products [including the copper-amino acid (AA) complex] generated from these reactions are illustrated as follows:



Chelation of Amino Acids with Metals

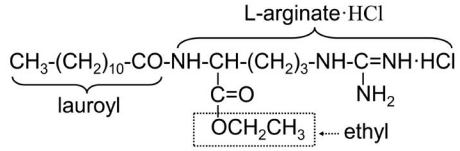
Chelates of amino acids with metals are used to efficiently supply an inorganic nutrient (e.g., Zn, Cu, and Fe) to animals. Amino acids have both ionizable carboxyl and amino groups, and therefore have the capacity to form metal complexes. This physicochemical property of amino acids was discovered in 1854 when A. Gössmann first prepared the copper-leucine chelate. Subsequently, the copper complex of glycine in the ratio of 1:2 [$(NH_2CH_2COO)_2Cu$] was made in 1904 by mixing glycine in a hot aqueous solution with an excess of copper carbonate. Amino acid-mineral chelates are widely used as supplements in animal feeds.

In the copper-glycine complex, a hydrogen atom in each glycine molecule is displaced by a single copper atom, and the metal forms coordinate covalent bonds with the amino groups of two glycine molecules (Yamauchi et al. 2002). In contrast to the salts of the alkaline metals with glycine, aqueous solutions of the copper-glycine complex have virtually no conductivity. It is now known that only α- and β-amino acids, but not γ- or δ-amino acids, may form stable copper complexes, and that their stability depends on the dissociation constant of the complexing N atom rather than simply the nature of the side chain. In addition to copper, α- and β-amino acids can also form chelates with Ni^{2+}, Zn^{2+}, Co^{2+}, Fe^{2+}, Mn^{2+}, Mg^{2+}, and Ca^{2+}. Examples include Zn-methionine, Cu-lysine, and Mn-glycine, which are now commercially available as feed additives for farm animals. Ca^{2+} ions can form covalent bonds with all their functional groups (i.e., carboxyl, amino, and hydroxyl groups) of serine, threonine, and tyrosine.

Esterification and N^{α}-dehydrogenation of α-Amino Acids

An example of the esterification and N^{α}-dehydrogenation of an α-amino acid is the chemical synthesis of lauric arginate (ethyl-N^{α}-lauroyl-L-arginate hydrochloride) from L-arginine-HCl, ethanol, and lauroyl chloride (Wu 2022). This reaction is initiated by thionyl chloride

($SOCl_2$)-catalyzed esterification of the carboxyl group of L-arginine-HCl with ethanol in the presence of a base (NaOH) to form ethyl arginine dihydrochloride, followed by its condensation with lauroyl chloride to finally yield lauric arginate. This substance is a novel cationic surfactant that has a potent antimicrobial effect. Thus, lauric arginate (e.g., 200 ppm) is now used as a safe preservative in the food and beverage industries. It may also be a useful feed additive to diets for animals (e.g., weanling pigs) to improve intestinal health and the efficiency of nutrient utilization.



Ethyl-N$^{\alpha}$-lauroyl-L-arginate hydrochloride (lauric arginate)

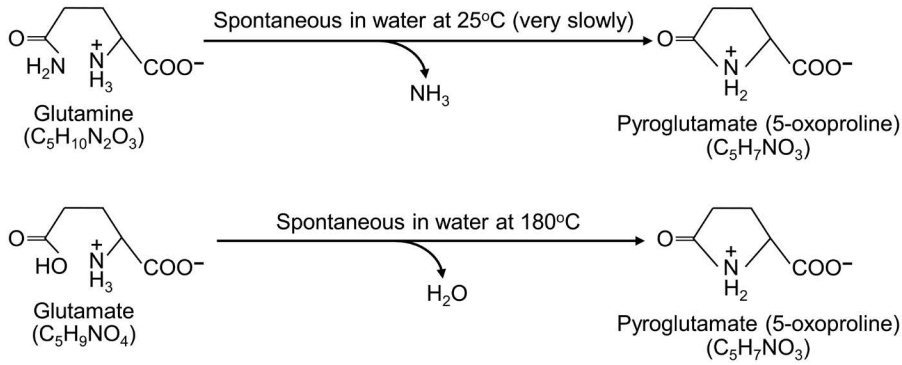
Oxidative Deamination (Decarboxylation) of Amino Acids

Oxidative deamination (decarboxylation) of an amino acid to form ammonia, CO_2, and aldehyde in response to a mixture of lead dioxide and dilute sulfuric acid was first recognized by J. von Liebig in 1849. Such a reaction also occurs when amino acids are treated with sodium hypochlorite or chloramine-T. The formation of the aldehyde is specific to the amino acid under consideration, whereas ammonia and CO_2 are generated nonspecifically from all amino acids. Another well-utilized oxidative deamination (decarboxylation) of an amino acid is its reaction with ninhydrin to yield colored products. This chemical reaction was discovered by S. Ruhemann in 1911, and its chemistry is very complex. First, an amino acid reacts with ninhydrin to form an intermediate amine, the corresponding aldehyde, CO_2, and ammonia. Interestingly, the initial products vary with individual amino acids. For example, aspartate and cystine yield 2 moles of CO_2, whereas proline and hydroxyproline do not generate ammonia. Second, the intermediate amine reacts with ninhydrin to yield indandione-2-*N*-2'-indanone enolate (Ruhemann's purple), hydrindantin (which can be detected by UV absorption), and ammonia. Third, hydrindantin reacts with ammonia to form Ruhemann's purple (blue-violet color with maximum absorption at 570 nm). Additionally, ammonia reacts with ninhydrin and the reduced ninhydrin to also yield Ruhemann's purple. Overall, the N atom of this enolate pigment arises from α-amino acids. On paper chromatography, the reaction of proline or hydroxyproline with ninhydrin gives a yellow-color product (maximum absorption at 440 nm), whereas a purple-blue color is formed for other amino acids. In addition to amino acids and ammonia, peptides can also react with ninhydrin to yield color products. To date, ninhydrin is often used to analyze amino acids.

$$\text{Ninhydrin} + \text{Amino acid} \rightarrow \text{RCHO (Aldehyde)} + CO_2 + H_2O + \text{Ruhemann's purple}$$

Intramolecular Cyclization Reactions Involving the Side Chain Group and the α-Amino Group of α-Amino Acids

As noted previously, glutamine in an aqueous solution at room temperature undergoes very slow spontaneous cyclization between the amide group and the α-amino group to yield pyroglutamate, with the associated release of ammonia. At 180°C, within the glutamate molecule, the side-chain carboxyl group cyclizes with the α-amino group to also form pyroglutamate, with the associated release of H_2O. These reactions are shown below.



Peptide and Protein Synthesis

A carboxyl group of one amino acid and the amino group of another amino acid can form a peptide bond (–CONH–) with the loss of one molecule of H_2O, which has the molecular weight of 18 Da (Figure 4.10). The average molecular weights of the 20 proteinogenic amino acids and their residues are 136.9 and 118.9 Da, respectively (Table 4.2). The average molecular weight of protein varies with its composition of amino acid residues. If the 20 amino acids contribute equally to the synthesis of protein (mol/mol), its molecular weight is 118.9 Da. Proteins of animal- and plant-sourced foods differ in amino acid composition and therefore molecular weight. Note that proteins with similar content of C, N, H, and O atoms may have very different composition of amino acids (Table 4.14).

In chemical synthesis, *N*-carboxy amino acid anhydride is an important activated derivative of an amino acid for the chemical synthesis of small and large peptides, as well

TABLE 4.14
Chemical Formulas and Molecular Weights of Animal- and Plant-Sourced Proteins

Animal or Plant	Tissue or Food	Average Molecular Weight (Da) of Mixed Proteins	General Chemical Formula of Mixed Proteins
Cattle	Skeletal muscle[a]	111.3	$C_{4.91}H_{7.83}O_{1.49}N_{1.38}S_{0.043}$
	Whole body	102.6	$C_{4.44}H_{7.83}O_{1.43}N_{1.32}S_{0.030}$
Sheep	Skeletal muscle[a]	110.5	$C_{4.93}H_{7.83}O_{1.49}N_{1.39}S_{0.042}$
	Whole body	102.7	$C_{4.46}H_{6.99}O_{1.43}N_{1.31}S_{0.030}$
Pig	Skeletal muscle[b]	110.5	$C_{4.88}H_{7.72}O_{1.49}N_{1.36}S_{0.041}$
	Whole body	102.2	$C_{4.44}H_{6.96}O_{1.43}N_{1.30}S_{0.028}$
Chicken	Skeletal muscle[b]	110.8	$C_{4.89}H_{7.75}O_{1.49}N_{1.37}S_{0.041}$
	Whole body	102.6	$C_{4.45}H_{6.98}O_{1.43}N_{1.31}S_{0.030}$
Hybrid striped bass	Skeletal muscle	111.4	$C_{4.91}H_{7.81}O_{1.51}N_{1.37}S_{0.041}$
	Whole body	105.8	$C_{4.56}H_{7.18}O_{1.46}N_{1.33}S_{0.038}$
Largemouth bass	Skeletal muscle	111.2	$C_{4.90}H_{7.79}O_{1.51}N_{1.37}S_{0.040}$
	Whole body	105.1	$C_{4.57}H_{7.18}O_{1.46}N_{1.32}S_{0.038}$
White rice	Grain	110.4	$C_{4.83}H_{7.56}O_{1.50}N_{1.40}S_{0.041}$
	Rice protein concentrate	112.4	$C_{4.97}H_{7.74}O_{1.48}N_{1.41}S_{0.050}$
Soybean	Grain	111.4	$C_{4.86}H_{7.57}O_{1.58}N_{1.38}S_{0.027}$
	Soybean meal	110.3	$C_{4.82}H_{7.48}O_{1.56}N_{1.37}S_{0.027}$

Source: Gilbreath et al. (2021), He et al. (2021), Li et al. (2021c,d), Wu et al. (2024), and Zhang et al. (2021).
[a] Longissimus lumborum muscle (formerly known as longissimus dorsi muscle).
[b] Gastrocnemius muscle.

as proteins. Liquid-phase peptide or protein synthesis is a classical method for peptide formation and is useful for large-scale production of peptides or proteins for industrial purposes. Solid-phase peptide or protein synthesis, pioneered by R.B. Merrifield in 1963, is now widely used to synthesize peptides and proteins in the laboratory (Marglin and Merrifield 1970). Unlike ribosomal protein synthesis, the chemical synthesis of peptides or proteins proceeds in a C-terminal to N-terminal manner, with the N-terminus being protected by either t-butyloxy carbamate (t-BOC) or 9-fluorenylmethyl chloroformate (FMOC).

In animal cells, polypeptide or protein synthesis requires mRNA templates, tRNAs, rRNAs, many enzymes, translation initiation factors, elongation factors, many enzymes, and biological energy (ATP and GTP). In contrast, the formation of special antioxidant dipeptides (e.g., anserine, balenine, carcinine, carnosine, homoanserine, and homocarnosine) is catalyzed by specific enzymes and does not require mRNAs, tRNAs or rRNAs (Figure 4.15). Likewise, a neuroactive dipeptide (kyotorphin) is synthesized from L-tyrosine and L-arginine by ATP-dependent kyotorphin synthetase in the brain. Furthermore, animal cells have

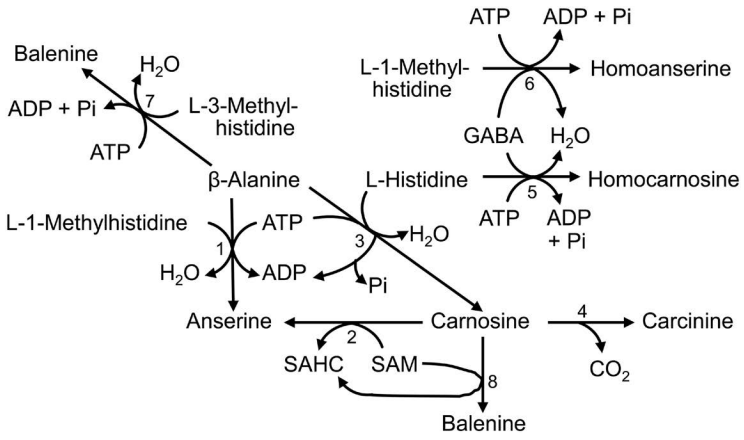


FIGURE 4.15 Synthesis of special antioxidant dipeptides in animal tissue. The enzymes that catalyze the indicated reactions are (1) anserine synthetase, (2) carnosine-N^1-methyltransferase, (3) carnosine synthetase, (4) carnosine decarboxylase, (5) homocarnosine synthetase, (6) homoserine synthetase, (7) balenine synthetase, and (8) carnosine-N^3-methyltransferase. The expression of these enzymes is species- and tissue-specific. GABA, γ-aminobutyrate. (Adapted from Wu 2022, with permission from CRC Press.)

TABLE 4.15
Dipeptides and Tripeptides in Animals[a]

Common Name	Composition	Storage in Major Tissues and Fluids
Dipeptides		
Anserine[b]	β-Alanyl-L-1-Methylhistidine	Skeletal muscle and brain in birds; Skeletal muscle in mammals
Balenine (Ophidine)	β-Alanyl-L-3-Methylhistidine	Skeletal muscle and brain
Carcinine	β-Alanyl-Histamine	Brain and heart of vertebrates; Crab muscle
Carnosine[c]	β-Alanyl-L-Histidine	Skeletal muscle and brain
Homocarnosine	γ-Aminobutyryl-L-Histidine	Brain of vertebrates
Homoanserine	γ-Aminobutyryl-L-1-Methylhistidine	Brain of vertebrates
Kyotorphin	L-Tyrosyl-L-Arginine	Mammalian brain
Tripeptides		
Glutathione	γ-Glu-Cys-Gly	Cells, bile acid, pancreatic juice, and uterine fluid
Collagen peptide	Gly-Pro-Hydroxyproline	Milk and plasma

[a] Present in both mammals and birds, unless indicated otherwise.
[b] Also known as methyl carnosine, not found in mammalian muscle or brain.
[c] Absent from birds.

high concentrations of glutathione (a tripeptide). The distribution of these dipeptides and glutathione varies among animal species and tissues (Table 4.15). For example, carnosine and anserine are the most abundant dipeptides in the skeletal muscles of mammals (Wu et al. 2016) and birds, respectively. Porcine skeletal muscle and avian retina contain high amounts of balenine and anserine, respectively. In mammalian brains, homocarnosine and homoanserine are more abundant than carnosine. Finally, carcinine is abundant in crab muscle, as well as the nervous tissue and heart of vertebrates.

CHEMICAL REACTIONS OF PROTEINS AND POLYPEPTIDES

Hydrolysis of the Peptide Bond in Protein and Polypeptides

The peptide bond in a protein can be broken down in the presence of strong acids (e.g., 6 M HCl) or bases (e.g., 4.2 M NaOH) and high temperature (e.g., 105–110°C). The degree of hydrolysis is time-dependent. Acid hydrolysis is the standard method for the determination of most amino acids in protein, whereas alkaline hydrolysis is the standard method for the determination of tryptophan in protein. The laboratory analysis of aspartate, asparagine, glutamate, and glutamine in foods and animal tissues requires a mixture of proteases and peptidases (Hou et al. 2019). The workflow of the complete analysis of free amino acids as well as amino acids in peptides and proteins is outlined in Figure 4.16.

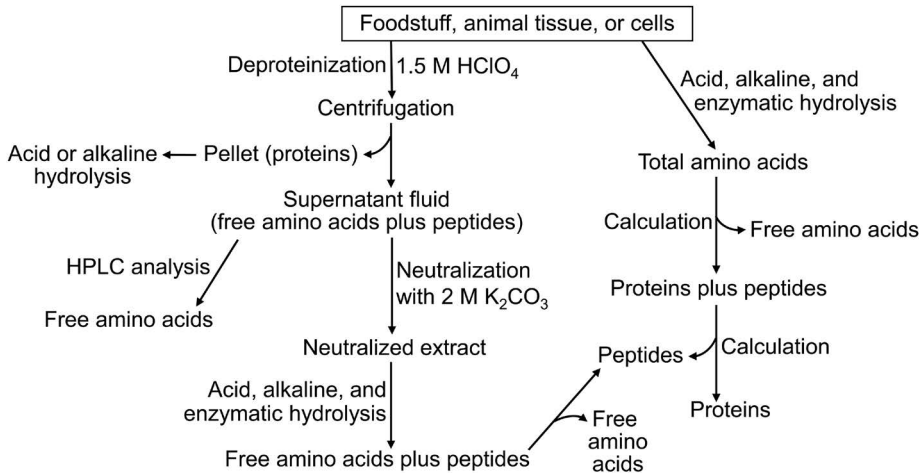


FIGURE 4.16 The workflow of the analysis of free amino acids, as well as amino acids in peptides and proteins. Currently, the most popular instrument is high-performance liquid chromatography (HPLC), but gas chromatography, ion-exchange chromatography, and mass spectrometry methods may also be used. A sample (e.g., foodstuff, animal tissue, or cells) is deproteinized with 1.5 M HClO4, (perchloric acid, PCA), followed by centrifugation ($600 \times g$ for 15 min) and the collection of the supernatant fluid for the analysis of free amino acids. Peptides in the neutralized supernatant fluid are hydrolyzed to free amino acids through an acid, alkaline, or enzymatic method for the calculation of the amounts of amino acids in peptides, namely the differences in amino acids before and after the peptide hydrolysis. To determine the amounts of amino acids in proteins, the sample (e.g., foodstuff, animal tissue, or cells) undergoes acid, alkaline, or enzymatic hydrolysis, followed by the analysis of total amino acids. Through the subtraction of free amino acids and amino acids in peptides, the amounts of amino acids in proteins can be calculated. (Adapted from Li et al. 2021d.)

In animals, dietary proteins are hydrolyzed by proteases in the gastrointestinal tract (extracellular proteolysis) and within all cell types (intracellular proteolysis).

$$\text{Protein} + H_2O \rightarrow \text{Amino Acids} \quad (6\ \text{M HCl}, 110°\text{C}, 24\ \text{h})$$
$$\text{Protein} + H_2O \rightarrow \text{Tryptophan} \quad (4.2\ \text{M NaOH}, 105°\text{C}, 20\ \text{h})$$

The molecular weight of an intact amino acid (e.g., aspartate, 133.10 Da) is greater by 18 Da (the molecular weight of H_2O) than that of its residue in protein (e.g., aspartate residue, 115.10 Da). Thus, care should be taken when true protein content in feedstuffs or animal tissues is calculated after their acid, alkaline, or enzymatic hydrolysis. In the animal body, the average molecular weight of tissue protein (the sum of the molecular weight of its constituent amino acid residues) is 100 Da (Table 4.16). This value is similar to the molecular weight (98.8 Da) of animal protein ($C_{4.3}H_7O_{1.4}N_{1.2}S_{0.03}$; Kleiber 1961). A total of 118 g of amino acids is required to produce 1 mol (100 g) of tissue proteins in animals, including pigs (Table 4.16), poultry, and ruminants (Wu 2022). If the molecular weights of intact amino acids were used to calculate the amount of protein, this would have overestimated the true quantity of protein in foods and animal tissues, possibly by 15–18%.

Dye-Binding of Protein and Polypeptides

Protein can bind the Coomassie dye under acidic conditions. The binding of protein to the dye results in a spectral shift, so that the color of the Coomassie solution changes from brown (absorbance maximum 465 nm) to blue (absorbance maximum 610 nm). The change in color density is read at 595 nm and is proportional to the protein concentration in the solution. This method is very useful in protein identification and quantification (e.g., proteomics).

Biuret Assay of Protein and Peptides

Under alkaline conditions, substances containing two or more peptide bonds form a purple complex with cupric

TABLE 4.16
Composition of Amino Acids (AAs) in Whole-Body Proteins of 30-Day-Old Growing Pigs

AA	Molecular Weight of Intact AA (Da)	Molecular Weight of AA Residue (Da)	g of AA Residue in 100 g Protein	mmol of AA Residue in 100 g Protein	g of AA Needed to Produce 100 g Protein	Energy of Intact AA (kJ/mol)	Energy (kJ) of Intact AA in 100 g Protein[a]
	A	B = (A – 18)	C	D = C × 1000/B	E = A × D/1000	F	G = D × F/1000
Ala	89.09	71.09	6.16	86.7	7.72	1577	136.6
Arg	174.2	156.2	7.14	45.7	7.96	3739	170.9
Asn	132.12	114.12	3.65	32.0	4.23	1928	61.7
Asp	133.1	115.1	4.36	37.9	5.04	1601	60.6
Cys	121.16	103.16	1.32	12.8	1.55	2249	28.8
Gln	146.14	128.14	5.28	41.2	6.02	2570	105.9
Glu	147.13	129.13	8.70	67.4	9.91	2244	151.2
Gly	75.07	57.07	10.5	184.0	13.81	973	179.0
His	155.15	137.15	2.16	15.7	2.44	3213	50.6
OH-Pro	131.13	113.13	3.85	34.0	4.46	2593	88.2
Ile	131.17	113.17	3.59	31.7	4.16	3581	113.6
Leu	131.17	113.17	6.93	61.2	8.03	3582	219.3
Lys	146.19	128.19	6.22	48.5	7.09	3683	178.7
Met	149.21	131.21	1.93	14.7	2.19	3245	47.7
Phe	165.19	147.19	3.59	24.4	4.03	4647	113.3
Pro	115.13	97.13	8.53	87.8	10.11	2730	239.7
Ser	105.09	87.09	4.31	49.5	5.20	1444	71.5
Thr	119.12	101.12	3.50	34.6	4.12	2053	71.1
Trp	204.22	186.22	1.19	6.4	1.31	5628	36.0
Tyr	181.19	163.19	2.86	17.5	3.18	4429	77.6
Val	117.15	99.15	4.20	42.4	4.96	2933	124.2
Mean	136.6	118.6	---	---	---	2888	---
Total	---	---	100	---	118	---	2326

Source: Adapted from Wu (2022) for amino acids [including 4-hydroxyproline (OH-Pro)].

[a] This value refers to the energy of intact amino acids in 100 g of animal protein. The combustion energy values of amino acid residues, which are lower than those of intact amino acids but are currently unknown, should be used to calculate the (ΔH) of animal protein. Because the (ΔH) of an average animal protein is 22.6 kJ/g, the average ΔH of its amino acid residues is estimated to be 97.2% of that for its intact amino acids.

ions (Cu^{2+}) in the reagent (9 g sodium potassium tartrate, 3 g copper sulfate·5 H_2O, and 5 g potassium iodide, all dissolved in order in 400 ml of 0.2 M NaOH, followed by the addition of water to a final volume of 1 L). The underlying principle is that Cu^{2+} ions chelate with the peptide bonds. The absorbance of the solution at 545 nm is measured. The intensity of the color produced is proportional to the protein and peptide concentrations in the solution. Note that this assay reagent does not actually contain biuret (a chemical compound with the chemical formula $C_2H_5N_3O_2$; also known as carbamylurea). The Biuret test is so named because both biuret and protein (or peptides) have the same response to the alkaline copper reagent.

Lowry Assay of Protein and Peptides

Under alkaline conditions, cupric ions (Cu^{2+}) chelate with the peptide bonds, resulting in the reduction of cupric ions (Cu^{2+}) to cuprous ions (Cu^{+}). The cuprous ions react with Folin Ciocalteu Reagent (phosphomolybdic/phosphotungstic acid) to produce a blue color, which can be read at 650–750 nm. The blue color of the solution is intensified by the oxidation of aromatic amino acid residues in protein and peptides. The intensity of the color produced is proportional to the amount of peptide bonds. This method is commonly known as the Lowry method for protein or peptide quantification.

Maillard Reaction of Protein and Peptides

The susceptibility of proteins or free amino acids to heat damage is increased in the presence of carbohydrates, owing to the Maillard reaction. This reaction occurs between the free amino groups of certain amino acids [particularly the ε-amino group ($–NH_2$) of a lysine residue in protein] and carbonyl compounds (–HC=O), notably reducing sugars (e.g., glucose, fructose, or ribose; Figure 4.17). The guanidino group of arginine residues or the side chain $–CH_2$ group of glutamine residues in protein can also react with the carbonyl compounds. Free lysine, arginine, and glutamine can also participate in the Maillard reaction.

The initial step is the formation of a Schiff base, followed by Amadori rearrangement of the Schiff's base to generate an Amadori compound. The formation of the Schiff base is reversible. However, further heating results in the production of melanoidin polymers that give a brown color. The dark coloration of overheated hays and silages is symptomatic of the Maillard reaction. The modified lysine in the form of melanoidin polymers is nutritionally unavailable to animals. Therefore, excess heating of feed proteins

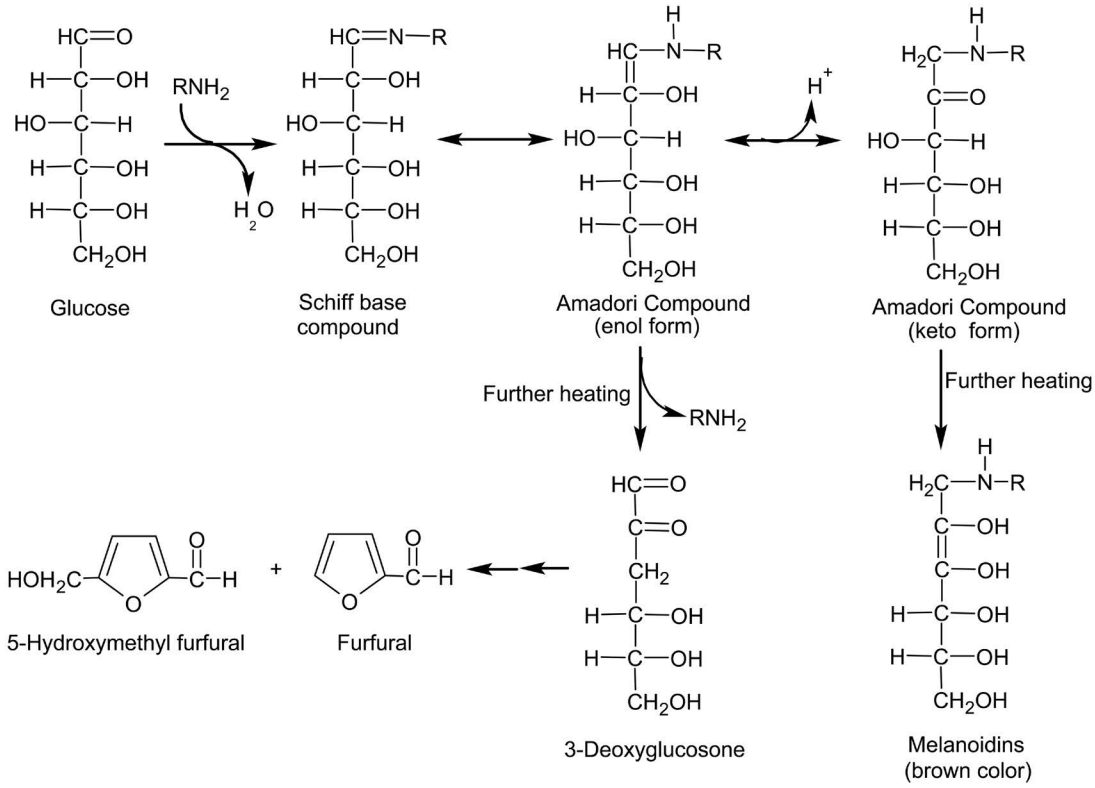


FIGURE 4.17 The Maillard reaction between amino acids and carbohydrates. RNH_2, a free amino acid or a peptide-bound amino acid (e.g., lysine, arginine, or glutamine) reacts with the carbonyl group (-HC=O) of a carbohydrate (e.g., starch, glucose, fructose, or ribose) to initially generate a Schiff base. The formation of the Schiff base is reversible. Through Amadori rearrangement, the Schiff base is converted into an Amadori compound (enol form). However, overheating results in the formation of melanoidin polymers, which are nutritionally unavailable to animals.

poses a problem. If the heated proteins escape breakdown by proteases in the rumen, these proteins may also escape breakdown by proteases in the abomasum and the small intestine. The Maillard reaction, which can occur in animals at a low rate under hyperglycemic conditions, also has important implications for their nutrition and health.

Buffering Reactions of Proteins

Proteins contain amino acid residues (e.g., histidine, arginine, lysine, and glycine) with ionizable groups, with each having different pKa values. Because of their high concentrations in blood and cells, proteins provide important buffering capacities to cells and organisms. A good example of intracellular buffering by a protein is hemoglobin, which is rich in histidine and abundant within red blood cells. Due to the permeability of the erythrocyte membrane to H^+, this buffering action also affects the pH of blood.

$$\text{H·Protein} \rightleftharpoons \text{H}^+ + \text{Protein}$$

Binding of Hemoglobin to O_2, CO_2, CO, and NO

In mammals, birds, and fishes, deoxygenated hemoglobin is the form of hemoglobin without the bound oxygen, whereas hemoglobin inside red blood cells carries oxygen to cells and CO_2 to the lungs. This protein is made up of four symmetrical subunits and four heme groups. Thus, one hemoglobin molecule transports 4 molecules of O_2. Ferrous iron (Fe^{2+}) associated with the heme binds O_2 to form oxyhemoglobin [Hb(Fe(II)-O_2)] in a cooperative manner. This process takes place in the pulmonary capillaries adjacent to the alveoli of the lungs. The O_2 carried by the oxyhemoglobin then travels through the blood stream to be released at cells and tissues where O_2 is utilized as a terminal electron acceptor. In those cells and tissues, where CO_2 is produced from energy-substrate oxidation, CO_2 binds to the terminal amino group of hemoglobin (4 molecules of CO_2 per molecule of hemoglobin) to form carbaminohemoglobin. The equilibrium of this reaction depends on whether hemoglobin binds to O_2, as binding to CO_2 increases when the dissolved O_2 decreases. Because the binding of CO_2 to hemoglobin is reversible, CO_2 freely dissociates from this protein when it reaches the capillaries in the lungs.

$$\text{Protein-NH}_2 + \text{CO}_2 \rightleftharpoons \text{Protein-NH-COO}^- + \text{H}^+$$
$$\text{Hemoglobin-NH}_3^+ \rightleftharpoons \text{Hemoglobin-NH}_2 + \text{H}^+$$
$$\text{Hemoglobin-NH}_2 + \text{CO}_2 \rightleftharpoons \text{Hemoglobin-NH-COOH}$$
$$\text{Hemoglobin-NH-COOH} \rightleftharpoons \text{Hemoglobin-NH-COO}^- + \text{H}^+$$

$$\underset{\substack{(Fe^{2+})\\ |\\ O_2}}{\text{Hemoblobin-NH}_2} + \text{CO}_2 \text{ <---------> } \underset{\substack{\\ |\\ COO^-}}{\text{Hemoglobin-NH}_2^+} + \text{O}_2$$

In addition to oxygen, hemoglobin also binds to competitive inhibitors, such as carbon monoxide (CO) and nitric oxide (NO). High levels of CO and NO are, therefore, toxic to animals. At a physiological level, NO is bound to the thiol group of cysteine residues in hemoglobin to form an *S*-nitrosothiol. The latter dissociates into free NO and thiol again, as the hemoglobin releases O_2 from its heme site. This assists in the transport of both O_2 and NO to tissues in the body. In the presence of CO, or when the heme Fe^{2+} is oxidized to the Fe^{3+} state, O_2 cannot bind to hemoglobin.

NO can readily react with oxyhemoglobin or oxymyoglobin to yield nitrate (NO_3^-) and methemoglobin or metmyoglobin, respectively. These two met forms of hemoglobin or myoglobin are the oxidized hemoproteins and cannot bind O_2. In addition, NO can react with the met and deoxy forms of hemoglobin or myoglobin to yield nitrite (NO_2^-) and the Hb(Fe^{2+})-NO complex. These reactions are summarized as follows.

$$\text{Hb}\left(\text{Fe}^{2+}\text{-O}_2\right) + \text{NO} \rightarrow \text{metHb}\left(\text{Fe}^{3+}\right) + \text{NO}_3^-$$
$$\text{metHb}\left(\text{Fe}^{3+}\right) + \text{NO} \rightarrow \text{deoxyHb}\left(\text{Fe}^{2+}\right) + \text{NO}_2^- + 2\text{H}^+$$
$$\text{deoxyHb}\left(\text{Fe}^{2+}\right) + \text{NO} \rightarrow \text{Hb}\left(\text{Fe}^{2+}\right)\text{-NO}$$

Protein Solubility in Water

Most proteins in an animal are soluble in water, but some (e.g., transmembrane proteins, mature collagens with covalent intermolecular cross-linkages, as well as α- and β-keratins) are not. Mature collagens can be isolated from connective tissue through their solubilization in 1% acetic acid, followed by dialysis against distilled water (Veis and Anesey 1965). Keratins can be dissolved in 0.5 M sodium sulfide at pH 10–13 to break their disulfide linkages, precipitated by 35% ammonium sulfate, and re-solubilized, like any other proteins, in 1 M NaOH (Gupta et al. 2012). This method is useful for isolating proteins from bird feathers, mammalian horns, and reptile scales.

SUMMARY

Proteins are macromolecules consisting of one or more polypeptide chains, with each chain composed of ~40 to over 1000 α-amino acids linked by peptide bonds. They are the most abundant DM in cells other than adipocytes. Proteins have four orders of structures: (a) a primary structure (the sequence of amino acids along the polypeptide chain), (b) a secondary structure (the conformation of the polypeptide backbone), (c) a tertiary structure (the three-dimensional arrangement of protein), and (d) a quaternary structure (the spatial arrangement of polypeptide subunits only found in proteins with more than one subunit). The primary sequence of amino acids in protein determines its other structures and biological functions. The forces stabilizing polypeptide aggregates are hydrogen and electrostatic bonds between amino acid residues. Proteins are stable in aqueous solutions at a physiological pH (7.0–7.4) and temperature (37°C in mammals and 40°C in birds). Unlike carbohydrates and fats, protein is the only macronutrient which provides N, C, and S to animals. As major components of animal tissues, proteins play important roles

in cell and tissue structure, enzyme-catalyzed reactions, muscle contraction, hormone-mediated effects, immune response, oxygen storage and transport, nutrition, metabolic regulation, buffering, and gene expression. Animal-sourced ingredients usually contain more proteins and more balanced ratios of amino acids relative to their composition in animals and, therefore, have higher nutritional values than plant-sourced ingredients. The digestion of dietary protein generates large peptides, which are further hydrolyzed to small peptides and free amino acids for absorption into enterocytes of the small intestine. Thus, animals have dietary requirements of amino acids but not protein.

Amino acids, except for glycine, taurine, β-alanine, and γ-aminobutyric acid, can exist in an L- and D-configuration. In animals, L-amino acids and glycine are substrates for protein synthesis, but some D-amino acids are also present in physiological fluids. The diversity of amino acids is also expressed by different side chains. Quantitatively, L-amino acids likely account for >99.98% of total amino acids (L-amino acids plus D-amino acids) in animal organisms. All amino acids are generally stable in the crystalline form at room temperature (e.g., 25°C). Except for cysteine (which is readily oxidized to cystine in an oxygenated solution) and glutamine (which spontaneously cyclizes to form pyroglutamate at a very slow rate), all amino acids are stable in water and physiological solutions at body temperature (e.g., 25–40°C). All amino acids, except for cysteine and glutamine, are stable in a 5% TCA or 0.75 M $HClO_4$ solution, or an alkaline solution (pH 8.4), at room temperature (e.g., 25°C) for at least 12 h or at –80°C for 2 months. Under the standard conditions of acid hydrolysis at high temperature (i.e., 6 M HCl, 110°C, and 24 h), all amino acids, except for glutamine, asparagine, tryptophan, and methionine, are highly stable. In contrast, most amino acids (including arginine, asparagine, cysteine, cystine, glutamine, and serine) are destroyed by alkaline hydrolysis at high temperature (e.g., 4.2 M NaOH at 105°C), but tryptophan is stable in alkaline solution without any detectable loss.

Free amino acids and peptide-bound amino acids participate in various reactions. They include (a) the reaction of the α-amino group of amino acids with a strong acid or reaction of the carboxyl group of amino acids with an alkaline; (b) the acetylation of the α-amino group of amino acids; (c) the conjugation of the α-amino group of the amino acid with a reagent; (d) the deamination, oxidative deamination, transamination, decarboxylation, oxymethylation, condensation, esterification, and intramolecular cyclization of amino acids; (e) chelation with metals; (f) dye binding; and (g) peptide synthesis (Wu 2022). Specific amino acids are also involved in certain reactions, such as the iodination of the phenol ring in tyrosine, the guanidination of the ε-NH2 group of lysine, and the Maillard reaction. The peptide bonds in protein and peptides can (a) be hydrolyzed by strong acids and bases, as well as proteases and peptidases; (b) stabilize amino acids; and (c) react with cupric ions (Cu^{2+}) to form colored products as the basis of protein/peptide analysis (Biuret and Lowry assays). Furthermore, hemoglobin binds to O_2, CO_2, and NO, thereby playing an important role in oxygenation and gas exchange. Collectively, protein is the most fundamental component of animals and exhibits versatile chemical reactions with enormous biological importance.

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5 Nutrition and Metabolism of Carbohydrates

Carbohydrates are the most abundant organic nutrients in the feedstuffs of land herbivores and omnivores, and are ultimately the most abundant metabolic fuels for these animals. In contrast, the diets for fish contain much less carbohydrates than those for most terrestrial animals, and the foods of obligate carnivores provide only a limited amount of carbohydrates. The digestion of polysaccharides differs markedly among species and, within a given species, the various parts of the digestive tract (McDonald et al. 2021). In nonruminants, starch and glycogen are the only complex carbohydrates that are digestible in the small intestine to yield free glucose, whereas dietary fibers are fermented by microbes in the large intestine to fatty acids [mainly short-chain fatty acids (SCFAs)], with the extent of fermentation varying tremendously among different species (Stevens and Hume 1998). By contrast, in ruminants, starch, glycogen, and dietary fibers are extensively fermented in the rumen to produce SCFAs and methane (CH_4), and dietary fibers that escape the forestomach are further degraded in the large intestine (Pond et al. 1995). Consequently, in ruminants fed diets without grain concentrates, there is little or no absorption of glucose from the small or large intestine into the blood circulation, but very active conversion of propionate into glucose in the liver (Thompson et al. 1975; Trotta et al. 2022). Likewise, conversion of glucose into fructose in the seminal vesicles and conceptus is vital for successful reproduction in ungulates (Kim et al. 2012). Thus, the diversity of carbohydrate metabolism is a strategy for animals to adapt to their environments, survive, and evolve.

Glucose and SCFAs are important for both ruminants and nonruminants. Specifically, glucose is almost the exclusive metabolic fuel for the brain of these animals in the fed or postabsorptive state when the concentrations of ketone bodies in plasma are low (e.g., <0.1 mM; Brosnan 1999). Under all physiological conditions, glucose is also the exclusive source of energy for mammalian red blood cells (RBCs) and is an important metabolic fuel in immunocytes, skeletal muscle, retina, and renal medulla (Swenson and Reece 1984). In all cell types, glucose is the major source of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) that is required by many enzymes, including nitric oxide (NO) synthase (Devlin 2011) and antioxidative enzymes (Fang et al. 2002; Krebs 1964). Thus, the maintenance of glucose homeostasis in the blood, which is achieved through the digestion of dietary carbohydrates as well as the endogenous synthesis and catabolism of glucose, is essential to the survival, growth, and development of mammals, birds, and fish. On the other hand, butyrate is the major metabolic fuel for epithelial cells of the large intestine and, therefore, dietary fiber plays an important role in gut health (Turner and Lupton 2021).

Dietary carbohydrates, particularly fiber, affect the digestion, absorption, and metabolism of nutrients in both ruminants and nonruminants. For example, a high intake of starch impairs the utilization of dietary protein by ruminal bacteria in ruminants (Bondi 1987), whereas excess consumption of dietary fiber reduces the digestion of protein, soluble carbohydrates, and total dry matter (DM) by the small intestine in nonruminants (Zhang et al. 2013). This is a conundrum in animal nutrition; namely how to take advantage of the benefits of nonstarch and nonglycogen complex carbohydrates on improving intestinal health, while alleviating their negative impacts on nutrient digestion and absorption in the small intestine. Toward a better understanding of such a nutritional dilemma, Chapter 5 highlights the digestion and absorption of carbohydrates in nonruminants, ruminants, and fish, as well as glucose metabolism through glycolysis, the Krebs cycle, the pentose cycle, uronic acid pathway, gluconeogenesis, glycogenesis, glycogenolysis, and other related pathways. In this chapter, pentoses and hexoses are physiological D-isomers, whereas lactate and amino acids (except for glycine) are physiological L-isomers, unless specifically indicated otherwise.

DIGESTION AND ABSORPTION OF CARBOHYDRATES IN OMNIVOROUS MAMMALS AND BIRDS

Starch content in typical diets for postweaning or post-hatching omnivores is 46–52%. These animals may ingest a small amount of glycogen from animal-sourced products. Amylase hydrolyzes starch and glycogen into shorter-chain saccharides. This enzyme is classified as α- or β-type according to the anomers of its products. The products of α- and β-amylases contain 2–3 glucose unit monosaccharides and β-maltose, respectively, in addition to oligosaccharides. α-Amylase produces α-saccharides, but β-amylase has both α-1,4-glucosidase and mutarotase activities to generate α-maltose and convert α-maltose into β-maltose. A third type of amylase was discovered after α- and β-amylases and is called γ-amylase. In omnivores (e.g., pig and chickens), α-amylase (α-1,4 endo-glucosidase) is released by both salivary glands and the pancreas, but these organs do not produce β-amylase or release a nutritionally significant amount of γ-amylase. Interestingly, the lysosomes of animal tissues (e.g., the small intestine, liver, and brain) contain acid γ-amylase that is not involved in the

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digestion of dietary carbohydrates. However, the microbes (e.g., bacteria and fungi) of the gastrointestinal tract (GIT) express α-amylase (α-1,4 endo-glucosidase), β-amylase (α-1,4 exo-glucosidase), and γ-amylase (α-1,4 and α-1,6 exo-glucosidase; also known as glucoamylase; Lemo et al. 2019). In the lumen of the small and large intestines, both animal- and microbe-derived amylo-1,6-glucosidase cleave the α-1,6 glycosidic bond to expose the main chain (α-1,4-glucose polymer), whereas oligosaccharidases convert oligosaccharides into monosaccharides. Omnivorous mammals and birds have high abilities to digest and use dietary starch and glycogen.

Animal or microbe-derived α-amylase is a metalloenzyme requiring Ca^{2+} for catalytic activity, has an optimum pH of 6.7–7.0 (depending on type), and is activated by Cl^-. This enzyme hydrolyzes α-1,4-glycosidic bonds within the internal region of starch, glycogen, and related polysaccharides, yielding dextrins, disaccharides, and trisaccharides. In contrast, β-amylase is a Ca^{2+}-independent enzyme, has an optimum pH of 4.0–5.5 (depending on type), and cleaves the second α-1,4 glycosidic linkage at the nonreducing end of starch, glycogen, and related polysaccharides to generate dextrins and maltose. Interestingly, neither α-amylase nor β-amylase acts on the α-1,6-glycosidic bond. Animal or microbe-derived γ-amylase has an optimum pH of 3.0 (the most active amylase in an acidic environment) and hydrolyzes the last α-1,4 bond in starch, glycogen and related polysaccharides, releasing glucose. Unlike α- and β-amylases, γ-amylase can also cleave the α-1,6 glycosidic bond in glycogen in animals, as well as starch, glycogen, and related polysaccharides.

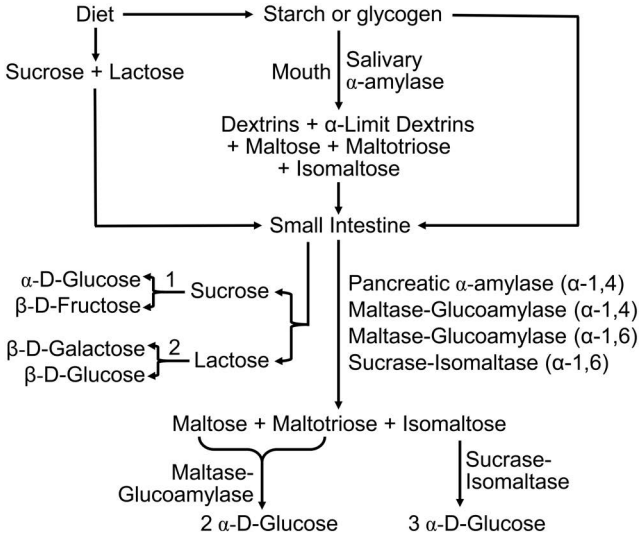


FIGURE 5.1 Digestion of starch, glycogen, disaccharides, and oligosaccharides in the digestive tract of nonruminants. Starch and glycogen are hydrolyzed by saliva-derived α-amylase in the mouth and by α-amylase (from the pancreas), maltase-glucoamylase (from enterocytes), and sucrase-isomaltase (from enterocytes) in the small intestine. Hydrolysis of maltose, maltotriose, and isomaltose to α-D-glucose is catalyzed, respectively, by maltase, α-1,4-glucosidase, and isomaltase (also known as oligo-α-1,6-glucosidase), which are all located on the outer brush border of the apical membrane of the enterocyte. Both salivary and pancreatic α-amylases require Ca^{2+} for enzymatic activity. The enzymes that catalyze the indicated reactions are (1) sucrase and (2) lactase.

Digestion of Starch and Glycogen in Omnivorous Mammals and Birds

Roles of α-Amylase in the Mouth and Stomach

Starch and glycogen are the only polysaccharides that are digestible by animal-sourced enzymes in the oral cavity and the small intestine of omnivorous mammals and birds. Digestion of homopolysaccharides begins in the mouth with the mechanical action of chewing and the chemical hydrolysis by salivary α-amylase. This enzyme is secreted from the salivary gland and is allosterically activated by Cl^-. The time and extent of hydrolysis of starch or glycogen in the mouth are limited. The enzymatic action of salivary α-amylase continues in the esophageal region of the stomach and ends in its fundus region (fluid pH < 3.6), but the gastric digestion of carbohydrates is limited (Yen 2001).

The products of salivary α-amylase in the mouth and stomach are dextrins, α-limit dextrins, maltose (dimer), maltotriose (trimer), and isomaltose (dimer; Figure 5.1). Dextrins are low-molecular-weight polymers of α-D-glucose, whereas α-limit dextrins are the short-chained, branched glucose polymer remnants (6 to 8 glucose units) of amylopectin or glycogen. Both dextrins and α-limit dextrins contain α-1,4 and α-1,6 glycosidic bonds. Note that α-amylase cannot hydrolyze the α-1,6 glycosidic bond. In contrast to noncarnivorous mammals, there is little or no salivary α-amylase activity in both cats and dogs, regardless of their body weight (BW; He et al. 2024).

Roles of Pancreatic α-Amylase and Apical Membrane Disaccharidases in the Small Intestine

Starch and glycogen, as well as their partial digestion products, move from the mouth and stomach into the upper part of the small intestine. The small intestine contains (a) α-amylase (α-1,4 endo-glucosidase) released from the pancreas, and (b) sucrase-isomaltase and maltase-glucoamylase (amylo-exoglucanase), which are bifunctional proteins. The latter two enzymes are secreted from the enterocytes of the small intestine and bound to the apical (brush border) membrane of these cells. Note that isomaltase is also known as palatinase. The biochemical properties of pancreatic α-amylase are the same as those for salivary α-amylase. As shown for mammals such as dogs (Dyck et al. 1974), cholecystokinin (CCK) and secretin stimulate the release of sucrase, maltase, and lactase by the mucosa of the small intestine. In nonruminants, the activities of carbohydrases in the small intestine increase proportionally to in response to the luminal flow of starch or oligosaccharides (Brannon 1990).

Like salivary α-amylase, pancreatic α-amylase has α-1,4 endo-glucosidase activity. The pancreatic α-amylase splits α-1,4 linkages in (a) the amyloses – linear chains

TABLE 5.1
Glucose Concentrations in the Lumen of the Small Intestine and in the Tissues of Animals[a]

Animal	Lumen of the SI	Plasma	Liver	Brain	Kidney	Skeletal Muscle	Heart	White Adipose Tissue
Rat[b]	42.8 ± 1.3	6.0 ± 0.2	7.4 ± 0.3	1.6 ± 0.1	3.3 ± 0.2	2.1 ± 0.1	0.65 ± 0.04	0.45 ± 0.02
Pig[c]	36.4 ± 1.0	5.9 ± 0.2	7.2 ± 0.4	1.7 ± 0.1	3.2 ± 0.2	2.0 ± 0.1	0.70 ± 0.05	0.48 ± 0.02
Chicken[d]	39.2 ± 1.6	12.4 ± 0.5	7.6 ± 0.4	1.9 ± 0.1	3.5 ± 0.3	1.6 ± 0.1	0.73 ± 0.05	0.51 ± 0.03
Sheep[e]	0.15 ± 0.01	3.4 ± 0.1	3.8 ± 0.2	1.0 ± 0.1	1.9 ± 0.1	1.2 ± 0.1	0.54 ± 0.03	0.37 ± 0.02

[a] Values, expressed as mM, are means ± SEM, n = 6. All animals were fed their conventional diets. All tissue samples and the digesta in the lumen of the proximal part of the jejunum were obtained from animals at 1 h after feeding. Glucose was measured using an enzymatic method (Wu et al. 1991).
[b] Three-month-old male rats.
[c] Thirty-five-day-old barrows.
[d] Thirty-five-day-old male broilers.
[e] Nine-month-old female sheep (nonpregnant and nonlactating).

of starch and glycogen to produce maltotriose and maltose and (b) the amylopectins – highly branched molecules of starch and glycogen to yield not only maltotriose and maltose but also α-limit dextrins. At the same time, the amylo-1,6-glucosidase activity of maltase-glucoamylase and sucrase-isomaltase breaks down α-1,6 branching bonds to expose the linear chains of starch and glycogen and facilitates the action of α-amylase. Collectively, these enzymatic reactions generate linear and branched glucose oligomers, such as maltose, maltotriose, and α-limit dextrins. The α-limit dextrins are further broken down into maltose, maltotriose, and isomaltose by maltase-glucoamylase. Maltose and maltotriose are then hydrolyzed by maltase-glucoamylase, and isomaltose by sucrase-isomaltase to form α-D-glucose. Thus, in post-weaning or post-hatching nonruminants, the digestion of starch and glycogen starts in the mouth and is completed in the small intestine, resulting in high concentrations of glucose in the intestinal lumen (Table 5.1).

In either fetuses or postnatal animals, the enterocytes of the small intestine do not express transporters for di-, tri-, or oligo-saccharides (Drozdowski and Thomson 2006). To fulfill their nutritional roles, these oligosaccharides must be degraded by the apical membrane sucrase-isomaltase and maltase-glucoamylase enzymes to monosaccharides for absorption into the enterocytes, as described previously.

Effects of the Structure of Starches on Their Digestion in the Small Intestine

The major site of the digestion of starches in the small intestine is dependent on their type. For example, in growing pigs, 82% of sticky rice starch, 47% of corn starch, and 30% of resistant starch are hydrolyzed in the anterior jejunum (Yin et al. 2010). In these animals, nearly 100% of sticky rice starch (23.6% amylose and 76.4% amylopectin), 93% of waxy corn starch (100% amylopectin and 0% amylose), and 67% of resistant starch (96.5% amylose and 3.5% amylopectin) are digested along the length of the entire small intestine, as determined through the collection of digesta from the terminal ileum. Thus, postweaning pigs have a high ability to digest dietary starch in the small intestine (e.g., 0.70–1.05 kg/day in a 30-kg barrow). Rapidly digestible starch yields a rapid increase in the concentrations of plasma glucose and insulin, and the opposite is true for slowly digestible starch. Thus, because of moderate glycemic and insulinemic responses, resistant starch may be beneficial for the nutritional control of glucose homeostasis in obese and diabetic animals.

The digestibilities of dietary starches are affected by their ratios of amylose to amylopectin, the length of their α-1,4 chains, and the frequency of their α-1,6 branching, which all vary among different plant species or varieties. The true digestibilities (%) of starch in most cereal grains and in field peas for postweaning swine are > 95% (ranging from 96% to 98%) and ~90%, respectively (Bach Knudsen 2001). Compared with cereal grains, some of the pea starch is entrapped within the cell wall and is not accessible to digestive enzymes, and pea starch contains a higher ratio of amylose to amylopectin. In chickens, the true digestibilities (%) of starch in most cereal grains range from 82% to 89% between 4 and 21 days of age (Noy and Sklan 1995) and reach ≥95% after 3 weeks of age (Svihus 2014). In horses, whose diets typically contain only 6–8% starch on the DM basis, the digestibility of starch in cereals is affected by feed processing methods: 20–30%, 45–80%, and 50–70%, respectively, in whole, ground, and pelleted corn; 25%, 80%, and 95%, respectively, in rolled, ground, and pelleted barley; and 55–85%, 50–90%, and 85–95%, respectively, in whole, rolled, and ground oat (Julliand et al. 2006).

Microbes in the Small Intestine

The small intestine of nonruminant mammals and birds contains microbes, which express α-, β-, and γ-amylases and amylo-1,6-glucosidase, as noted previously. These enzymes can contribute to the digestion and utilization of dietary carbohydrates. The number of bacteria (mainly *Streptococci* and *Lactobacilli*) in the fluid of the canine duodenum and jejunum rarely exceeds 10^4/ml (Suchodolski 2011). In the duodenum of healthy dogs, Firmicutes is the most abundant

microbial species (46.4%), followed by *Proteobacteria* (26.6%), *Bacteroidetes* (11.2%), *Spirochaetes* (10.3%), *Fusobacteria* (3.6%), and *Actinobacteria* (1%; Xenoulis et al. 2008). In the jejunum of healthy adult dogs, Proteobacteria is the most abundant microbial species (46%), followed by Firmicutes (15%), *Actinobacteria* (11.2%), *Spirochaetes* (14.2%), *Bacteroidetes* (6.2%), and *Fusobacteria* (5.4%; Suchodolski et al. 2009). The number of bacteria (mainly *E. coli* and anaerobic bacteria) in the canine ileum is ~10^6/ml (NRC 2006). These data extend the report of Johnston et al. (1993) that, in the canine duodenum, anaerobic bacteria are mainly *Bacteroides* spp. (species plural), *Fusobacterium* spp., *Eubacterium*, and aerobic bacteria are mainly *Pasteurella* species. In dogs, the abundance of bacteria increases along the length of the GIT (namely ileum> jejunum > duodenum), and the microbiota in the small intestine inhibits the colonization of pathogenic microbes. Compared with dogs, the feline small intestine contains much more bacteria. For example, the duodenum fluid of cats fed a canned diet contains 2.2×10^5 to 1.6×10^8 bacteria, with anaerobic bacteria being 7.5×10^4 to 1.1×10^8 (Johnston et al. 1993). In addition, anaerobic bacteria of the feline duodenum are mainly *Bacteroides* spp., *Fusobacterium* spp., *Eubacterium*, and aerobic bacteria are mainly *Pasteurella* species (Johnston et al. 1993).

Digestion of Milk- and Plant-Sourced Di- and Oligosaccharides in Omnivorous Mammals and Birds

Milk- and/or plant-sourced disaccharides are hydrolyzed by disaccharidases in the small intestine of omnivores into their constituent monosaccharides. These enzymes are synthesized by enterocytes and bound to their apical membrane. For example, lactose is hydrolyzed by lactase (a β-galactosidase, also known as lactase-phlorizin hydrolase) into β-D-galactose and β-D-glucose, whereas sucrose is hydrolyzed by the apical membrane-bound sucrose-isomaltase into α-D-glucose and β-D-fructose. In *E. coli* (e.g., intestinal bacteria), the β-galactosidase gene (*lacZ* gene) is expressed as part of the inducible system *lac* operon, which is activated by lactose when glucose concentration is low. Oligosaccharides in α-1,4- and α-1,6-glycosidic linkages are hydrolyzed by α-1,4-glucosidase and oligo-1,6-glucosidase, respectively, to produce their constituent monosaccharides. The digestibility of dietary lactose by neonates (e.g., piglets) is nearly 100% (Manners 1976).

Substrate Specificity of Carbohydrases in Omnivorous Mammals and Birds

Except for trehalase and lactase, the saccharidases secreted by the small-intestinal mucosa are not specific for one substrate, but rather they have a broad specificity for a group of substrates with a similar chemical structure. Trehalase, which is bound to the apical membrane of the enterocyte, specifically hydrolyzes 1 mol of trehalose into 2 mol of glucose and does not act on other substrates. As alluded to previously, lactase only hydrolyzes lactose and does not use other substances as substrates. In contrast, sucrase-isomaltase can hydrolyze starch, glycogen, and isomaltose, whereas maltase-glucoamylase can hydrolyze maltose, maltotriose, and low-molecular-weight glucose oligomers (e.g., α-limit dextrins), as noted previously.

Developmental Changes of Carbohydrases in Omnivorous Mammals and Birds

Omnivorous Mammals

Developmental changes of carbohydrases result from changes in (a) transport activity per cell or unit of mucosal mass and (b) the total number of cells or intestinal mass. The specific activity of substrate transport refers to the amount of transported substance per cell or unit of mucosal mass, whereas the total activity of substrate transport is defined as the amount of transported substance per animal or the small intestine. In mammals, except for lactase (β-galactosidase), specific (μmol product/g tissue) and total (μmol product/total tissue) activities of α-amylase, sucrase-isomaltase, and maltase-glucoamylase are very low or absent at birth and then increase with advancing age until the small intestine becomes mature (Aumaitre and Corring 1978; Manners 1976). For example, in pigs, specific and total activities of maltase are minimal at birth, increase from birth to 56 days of age, and then decline moderately, whereas both sucrase-isomaltase and trehalase are completely absent from the small-intestinal mucosa at birth and are expressed after 7 days of age; specific and total activities of these two enzymes increase markedly until 56 days of age. Thus, feeding large amounts of sucrose to young mammals (e.g., 0- to 7-day-old pigs) results in severe diarrhea, weight loss, and high mortality because of a complete lack or an insufficiency of sucrase to hydrolyze sucrose. Two-week-old piglets can digest a small amount of dietary sucrose. In contrast, specific and total lactase activities in the mammalian small-intestinal mucosa peak at birth, remain high until weaning, and decline markedly thereafter. These changes in intestinal enzymes prepare mammals for transition from the milk containing lactose to the solid foods containing starch or glycogen. Glucocorticoids play an important role in increasing the expression of these digestive enzymes through mechanisms involving the binding of glucocorticoids to cytosolic glucocorticoid receptors (GRs), followed by the translocation of the steroid-GR complexes to the nucleus, where they bind to glucocorticoid-response elements in the promoter region of glucocorticoid-sensitive genes and then promoting the transcription of the genes (Adcock and Mumby 2017).

In omnivorous mammals that exhibit sucrose or lactose intolerance, the osmotic effect of undigested disaccharides attracts water from the small-intestinal mucosa and the blood into the lumen of the small intestine, leading to the formation of the watery digesta. Similarly, humans with a low activity of lactase in their small intestines have a reduced capacity for utilizing dietary lactose, and the affected individuals cannot tolerate a large amount of milk. Many cheeses are well tolerated by these individuals

because the bacteria used in cheese production have degraded most of the lactose that was originally present in mammalian milk.

Birds

Birds exhibit a different pattern of developmental changes in gastrointestinal carbohydrases than mammals (Chotinsky et al. 2001). Significant activities of α-amylase and sucrase-isomaltase are present on the apical membrane of the enterocyte in newly hatched broiler chickens. This, along with the grinding function of the gizzard, provides the basis for poultry to utilize a corn- and soybean-meal (SBM)-based diet immediately after hatching. The specific activities of these two enzymes increase 2- to 3-fold between hatching and 35 days of age and decrease thereafter. In contrast, the specific activities of maltase-glucoamylase, lactase, and trehalase are greatest on day 18 of embryonic development and then gradually decrease. The specific activity of maltase-glucoamylase is high at hatching, declines until 18 days of age, and remains at the reduced level until 56 days of age. Lactase and trehalase are barely detectable in the proventriculus, the pancreas, and the mucosa (including enterocytes) of 1- to 7-day-old or older chickens. Thus, post-hatching chickens cannot digest lactose or trehalose in their stomach and small intestine and therefore cannot tolerate a large amount of those carbohydrates in diets. When fed a diet containing a high level of lactose (e.g., >20%, as-fed basis) or milk, chicks exhibit diarrhea as explained previously, leading to impaired growth and even death. However, an appropriate amount of dietary lactose (e.g., 0.5–4% in a diet) has been used as an effective prebiotic for poultry (e.g., broilers and turkey) without affecting their growth performance (Douglas et al. 2003; Simoyi et al. 2006). In the avian hindgut, the fermentation of ingested lactose by microbes produces lactic acid and SCFAs to lower the pH of the intestinal lumen, thereby inhibiting pathogenic bacteria, reducing infection, increasing the provision of energy substrates to the epithelial cells of the large intestine, and improving the growth performance of the birds.

Absorption of Monosaccharides by the Small Intestine in Omnivorous Mammals and Birds

Role of Glucose and Fructose Transporters in the Apical Membrane of Enterocytes

In omnivorous mammals and birds, monosaccharides are absorbed from the lumen of the small intestine into its enterocytes through specific carriers localized in the apical membrane of the cells (Table 5.2): (a) one or more of the 14 facilitative glucose transporters (GLUTs, the SLC2A family of proteins) and (b) sodium-glucose linked transporter-1 (SGLT1). They are all transmembrane proteins, with GLUTs 1–12 being Na^+-independent uniporters and SGLTs being Na^+-dependent symporters (Deng and Yan 2016). GLUT1 is ubiquitously expressed in animal cells, and GLUT3 is widely distributed in animal cells. These two transporters provide the cells with their basal glucose needs. In addition to its predominant localization in the basolateral membrane, GLUT2 is also present at a low level in the apical membranes of the enterocyte for glucose

TABLE 5.2
Facilitative Glucose Transporters (GLUTs) in Animal Cells

GLUT[a]	Distribution in Animal Cells and Tissues
GLUT-1	Ubiquitously expressed in cells; particularly abundant in blood-brain barrier, glial cells, red blood cells, placenta, and mammary tissue; provides cells with their basal glucose requirement.
GLUT-2	The major glucose transporter isoform expressed in hepatocytes, pancreatic ß-cells, and the basolateral membrane of enterocytes; also found to a lesser extent in kidney; also present in the apical membrane of enterocytes when intraluminal concentrations of glucose and Ca^{2+} in the small intestine rise; responsible for the unidirectional transport of glucose, galactose, and fructose from enterocytes into the lamina propria and for the bidirectional transport of glucose, galactose, and fructose by the liver; absorbs dietary dehydroascorbic acid.
GLUT-3	Distributed in many tissues; particularly abundant in placenta, neurons of brain, kidneys, and fetal skeletal muscle; low levels in adult skeletal muscle.
GLUT-4	The major glucose transporter isoform in tissues that exhibit insulin-stimulated glucose uptake, such as skeletal muscle, heart, and adipose tissue; also present in brown adipose tissue; absent from avian skeletal muscle.
GLUT-5	A fructose transporter that is abundant in spermatozoa, testes, and small intestine; expressed to a lesser extent in adipose tissue, skeletal muscle, heart, brain, kidneys, mammary tissue, and placenta (unidirectional from maternal site to fetus); nearly absent from hepatocytes of nonruminant mammals; significant mRNA levels in avian and bovine livers.
GLUT-6	Present in spleen, leukocytes, and brain.
GLUT-7	Present in the endoplasmic reticulum membrane of hepatocytes, and in brain, small intestine, colon, and testis.
GLUT-8	Present in testes, blastocytes, brain, skeletal muscle, heart, adipocytes, and the apical membrane of enterocytes; transport trehalose in mammalian hepatocytes; absorbs dietary dehydroascorbic acid.
GLUT-9	Present in liver and kidneys.
GLUT-10	Present in liver and pancreas.
GLUT-11	Present in heart and skeletal muscle.
GLUT-12	Present in mammary tissue, skeletal muscle, heart, adipose tissue, and small intestine.
GLUT13	Present in animal cells for myo-inositol transport.
GLUT14	Present in animal cells for glucose and fructose transport.

Source: Aschenbach et al. (2009), Deng and Yan (2016), Gilbert et al. (2007), and Zhao and Keating (2007).

[a] Glucose transporters (GLUTs) belong to the SLC2A family of solute carriers. Their corresponding gene names are: SLC2A1 for GLUT-1 to SLC2A14 for GLUT-14.

and fructose transport into or out of the cell. The GLUT2 is recruited into the apical membrane when intraluminal concentrations of glucose and Ca^{2+} in the small intestine rise (e.g., after a meal). However, the role of GLUT2 in the transport of glucose from the intestinal lumen into enterocytes remains minor in comparison with SGLT1, which is responsible for the intestinal absorption of most diet-derived glucose (Ferraris et al. 1999). Except for GLUT5, all apical-membrane GLUTs transport glucose, galactose, and xylose from the lumen of the small intestine into the enterocytes. GLUT5 is a specific transporter of fructose in the apical membrane of the small intestine, as well as in the seminal vesicles, sperm, conceptus, and muscles.

As noted previously, the apical-membrane SGLT1 is the major transporter of glucose and galactose on the luminal surface of the small intestine for their absorption into the enterocytes (Table 5.3). This transporter also transports Na^+ into the cells. Na^+ exits the enterocyte into the lamina propria through its basolateral Na^+-K^+ pump (an ATPase), which transports 3 Na^+ ions out of the cell in exchange for the entry of 2 K^+ ions into the cell (Chapter 1). One Cl^- ion is pumped out of the enterocyte by the Na^+-K^+ ATPase into the lamina propria to maintain intracellular electroneutrality. The Na^+-dependent glucose transport system affects electric potential across the plasma membrane. Thus, when α-D-glucose is added to the mucosal side of the small intestine, the electric current of the plasma membrane is substantially increased as the sugar moves through the enterocyte. For comparison, sodium-glucose cotransporter-2 (SGLT2) is responsible for glucose reabsorption from the lumen of the proximal tubule of the nephron into the blood. Intracellular redox status can modulate intestinal glucose absorption because increasing intracellular glutathione concentration stimulates the uptake of luminal glucose by the enterocyte via the apical Na^+-dependent glucose active transporter (Iantomasi et al. 1998). Mizagliflozin and canagliflozin are potent inhibitors of SGLT1 and SGLT2, respectively, and can therefore be used to treat hyperglycemia in humans and other animals (Kshirsagar et al. 2020).

TABLE 5.3
Sodium-Glucose Linked Transporters (SGLTs) in Animal Cells

SGLT	Distribution in Animal Cells and Tissues
SGLT1	Apical membrane of the enterocyte in the small intestine (major glucose transporter for absorbing glucose from the lumen); apical membrane of the late proximal kidney tubule for the absorption of glucose into blood
SGLT2	Luminal membrane of the early proximal renal tubules (major glucose transporter for absorbing glucose from kidney tubules into blood)
SGLT3	Cholinergic neurons of the submucosal and myenteric plexi, and in skeletal muscle (possibly serving as a glucose sensor)
SGLT4	Apical membrane of the enterocyte in the small intestine and luminal membrane of the proximal renal tubules (monosaccharide transporter for absorbing mannose and fructose from the intestinal lumen into the enterocyte, or from kidney tubules into blood)
SGLT5	Apical membrane of the enterocyte in the small intestine and luminal membrane of the proximal renal tubules (monosaccharide transporter for absorbing mannose, fructose, glucose and galactose from the intestinal lumen into the enterocyte, or from kidney tubules into blood)
SGLT6	Apical membrane of the enterocyte in the small intestine and luminal membrane of the proximal renal tubules (monosaccharide transporter for absorbing myoinositol from the intestinal lumen into the enterocyte, or from kidney tubules into blood)

Sources: Deng and Yan (2016) and Gilbert et al. (2007).

Role of Basolateral Membrane GLUT2 in the Exit of Monosaccharides from Enterocytes into the Lamina Propria

GLUT2 is the major GLUT isoform in the tissues of the digestive system. In the small intestine, GLUT2 is constitutively localized mainly in the basolateral membrane of the enterocyte. After glucose, galactose and fructose enter the enterocytes from the intestinal lumen, they are transported by the basolateral-membrane GLUT2 from the cytoplasm of the cells into the extracellular space (interstitial fluid) of the lamina propria. Because GLUT2 is a high-capacity glucose transporter, it allows for a large flux of glucose from the enterocytes into the lamina propria. The basolateral membrane GLUT2 also transports mannose and 2-deoxy-D-glucose from the enterocytes into the lamina propria (Gilbert et al. 2007; Deng and Yan 2016). The various cell types of the lamina propria metabolize only a small amount of dietary glucose and other monosaccharides.

Trafficking of Monosaccharides from the Lamina Propria into the Liver

Monosaccharides (e.g., glucose, galactose, fructose, mannose, and 2-deoxy-D-glucose) present in the interstitial fluid of the lamina propria traffic from the lumen of the small intestine into the intestinal venule via a series of coordinated steps, as alluded to previously. Through blood circulation, these simple sugars ultimately enter the portal vein for uptake by hepatocytes. In the liver, GLUT2 is primarily responsible for both taking up and releasing glucose. The net flux of glucose through this organ depends on the glucose concentration gradient across the plasma membrane. In the fed state, when the concentrations of glucose in the plasma are greater than the intracellular concentrations of glucose in the liver, glucose is taken up by hepatocytes. Under this condition, only a relatively small percentage of glucose in the blood is utilized by the liver (Kristensen and Wu 2012). Conversely, under fasting conditions, when the intracellular concentrations of glucose in the liver are greater than the concentrations of glucose in the plasma, glucose is released into the venous blood (Williamson and Brosnan 1974).

In omnivores (e.g., humans, pigs, and chickens), ~5% of diet-derived glucose is catabolized in the small intestine, and the remaining 95% enters the portal vein (Watford 1988). Glucose metabolism provides only 5–10% of energy for this tissue in the fed state. As noted previously, ~10–15% of glucose in the portal vein blood is extracted by the liver for glycogen synthesis and limited catabolism, and the remaining portion (85–90%) enters systematic circulation for metabolism in extrahepatic tissues (e.g., the heart, brain, skeletal muscle, and lymph nodes). Catabolism of glucose is necessary to provide NADPH that is required for syntheses of amino acids and fatty acids in the small intestine.

Developmental Changes of Intestinal Monosaccharide Transport in Omnivorous Mammals and Birds

Omnivorous Mammals

Proteins for apical membrane (GLUT1 and SGLT1) and basolateral membrane (GLUT2) monosaccharide transport are present in the fetal small intestine during the first trimester in the mammals with a long gestation period (e.g., humans and pigs) or during the last trimester in the mammals with a short gestation period (e.g., rats and mice; Ferraris et al. 1999). These glucose transporters function to absorb glucose, galactose, and xylose before and immediately after birth. The specific activities (the amount of glucose transported per unit mucosal mass) of intestinal glucose transporters in nonruminant mammals are highest at birth and then progressively decline to lower levels at 21 days of age, i.e., weaning [30% and 50% of values at birth for pigs and rats, respectively (Ferraris et al. 1999)]. However, the total activities of the intestinal glucose transporters increase progressively during the suckling period because of a marked increase in the intestinal mucosal mass (Drozdowski et al. 2010). Likewise, basolateral membrane GLUT2 is present in the mammalian small intestine at birth, and its protein abundance is enhanced 2- to 3-fold in response to the onset of suckling. The total activity of the basolateral membrane GLUT2 increases between birth and weaning to transport glucose and galactose from the enterocytes to the interstitial space of the lamina propria.

Intestinal GLUT5 is virtually absent from the mammalian gut at birth, and is expressed at a low level during the first week of postnatal life to absorb a small amount of fructose from the small-intestinal lumen. For example, in rats, apical membrane GLUT 5 is not detected in the small intestine until weaning. In piglets, apical membrane GLUT5 is barely detectable in the small intestine at 0 to 7 days of age and is expressed at a low level at 2-weeks of age. Thus, much of orally administered fructose is not absorbed by the neonatal small intestine before weaning. Consequently, feeding a large amount of fructose to mammalian neonates (e.g., piglets) can result in intestinal dysfunction, malabsorption, severe diarrhea, weight loss, and high mortality. This is called the toxicity of fructose, like the toxicity of sucrose for mammalian neonates. After weaning, mammals have a high capacity to absorb dietary fructose due to the increased expression of the apical membrane GLUT5.

Birds

In chickens, apical membrane GLUT5 and SGLT1 are expressed in the small intestine five days before hatching, and their mRNA levels increase 2- to 3-fold between birth and 14 days (Ferraris et al. 1999; Gilbert et al. 2007). The expression of these two transporters allows for the absorption of luminal fructose, glucose, galactose, and xylose by the avian small intestine immediately after birth. In contrast, intestinal mRNA levels for apical membrane SGLT5, which is expressed on embryonic day 18 and then is increased by 2-fold at hatching, do not differ between birth and 14 days of age. Of note, basolateral membrane GLUT2 is expressed on embryonic day 18, increased 10-fold at hatching, and further augmented 2-fold between birth and 14 days (Gilbert et al. 2007). The changes in mRNA levels do not always match the changes in monosaccharide transport. For example, in chickens, the specific activity of intestinal glucose transport (amount/g of intestinal mucosal mass) increases approximately 3-fold between birth and 1 day of age, then progressively declines to ~85% and 35% of the peak value (at 1 day of age) by 7 and 21 days of age, respectively, and remains at the reduced level throughout the adult life (Ferraris et al. 1999). This illustrates the physiological and nutritional importance of measuring the actual rate of substrate transport by the intestine rather than merely relying on data on the mRNA levels for transport proteins. Because the small-intestinal mucosal mass increases more than 10-fold between birth and 6 weeks of age, the total transport of glucose, galactose, and fructose by the gut increases during this period of growth. The ability of the avian small intestine to absorb fructose immediately after hatching helps to explain the interesting finding that dietary supplementation with fructose (as 15% high-fructose corn syrup) increases the growth performance of broilers without affecting their feed intake (Miles et al. 1987).

DIGESTION AND ABSORPTION OF CARBOHYDRATES IN NONRUMINANT HERBIVOROUS MAMMALS

Nonruminant herbivorous mammals are very diverse in species, but they all consume plants as their sole source of carbohydrates. Their foods differ in the content of starch and nonstarch polysaccharides (NSPs). Interestingly, salivary α-amylase activity varies among these animals (Boehlke et al. 2015, 2021). Among marsupials studied, salivary α-amylase activity is present in the red kangaroo and the common wombat, but is absent in the koala. A relatively low activity of α-amylase occurs in the saliva of guinea pigs, rabbits, horses, sloths, and elephants but is likely to play only a minor role in the digestion of starch.

Nonruminant herbivorous mammals that are hindgut fermenters (e.g., horses and rabbits) have a limited ability

to digest NSPs in the stomach or small intestine. In these animals, the pancreas secretes a nutritionally significant amount of α-amylase into the lumen of the duodenum for the hydrolysis of starch to glucose by the small intestine. This process may be enhanced by α-amylase released from intestinal microbes. The absorption of glucose into the enterocytes of the small intestine is the same as described above for omnivorous mammals.

Nonruminant herbivorous mammals that are foregut fermenters have very specialized stomachs, such as the sacciform chamber of the stomach in kangaroos and the multi-chambers of the stomach in sloths. These specialized chambers contain many bacteria, fungi, and protozoa that can break down starch and NSPs to monosaccharides and ferment them to SCFAs. The latter are absorbed by the stomach and the small intestine into the blood circulation. These processes are the same as those in the rumen of ruminants (see below). At present, little is known about whether the pancreas of these animals secretes α-amylase into the duodenum for starch digestion in the small intestine.

DIGESTION AND ABSORPTION OF CARBOHYDRATES IN CARNIVORES

Mechanisms responsible for the actions of α-amylase and other carbohydrases, as well as the intestinal absorption of monosaccharides in carnivores, are the same as those in omnivorous mammals. However, the activities of carbohydrases and the transport of sugars by the small intestine are much lower in carnivores than in omnivores (He et al. 2024). Specifically, cats and dogs have little or no salivary α-amylase activity (McGeachin and Akin 1979). In addition, pancreatic α-amylase activity is low in cats but high in dogs (He et al. 2024). For example, pancreatic α-amylase activity in adult cats is only 2.3% and 2% of that in adult dogs and pigs, respectively (Kienzle 1993). Thus, the feline small intestine can digest only a small amount of glycogen in meat but cannot digest a large amount of cooked or uncooked starch. Nonetheless, cats can tolerate a low proportion of starch-rich ingredients (e.g., grains and potatoes; providing less than 10% starch in diets, on a DM basis) used for pet food processing, but a high intake of starch can cause intestinal inflammation and diarrhea in cats (Morris et al. 1977). In contrast, dogs have a much greater ability to digest dietary starch (e.g., up to 40% starch, on a DM basis) than cats and wolves due to higher activities of enzymes for hydrolyzing starch and disaccharides (MacDonald et al. 1984). For example, mRNA levels for the intestinal maltase-glucoamylase gene (*MGAM*) and maltase-glucoamylase activity in dogs are 12- and 2-fold greater than those in wolves, respectively (Axelsson et al. 2013). In addition, dogs have 3.1- and 1.6-fold greater lactase, 4.4- and 2.9-fold greater sucrase, and 4.6- and 3.1-fold greater maltase activities in the proximal and middle regions of the small intestine, respectively, when compared to cats. Furthermore, SGLT1 (a brush border transport protein responsible for most of the glucose uptake by enterocytes) is expressed in the small intestine of dogs but is absent in 18 out of 19 wolves (Batchelor et al. 2011). For comparison, SGLT1 is expressed in feline enterocytes; however, mRNA and protein abundances for SGLT1 in the proximal, middle, and distal segments of the small intestine are about 2-fold greater in dogs than in cats. Therefore, dogs have evolved to consume grain-based rations with high starch content, but cats have a much lower ability to digest and use dietary starch than dogs. Consequently, the consumption of carbohydrate-rich diets by cats can result in intestinal disorders, including inflammation and diarrhea (MacDonald et al. 1984).

DIGESTION AND ABSORPTION OF CARBOHYDRATES IN PRERUMINANTS

Digestion of Carbohydrates in Preruminants

When preruminants (e.g., preweaning calves and lambs) consume a liquid diet (e.g., milk), almost all of it bypasses the reticulorumen and instead passes through the omasum into the abomasum as a result of the reflex closure of the esophageal groove (Chapter 1). Like young monogastric mammals, preruminants rapidly digest milk lactose in the small intestine, with the lactose digestibility being 99%. However, these animals have a limited or no ability to hydrolyze sucrose due to the underdevelopment of the small-intestinal sucrase. In addition, preruminants cannot break down the cell walls of cereal grains due to the lack of enzymes to degrade cellulose and hemicellulose. Likewise, because of little or low secretion of salivary and pancreatic α-amylase, young preruminants cannot use starch in either purified diets or unprocessed cereal grains (Porter 1969). Thus, oral administration of sucrose or starch to preruminant calves does not increase the concentrations of glucose or fructose in the plasma. As the animals grow older (e.g., 80-kg calves), they can digest a small amount of dietary pregelatinized starch (e.g., less than 5% in the diet) with a high digestibility coefficient (e.g., 95%; Gerrits et al. 1997).

Absorption of Monosaccharides in Preruminants

Glucose and galactose (products of lactose hydrolysis), as well as xylose, are absorbed rapidly into the enterocytes of the small intestine in all preruminants, as in preweaning monogastric mammals. In sheep, the protein abundance of the apical membrane SLGT1 per unit of the small-intestinal mucosa is high at birth but is decreased by 50% at 3 weeks of age and to a minimal level at 11 weeks of age (Ferraris et al. 1999). This corresponds to a large flux of glucose and galactose from the lumen of the small intestine into the enterocytes during the suckling period in lambs. Generally, except for GLUT5, intestinal SGLT1, GLUT1, and GLUT2 transport glucose at higher rates than other simple sugars. In calves, GLUT5 is virtually absent from

the small intestine at 0–10 days of age, but is expressed at a low level at 30–50 days of age (Porter 1969). Thus, preruminant calves cannot tolerate a large amount of fructose in their diets. Consequently, much of orally administered fructose is unabsorbed and just passes through the small intestine. Consequently, like sucrose, feeding fructose to young calves can result in severe scouring.

DIGESTION AND ABSORPTION OF CARBOHYDRATES IN RUMINANTS

Major Dietary Complex Carbohydrates

Plant-sourced carbohydrates can be broadly classified as structural and nonstructural. Structural carbohydrates (e.g., cellulose and hemicellulose) are less digestible in the ruminant digestive tract than nonstructural carbohydrates (NSC), including sugars, starches, and other carbohydrates found inside the cells of plants (Harmon and Swanson 2019). Finer particle size (e.g., ≤2 mm) confers higher digestibilities of dietary carbohydrates than larger particle size (e.g., >2 mm) by increasing surface areas for extracellular enzymatic digestion. Carbohydrates usually comprise 60–70% of the total diet for postweaning ruminants and are the major source of energy for these animals and their ruminal microbes. The composition of carbohydrates [e.g., neutral detergent fiber (NDF) and acid detergent fiber (ADF)] differs among feedstuffs (Chapter 2). In general, roughages contain more NDF than nonfiber carbohydrates (e.g., starch), and vice versa for grains, barley, and SBM. Pectin is the most abundant soluble NSP in roughages. Grains, corn silage and barley contain much more starch than free sugars and pectin.

In forages, plant cell walls are composed of β-1-4-linked polysaccharides, which are complexed with lignin. Mature cereal grains consist of the outer surface pericarp (bran; 3–8% of kernel weight; containing β-1-4-linked cellulose and pentosans), the starchy endosperm (60–90% of kernel weight), and the germ (embryo; 2–3% of kernel weight). In addition to the pericarp, barley and oats are surrounded by a fibrous hull (90% fiber and highly lignified; up to 25% of total kernel weight). The cell walls are composed of a cellulose skeleton filled with arabinoxylans, xylans, and β-glucans (e.g., mainly arabinoxylans in wheat and corn, but primarily β-glucans oats and barley). Within the endosperm, starch granules are embedded in a protein matrix. Starch granules are tightly associated with protein in corn, but only loosely in barley and wheat.

The pericarp of cereal grains is the foremost barrier to their digestion in the rumen. Thus, refining or destruction of the pericarp through physical processing (e.g., grinding and rolling) or mastication is essential for efficient starch utilization in ruminants. After the endosperm is exposed, endosperm cell walls can be readily digested in the rumen. Once starch granules are dissociated from the protein matrix through proteolysis, there is little difference in the fermentative digestion of starch granules in the rumen among different grain types.

Overview of Digestion and Absorption of Complex Carbohydrates

Ruminants appear to contain no or very low salivary α-amylase activity (Boehlke et al. 2015). Dietary fiber and starch are degraded extracellularly by microbe-derived enzymes in the rumen, but pancreatic- and possibly intestinal α-amylases act only on starch in the small intestine. The breakdown of the fiber- and starch-protein complexes is a prerequisite for enzymic attack on the fiber and starch granules, respectively. The products of various carbohydrases in the rumen include pyruvate, lactate, and SCFAs, but glucose is formed from the intestinal hydrolysis of starch and oligosaccharides.

The digestion of complex carbohydrates in the GIT is influenced by a plethora of factors, including the activities of ruminal microbe- and animal-derived carbohydrases and related enzymes; the composition of diet and digesta, the rates of passage of feed particles and digesta through the rumen and the intestines; the luminal flow of nutrients (e.g., glucose and amino acids) and the hydrolysis of di-, tri-, and short-chain oligosaccharides in the small intestine; and the extent of nutrient disappearance in the small intestine, particularly the rate of intestinal absorption of glucose. For example, the postruminal administration of casein (200 or 400 g/day) increases the disappearance of duodenally infused cornstarch in the small intestine due to the actions of nutritionally nonessential amino acids [including glutamate (Blom et al. 2016; Brake et al. 2014] and some nutritionally essential amino acids [including leucine, phenylalanine, and tryptophan (Trotta et al. 2022)]. In addition, restricted feeding reduces the secretion and activity of pancreatic α-amylase in nonpregnant ewes, pregnant ewes, and pregnant beef cows, as well as microbial activities (Trotta et al. 2022).

Because starch supplements (e.g., barley, cassava, corn, and wheat) are important sources of dietary energy for high-producing dairy cows and feedlot beef cattle to enhance microbial nitrogen (N) utilization in the rumen, there is increasing interest in this area of nutrition research. Evidence from studies with cattle, dairy cows, and sheep reveals that (a) the rates of disappearance of starch in the small intestine are only 55–60% when fed high-concentrate (e.g., 30% starch) diets, (b) the intestinal hydrolysis of starch to glucose is far from complete, (c) the intestinal absorption of glucose is suboptimal, and (d) unlike nonruminants, pancreatic α-amylase activity in ruminants decreases in response to increasing luminal starch flow (Trotta et al. 2022). Consistent with this view, approximately 65% and 25% of abomasally infused glucose and cornstarch appeared, respectively, in the portal blood of lactating dairy cows and beef heifers (Huntington and Reynolds 1986). Likewise, abomasal infusions of glucose, cornstarch, or corn dextrin to Holstein steers at 60 g/h resulted in their disappearance at the rates of 94%, 38%, and 29%, respectively, in the small intestine that was accounted for as glucose in the portal blood (Kreikemeier et al. 1991).

Nonetheless, despite a low efficiency in the release of glucose from starch, an increase in intestinal starch flow is positively proportionate to an increase in net portal glucose flux in cattle, sheep, and goats. Unless fed a high-starch diet, there is little or no absorption of glucose by the small intestine of ruminants with a functional rumen (Loncke et al. 2020).

Retention Times of Feed Particles and Carbohydrates in the Rumen

The rumen exhibits selective retention of ingested feed particles. The rate of passage of feed particles through the rumen is inversely related to their retention time in the rumen and, therefore, the rate of their digestion by ruminants. The greater the ruminal retention time is, the greater the rate of DM (including fiber) digestion. Ruminal retention times of dietary carbohydrates are affected by many factors, including lag-rumination pool, feed intake, dietary nutrient content, the size of feed particles, the rate of escape of feed particles from the rumen, ruminal microbial ecosystems for nutrient fermentation, and animal species (Ellis et al. 1979). The mean retention time of fluid in the rumen of forage-fed cattle, sheep, and goats is ~10 h (Lechner-Doll et al. 1991). The mean retention times of feed particles in the rumen of roughage-fed ruminants are ~10 h for a large particle pool and ~25 h for a small particle pool (~28 h for cattle and 20 h for sheep and goats; Lechner-Doll et al. 1991). For comparison, the transit times of digesta along the other parts of the GIT in ruminants are as follows: omasum, 4 h (mixing flow); abomasum and small intestine, 4 h (tubular flow); cecum and proximal colon, 5 h (mixing flow); and distal colon, 4 h (tubular flow) (Huhtanen et al. 2008).

Extracellular Hydrolysis of Complex Carbohydrates into Monosaccharides by Ruminal Microbes

Bacteria play the predominant role in the extracellular hydrolysis of dietary complex carbohydrates in the rumen, but fungi and protozoa also contribute to this process. Specifically, the granules and soluble forms of starches, as well as related polysaccharides are degraded extracellularly by microbial α-, β-, and γ-amylases and oligosaccharidases, as described previously. These enzymes are of primarily bacterial origin but are also produced by fungi (Mountfort and Asher 1988).

Complex carbohydrates in the plant cell walls are hydrolyzed extracellularly by cellulases, hemicellulases, and xylanases to their monosaccharides. These enzymes are of primarily bacterial origin, and are also released at high levels by the anaerobic fungi (Akin and Borneman 1990; Weimer 2022). The main cellulolytic bacteria are *Fibrobacter succinogenes, Ruminococcus flavefacians,* and *R. albus* (Table 5.4). Thus, the ruminal bacteria and fungi are essential in breaking down plant cell walls through the hydrolysis of β-1-4 glycosidic bonds in membrane polysaccharides. The lysis of the plant cell walls releases cellular soluble carbohydrates (e.g., monosaccharides) and

TABLE 5.4
Fermentative Properties of Ruminal Bacteria

Bacterial Species	Function	Product
Fibrobacter succinogenes[a]	Cellulolytic, Amylolytic	Formate, acetate, succinate
Ruminococcus albus	Cellulolytic, Xylanolytic	Formate, acetate, ethanol, H_2, CO_2
Ruminococcus flavefaciens	Cellulolytic, Xylanolytic	Formate, acetate, succinate, H_2
Butyrivibrio fibrisolvens	Cellulolytic, Xylanolytic, PR	Formate, acetate, butyrate, lactate, ethanol, H_2, CO_2
Clostridium lochheadii	Cellulolytic, PR	Formate, acetate, butyrate, ethanol, H_2, CO_2
Streptococcus bovis	Amylolytic, PR, SS	Formate, acetate, lactate
B. amylophilus	Amylolytic, Pectinolytic, PR	Formate, acetate, succinate
B. ruminicola	Amylolytic, Pectinolytic Xylanolytic, PR	Formate, acetate, succinate, propionate
Succinimonas amylolytica	Amylolytic, Dextrinolytic	Acetate, succinate
Selenomonas ruminantium	Amylolytic, PR, SS, GU, LU	Acetate, lactate, propionate, H_2, CO_2
Lachnospira multiparus	Amylolytic, Pectinolytic, PR	Formate, acetate, lactate, ethanol, H_2, CO_2
Succinivibrio dextrinosolvens	Pectinolytic, Dextrinolytic	Formate, acetate, succinate, lactate
Methanobrevibacter ruminantium	Methanogenic, H_2-utilizer	Methane
Methanosarcina barkeri	Methanogenic, H_2-utilizer	Methane, CO_2
Spirochete species	Pectinolytic, SS	Formate, acetate, lactate, succinate, ethanol
Megasphaera elsdenii	SS, LU	Acetate, propionate, butyrate, valerate, caproate, H_2, CO_2
Lactobacillus sp.	SS	Lactate
Anaerovibrio lipolytica	Lipolytic, GU	Acetate, priopionate, succinate
Eubacterium ruminantium	SS	Formate, acetate, butyrate, CO_2

Source: Adapted from Stevens and Hume (1998).
[a] Previously known as *Bacteroides succinogenes*.
Note: GU, glycerol-utilizing; LU, lactate-utilizing; PR, proteolytic; SS, soluble sugar fermenter.

also exposes the protein-starch granule matrix of grains. The rhizoids in ruminal fungi (e.g., *Orpinomyces joyonii, Neocallimastix patriciarum*, and *Piromyces communis*) can penetrate directly through the protein matrix to allow for enhanced microbial colonization and enhanced proteolysis to release the encased starch granules (McAllister et al. 1993). In the rumen, amino acids stimulate the growth of fibrolytic bacteria, particularly those involved in the degradation of hemicelluloses (Firkins et al. 2007). This indicates an important role for protein nutrition in ruminal utilization of dietary fiber and the efficiency of ruminant production.

Extracellular hydrolysis of NSPs in the rumen requires a series of enzymes. Specifically, cellulose is broken down by the cellulase complex that consists of three physically and enzymatically distinct proteins (monomers): endoglucanase, exoglucanase, and β-glucosidase [also known as cellobiase] (Figure 5.2). Endoglucanase randomly cleaves the internal glycosidic bonds within the cellulose chain, releasing cellobiose (consisting of two units of β-D-glucose) and cello-oligosaccharides. The latter are degraded by exoglucanase to cellobiose. Finally, β-glucosidase hydrolyzes cellobiose to β-D-glucose, which is at equilibrium with α-D-glucose.

A series of enzymes is required to degrade hemicelluloses. Hemicellulases (e.g., D-galactanases, D-mannanases, and D-xylanases) hydrolyze the hemicellulose polymer backbone (Figure 5.2). Before or in conjunction with depolymerization of the hemicellulose backbone, many different debranching enzymes release its side chains, depending on

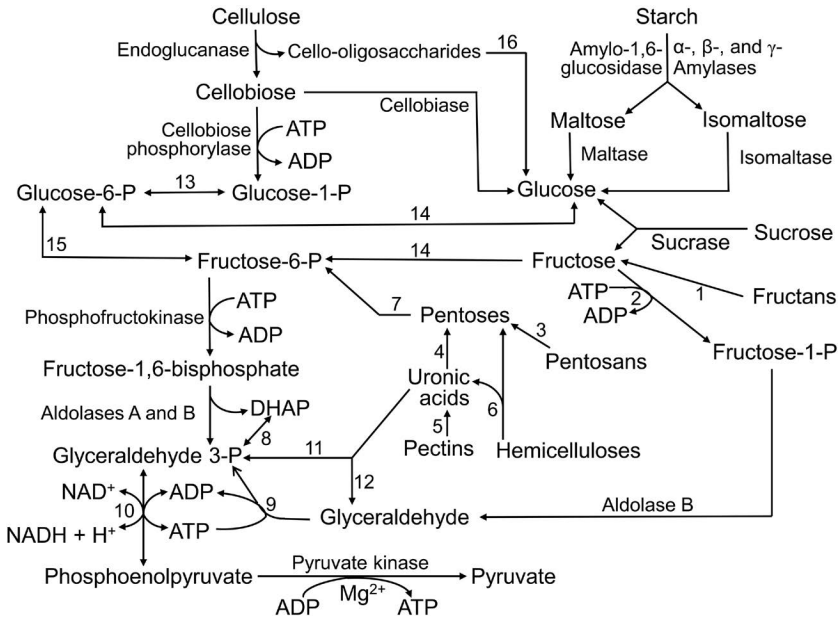


FIGURE 5.2 Fermentation of complex carbohydrates to pyruvate in the rumen. The degradation of cellulose is initiated by the cellulase complex, which consists of endoglucanase, exoglucanase, and β-glucosidase (also known as cellobiase). The degradation of starch is initiated by α-amylase (including amylo-1-6 glucosidase, as well as α-, β-, and γ-amylases). The enzymes that catalyze the indicated reactions are (1) β-fructan fructanohydrolases (e.g., exoinulinase and endoinulinase); (2) fructokinase (ketohexokinase); (3) enzymes that hydrolyze the polymers of pentoses (e.g., xylose and arabinose), including arabinosidase (hydrolyzing arabinoxylan to arabinose and xylan), endoxylanase (hydrolyzing xylan dextrins to xylose), exoxylanase (hydrolyzing xylan to xylose), and β-xylopyranosidase (hydrolyzing xylobiose to xylose); (4) the enzymes that degrade galacturonic acid (the main constituent of pectin) to pentose (2-keto-glutarate) are D-galacturonate dehydrogenase, galactarate dehydratase, 5-dehydro-4-deoxyglucarate dehydratase (decarboxylating), and 2,5-dioxovalerate dehydrogenase; (5) pectinases (e.g., pectin lyases) and polygalacturonase (pectin hydrolase that hydrolyzes the α-1,4 glycosidic bonds between galacturonic acid residues); (6) hemicellulases, including endo-β-1,-4-xylanase [xylanase] (hydrolyzing the interior β-1,-4 glycosidic linkages of the β-D xylan backbone in hemicellulose to oligomers of different lengths); β-mannanase (hydrolyzing the β-D-mannan backbone in hemicellulose); arabinoxylanase (hydrolyzing arabinoxylans to xylo-oligosaccharides and arabinoxylo-oligosaccharides); β-xylosidases (hydrolyzing the xylose oligomers to xylose); arabinase (hydrolyzing the polymers of arabinose); arabinosidase [α-L-arabinofuranosidase] (hydrolyzing arabinose-containing polysaccharides); β-glucanase (hydrolyzing the polymers of β-D-glucose); endo-(1,3-1,4)-β-D-glucanase (hydrolyzing (1,3;1,4)-β-glucans); glucuronidase (releasing glucuronic acid from glucuronoxylans); acetylxylan esterase (hydrolyzing acetyl ester bonds in acetylxylans); ferulic acid esterase (hydrolyzing lignin-feruloylester bonds in xylans); (7) enzymes of the pentose cycle; (8) phosphotriose isomerase; (9) glyceraldehyde kinase; (10) enzymes of glycolysis, including glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase (Mg^{2+}), phosphoglycerate mutase, and enolase (Mg^{2+}); (11) the enzymes that convert galacturonic acid to D-glyceraldehyde-3-phosphate are uronate isomerase, D-tagaturonate reductase, D-altronate dehydratase, 2-keto-3-deoxy-D-gluconate kinase, and 2-keto-3-deoxy-6-phosphogluconate aldolase; (12) the enzymes that convert galacturonic acid to 2-keto-glutarate are D-galacturonate reductase, L-galactonate dehydratase, and 3-deoxy-L-*threo*hex-2-ulosonate aldolase; (13) phosphoglucomutase; (14) hexokinase and fructokinase; (15) phosphohexose isomerase; and (16) exoglucanase. *Note*: DHAP, dihydroxyacetone phosphate. (Adapted from Downs 2006; Kandler 1983; McDonald et al. 2021.)

the specific type of hemicellulose. The debranching enzymes include arabinofuranosidase, feruloyl and coumaric esterases, acetylxylan esterase, α-glucuronidase, and xylosidases. Other enzymes play important roles in the hydrolysis of ester, ether, and glycosidic linkages between hemicellulose and lignin to release lignin. For example, glucuronoyl esterases hydrolyze the ester bond between the D-glucuronic acid or 4-*O*-methyl-D-glucuronic acid of glucuronoxylan and the hydroxyl groups of lignin (Biely 2016). Efficient hydrolysis of hemicelluloses by digestive enzymes in the GIT is essential for the utilization of dietary fiber by ruminants.

Intracellular Hydrolysis of Complex Carbohydrates into Monosaccharides by Ruminal Protozoa

Ruminal protozoa also produce cellulases, hemicellulases, xylanases, and α-amylase, as do bacteria and fungi. The protozoa engulf bacteria, as well as water-insoluble feed particles (including fiber) and starch granules through pinocytosis. The engulfment of bacteria by protozoa is a way for the rumen to control the number of its bacteria (Newbold et al. 2015). Within the protozoa, the above enzymes act in concert to hydrolyze the polysaccharides in the engulfed materials to the constituent monosaccharides. The number of protozoa in the rumen increases when the content of grains in the diet is augmented from 0% to 40%. Because of their ability to take up starch granules and predate on amylolytic bacteria, protozoa can substantially reduce the rate of starch digestion in the rumen (which may take up to 36 h) and, therefore, the risk for the development of clinical or subclinical acidosis in the host. Jouany and Ushida (1999) estimated that protozoa may be responsible for as much as 40% of the starch digestion in the rumen.

Intracellular Degradation of Monosaccharides in Ruminal Microbes

Ruminal microbes (bacteria, fungi, and protozoa) take up extracellular monosaccharides (e.g., glucose, fructose, and xylose) through specific transporters. Both extracellular and intracellularly generated monosaccharides undergo extensive fermentation by a series of enzymes to produce pyruvate, as well as CO_2 and H_2 in the rumen (Figure 5.2). $2H^+$ ions are reduced to H_2 by hydrogenases, which are classified into three types based on their catalytic requirement for metal cofactors at active sites: [Fe]-, [FeFe]-, and [NiFe]-hydrogenases. [Fe]-hydrogenases without nickel or Fe-S clusters occur exclusively in methanogenic archaea. Prototypical hydrogenases and bifurcating hydrogenases contain [FeFe] active sites. Energy-converting (Ech) hydrogenases contain [NiFe] active sites. Both [FeFe]- and [NiFe]-hydrogenases are deactivated by O_2. Electron donors for H^+ reduction include ferredoxin, quinone, and cytochromes.

$$2H^+ + 2e^- \rightleftharpoons H_2 \left(\text{Prototypical hydrogenases, Bifurcating hydrogenases, and Ech hydrogenase}\right)$$

Among microbes, bacteria are the principal microbes to metabolize monosaccharides to phosphoenolpyruvate (PEP), pyruvate (Pyr), and oxaloacetate (OAA). These three metabolites are interconvertible and form the PEP-Pyruvate-OAA (PPO) node, which provides an integrated metabolic network among different microbial species (Koendjbiharie et al. 2021). The PEP-Pyr-OAA node is important for cellular energetics because some bacteria (e.g., 27% of 48 ruminal bacterial species) have no or incomplete glycolysis for glucose metabolism (Hackmann et al. 2017). Key reactions in the PPO node along with key products (lactate and malate) are illustrated in Figure 5.3. Pyruvate

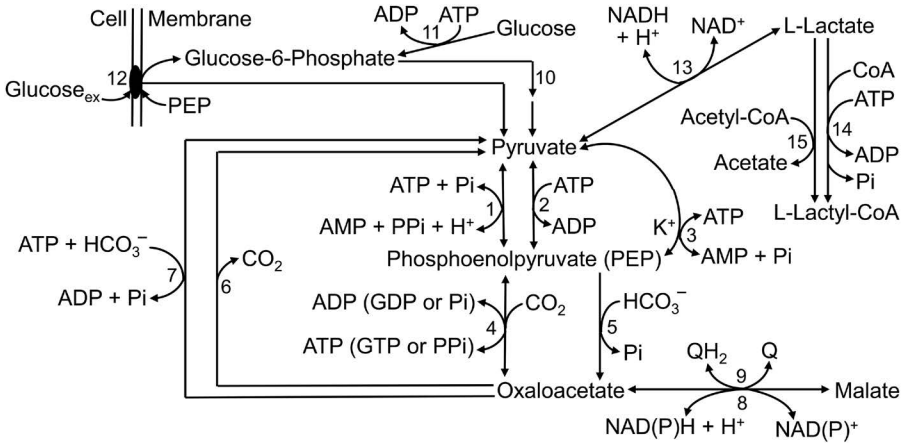


FIGURE 5.3 The phosphoenolpyruvate (PEP), pyruvate, and oxaloacetate (OAA) node in microbes. These three metabolites are interconvertible and form the PEP-Pyruvate-OAA (PPO) node. No single organism possesses all the required enzymes of the PPO node, but different microbial species cooperate to form an integrated network that is crucial for glucose metabolism and cellular energetics. The enzymes that catalyze the indicated reactions are (1) pyruvate phosphate dikinase; (2) pyruvate kinase; (3) PEP synthase (Ca^{2+}); (4) PEP carboxykinase (using ADP, GDP, or Pi as a cofactor); (5) PEP carboxylase; (6) OAA decarboxylase; (7) pyruvate carboxylase; (8) malate dehydrogenase (using NAD^+ or $NADP^+$ as a cofactor); (9) malate:quinone oxidoreductase; (10) enzymes of glycolysis; (11) hexokinase (Mg^{2+}); (12) phosphotransferase system (extracellular glucose reacts with intracellular PEP to form glucose-6-phosphate and pyruvate); (13) lactate dehydrogenase; (14) lactyl-CoA synthetase; and (15) CoA transferase. Q, quinone. QH_2, reduced quinone.

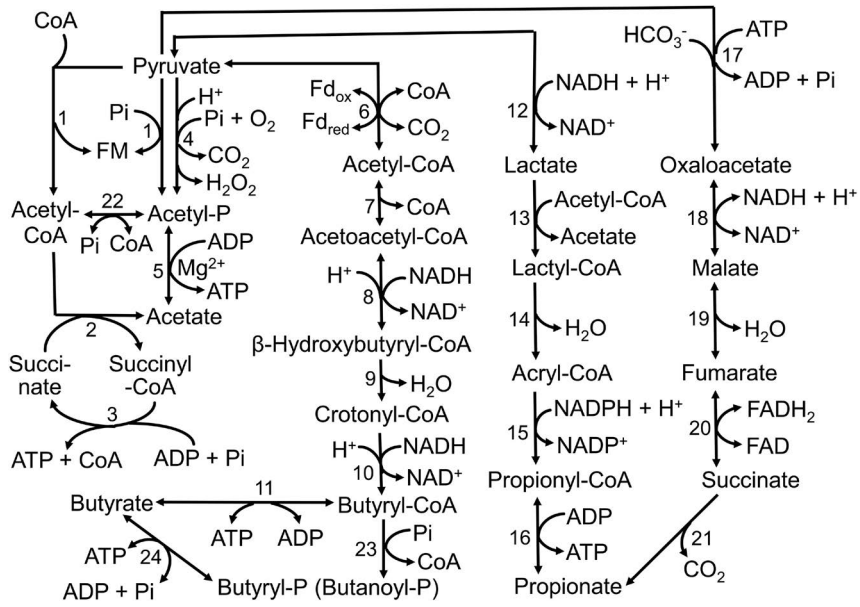


FIGURE 5.4 Formation of formate (FM) and short-chain fatty acids from pyruvate in ruminal bacteria. The enzymes that catalyze the indicated reactions are: (1) pyruvate formate-lyase (acetyl-CoA:formate C-acetyltransferase; ATP-dependent enzyme); (2) acetate:succinate CoA transferase; (3) succinate thiokinase; (4) pyruvate oxidase (FAD; an insignificant enzyme in the rumen); (5) acetate kinase; (6) pyruvate:ferredoxin oxidoreductase (pyruvate synthase; thiamine pyrophosphate-dependent enzyme); (7) thiolase; (8) β-hydroxybutyryl-CoA dehydrogenase; (9) β-hydroxybutyryl-CoA dehydratase; (10) crotonyl-CoA reductase; (11) butyrate kinase; (12) lactate dehydrogenase; (13) CoA transferase; (14) lactyl-CoA dehydratase; (15) acrylyl-CoA reductase; (16) propionate kinase; (17) pyruvate carboxylase; (18) malate dehydrogenase; (19) fumarase; (20) succinate dehydrogenase; (21) succinate decarboxylase; and (22) phosphotransacetylase; (23) phosphate butyryltransferase; (24) butyrate kinase. CoA, coenzyme A; Fd_{ox}, oxidized ferredoxin; Fd_{red}, reduced ferredoxin; Pi, inorganic phosphate. (Adapted from Downs 2006; Kandler 1983; McDonald et al. 2021; Ragsdale 2003.)

is metabolized to SCFAs by ruminal bacteria via a series of enzymes, but fungi also possess the enzymes required for these intracellular biochemical processes (Figure 5.4). In addition, protozoa can modulate the production of SCFAs in the rumen by engulfing and controlling the number of bacteria (Newbold et al. 2015). Degradation and fermentation are the only ways to digest dietary fibers in the rumen.

In adult ruminants fed either only hay (Heald 1951) or a maintenance diet with a mixture of 50% hay and 50% grain (Bergman et al. 1974), little carbohydrates (including glucose), except for small amounts of cellulose, reach the duodenum (Heald 1951; Macrae and Armstrong 1969). However, in adult cattle and sheep fed all-concentrate diets, as much as 30% of starches may escape ruminal fermentation and enter the small intestine (MacRae and Armstrong 1969; Symonds and Baird 1975). This may result from the reduced ability of ruminal microbes to degrade dietary carbohydrates because of changes in ruminal pH, as well as the numbers and species of ruminal bacteria.

Generation and Utilization of NADH and NADPH in the Rumen

In ruminal microbes, starch- and fiber-derived glucose, fructose, and pentoses are metabolized to pyruvate through the enzymes of the glycolysis pathway, with the production of NADH and H^+ (Downs 2006). Through the pentose cycle, glucose is metabolized to produce NADPH and H^+. In addition, the NADP-linked malic enzyme converts malate into pyruvate and forms NADPH. The NADH is used by specific microbes to generate H_2 and methane (see the following sections), reduce pyruvate into lactate, and convert acetate into butyrate, thereby regenerating NAD^+ to allow for glycolysis (Kandler 1983). The NADPH is required to reduce unsaturated fatty acids into saturated fatty acids, sulfate into sulfide, oxidized glutathione into reduced glutathione, nitrate into nitrite, and nitrite into ammonia; these reactions regenerate $NADP^+$, permitting the pentose cycle to proceed. Rates of NADH and NADPH production must be equal to rates of their utilization to maintain ruminal homeostasis. Because of the extensive fermentation of carbohydrates in the rumen to SCFAs, there is little glucose present in the lumen of the small intestine of ruminants (Table 5.1).

Production of SCFAs in the Rumen

There are fundamental differences between ruminants and nonruminants in carbohydrate digestion (Weimer 1998). This is because the rumen provides a continuous culture system for large amounts of anaerobic bacteria, protozoa, and fungi. Fermentative properties of ruminal bacteria are summarized in Table 5.4. Most of the dietary carbohydrates are fermented in the rumen to form SCFAs, namely, acetic acid, propionic acid, and butyric acid, which are the most

prevalent hydrocarbons produced by microbial enzymes. Acetate, propionate, and butyrate together account for ~95% of the total SCFAs in the rumen.

The breakdown of carbohydrates to SCFAs in the rumen can be divided into two stages: (I) the extracellular hydrolysis of carbohydrates (e.g., starch, cellulose, hemicellulose, and pectin) to monosaccharides, including pyruvate, as noted previously; and (II) the intracellular conversion of the monosaccharides into pyruvate (Figure 5.2) and its intracellular metabolism to form SCFAs (Figure 5.4). All these reactions require specific enzymes, including those that hydrolyze β-1,4- and β-1,6-glycosidic linkages, as well as acetylester and lignin-feruloylester bonds (Richard and Hilditch 2009; Tanaka and Johnson 1971). In the rumen of cattle fed a roughage diet, rates of fermentation for starch, pectin, and cellulose (mmol/h) peak around 2.5, 5, and 10 h after the initiation of feeding, respectively (Baldwin et al. 1977). The ruminal fermentation of starch and pectin is completed by 5 and 15 h after the initiation of feeding. In contrast, rates of fermentation for cellulose (mmol/h) remain constant between 10 and 15 h after the initiation of feeding and decline slightly thereafter (Baldwin et al. 1977). Based on dietary carbohydrate content (75% DM) and the assumption that 75% of dietary carbohydrates is converted into SCFAs, it can be estimated that dietary carbohydrates provide nearly all (96–98%) of the SCFAs produced in the rumen under normal feeding conditions (dietary CP and lipid content = 10–18% and 2–5% of DM, respectively).

Acetate is the major SCFA in the rumen of ruminants fed on a variety of roughages and concentrates (Table 5.5). Concentrations of SCFAs in the rumen depend on the balance between their rates of production and absorption into the ruminal epithelial cells. Total amounts of SCFAs in the rumen vary widely with the diets of animals and with the time that has elapsed since the previous meal, but are normally in the range of 75 to 150 mM (Baldwin et al. 1977). The relative proportions of SCFAs are affected by the ratio of soluble carbohydrates to insoluble fibers in the diet. For example, mature fibrous forages give rise to a mixture of SCFAs containing a high proportion (~70%) of acetate, 20% propionate and 10% butyrate, but less mature forages tend to yield a lower proportion of acetate and a higher proportion of propionate. The addition of feed concentrates to a forage diet also increases the proportion of propionate at the expense of acetate. For example, propionate represents 35–45 or 15–20 mol/100 mol of SCFAs in ruminal fluid when starch or cellulose/hemicellulose is fermented in the rumen, respectively (Ørskov 1986), which ensures that hydrogen is captured in a metabolizable form rather than lost as methane (Figure 5.5). With all-concentrate diets, the proportion of propionate may exceed that of acetate in the rumen. The underlying biochemical mechanisms are complex but involve changes in the ruminal bacteria and their metabolism. Specifically, rapid fermentation of starch and monosaccharides in concentrate diets results in a sudden drop in ruminal fluid pH due to lactic acid production via glycolysis, possibly causing metabolic acidosis (ruminal fluid pH <5.5). A low ruminal pH inhibits the growth of ruminal cellulolytic bacteria and acetate-producing bacteria but promotes the growth of propionate-producing

TABLE 5.5
Concentrations of Short-Chain Fatty Acids (SCFAs) in the Rumen of Ruminants Fed Various Diets

		Molar Ratios (mol/mol)		
Diet	**Total (mM)**	**Acetate**	**Propionate**	**Butyrate**
Cattle fed roughages or concentrates				
Mature ryegrass herbage	137	0.64	0.22	0.11
Grass silage	108	0.74	0.17	0.07
Barley (no ciliate protozoa in rumen)	146	0.48	0.28	0.14
Barley (ciliates present in rumen)	105	0.62	0.14	0.18
Long hay/Concentrates (0.4:0.6; g/g)	96	0.61	0.18	0.13
Pelleted hay/Concentrates (0.4:0.6; g/g)	140	0.50	0.30	0.11
Hay/Concentrates (g/g)				
1:0	97	0.66	0.22	0.09
0.8:0.2	80	0.61	0.25	0.11
0.6:0.4	87	0.61	0.23	0.13
0.4:0.6	76	0.52	0.34	0.12
0.2:0.8	70	0.40	0.40	0.15
Sheep fed roughages				
Young ryegrass herbage	107	0.60	0.24	0.12
Chopper lucerne hay	113	0.63	0.23	0.10
Ground lucerne hay	105	0.65	0.19	0.11

Source: Adapted from McDonald et al. (2021).

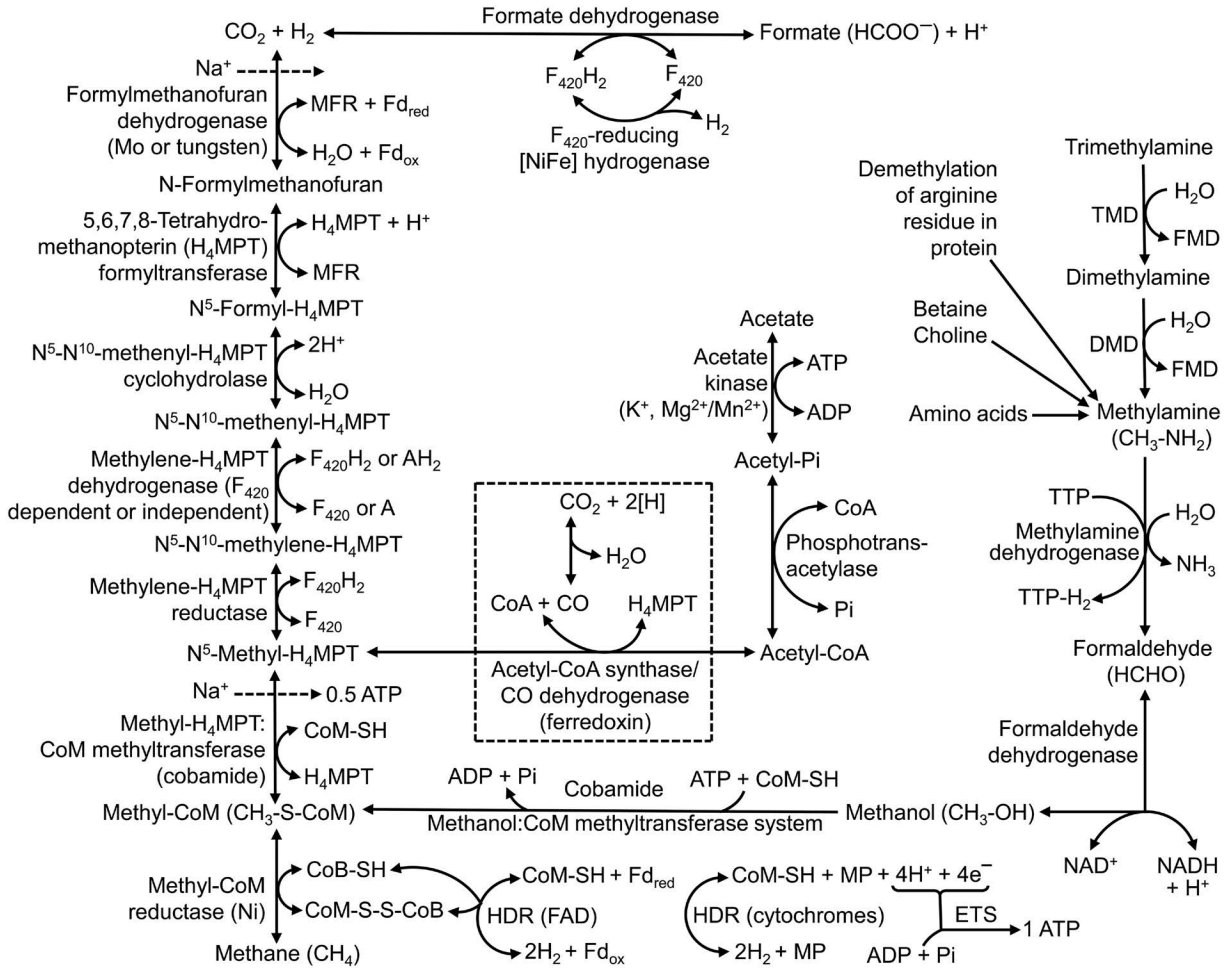


FIGURE 5.5 Production of methane from dissolved CO_2 and H_2, and from acetate, methanol, formate, and methylamines by ruminal archaea with or without cytochromes. The metabolic pathways are similar between both classes of methanogenic archaea, with the major exception of the mechanisms for electron transfers during the reduction of CoM-S-S-CoB to CoM-SH and CoB-SH (the last step of methane formation) by heterodisulfide reductase (HDR). In methanogenic archaea with cytochromes, methanophenazine (MP)-reducing [NiFe] hydrogenase converts H_2 and MP into MPH_2 (i.e., $H_2 + MP \rightleftharpoons MPH_2$); the MP-dependent HDR transfers two electrons from MPH_2 to cytochrome b_2, which then donates two electrons to CoM-S-S-CoB for its reduction to regenerate CoM-SH and CoB-SH (i.e., $MPH_2 + CoM\text{-}S\text{-}S\text{-}CoB \rightleftharpoons HS\text{-}CoM + HS\text{-}CoB$) for the continuation of methane production; the cell-membrane electron transport system pumps four protons from the cell, generating a proton gradient that drives the synthesis of 1 mol of ATP from 1 mol of ADP + 1 mol of Pi by ATP synthase and the pumping of cytosolic Na^+ across the cell membrane for coupling with the conversion of CO_2, H_2, and methanofuran into *N*-formylmethanofuran (the first step of methane formation). By contrast, in methanogenic archaea without cytochromes, HDR transfers two electrons and two protons from H_2 to FAD, forming $FADH_2$; the latter bifurcates the two electrons into one high-potential electron and one low-potential electron; the high-potential electron and the low-potential electron are transferred to CoM-S-S-CoB (for its reduction to CoM-SH and CoB-SH) and ferredoxin (for its oxidation), respectively; and the HDR reaction is energetically and chemically coupled with the first step of methane formation without ATP synthesis. In methanogenic archaea with or without cytochromes, the formation of methyl-CoM from N^5-methyl-tetrahydromethanopterin (H_4MPT) is coupled with the vectorial transport of cytosolic Na^+ to the cell exterior, generating a Na^+ gradient across the cell membrane that drives the synthesis of 1 mol of ATP from 1 mol of ADP + 1 mol of Pi by ATP synthase. Thus, the ATP yields of methanogenesis (mol/mol methane) are 1.5 and 0.5 for methanogenic archaea with and without cytochromes, respectively. CoB (coenzyme B, also known as 7-mercaptoheptanoylthreoninephosphate); CoM (coenzyme M, also known as 2-sulfanylethanesulfonate or 2-mercaptoethanesulfonate; $HS\text{-}CH_2\text{-}CH_2\text{-}SO_3^-$) is a coenzyme required for methyl transfer reactions. CoB (coenzyme B; 7-mercapto-heptanoyl-threonine-phosphate) and F_{420} (8-hydroxy-5-deazaflavin) are coenzymes required for redox reactions. Cobamide is a vitamin B_{12} derivative containing cobalt; A, a receptor [e.g., NAD(P)$^+$] of electrons; DMD, dimethylamine dehydrogenase (flavoprotein); ETS, cell-membrane electron transport system; Fd_{ox}, oxidized ferredoxin; Fd_{red}, reduced ferredoxin; FMD, formaldehyde; $F_{420}H_2$, reduced F_{420} (a flavin derivative with the maximum absorbance at 420 nm); HDR (FAD), heterodisulfide reductase (flavoprotein containing FAD) in methanogenic archaea without cytochromes; HDR (cytochromes), heterodisulfide reductase in methagenic archaea with cytochromes; H_4MPT, 5,6,7,8-tetrahydro-methanopterin; MFR, methanofuran; MP, methanophenazine; TMD, trimethylamine dehydrogenase (flavoprotein); TTP, tryptophan tryptophylquinone. (Adapted from Hook et al. 2010; Shima et al. 2002.)

bacteria, thereby reducing the digestibility of roughages and acetate concentration but stimulating propionate production (Chesson and Forsberg 1997). The opposite is true when ruminants are fed a diet containing a high percentage of fiber and a low percentage of starch. Thus, the NRC (2001) recommends a maximum content of dietary nonfiber carbohydrates to be 44% for ruminants. In addition to the ratio of forage to concentrate, the ruminal production of SCFAs is influenced by many other factors (e.g., the physical forms, water and fat content, and intake of the feed, as well as feeding methods) through their effects on physical fill and rumination (regurgitation, chewing, salivation, and swallowing; Weimer 1998).

It is noteworthy that when fed processed barley grain, fattening lambs and goats develop a very soft and unattractive subcutaneous fat. This is due to increases in the formation and concentration of branched-chain fatty acids with a low melting point in the white adipose tissue (WAT). Specifically, propionyl-CoA (C_3) and acetyl-CoA (C_2) are precursors of odd- and even-numbered fatty acids, respectively. In addition, propionyl-CoA and its derivative (methyl-malonyl-CoA) may compete with malonyl-CoA (the intermediate precursor of even-numbered fatty acids) for β-keto-acyl carrier protein synthase, thereby reducing the synthesis of even-numbered fatty acid synthesis in the WAT. The severity of the low quality of meat can be alleviated by feeding unprocessed whole grain, which is fermented at a slower rate than processed grain. Interestingly, this nutritional and metabolic problem does not occur in cattle, possibly due to a greater rate of utilization of propionate via hepatic gluconeogenesis and oxidation in these animals than in sheep and goats.

Entry of SCFAs from the Rumen into Blood

The SCFAs produced in the rumen enter the blood primarily through three routes: ~75% are absorbed directly through the ruminal wall, 20% from the omasum and abomasum, and 5% pass along with the digesta into the small intestine, where they are absorbed into the portal vein. The absorption of SCFAs occurs through passive transport, and its rate depends on a concentration gradient. The uptake of SCFAs by the cell is accompanied by the efflux of intracellular bicarbonate via an antiporter (also called an exchanger). The rate of absorption of SCFAs from the rumen into the blood increases as the pH of the ruminal fluid decreases. This has led to the concept that nondissociated acids pass through the ruminal wall more rapidly than their anions. During their passage from the rumen into the blood, some of the butyrate and propionate are metabolized to form β-hydroxybutyrate and lactate, respectively, in the ruminal epithelium.

SCFAs play an important role in ruminant nutrition. First, acetate, propionate, and butyrate are the major sources of energy in ruminants. As noted previously, butyrate is the major substrate for ATP production in the epithelial cells of the large intestine and thus for maintaining the health of the hindgut in both ruminants and nonruminants. The rumen of the cow or sheep produces 3–4 kg or 0.3–0.4 kg SCFAs per day, respectively. Thus, SCFAs can effectively replace a bulk of glucose and long-chain fatty acids (LCFAs) as metabolic fuels in the large intestine, liver, skeletal muscle, and kidneys. Similarly, SCFAs can substitute substantial amounts of glucose as oxidative substrates in the brains of ruminants. Second, propionate is used to synthesize glucose in the livers of ruminants. Third, acetate and butyrate are converted into acetoacetate and β-hydroxybutyrate in the ruminal wall and colonocytes. These ketone bodies are utilized as metabolic fuel by extrahepatic tissues, including the brain, small intestine, heart, skeletal muscle, and kidneys. Third, SCFAs, along with lactic acid, CO_2/HCO_3^-, and NH_3, play important roles in maintaining ruminal fluid pH at 5.5–6.8. This is critical for bacterial growth and metabolism in the rumen.

Production of Methane in the Rumen

Methane is produced by microbes known as methanogens, which belong to the domain *Archaea* and the phylum *Euryarchaeota* (a procaryote) that is distinct from bacteria (another type of procaryote) and eucaryotes (Thauer et al. 2008). Methanogens are much more abundant in the rumen than in the hindgut. Although methane can be generated by *Euryarchaeota* in the lower GIT (as in nonruminants), the rumen and hindgut contribute to approximately 90% and 10% of the methane emitted from ruminants, respectively (Hook et al. 2010). Note that bacteria, fungi, and protozoa do not directly form methane but play an important role in methane production though proving its precursors substrates through metabolism. CO_2 is a substrate for the production of methane by methanogens, whereas H_2 and formate provide both precursors and energy for this biochemical pathway.

Major Metabolic Pathways for Methane Production

Figure 5.5 illustrates the biochemical pathways for the production of methane from dissolved CO_2 and H_2 by ruminal archaea. Cytochromes are either present or absent in these cells. The net reaction for methanogenesis is as follows. Methane production represents a significant loss of dietary energy from ruminants because CH_4 is not used to meet their metabolic needs and is usually expelled from the bodies.

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad \begin{pmatrix}\text{Net reaction of} \\ \text{methanogenesis in archaea}\end{pmatrix}$$

The metabolic pathways of methanogenesis are similar between archaea with and without cytochromes, with the major exception of electron transfers during the reduction of CoM-S-S-CoB to CoM-SH and CoB-SH (the last step of methane formation) by heterodisulfide reductase (HDR). In methanogenic archaea with cytochromes, methanophenazine (MP)-reducing [NiFe] hydrogenase converts H_2 and MP into MPH_2 (i.e., $H_2 + MP \rightleftharpoons MPH_2$); the MP-dependent HDR transfers two electrons from MPH_2 to cytochrome b_2, which then donates two electrons to CoM-S-S-CoB for its reduction to regenerate CoM-SH and CoB-SH (i.e.,

MPH_2 + CoM-S-S-CoB ⇌ HS-CoM + HS-CoB) for the continuation of methane production; the cell-membrane electron transport system pumps four protons from the cell, generating a proton gradient that drives the synthesis of 1 mol of ATP from 1 mol of ADP + 1 mol of Pi by ATP synthase and the pumping of cytosolic Na^+ across the cell membrane for coupling with the conversion of CO_2, H_2, and methanofuran into *N*-formylmethanofuran (the first step of methane formation; Figure 5.5). By contrast, in methanogenic archaea without cytochromes, HDR transfers two electrons and two protons from H_2 to FAD, forming $FADH_2$; the latter bifurcates the two electrons into one high-potential electron and one low-potential electron; the high-potential electron and the low-potential electron are transferred to CoM-S-S-CoB (for its reduction to CoM-SH and CoB-SH) and ferredoxin (for its oxidation), respectively; and the HDR reaction is energetically and chemically coupled with the first step of methane formation without ATP synthesis. In methanogenic archaea with or without cytochromes, the formation of methyl-CoM from N^5-methyl-tetrahydromethanopterin (H_4MPT) is coupled with the vectorial transport of cytosolic Na^+ to the cell exterior, generating a Na^+ gradient across the cell membrane that drives the synthesis of 1 mol of ATP from 1 mol of ADP + 1 mol of Pi by ATP synthase. Thus, the ATP yields of methanogenesis (mol/mol methane) from CO_2 + H_2 are 1.5 and 0.5 for methanogenic archaea with and without cytochromes, respectively. This helps to reduce (or partially compensate for) the loss of dietary energy via CH_4 production.

The formation of methane does not require O_2 and is actually inhibited by O_2. Notably, the conversion of CO_2 + H_2O to methane requires several unique cofactors (e.g., coenzymes M, G, and F_{420}, and tetrahydromethanopterin) and a series of complex reactions catalyzed by 10 enzymes (Figure 5.5). These coenzymes are either synthesized by the bacteria or supplied from an external source. As indicated in Figure 5.4, the formation of acetate and butyrate from pyruvate results in the production of H_2 and metabolic hydrogen in the form of reducing equivalents (e.g., NADH and NADPH), whereas propionate synthesis consumes the reducing equivalents and, therefore, is a competitive pathway for hydrogen utilization in the rumen. Thus, excessive H_2 is generated along with the production of acetate and butyrate, but not with that of propionate. Diets which have a lower proportion of fiber, but a higher proportion of starch can increase the proportion of propionate among SCFAs in the rumen, resulting in reduced production of methane per mole of substrate fermented.

Minor Metabolic Pathways for Methane Production

In the rumen, the reduction of CO_2 by H_2 is the major pathway for methane production, where carbon is the terminal electron receptor. A smaller amount of methane is formed from acetate in the rumen (Figure 5.5). Acetate is first activated to acetyl-phosphate, which is then converted into acetyl-CoA by phosphotransacetylase. Acetyl-CoA reacts with H_4MPT to form N^5-methyl-H_4MPT, which is metabolized to methane via the formation of methyl-CoM, as described previously (Figure 5.5). In addition, some methanogens of the genus *Methanosarcina*, which grow slowly on H_2 and CO_2 but utilize methanol and methylamines to produce methane via the formation of methyl-CoM (Thauer et al. 2008).

Sources of Substrates for Methane Production in the Rumen

Carbohydrate fermentation by bacteria is the major source of both CO_2 and H_2 in the rumen, but these two gases are also generated, to a much lesser extent, by protozoa and fungi. In addition, both CO_2 and H_2 are produced from the cleavage of formate by F_{420}-dependent formate dehydrogenase (FAD and molybdenum as cofactors) or NAD^+-dependent formate dehydrogenase. F_{420}, a coenzyme for hydride transfer in redox reactions, is a deazaflavin derivative exhibiting blue-green fluorescence when irradiated with UV light. Acetate is generated from the decarboxylation of pyruvate, which is derived primarily from carbohydrates and, to a lesser extent, amino acids. Furthermore, a source of CO_2 in the rumen is HCO_3^- in the saliva and blood.

In the rumen, methanol is produced mainly from the metabolism of (a) the methyl esters of pectins, (b) the methyl group of methionine, and (c) formaldehyde (CH_2O; a product of some metabolic pathways such as glycine synthesis). Formaldehyde can be generated from formate via activation into formyl-CoA (the formyl-CoA route), the condensation with glycine into serine (the serine shunt), and the formation of formyl-phosphate (the formyl-phosphate route) in microbes (Schann and Wenk 2024). Methylamines are produced from the degradation of amino acids, betaine, and choline by bacteria, as well as the demethylation of arginine residues in protein. Amino acids, formate, betaine, and choline are supplied from diets and endogenous formation.

Formate + ATP + CoA → Formyl-CoA + AMP + PPi;
Formyl-CoA + NADPH → CH_2O + $NADP^+$ + CoA

Formate + Gly + NADPH + ATP + THF → Serine + $NADP^+$ + ADP + Pi + THF; Serine → CH_2O + Gly

Formate + ATP → Formyl-phosphate + ADP;
Formyl-phosphate + NADPH → CH_2O + $NADP^+$ + Pi

Nutritional and Environmental Impacts of Methanogenesis

Elevated concentrations of H_2 in ruminal fluid inhibit microbial fermentation. If H_2 is not used for metabolism in the rumen and the GIT, its energy would be completely lost, and gastrointestinal function would be impaired. As noted previously, methanogenesis from CO_2 + H_2 generates ATP, thereby compensating, albeit to a small but significant extent, for the loss of dietary energy and sustaining ruminal activity. However, methane cannot be utilized by animal tissues and is exhaled from ruminants into the environment. As a potent greenhouse gas, the warming effect of methane in the atmosphere is 28 times greater than that

of CO_2 over a period of 100 years (EPA 2025). Although ATP is generated from the formation of methane from $CO_2 + H_2$, the diversion of amino acids, pyruvate, acetate, other substrates, and reducing equivalents into the production of methane results in an irreversible loss of dietary energy. Thus, methanogenesis from dietary amino acids and carbohydrates reduces the conversion of dietary energy and nutrients into milk, skeletal muscle, or wool in ruminants. In cattle and sheep, the fermentation of carbohydrates results in inevitable losses as heat and methane, accounting for 12–20% of the ingested energy (Ørskov 1986), with methane production representing 2–12% of the ingested energy, depending upon diets (Hook et al. 2010). Thus, decreasing methane production is an important means to improve the efficiency of energy utilization in ruminant production. In addition, because methane is a contributor to the greenhouse effect, mitigation of methane emission is environmentally sustainable in animal agriculture.

Methods for inhibiting methanogenesis include (a) direct inhibition by halogenated methane analogues and related compounds [e.g., chloroform (not practical), chloral hydrate (not practical), amichloral (safe for in vivo feeding), trichloroacetamide, trichloroethyl adipate, bromochloromethane, 2-bromoethanesulfonic acid, 9,10-anthraquinone, and bromoform]; (b) the use of ionophores (e.g., monensin and salinomycin) to alter bacterial population; (c) enhancers of propionate production (e.g., malate, fumarate); (d) the re-channeling of substrates for methane production into the formation of alternative products by reducing the availability of cofactors, prosthetic groups, reducing equivalents (e.g., through biohydrogenation with polyunsaturated fatty acids as hydrogen acceptors), electron acceptors (e.g., nitrate and carbon sources), and electron carriers (e.g., cytochromes); (e) the removal of protozoa from the rumen (defaunation) with use of phytochemicals (e.g., saponins); and (f) the manipulation of microbial population and activity by fatty acids (e.g., propionic, medium-chain, and long-chain polyunsaturated or saturated fatty acids), plant extracts (e.g., condensed tannins and essential oils), probiotics (e.g., yeast and fungal probiotics), and other substances (e.g., formate). Over the past three decades, there has been growing interest in the use of nitrate (NO_3^-) as a feed additive to reduce the production of methane by ruminants. Nitrate can compete with methanogens for H_2 via enzyme-catalyzed NO_3^- hydrogenation, thereby acting as an alternative H_2 sink in the rumen of ruminants. Significant challenges are that ruminal bacteria may adapt to these manipulation methods and render their effects only transient in vivo. For all these reasons, it would be advantageous to promote the postruminal digestion of starch for the direct release of glucose and its subsequent absorption by the small intestine.

$$NO_3^-\,(\text{Nitrate}) + H_2 \rightarrow NO_2^-\,(\text{Nitrite}) + H_2O$$
$$(\text{Nitrate reductase};\ \Delta G = -130\ \text{kJ/mol}\ H_2)$$
$$NO_2^- + 3H_2 + 2H^+ \rightarrow NH_4^+ + 2H_2O$$
$$(\text{Nitrite reductase};\ \Delta G = -371\ \text{kJ mol}\ H_2)$$
$$NO_3^- + 4H_2 + 2H^+ \rightarrow NH_4^+ + 3H_2O$$

Ruminal Metabolic Disorders

1. *Lactic acidosis.* A small proportion of grain (e.g., 20–30%) in diets normally does not cause depression in the intake or digestibility of roughages in ruminants (Ørskov 1986). However, when a larger amount of highly fermentable carbohydrates is fed to ruminants, the rate of glycolysis to yield lactic acid in the rumen is much greater than the rate of lactic acid utilization, resulting in a rapid drop in ruminal fluid pH and the onset of lactic acidosis. In the rumen, major lactate-producing bacteria (e.g., *Streptococcus bovis*) are tolerant to low pH, but the growth and metabolic activity of major lactic acid utilizers (e.g., *Megasphaera elsdenii* and *Selenomonads*) are inhibited by low pH (Fellner 2002). Thus, there is a lack of effective feedback inhibition of glycolysis in the rumen, such that ruminal lactic acidosis develops and becomes more severe as the dietary intake of concentrate increases markedly beyond its maximum threshold (e.g., 50% concentrate in the diets of lactating cows; DM basis). In addition, a low ruminal pH inhibits the growth of cellulolytic bacteria and acetate-producing bacteria but promotes the growth of propionate-producing bacteria in the rumen, thereby reducing roughage digestion and acetate production while stimulating propionate formation. Thus, a minimum of 50% roughage in the diet (DM basis) is recommended for feeding ruminants. Adapting the rumen to the increased concentrations of grains in diets over a period of weeks can reduce the incidence of acidosis in feeding ruminants (e.g., lactating dairy cows and feedlot beef). This is because the gradual adaptation to dietary concentrates can allow for the simultaneous growth of both lactic acid-producing and utilizing microbes in the rumen. In addition, dietary supplementation with anti-microbial agents (e.g., ionophores) can also be effective in limiting the growth of lactic acid-producing bacteria in the rumen and alleviating lactic acidosis in ruminants. Because ruminal acidosis (ruminal pH < 5.5) reduces feed intake, growth performance, milk production, and profitability while increasing culling rate and death loss, this metabolic disorder must be avoided in ruminant nutrition.
2. *Ruminal bloat in ruminants.* Under normal conditions, nearly all the methane and H_2, as well as most of the CO_2 produced in the rumen, are removed by eructation. When the rate of gas production greatly exceeds the rate of eructation, normal elimination of the ruminal gases is impaired. This results in a disorder called bloat, which occurs in the form of a persistent foam mixed with either the ruminal contents (called primary bloat) or gases separated from the digesta (called secondary bloat). Bloat is characterized by severe distention of the abdomen,

resulting in severe compression of the heart and lungs, and therefore impairment of blood circulation. With this metabolic disease, death rates can be up to 20% in cattle grazing bloat-prone pastures. The susceptibility of individual animals to bloat is affected by their genotypes and diets. For example, this disorder occurs primarily in cattle and, to a lesser extent, in sheep and goats. Pasture bloat is often observed in cattle grazing on legume pastures. This is possibly because products of rapid degradation of high amounts of soluble plant proteins (e.g., ammonia, CO_2 and H_2S), along with the high content of phospholipids in legumes, contribute to the formation and stabilization of the foam in the rumen. In contrast, feedlot bloat in cattle that are fed diets consisting of high percentages of grain concentrates and alfalfa or clovers results from the production of soft and adhesive slime by amylolytic bacteria, which leads to the formation of stable foam. Feedlot bloat in cattle fed high-concentrate or all-concentrate rations can be reduced through dietary management by the addition of coarsely chopped roughage. Of note, poloxalene, a nonionic surfactant, can be fed at the dose of 1 g/100 lb to beef cattle and lactating dairy cows on a legume or high grain ration to prevent bloat (Bezeau et al. 1967).

Differences and Similarities in Carbohydrate Digestion among Ruminants

There are species differences in the digestion of carbohydrates among ruminants. For example, the digestibility of DM (including carbohydrates) in a moderately high fiber diet is greater for cattle (64.3%) than deer (55.7%), with the values for sheep (60.6%) and goats (58.8%) being intermediate (Huston et al. 1986). The digestibility of alfalfa hay fiber is 46% in dairy cows, 44% in cattle, 45% in sheep, and 41% in goats. When fed high-concentrate diets, the rates of disappearance of starch in the small intestine of beef cattle and dairy cows are 55% and 60%, respectively (Trotta et al. 2022). Within the same species, tremendous differences occur in the digestion of carbohydrates by the GIT among individual animals. Species or individual differences in gastrointestinal dynamics of carbohydrate digestion are a major factor affecting the utilization of forages by ruminants.

As noted previously, ruminants share the same characteristics for some aspects of carbohydrate digestion in the GIT. First, the digestibility of starch in the GIT is much higher than that of fibers in ruminants as in nonruminants. In all ruminants, the digestibility of starch (% of intake) decreases with increasing its intake. Second, the digestibility of total carbohydrates in ruminants varies widely with the type of diet and dietary content, ranging from 95% for molasses, to 75% for grass, 60% for hay, and 40% for straw. Third, the rate of digestion of DM (including complex carbohydrates) is influenced more by diet than animal species, such that ruminal residence time is shorter for concentrate particles than that for forages. Fourth, due to the actions of ruminal microbes, the digestibility of raw starch in the rumen of cattle, sheep, and goats is ≥90% for some grains (e.g., low amounts of oats, barley, or wheat as whole or crushed grain) except corn and sorghum and is ≤70% for corn or sorghum (present in low amounts in diets; Ørskov 1986). Thus, the total tract digestibility of certain raw starch is very high (90–100%) in ruminants fed low-starch, common feedstuffs. Interestingly, steaming or cooking cornstarch before feeding increases its digestibility in the rumen to nearly 100%. In contrast, when ruminants consume diets containing high levels of starch (e.g., 30% on a DM basis), approximately 60% of the starch is fermented in the rumen. In all ruminants, excess amounts of fibrous roughages in diets increase the escape of starch from the rumen. Fifth, the small intestine of ruminants has a relatively limited ability to digest starch (e.g., 100–200 g raw starch and 200–300 g gelatinized starch in the 30-kg sheep, only ~10–15% of the value for the 30-kg pig; Ørskov 1986) because of insufficient activities of pancreatic α-amylase and small-intestinal carbohydrases, the absence of sucrase activity, and suboptimal glucose absorption (Trotta et al. 2022). Thus, due to physiological constraints, excessive dietary starch (e.g., >35% on a DM basis) is unlikely to be beneficial for ruminants and rather may cause metabolic and digestive problems.

Absorption of Monosaccharides by the Small Intestine in Ruminants

The enterocytes of the ruminant small intestine express glucose and fructose transporters (Chapter 1) and, therefore, have an ability to absorb glucose, galactose, and fructose present in the lumen of the small intestine. The mechanisms of intestinal monosaccharide transport in ruminants are the same as those in nonruminants. However, in forage-fed ruminants, little glucose enters the lumen of the small intestine from the stomach due to the extensive ruminal fermentation. Thus, under normal feeding conditions, virtually no glucose is absorbed from the small intestine into the portal vein in cattle, sheep, goats, and other ruminant species. Based on studies of abomasal infusions of glucose, the small intestine of the 30-kg sheep can absorb 300–400 g of this sugar per day (Ørskov 1986). Thus, capacity for the absorption of glucose may ultimately limit starch digestion in the small intestine. In ruminants fed grain-based diets containing high percentages of starch (e.g., 25–30%), up to 40% of the starch may escape the rumen into the small intestine for further digestion, and only a small amount of the escaped starch is hydrolyzed or absorbed to the portal vein as glucose. Results of recent studies show the lack of an adaptive response of glucose transporter activities or protein abundances to dietary substrates, as well as the disproportional relationship between the disappearance of glucose from the small intestine and the appearance of glucose in the portal vein (Trotta et al. 2022), indicating a suboptimal capacity for glucose absorption in ruminants with a functional rumen.

Considering that 5% of absorbed glucose is used by the intestinal mucosa, ~20% of starch appears as glucose in the portal vein of ruminants fed high-starch (25–35%) diets.

The rate of glucose utilization by the small intestine is low in ruminants. Thus, ≥ 95% of absorbed glucose enters the portal vein. Furthermore, there is a very low rate of extraction of blood glucose by the liver in ruminants. For example, in nonpregnant, nonlactating adult sheep fed lucerne hay at 0.5-h intervals, the arterial blood supplies 56.7 g glucose/h to the portal-drained viscera (primarily the small intestine) but only 0.6 g glucose/h is extracted, and the portal vein and the hepatic artery supply 56.1 and 9.5 g glucose/h to the liver, respectively, but only 0.8 g glucose/h is extracted (Bergman 1983). Thus, in contrast to nonruminants, only 1.4% of the glucose in portal vein blood is extracted by the liver in ruminants, and the remaining portion (98.6%) enters the systematic circulation for metabolism in extrahepatic tissues.

DEGRADATION OF CARBOHYDRATES IN THE LARGE INTESTINE OF NONRUMINANTS AND RUMINANTS

The large intestine contains relatively large amounts of microbes (including bacteria and protozoa), compared with the small intestine. For example, the large intestine of dogs contains much more bacteria than their small intestine. Specifically, bacterial counts in the colon of dogs are 10^9 to 10^{11}/ml, which help to digest fiber and other nutrients (Suchodolski 2011). The main bacteria in their hindgut are Fusobacteria (24–40%), Bacteroidetes (32–34%), and Firmicutes (15–28%), whereas Proteobacteria (5–6%) and Actinobacteria (0.8–1.4%) are quantitatively minor microbial species (Maskell and Johnson 1993; Suchodolski et al. 2008). For comparison, the main bacteria in the hindgut of healthy pigs are Firmicutes (78%), followed by Bacteroidetes (14%), Actinobacteria (1.6%), and Proteobacteria (0.2%) (Hoogeveen et al. 2020). The large intestine of 28-day-old pigs weaned at 21 days of age contained only 0.01% Fusobacteria (Meng et al. 2020). Thus, the composition of bacteria in the hindgut differs greatly between dogs (facultative carnivores) and omnivores. The large intestine of the cat has the same predominating bacterial species and the same pH (Maskell and Johnson 1993; Suchodolski 2011). The fermentation of carbohydrates (particularly starch) in the hindgut promotes the loss of N due to the increased synthesis of microbial proteins that are excreted in the feces.

If starch grains and dietary fibers escape the small intestine, they will ferment in the large intestine. Up to 100 g of starch can be fermented daily in the cecum and colon of the 30-kg sheep (Ørskov 1986). The processes for microbial fermentation and absorption of carbohydrates in the large intestine of nonruminants and ruminants are similar to those in the rumen of ruminants. However, fermentation in the hindgut is less effective than that in the rumen. This is because there are fewer microbes and the transition time of chyme is much shorter in the large intestine than in the rumen. Nonetheless, concentrations of total SCFAs are found up to 150 mM in the lumen of the cecum and colon. Butyrate is the major source of energy for the epithelial cells of the large intestine and, therefore, is important for maintaining the health of the hindgut in ruminants and nonruminants.

In hindgut fermenters (e.g., horses and rabbits), the enlarged colon and cecum contain microbes that ferment food constituents (including dietary fibers) via biochemical reactions similar to those in the rumen. In horses, 4–30% of the ingested grain starch can escape prececal digestion and enter the large intestine, where its digestion is almost complete (Julliand et al. 2006). Regardless of the methods used to process grains, the apparent digestibilities of starch in the total GIT of horses are 98.8%, 96.8%, 97.9%, and 99.5%, respectively, for corn, oats, barley, and wheat (Julliand et al. 2006). The hindgut fermentation contributes to the utilization of 15–30% of dietary soluble carbohydrates and 75–85% of cell wall carbohydrates. However, high intakes of sugar and starch by horses (e.g., >12 and 20% NSCs for sensitive and less sensitive horses, respectively) may result in high rates of glycolysis in the hindgut to generate excess lactic acid, thereby adversely disturbing bacterial and protozoal activities (including the fermentation of dietary fiber) (Brøkner et al. 2012; Jensen et al. 2014). Thus, horses cannot tolerate large amounts of NSCs in one meal, and frequent feeding of small amounts of high sugar/starch diets (grains and concentrates) is recommended to prevent metabolic and intestinal disorders (e.g., colic or laminitis). With hay and concentrates, the entire digestive tract of horses can digest ~85% of the organic matter (including carbohydrates, protein, and fats) in their diets (Brøkner et al. 2016; Jensen et al. 2014). As a result, postweaning horses can grow by consuming forages, although their growth rate may be suboptimal. In contrast, the fermentability of dietary fiber in the large intestine of rabbits is not particularly high (e.g., only 14% for alfalfa hay fiber) (Yu and Chiou 1996), but their very high capacity to consume forages provides the physiological basis for them to survive and grow on grass.

In pigs, the hindgut is less enlarged in comparison with horses, and forages are poorly fermented (e.g., the digestibility of alfalfa hay fiber in diets with 5% alfalfa being only ~20%). However, postweaning pigs can ferment up to 50% of dietary cellulose and hemicellulose in young plants, cereal grains, and their byproducts when they are included as ingredients of conventional diets. Thus, green leafy vegetables can be used to feed pigs. In the large intestine of growing-finishing pigs, fermentable fiber provides 8 kJ/g [e.g., resistant starch (8.8 kJ/g), fructo-oligosaccharides (8.4 kJ/g), and inulin (8.8 kJ/g)] (Eswaran et al. 2013).

As in other mammals, SCFAs are highly abundant in the large intestine of dogs and cats and are the major sources of energy for their colonocytes (He et al. 2024). The content of acetate, propionate, and butyrate in the feces (35% DM) of healthy adult dogs fed a 35%-CP diet was 280, 93, and 50 mmol/kg DM (Beloshapka et al. 2012), whereas the content of acetate, propionate, and butyrate in the feces (35% DM) of healthy adult cats was 130, 42, and 24 mmol/kg

feces (Summers et al. 2020). Thus, inclusion of appropriate amounts of plant fiber fermentable fibers in canine and feline diets is beneficial for the health of their hindgut. In support of this view, dietary supplementation with fructans (highly fermentable substrates) decreases pH while increasing concentrations of SCFAs and the abundance of *Bifidobacterium* spp. and *Lactobacilli* spp. in the feces of healthy adult dogs (He et al. 2024). Similar effects of dietary supplementation with Brewer's spent grains and carrot pomace (Eisenhauer et al. 2019) or dried apple pomace (De Brito et al. 2021) have been reported for dogs. Although plant-based foods are normally not fed to cats, inclusion of fibers (water-soluble and insoluble) in feline diets plays an important role in stimulating intestinal motility and maintaining GIT homeostasis (Moreno et al. 2022). An ideal proportion of soluble (e.g., fructans and galactans) to insoluble (e.g., cellulose and hemicellulose) fibers in feline diets is 1:3, with the total content of dietary fiber being <5% of DM (He et al. 2024). Thus, diets have great impacts on the gastrointestinal microbiome of cats and dogs.

DIGESTION AND ABSORPTION OF CARBOHYDRATES IN FISH AND CRUSTACEANS

Dietary Carbohydrates for Fish and Crustaceans

The use of dietary carbohydrates by fish depends on species. Omnivorous fish (e.g., the common carp and channel catfish) can digest relatively large amounts of starch and glycogen, and herbivorous fish (e.g., the grass carp) can utilize a primarily vegetarian diet. In addition, hybrid striped bass can efficiently use at least 25% starch in the diet as a source of glucose and energy (Wu et al. 2015). In contrast, carnivorous fish (e.g., the Atlantic salmon and the Japanese yellowtail) consume little starch, but they do ingest a small amount of glycogen from their diets. Carnivorous fish digest carbohydrates less efficiently than omnivorous or herbivorous fish, and cannot tolerate significant quantities of dietary complex carbohydrates. In addition to nutritional and metabolic functions, carbohydrates are used to manufacture fish diets because (a) starch enhances floatability and integrity of extruded pellets due to their expansion and gelatinization during extrusion and (b) water-insoluble fiber (e.g., cellulose, hemicellulose, or their derivatives) is an effective binding agent to keep feed particles together and maintain pellet quality.

Crustaceans are a very diverse group of invertebrates and have evolved to consume a wide variety of foods. Most of the agriculturally important crustaceans are omnivores [e.g., lobsters, crabs, crayfish, the Indian prawn (Indian white shrimp), and Pacific white shrimp], but some are carnivores [e.g., black tiger shrimp (giant tiger prawn or Asian tiger shrimp) and Mantis shrimp]. There are also many species of herbivorous crustaceans in the ocean and fresh water. Both omnivorous and herbivorous crustaceans consume foods containing carbohydrates such as starch, fiber (e.g., cellulose and hemicellulose), and sugars. Different species of crustaceans tolerate different types and amounts of carbohydrates (Wang et al. 2016b). For example, swimming crabs can effectively use 20% corn starch, wheat starch, and dextrin in a diet that have amylose/amylopectin ratios of 14.18%, 15.07%, and 6.55%, respectively, but not potato starch that has an amylose/amylopectin ratio of 10.64% (Zhang et al. 2021). In addition, black tiger shrimp ingest a small amount of glycogen but no starch or fiber. Overall, crustaceans have a limited ability to utilize dietary carbohydrates and poorly adapt to high levels of dietary complex or simple carbohydrates, although these animals tolerate disaccharides or more complex carbohydrates better than monosaccharides in their foods. For example, black tiger shrimp have a greater survival rate when fed trehalose, sucrose, starch, and dextrin compared with diets high in glucose (Alava and Pascual 1987).

Digestion of Carbohydrates in Fish and Crustaceans

Digestion of Starch and Glycogen

Fish do not have salivary glands or salivary α-amylase and, thus, there is no digestion of starch or glycogen in their mouth (Rønnestad et al. 2013). In all species of fish, the pancreas synthesizes and secretes α-amylase into the lumen of the intestine to digest starch and glycogen, as in terrestrial animals. However, both the secretion of α-amylase by the pancreas and its enzymatic activity differ greatly among fish species (Bakke et al. 2011). For example, the activity of α-amylase is generally greatest in herbivorous fish, where it is widespread throughout the entire digestive tract. In carnivorous fish (e.g., the Atlantic salmon, rainbow trout, and sea perch), α-amylase activity is low, possibly because of its gene mutation. In carnivorous, herbivorous, and omnivorous fishes, the intestinal mucosa does not synthesize or release α-amylase, but intestinal bacteria may be a source of this enzyme in the gut lumen. Of note, Frøystad et al. (2006) reported that a peptide segment is deleted from the Atlantic salmon α-amylase and that its specific activity in the intestine is only 0.67% of that for the common carp. In omnivorous fish (e.g., most types of freshwater tilapia), α-amylase activity is generally intermediate between herbivorous and carnivorous fish, and its activity is much greater in the pancreas than in the upper part of the intestine. In addition to the pancreas, intestinal bacteria may be a source of α-amylase in the gut of aquatic animals. As for mammals, the products of carbohydrate digestion by α-amylase in fish are di- and oligosaccharides, which are further hydrolyzed by sucrase-isomaltase and maltase-glucoamylase to form monosaccharides (e.g., glucose, fructose, galactose, mannose, xylose, and arabinose).

Dietary intake of starch increases the secretion of pancreatic α-amylase in herbivorous and omnivorous fish but has little effect in carnivorous fish (Bakke et al. 2011). The digestion of starch and dextrin by carnivorous fish decreases progressively if the content of dietary carbohydrates is more than 20%. However, some carnivores can utilize up to 60% glucose, sucrose, or lactose in their diets, indicating high

disaccharidase activity in their intestine. Thus, like herbivorous and omnivorous fish, some carnivorous fish can efficiently digest disaccharides. As reported for terrestrial animals, studies with channel catfish and rainbow trout have shown that fish have a higher ability to digest dietary carbohydrates after the ingredients (e.g., corn, wheat) are cooked to alter their structures and matrixes, resulting in complete hydration and swelling. Of note, largemouth bass (a carnivorous fish) cannot tolerate more than 10% starch in their diets (Li et al. 2020). Taken together, it is evident that pancreatic α-amylase activity is the major factor limiting the digestion of dietary starch and glycogen in fish.

In crustaceans, the hepatopancreas is the major site for the digestion of starch, glycogen, and oligosaccharides. Their pancreas (F-cells) synthesizes and releases α-amylase. In addition, sucrase-isomaltase and maltase-glucoamylase are synthesized and released from the enterocytes of the gut to hydrolyze disaccharides. There is no evidence for the synthesis or secretion of α-amylase by the intestinal mucosa of crustaceans, but this enzyme may be derived from intestinal bacteria. The digestibilities of starches vary among different types and animal species (see below).

Digestion of β-(1-4)-Linked Carbohydrates

In some species of fish and crustaceans (e.g., carnivores), the gastric and intestinal mucosae synthesize and secrete chitinase (Bakke et al. 2011). This enzyme may also be produced by bacteria present in the GIT. Chitinase cleaves β-(1-4) linkages in chitin to release *N*-acetyl-D-glucosamine. The presence of endogenous chitinase provides an adaptation for certain fish to break down chitin-containing exoskeletons of their prey animals.

Cellulase and β-galactosidase of bacterial origin are present in the gut of most species of carps and crustaceans. However, all fish are virtually incapable of hydrolyzing dietary fiber because they lack cellulase and β-galactosidase in the intestine, just like the stomach and the small intestine of terrestrial animals. In contrast, the pancreas of crustaceans synthesizes and releases a small amount of cellulase, which degrades some dietary cellulose in its different forms, such as cellulose, carboxymethyl cellulose, and cellobiose. This explains the poor ability of fish and crustaceans to digest dietary SBM that contains significant levels of the galactosidic oligosaccharides (e.g., raffinose and stachyose). The nutritive value of SBM and other legume seeds can be enhanced if they undergo enzymatic hydrolysis before meal production. It should also be pointed out that the nutritive value of pulses and other legume seeds can likewise be improved for fish and crustaceans because oligosaccharides constitute a large portion of the carbohydrates in legume seeds.

Overall Digestibility of Starch

In fish and crustaceans, the digestibility of dietary starch is affected by many factors, including (a) its molecular complexity, origin, degree of gelatinization, and content; (b) species, breeds, age, and health of fish; and (c) environmental temperatures. For example, in 10- to 25-g rainbow trout raised at 15°C fresh water, the apparent digestibilities of 20% potato starch and 20% dextrin in diets are 69% and 77%, respectively; in 80-g Atlantic salmon raised at 10.2°C seawater, the apparent digestibilities of 10.5%, 14.5%, and 21.3% wheat starch are 88%, 82%, and 75%, respectively; and in 28-g European sea bass raised at 25°C seawater, the apparent digestibilities of 20% raw starch and 20% gelatinized starch in diets are 66% and 95%, respectively (NRC 2011).

The apparent digestibilities of wheat, rice, and corn starches in spiny lobsters were 90.7%, 81.4%, and 60.1%, respectively (Rodríguez-Viera et al. 2014). Thus, in contrast to swimming crabs, corn starch is less digestible in spiny lobsters than all other starches studied, and its inclusion in diets reduces protein and DM digestibilities. Zhang et al. (2021) reported that in swimming crabs fed practical diets (containing 45.4% CP and 9.0% crude lipids, as well as 20% corn, wheat starches, or dextrin but not potato starch) are well digested. In Pacific white shrimp, the apparent digestibilities (%) of starch from the following sources were as follows: native corn, 85.1; high amylose corn, 63.1; waxy corn, 85.2; gelatinized corn starch, 93.9; gelatinized waxy corn starch, 95.8; native potato, 72.4; gelatinized potato starch, 93.1; and native wheat, 92.4 (Cousin et al. 1996). Thus, gelatinization improves the digestion of starch by shrimp, as reported for fish.

Absorption of Monosaccharides by the Intestine of Fish and Crustaceans

Monosaccharides released from the hydrolysis of starch, glycogen, and other complex carbohydrates in the gut are absorbed through transmembrane glucose or fructose transporters into the intestinal epithelial cells in fish, as in mammals (Ferraris 2001). For example, as shown for carnivorous fish (Polakof and Soengas 2013), the gilthead sea bream (Sala-Rabanal et al. 2004), and zebrafish (Castillo et al. 2009), the apical membrane SGLT1 plays a major role in the intestinal absorption of glucose, galactose, xylose, and arabinose into enterocytes. As in mammals, GLUT1 is also expressed in the apical membrane of fish intestinal enterocytes to absorb some of the luminal glucose (Teerijoki et al. 2000). GLUT5 has been reported to be expressed in the apical membrane of the intestinal enterocytes to absorb fructose from the intestinal lumen in fish (Sundell and Rønnestad 2011). The absorbed monosaccharides (e.g., glucose, fructose, galactose, mannose, xylose, or arabinose) exit the enterocytes through their basolateral membrane GLUT2 into the lamina propria where they are taken up into the intestinal venule. Through the blood circulation, the simple sugars move from the venule to the small vein and then to the portal vein. Depending on the species of fish as well as their developmental stage, living environment, and dietary composition, the efficiency of absorption of the monosaccharides from the intestinal lumen into the enterocytes is 95–99% (Li et al. 2022).

The processes of absorption of monosaccharides in crustaceans are the same as those in vertebrates described previously, except for the following. First, in crustaceans, the hepatopancreas synthesizes and releases cellulase for the hydrolysis of cellulose and its derivatives; therefore, these animals can digest a small amount of dietary fiber.

TABLE 5.6
Rates of Whole-Body Glucose Turnover in Animals[a]

Animal Species	Body Weight (kg)	Concentration of Glucose in Plasma (mM)	Whole-Body Glucose Turnover		
			g/h	g/h/kg BW	g/h/kg $BW^{0.75}$
Rat	0.2	5.5	0.9	4.5	3.01
Laying hen	2	12	1.6	0.80	0.95
Dog	15	5.0	2.5	0.17	0.33
Sheep	40	3.3	4.4	0.11	0.28
Pig	59	5.5	11.8	0.20	0.55
Human	71	5.0	9.1	0.13	0.37
Horse	186	5.0	15.3	0.082	0.30
Cow	500	3.3	30.0	0.060	0.28

Source: Adapted from Bergman et al. (1974, 1983).
[a] The values were obtained from nonruminant mammals and laying hens in the post-absorptive state or from sheep and cows in the fed state.
Note: BW, body weight; $BW^{0.75}$, metabolic body weight

Second, in crustaceans, the digestion of dietary carbohydrates is initiated and completed in the pyloric region of the stomach, where carbohydrases (released primarily by the F-cells of the hepatopancreas) act and are stored. Third, in crustaceans, the products of gastric carbohydrate digestion move to the hepatopancreas for absorption into its R-cells and then into the hemolymph. Fourth, in crustaceans, the midgut tube and hindgut play a minor role in the digestion of dietary carbohydrates or the absorption of its products.

GLUCOSE METABOLISM IN ANIMAL TISSUES

Glucose Turnover in the Whole Body

Glucose is actively utilized by all animals. This sugar enters different tissues at different rates and through different major transporters [e.g., GLUT4 in skeletal muscle (except for birds), heart, and adipose tissue; GLUT3 in the placenta; and GLUT2 in the liver and pancreas]. At a physiologically steady state, the rate of whole-body glucose utilization is equal to the rate of whole-body glucose synthesis. The continuous utilization and synthesis of glucose in the body is known as glucose turnover. Expressed per metabolic BW ($kg^{0.75}$), rates of whole-body glucose turnover are fairly constant among mammals but relatively high in avian species (Table 5.6). Rates of whole-body glucose turnover are reduced in response to food deprivation because of the lack of exogenous provision of substrates, but are increased under the conditions of pregnancy and lactation due to augmented demands for glucose. This is graphically illustrated from studies with sheep (Table 5.7).

Pathways for glucose metabolism in tissues are the same in animals (including ruminants, nonruminants, and fish), and thus these pathways are highlighted in this section.

TABLE 5.7
Effects of Fasting, Pregnancy, or Lactation on Whole-Body Glucose Turnover in Sheep[a]

Sheep	Body Weight (kg)	Concentrations of Glucose in Plasma (mM)	Glucose Turnover in the Whole Body		
			(g/h)	(g/h/kg BW)	(g/h/kg $BW^{0.75}$)
Nonpregnant					
Fed	53	3.3	4.6	0.087	0.23
Fasted[b]	53	3.0	3.0	0.057	0.15
Pregnant[c]					
Fed	68	2.6	7.5	0.110	0.32
Fasted[b]	71	1.6	4.6	0.064	0.19
Lactating (wk 2–4)					
Fed	61	3.2	13.3	0.218	0.61

Source: Adapted from Bergman et al. (1974, 1983).
[a] Nonpregnant or pregnant sheep were not in the lactating state.
[b] Fasted for 3 to 4 days.
[c] During the last month of twin pregnancy.
Note: BW, body weight; $BW^{0.75}$, metabolic body weight; wk, weeks

Catabolism refers to nutrient degradation, whereas anabolism is characterized by nutrient biosynthesis. However, a degradation pathway for one substance can be viewed as a synthetic pathway for another substance. For example, glutamine catabolism in the liver or kidney results in glucose synthesis. Although the metabolic pathways of glycolysis, glycogen synthesis, and the pentose cycle are present in all tissues, gluconeogenesis is restricted to the liver and kidneys in animals. At present, the quantitative aspects of glucose metabolism in fish are poorly understood, although glucose does not appear to be a major energy source for fish.

Pathway of Glycolysis

Definition of Glycolysis

Glycolysis is defined as the conversion of glucose into pyruvate. This pathway was discovered by Otto F. Meyerhof, who received the Nobel Prize in Physiology or Medicine in 1922. Glycolysis occurs in the cytosol of all animal cells. In the liver, glycolysis takes place primarily in perivenous hepatocytes. In cells without mitochondria (e.g., mammalian RBCs and bacteria), pyruvate is reduced by NADH + H^+ to lactate (Murray et al. 2001). Under conditions of limited or insufficient O_2 provision (e.g., the hypoxic uterine microenvironment during pregnancy, the hypoxic tumor microenvironment, and extensive exercise), large amounts of lactate are produced in cells with mitochondria. This is also true for certain cells (e.g., lymphocytes, macrophages, and tumors) even though O_2 is not limiting. In animals, pyruvate is oxidized to CO_2 and water.

Entry of Glucose into Cells via Different Transporters

Glucose transport is the first step in glucose utilization by the cell. Thus, it is important to understand the systems of glucose transport as described previously. Specifically, the entry of glucose into animal cells occurs via facilitated diffusion, which is an energy-independent process down a concentration gradient. Among twelve distinct facilitative glucose transporters (GLUT1 to GLUT12), GLUT2, GLUT3, and GLUT4 are known to be tissue specific (Chadt and Al-Hasani 2020). In polarized epithelial cells such as the enterocyte of the small intestine and the proximal tubule of the kidney, glucose transport across the apical membrane (facing the lumen) into the cell is also carried out by SGLT1 and SGLT2, respectively. In the small intestine and kidneys, glucose is transported from the cytoplasm into the extracellular space mainly by GLUT2, which is located on the basolateral membrane. As a result, SGLT1 and GLUT2 work together to efficiently absorb luminal glucose into venous blood circulation in the small intestine and SGLT2 and GLU2 to efficiently reabsorb the filtered luminal glucose back to the venous blood in the kidneys. In the livers of animals [including mammals, birds, and fish (Ferraris 2001; Krasnov et al. 2001)], GLUT2 is primarily responsible for both taking up and releasing glucose, depending on the glucose concentration gradient across the plasma membrane. GLUT3 is the major transporter of glucose in the placenta and the neurons of the brain but is also distributed in some animal tissues (e.g., brain, liver, heart, skeletal muscle, and kidneys). GLUT4 is the major GLUT isoform in the skeletal muscle, heart, and WAT of mammals and fish (Hall et al. 2006). In these tissues, GLUT4 expression is regulated by insulin at the mRNA and protein levels, and the GLUT4 protein translocates to the plasma membrane in response to insulin. Interestingly, GLUT4 is absent from the skeletal muscle of birds, contributing to elevated concentrations of glucose in their plasma. Furthermore, in calves, the expression of the GLUT4 gene and protein in skeletal muscle and WAT declines markedly after birth, indicating the development of insulin resistance in glucose utilization as cattle transition from suckling to functional ruminants (Smith 2017). Such a developmental change in GLUT4 activity may help to conserve glucose in ruminants. GLUT-5 (the fructose transporter) is expressed primarily in the small intestine, testes, and placenta but also present in skeletal muscle, kidneys, brain, and WAT.

It is difficult to determine the kinetics of glucose transport by individual transport isoforms because of the lack of mammalian cells expressing only a single isoform, with the exception of human erythrocytes. Human erythrocytes express only GLUT1. To overcome the problem of having multiple isoforms of GLUT present in most mammalian cells, *Xenopus laevis oocytes*, which have low levels of endogenous glucose transport and are amenable to the expression of specific transport proteins, have been widely used for measuring the kinetics of glucose transport catalyzed by a single GLUT isoform. Because glucose is readily metabolized by mammalian cells, various nonmetabolizable glucose analogues have been used to measure glucose transport kinetics. For example, 2-deoxyglucose, which can be phosphorylated by hexokinase but not further metabolized, is useful under conditions where phosphorylation is not rate-limiting. 3-*O*-Methylglucose is neither phosphorylated nor metabolized by mammalian cells and thus is the preferred substrate for kinetics analyses. The K_M values (mM) of various glucose transporters for 3-*O*-methylglucose are as follows: GLUT1, 16.9–26.2; GLUT2, 40; GLUT3, 10.6; GLUT4, 1.8–4.8 (Olson and Pessin 1996). Although K_M measurements in oocytes are quantitatively different from those determined for the endogenous glucose transporters, the above data suggest that GLUT3 and GLUT4 have a higher affinity for glucose than GLUT1 and GLUT2. Among the known GLUTs, GLUT2 has the highest K_M for glucose, which allows for this transporter to function as a glucose sensor.

Pathway of Glycolysis

The pathway of glycolysis (also known as the Embden-Meyerhof pathway) is outlined in Figure 5.6. The nonequilibrium reactions of glycolysis are catalyzed by the glucose transport system, hexokinase, phosphofructokinase-1, and pyruvate kinase. The flux-generating step for glycolysis has been considered to be the absorption of glucose from the

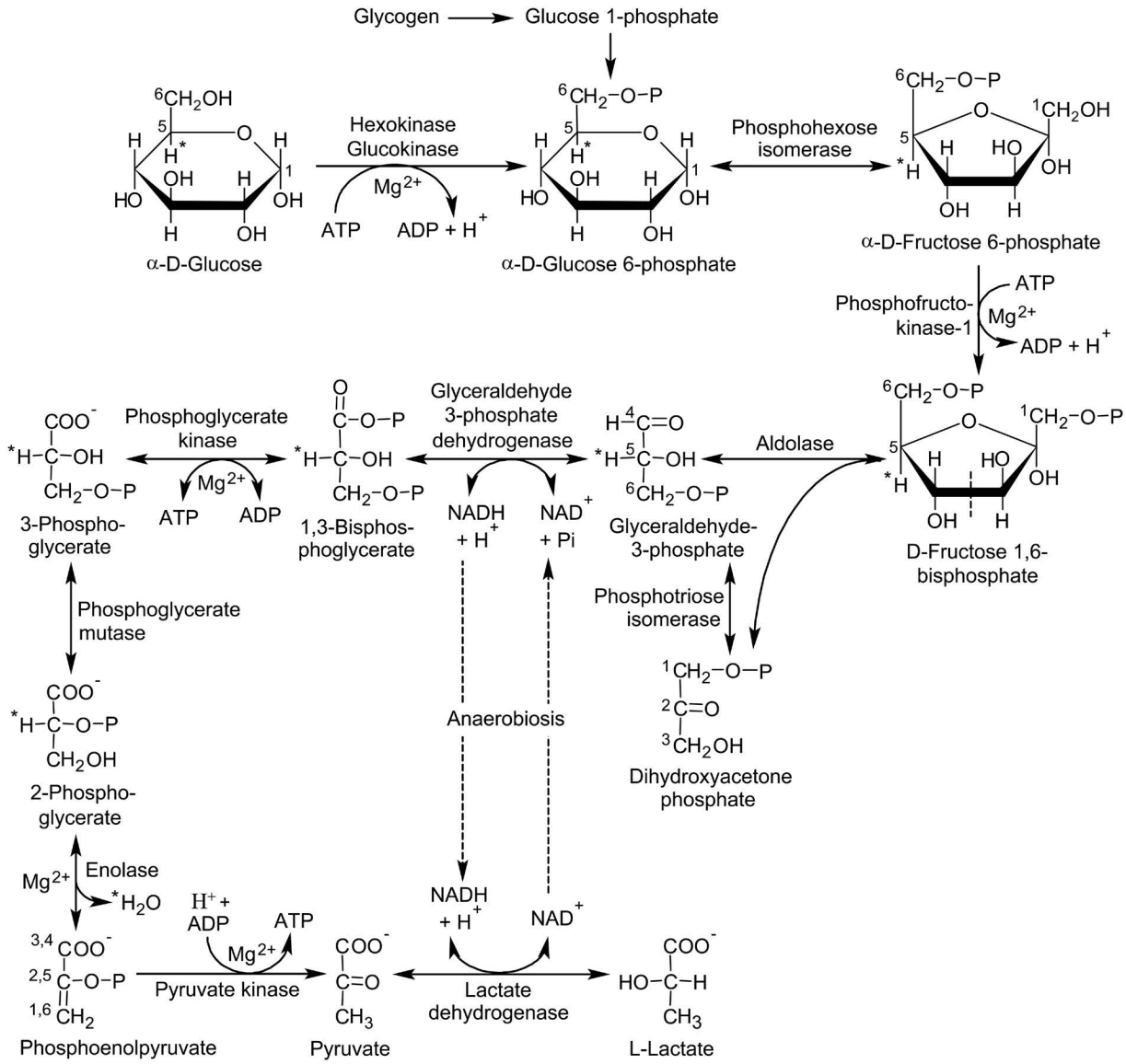


FIGURE 5.6 The pathway of glycolysis in animal cells. Glycolysis occurs in the cytosol of all animal cells or microbes. The conversion of pyruvate to lactate in the cytosol or the oxidation of pyruvate to acetyl-CoA in mitochondria is required to regenerate NAD^+ so that glycolysis can proceed. Glycolysis is the sole source of ATP for mammalian red blood cells and a major source of ATP for some animal cells (e.g., cells of the immune system) under aerobic conditions, while providing significant amounts of energy for all animal cells under anaerobic conditions. This pathway is obligatory for complete oxidation of glucose to CO_2 and water. Note that glucokinase is also known as hexokinase IV, which is present in the livers of humans, pigs, rats, and many other single-stomached mammals but is virtually absent from the livers of ruminants, many birds (including chickens), and certain fish species. The numbers 1–6 indicate the position of carbons in molecules. The sign "*" denotes a specific hydrogen atom. (Adapted from Devlin 2011.)

small intestine or, when glucose arises endogenously, the breakdown of glycogen in the liver. Key regulatory steps of glycolysis are outlined as follows.

1. *Hexokinase*. In mammalian cells, the phosphorylation of glucose to glucose-6-P is catalyzed by a family of closely related enzymes called hexokinases. Mg^{2+} is a cofactor for all hexokinase isozymes. Four mammalian hexokinases have been characterized. They are designated as hexokinase (HK) I, II, III, and IV based on their relative mobility following starch gel electrophoresis (Table 5.8). HK-I is ubiquitous in animal cells, whereas HK-II is primarily present in the skeletal muscle, heart, and adipose tissue. Expression of HK-III is relatively low in most tissues, including the lungs, kidneys, and liver. HK I, II, and III can phosphorylate both glucose and fructose, but their enzymatic activity toward fructose is 88% of that toward glucose (Weinhouse 1976).

TABLE 5.8
Comparison of Enzymatic Kinetics among Hexokinases

Variable	GK (HK IV)	HK I, II, and III
K_M for glucose	5–12 mM	0.02–0.13 mM
K_M for ATP	~ 0.5 mM	0.2–0.5 mM
Ki for glucose-6-phosphate	60 mM	0.2–0.9 mM
Molecular weight	52 kDa	~ 100 kDa
Substrate preference		
Glucose	1	1
Mannose	0.8	1–1.2
2-Deoxyglucose	0.4	1–1.4
Fructose	0.2	1.1–1.3

Source: Olson and Pessin (1996).
Note: GK, glucokinase; HK, hexokinase.

HK-IV (also known as glucokinase) is specifically expressed in the liver and pancreatic ß-cells. This enzyme converts glucose into glucose-6-P (as do HK I, II, and III), has very low activity toward fructose (Table 5.8), and is inactive toward galactose, allose, sorbitol, xylose, 3-methylglucose, and glucosamine (Weinhouse 1976). There are species differences in the hepatic expression of glucokinase: present at relatively high levels of activity in the livers of common frogs, dogs, guinea pigs, hamsters, humans, mice, monkeys, pigs, rabbits, rats, and turtles; very low or negligible in the livers of cats, goldfish, and finch; and absent from the livers of rattlesnakes, rainbow trout, ruminants (e.g., cattle and sheep), chickens, and nine other species of birds (Weinhouse 1976). Of note, the hepatic activity of glucokinase in adult rats is 3.4 times that of hexokinase (Vinuela et al. 1963). In those animals that have negligible or no glucokinase activity, their livers have a low ability to catabolize glucose under physiological conditions. For example, only 1.2% of the glucose supplied to the ruminant liver by the combined blood of the portal vein and the hepatic artery is extracted by this organ even under fed conditions (Bergman 1983). This is nutritionally significant for sparing glucose in ruminants, which have no absorption of glucose by the small intestine when fed a roughage diet (Loncke et al. 2020).

HK I, II, and III have relatively low K_M values (<0.2 mM) for glucose and would be saturated by physiological concentrations of this sugar in most cell types, whereas HK-IV has a high K_M value (5–12 mM) for glucose and would respond to elevated glucose concentrations in the plasma after feeding. The differences in tissue expression and kinetics of HK I-IV suggest that each isozyme has a distinct metabolic role. For example, in cattle and sheep, the lack of glucokinase in hepatocytes results in the negligible uptake of glucose by the liver. For nonruminants, because the K_M value of HK-IV for glucose is much higher than that of HK I, II, and III, rates of glucose utilization by the liver are lower than those in skeletal muscle. Thus, when physiological plasma concentration rises in response to feeding, intracellular glucose in skeletal muscle, heart, and adipose tissue is readily phosphorylated by HK-II to form glucose-6-P. This reaction traps glucose inside the cell because the plasma membrane is not permeable to glucose-6-P. Similarly, during and immediately after consumption of a carbohydrate meal, HK-IV converts glucose into glucose-6-P in the liver of nonruminants. In contrast, when the plasma glucose concentration is reduced during fasting, hepatic HK-IV activity is reduced and the liver is a net producer of glucose from glucogenic substrates. Therefore, HK-IV is a "glucose sensor" in liver because GLUT2 is in liver and gastrointestinal tissues. This is also true in pancreatic ß-cells.

2. *Phosphofructokinase-1 (PFK-1).* This enzyme catalyzes the conversion of fructose-6-P to fructose-1,6-bisphosphate and plays a major role in the regulation of the rate of glycolysis (Han et al. 2016). PFK-1 is an allosteric and inducible enzyme. As noted in Chapter 1, an allosteric enzyme is an enzyme whose activity at the catalytic site may be modulated by the presence of allosteric effectors at an allosteric site. "Allosteric" means "occupy another space."
3. *Pyruvate kinase.* Pyruvate kinase catalyzes the transfer of a phosphate group from PEP to ADP, generating pyruvate and ATP. This enzyme requires Mg^{2+} as a cofactor and has two isoforms: type M (muscle) and type L/R (liver and erythrocyte). The isozymes differ in their primary structures.
4. *Lactate dehydrogenase (LDH).* LDH has two isoforms: A and B. Both isozymes are widespread in animal cells and catalyze the reversible interconversion of pyruvate into lactate, but LDH A and B favor the formation of lactate and pyruvate, respectively. The expression levels of these two isozymes vary among tissues. Lactate-generating tissues and cells have a relatively high abundance of LDH-A, but lactate-using tissues and cells have a relatively high abundance of LDH-B. For example, LDH-A is more abundant than LDH-B in skeletal muscle, immunocytes, erythrocytes, conceptuses, and distal renal tubules; they release lactate as a vehicle for the interorgan transport of glucose carbons. In contrast, LDH-B is more abundant than LDH-A

in the heart, brain, periportal hepatocytes, and proximal renal tubules; they contribute to glucose synthesis or acetyl-CoA (ATP) production via the formation of pyruvate in a cell- and tissue-specific manner, depending on physiological and nutritional conditions.

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \rightleftharpoons \text{L-Lactate} + \text{NAD}^+$$

Energetics of Glycolysis

The conversion of 1 mol of glucose to 2 mol of pyruvate is accompanied by the net production of 2 mol of ATP (see the calculations below) and by the reduction of 2 mol of NAD^+ to NADH + H^+. As noted previously, glycolysis is the only metabolic pathway for ATP production by all cells without mitochondria regardless of oxygen supply. The energetic efficiency of glycolysis is low. Thus, the use of large amounts of glucose via this metabolic pathway under pathological conditions such as cancer results in wasting.

The overall reaction of glycolysis:

$$\text{Glucose} + 2\text{P}_\text{i} + 2\text{ADP} + 2\text{NAD}^+ \rightarrow 2\text{Pyruvate} + 2\text{NADH} + 2\text{H}^+ + 2\text{ATP} + 2\text{H}_2\text{O}$$

1 mol of ATP is required for phosphorylation of glucose:

$$\text{Glucose} + \text{ATP} \rightarrow \text{Glucose-6-P} + \text{ADP} + \text{H}^+ \left(\text{Hexokinase; } \text{Mg}^{2+}\right)$$

1 mol of ATP is required for phosphorylation of fructose-6-P:

$$\text{Fructose-6-P} + \text{ATP} \rightarrow \text{Fructose-1,6-bisphosphate} + \text{ADP} + \text{H}^+ \left(\text{PFK-1}\right)$$

2 mol of ATP are produced from conversion of 2 mol of 1,3-bisphosphoglycerate to 3 phosphoglycerate. This reaction is catalyzed by phosphoglycerate kinase as follows:

$$2\left[\text{1,3-Bisphosphoglycerate}\right] + 2\text{ADP} + 2\text{H}^+ \rightleftharpoons 2\left[\text{3-Phosphoglycerate}\right] + 2\text{ATP}$$

2 mol of ATP are produced from conversion of 2 mol of PEP to pyruvate (catalyzed by pyruvate kinase):

$$2\text{PEP} + 2\text{ADP} \rightarrow 2\text{Pyruvate} + 2\text{ATP}$$

Net production of ATP from the conversion of 1 mol of glucose to 2 mol of pyruvate:

$$2 + 2 - 1 - 1 = 2 \text{ mol of ATP}$$

In biochemcal reactions, the electric charges of reactnts and products must be balanced. At pH 7.0, ATP, ADP, AMP, inorganic phosphate (Pi), and inorganic pyrophosphate (PPi) exist mostly as ATP^{4-}, ADP^{3-}, AMP^{2-}, HPO_4^{2-}, and $P_2O_7^{4-}$, respectively. Likewise, the electric charges of some metabolites are as follows: glucose-6-P, –2; fructose-1,6-bisphosphate, –4; phosphoglycerate, –2; 1,3-bisphosphoglycerate, –4; PEP, –2; pyruvate, –1; and lactate, –1.

Conversion of Pyruvate into Lactate and the Cori Cycle

In cells without mitochondria, pyruvate is converted into lactate by LDH, with the oxidation of NADH to NAD^+. This reaction also takes place in mitochondria-containing cells (e.g., immunocytes and tumors) under anaerobic conditions. The conversion of pyruvate to lactate allows for the regeneration of NAD^+ so that glycolysis proceeds in cells.

$$\text{D-Glucose} + 2\text{ADP} + 2\text{P}_\text{i} \rightarrow 2\text{L-Lactate} + 2\text{ATP} + 2\text{H}_2\text{O}$$

In 1929, G.F. Cori and G. Cori proposed that lactate is released from skeletal muscle and is taken up by the liver, where it is converted to glucose. The lactate-derived glucose is taken up by the muscle. This pathway was named the Cori cycle and recognized with the Nobel Prize in Physiology or Medicine in 1947. It is now known that cells other than skeletal muscle, including lymphocytes and macrophages, also actively convert glucose into lactate and release lactate. There is no net production of glucose from lactate via the Cori cycle.

Conversion of Pyruvate into Lactate or Ethanol

Under anaerobic conditions, pyruvate is converted to lactate by LDH, and to ethanol by pyruvate decarboxylase (cytosolic and mitochondrial) and alcohol dehydrogenase in yeast, with the oxidation of NADH to NAD^+. The conversion of pyruvate to ethanol allows for the regeneration of NAD^+ so that glycolysis proceeds to increase ethanol production.

$$\text{Pyruvate} \rightarrow \text{Acetaldehyde} + \text{CO}_2 \left(\text{Yeast}\right)$$

$$\text{Acetaldehyde} + \text{NADH} + \text{H}^+ \rightleftharpoons \text{Ethanol} + \text{NAD}^+ \left(\text{Yeast}\right)$$

Cytosolic Redox State in Animal Cells

LDH (a cytosolic enzyme) catalyzes the interconversion of lactate and pyruvate. The equilibrium constant (K_{eq}) of this reaction is calculated as follows.

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \rightleftharpoons \text{L-Lactate} + \text{NAD}^+$$

$$K_{eq} = \left(\left[\text{L-Lactate}\right] \times \left[\text{NAD}^+\right]\right) / \left(\left[\text{Pyruvate}\right] \times \left[\text{NADH}\right] \times \left[\text{H}^+\right]\right)$$

Because K_{eq} is a constant and intracellular pH is constant under normal physiological conditions, an increase in the ratio of [lactate]/[pyruvate] is equivalent to that of [NADH]/[NAD^+]. Because this reaction occurs in the cytosol, the [lactate]/[pyruvate] ratio is a useful indicator of the cytosolic redox state (i.e., [NADH]/[NAD^+] ratio) in animal

cells. When intracellular pH fluctuates considerably, $[H^+]$ must be included in the calculation of K_{eq}.

Glycolysis and Cell Proliferation

All cultured cells have a high rate of glycolysis even in the presence of adequate amounts of oxygen, which is referred to as aerobic glycolysis. Cell proliferation is associated with an enhanced rate of glycolysis, and therefore H^+ production. Cells cannot divide or grow in the absence of glucose. In mitogen-stimulated lymphocytes (e.g., thymocytes) with a rapid rate of proliferation, increased cytosolic regeneration of NAD^+ by enhanced expression of LDH effectively competes with the transport of NADH into mitochondria. Because glycolysis does not generate reactive oxygen species (ROS), highly proliferating cells can avoid oxidative stress.

Pasteur Effect in Animal Cells

The Pasteur effect refers to the inhibition of glycolysis by aerobic oxidation in mitochondria-containing cells. This phenomenon, which was discovered in 1857 by Louis Pasteur, results from the inhibition of PFK-1 by ATP and citrate that are produced by aerobic oxidation via the Krebs cycle. In other words, when oxygen concentration is low, glucose-derived pyruvate is converted into lactate (or ethanol plus CO_2). When oxygen concentration is high, pyruvate is oxidized to acetyl-CoA that enters the Krebs cycle for oxidation to CO_2 and water, thereby inhibiting glycolysis.

Warburg Effect

Some cells (e.g., tumors, activated macrophages, and proliferating lymphocytes) prefer to metabolize glucose through glycolysis even in the presence of ample oxygen. This phenomenon is known as the Warburg effect. In cancer cells, drivers for the metabolic derangements include (a) adaptation to the extracellular microenvironment (low pH and low pO_2); and/or (b) aberrant signaling of oncogene activation (p53, Myc, Ras, Akt, and HIF). Metabolic advantages are to (a) increase rates of biosynthesis of nucleotides, lipids, and proteins; (b) avoid apoptosis by reducing the production of ROS through the mitochondrial electron transport system; and (c) participate in local metabolite-based paracrine and autocrine signaling. Adverse outcomes of the Warburg effect are the production of large amounts of metabolites (e.g., lactate from glucose) and the wasting of body energy reserves (e.g., formation of glucose from proteins and amino acids, such as glutamine and alanine).

Quantification of Glycolysis in Animal Cells

Under anaerobic conditions, the accumulation of lactate is a valid indicator of the rate of glycolysis. Under aerobic conditions, glycolysis is usually measured by using [5-^{3}H] glucose (Wu and Thompson 1988). This is based on the principle that (a) 1 mol of ^3H_2O is produced from 1 mol of [5-^{3}H]glucose via glycolysis, and (b) the ^3H_2O produced is extensively diluted by intracellular and extracellular H_2O, so that re-incorporation of ^3H_2O into metabolic pathways is negligible. Note that the use of [2-^{3}H]glucose to measure glycolysis is invalid because [2-^{3}H]glucose-6-P loses its ^{3}H in its conversion to fructose-6-P catalyzed by phosphoglucoisomerase. Indeed, the rate of detritiation of [2-^{3}H] glucose is commonly used to estimate the rate of glucose phosphorylation in intact cells (Van Schaftingen 1995).

Nutritional and Physiological Significance of Glycolysis

Starch (the polymer of glucose) is the most abundant non-water nutrient in the diets of omnivores and vegetarians. In addition, glucose is the predominant sugar in the blood of animals. Thus, glycolysis is of nutritional and physiological significance for animals.

ATP production in Animal Cells

Glycolysis provides ATP for (a) all animal cells (including myocytes, trophoblasts, and cells of the immune system) under both aerobic and hypoxic conditions, (b) animal cells without mitochondria (including the RBCs of mammals and the mature lens fiber cells of the eyes in vertebrates) as the sole source of energy, (c) anaerobic microbes, and (d) tumors regardless of O_2 provision. Of note, glycolysis is the major source of energy for the kidney medulla that receives low O_2 supply and function at the verge of hypoxia, as well as for certain cells (e.g., the basal cells of the corneal epithelium and the retinal Müller glial cells in the eyes) with few mitochondria or limited mitochondrial activity. Note that the presence of mitochondria (that are typically reddish-brown in color) in the mature lens fiber cells of the eyes would hinder the transparency of light to the retina.

An Essential Metabolic Pathway

In mitochondria-containing cells, glycolysis allows for the catabolism of glucose into pyruvate under both normoxic and hypoxic conditions. Glycolysis is obligatory for the complete oxidation of glucose to O_2 and water, and plays a role in regulating glucose homeostasis in all of these cells. Furthermore, 2,3-bisphosphoglycerate, an intermediate in glycolysis, stabilizes the tetrameric structure of hemoglobin and modulates its binding affinity for O_2 in RBCs. This helps sustain a relatively long half-life of RBCs (e.g., ~120 and 80–500 days for mammalian and fish RBCs, respectively; Götting and Nikinmaa 2017).

An Amphibolic Role in Cell Metabolism

Glycolysis plays an important role in both catabolic and synthetic processes, which are collectively known as an amphibolic role. Specifically, glycolysis supplies intermediates for synthetic pathways: (a) glucose-6-P, which is a precursor of ribose-5-P required for synthesis of purine and pyrimidine nucleotides; (b) glycerol-3-P and dihydroxyacetone-P, which are precursors for the synthesis of triacylglycerols (TAGs) and phosphoglycerols; and (c) fructose-6-P, which is a precursor for the synthesis of glucosamine-6-P by glutamine:fructose-6-P amidotransferase (cytosolic). This enzyme is now referred to as glutamine:fructose-6-P transaminase. Glucosamine-6-P is the substrate for the synthesis of UDP-N-acetylglucosamine, a precursor for the formation of all macromolecules containing amino sugars

in cells. In postnatal animals, glucosamine-6-P may mediate tissue insensitivity to insulin in type-II diabetes mellitus and diabetes-associated cardiovascular abnormalities.

$$\text{Fructose-6-P} + \text{Glutamine} \rightarrow \text{Glucosamine 6-P} + \text{Glutamate}$$

Lack of Production of Oxidants

The production of lactate from glucose via glycolysis does not generate ROS, therefore protecting cells and tissues (e.g., immunocytes, RBCs, and tumors) from oxidative stress. In addition, the ratio of lactate/pyruvate aids in maintaining proper redox balance in the cytosol. These phenomena are of physiological importance in vertebrate and invertebrate cells, as well as gastrointestinal microbes. However, tumors take advantage of this biochemical property to avoid being killed and sustain rapid growth in the hosts.

Lactate as a Signaling Molecule

Lactate has long been considered a metabolic waste since its discovery in 1780 by Carl Wilhelm Scheele. However, lactate has now been recognized as a signaling molecule in animals during both prenatal and postnatal growth by regulating energy supply, cell differentiation, and tissue development (Johnson et al. 2023; Moses et al. 2024b; Vavřička et al. 2024). Lactate acts by binding to the transmembrane G-protein-coupled receptors (GPRs) such as GPR81 (hydroxycarboxylic receptor 1), GPR109A (hydroxycarboxylic receptor 2), and GPR109B (hydroxycarboxylic receptor 3) that mediate down-stream signal transduction and physiological responses such as the induction of hypoxia-inducible and angiogenic factors (Hilger et al. 2018). Thus, through mediating cell signaling, lactate may play important roles in improving cardiovascular, metabolic, endocrine, reproductive, immune, musculoskeletal, and neurological health.

Lactate as an Allosteric Regulator of Enzymatic Activity

Lactate binds to intestinal proline oxidase and is a noncompetitive inhibitor of this enzyme (decreased maximal velocity and unaltered Michaelis constant; Dillon et al. 1999). Thus, addition of 1, 5, or 10 mM lactate to the medium for the incubation of porcine enterocytes decreases the synthesis of ornithine, citrulline, and arginine from proline in a concentration-dependent manner. These results explain why hypocitrullinemia and hypoargininemia but hyperprolinemia occurs in infants with elevated plasma concentrations of lactate (up to 14 mM). Likewise, lactate (e.g., at 10 mM) is an allosteric inhibitor of hexokinase and PFK-1 in diverse tissues (including skeletal muscle), thereby reducing the rate of glycolysis and preserving glucose (Leite et al. 2011). In contrast, at elevated concentrations (e.g., 10 mM), lactate binds cytosolic acetyl-CoA carboxylase and activates this enzyme through its confirmational changes to promote fatty acid synthesis in T helper cells (also known as $CD4^+$ T cells), thereby possibly acting as a pro-inflammatory signal in inflamed tissues (Pucino et al. 2019). Furthermore, as extracellular concentrations of lactate increase, it enters the mitochondrial matrix, binds to enzymes/proteins in the mitochondrial electron transport system, and stimulates its activity for substrate oxidation in tumors and primary T cells (Cai et al. 2023). This results in a decrease in glycolysis but an increase in mitochondrial ATP synthesis from the oxidation of pyruvate and amino acids to support rapid cell proliferation. Thus, lactate may act as an allosteric inhibitor or activator of enzymes/proteins in animal cells, depending on physiological needs and pathological states.

Lactylation

Lactylation is a form of post-translational modification of proteins by lactate. Using macrophage polarization as a model, Zhang et al. (2019) found that M1 macrophages (proinflammatory cells that have active aerobic glycolysis) have high levels of lactylation in histone's lysine residues and that during their transition to a M2 subtype (anti-inflammatory cells), lactylation (occurring specifically in genes for M2 phenotypes) directly stimulated gene transcription. Subsequently, Galle et al. (2022) reported that histone H3K18 lactylation in mouse embryonic stem cells, macrophages, and white adipocytes, as well as mouse and human skeletal muscles, could mark both promoters and active enhancers in a tissue-specific manner, further supporting a role for lactate in epigenetic regulation. Furthermore, there is evidence that lactylation shapes the epigenomic landscape to promote the expression of genes that are essential for the development of cells such as neural crest stem cells (Merkuri et al. 2024). These cells have high rates of glycolysis when they proliferate and maintain pluripotency, but exhibit a decrease in glycolysis when they start to differentiate into specific cell types.

Regulation of Glycolysis

Glycolysis can be regulated at the steps of glucose transport, hexokinase, PFK-1, and pyruvate kinase (Table 5.9). All these enzymes are inhibited by medium- and long-chain fatty acids. This is an excellent example of the concept of "distributive metabolic control." For example, fructose-1,6-bisphosphate is an allosteric activator of pyruvate kinase in tissues (e.g., liver and skeletal muscle), therefore serving as a positive feed-forward regulator. Because a persistent high concentration of glucose in the plasma of fasting subjects (hyperglycemia, >6.1 mM) is a diagnostic indicator of diabetes mellitus (a serious metabolic disease), extensive research has been conducted on glucose metabolism in mammalian cells. The near absence of glucose-stimulated insulin secretion is the common feature of insulin-dependent (type-I) diabetes mellitus (IDDM), whereas patients with noninsulin-dependent (type-II) diabetes mellitus (NIDDM) can still produce insulin or even more insulin early in the disease. The discovery of insulin and its role in stimulating glycolysis led to the award of the Nobel Prize in Physiology or Medicine to Frederick G. Banting in 1921.

Results of recent studies have revealed an important role for AMP-activated protein kinase (AMPK) in the regulation

TABLE 5.9
Regulation of Glycolysis in Animal Cells

Target of Action	Activation or Induction	Inhibition or Suppression
GLUT-1	AMPK, phorbol esters, sulfonylureas, glucose, butyrate, vanadate, hypoxia, cAMP, serum, thyroid hormones, insulin, IGF-1, PDFG, FGF, growth hormone, TGF-ß, oncogenes	Rubusoside (a phytochemical), forskolin, cytochalasin B
GLUT-2	Glucose, protein kinase C, AMPK	Diabetes, forskolin, cytochalasin B
GLUT-4	Insulin, muscle contraction, cAMP, AMPK	Diabetes, food deprivation, forskolin, cytochalasin B
GLUT-5	Fructose, glucocorticoids, AMPK, thyroid hormones	Phytochemicals (rubusoside and astragalin-6-glucoside)
HK-1, 2, 3	Hypoxia-inducible factors, Akt, MTOR	Glucose-6-P and Pi
Glucokinase	Insulin, carbohydrate feeding	Glucagon, cAMP, diabetes, food deprivation, glucosamine
PFK-1	Insulin, carbohydrate feeding, AMP, ADP, Pi, F-6-P, F-2,6-bisphosphate, MTOR[a]	Citrate, ATP, low pH, cAMP, PEP, glucagon, diabetes, fast
Pyruvate kinase	Insulin, carbohydrate feeding, fructose, fructose-1-6-bisphosphate	ATP, alanine, glucagon, diabetes, food deprivation

[a] The MTOR phosphorylates phosphofructose kinase 2 (PFK-2), which increases the conversion of fructose-6-P into fructose-2,6-bisphosphate, an allosteric activator of PFK-1.

Note: Akt, protein kinase B; AMPK, AMP-activated protein kinase; F-6-P, fructose-6-P ; F-2-6-P, fructose-2,6-bisphosphate; IGF-1, insulin-like growth factor-1; PDFG, platelet-derived growth factor; FGF, fibroblast growth factor; MTOR, mechanistic target of rapamycin, PEP, phosphoenolpyruvate; TGF-ß, transforming growth factor-ß.

of glycolysis in animal cells. Specifically, AMPK increases the expression and activity of GLUT-1, GLUT-2, GLUT-4, GLUT-5, and hexokinase-2 to promote the transmembrane transport of glucose and fructose by cells. In addition, the mechanistic target of rapamycin (MTOR) phosphorylates phosphofructose kinase 2 (PFK-2), the enzyme that catalyzes the conversion of fructose-6-P into fructose-2,6-bisphosphate, an allosteric activator of PFK-1. Of note, canagliflozin, an inhibitor of SGLT2 (the protein responsible for the reabsorption of glucose by kidney tubules, as noted previously), can activate AMPK in a manner similar to the action of metformin, i.e., the promotion of formation of the AMPK αβγ heterotrimeric complex (Steinberg and Carling 2019). Furthermore, physiological levels of NO mediate insulin-stimulated glucose uptake by skeletal muscle (Dai et al. 2013).

Transfer of NADH from the Cytosol into Mitochondria

Under aerobic conditions that favor the oxidation of pyruvate, the NADH generated from the pathway of glycolysis enters mitochondria for oxidation. Because NADH cannot penetrate the inner mitochondrial membrane, it is transported into the mitochondria via the glycerophosphate shuttle (Figure 5.7) and the malate shuttle (Figure 5.8). In the glycerophosphate shuttle, mitochondrial glycerol-3-P dehydrogenase is a flavoprotein with FAD rather than NAD as the cofactor, and the formation of $FADH_2$ from FAD is coupled with the conversion of glycerol-3-P to dihydroxyacetone-P. As a result, only 1.5 rather than 2.5 mol of ATP are produced for oxidation of 1 mol of cytosolic NADH. This shuttle is present at high activity in insect flight muscles. However, in mammals and birds, the expression of mitochondrial glycerol-3-P dehydrogenase is highly variable in tissues because the activity of this enzyme is present in the brain, brown adipose tissue (BAT), liver, and white skeletal muscle but is deficient in the heart and many other tissues (Mráček et al. 2013). The malate shuttle involves malate, glutamate, and aspartate, and the complexity of this system is due to the impermeability of the mitochondrial membrane to OAA. The malate shuttle is widely present in cells and tissues of all animals and is considered to be of more universal utility than the glycerophosphate shuttle in vertebrates

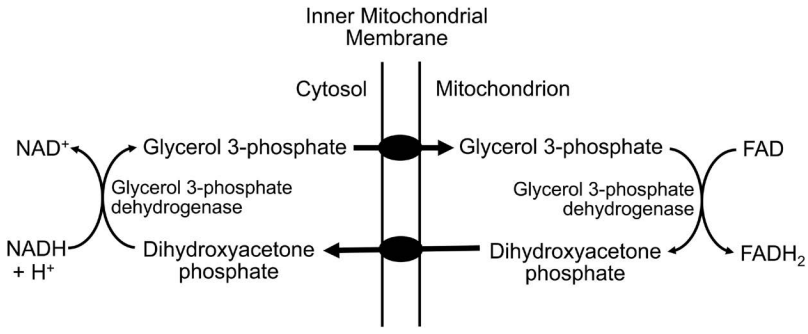


FIGURE 5.7 The glycerophosphate shuttle for the transport of NADH from the cytosol into mitochondria in animal cells. This shuttle requires cytosolic and mitochondrial glycerol-3-phosphate dehydrogenases, which are different proteins and are encoded by different genes. The glycerophosphate shuttle is highly active in insect flight muscles but occurs in mammals and birds in a tissue-specific manner. In these vertebrates, the expression of mitochondrial glycerol-3-phosphate dehydrogenase is highly variable, with its enzyme activity being present in the liver, brain, brown adipose tissue, white adipose tissue, and white skeletal muscle but being deficient in the heart and many other tissues.

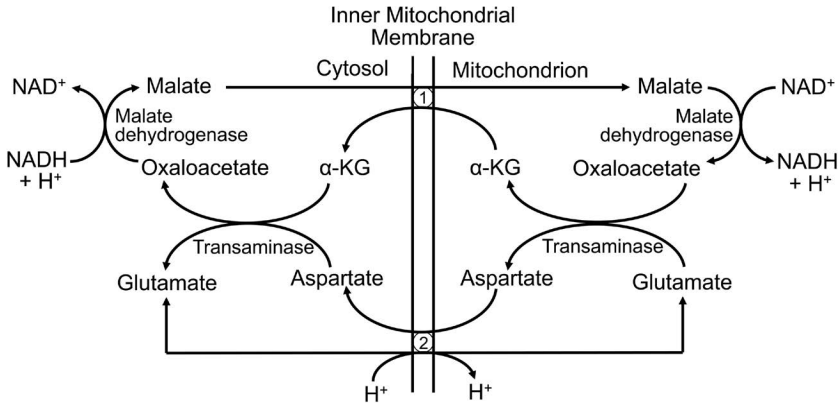


FIGURE 5.8 The malate shuttle for the transport of NADH from the cytosol into mitochondria in animal cells. This shuttle requires glutamate and aspartate, cytosolic and mitochondrial malate dehydrogenases (MDH), as well as transporters for malate, α-ketoglutarate (α-KG), and aspartate. The malate shuttle is widely present at high activity in the cells of all animals and is of more universal utility than the glycerophosphate shuttle for transporting NADH from the cytosol into mitochondria in vertebrates (e.g., mammals and birds).

(Ying 2008). In the heart, the malate shuttle is the dominant pathway for transporting NADH from the cytosol into mitochondria, with its activity being 10-fold or greater than that of the glycerophosphate shuttle. When NADH is transported into mitochondria via the malate shuttle, it is oxidized via the electron transport system to produce 2.5 mol of ATP/mol NADH. However, when NADH is transported into mitochondria via the glycerophosphate shuttle, it is oxidized via the electron transport system to produce 1.5 mol of ATP/mol NADH.

Mitochondrial Oxidation of Pyruvate to Acetyl-CoA

Pyruvate can be produced from glucose, lactate, and most amino acids (e.g., alanine, serine, cysteine, threonine, glycine, glutamine, glutamate, aspartate, and asparagine). If pyruvate is formed in the cytosol (e.g., in glycolysis), it must be transported into the mitochondria via a pyruvate or monocarboxylic acid transporter. Within the mitochondria, pyruvate undergoes oxidative decarboxylation to acetyl-CoA by the pyruvate dehydrogenase complex (often simply referred to as pyruvate dehydrogenase, PDH), which is attached to the inner side of the inner mitochondrial membrane. The pyruvate dehydrogenase complex consists of three enzymes (pyruvate dehydrogenase, dihydrolipoyl transacetylase, and dihydrolipoyl dehydrogenase) that work sequentially to produce CO_2 (from carbons 3 and 4 of glucose) and acetyl-CoA (Figure 5.9). The carbon 2 (i.e., the methyl carbon) of acetyl-CoA is derived from carbons 1 and 6 of glucose, whereas the carbon-1 of acetyl-CoA is derived from carbons 2 and 5 of glucose.

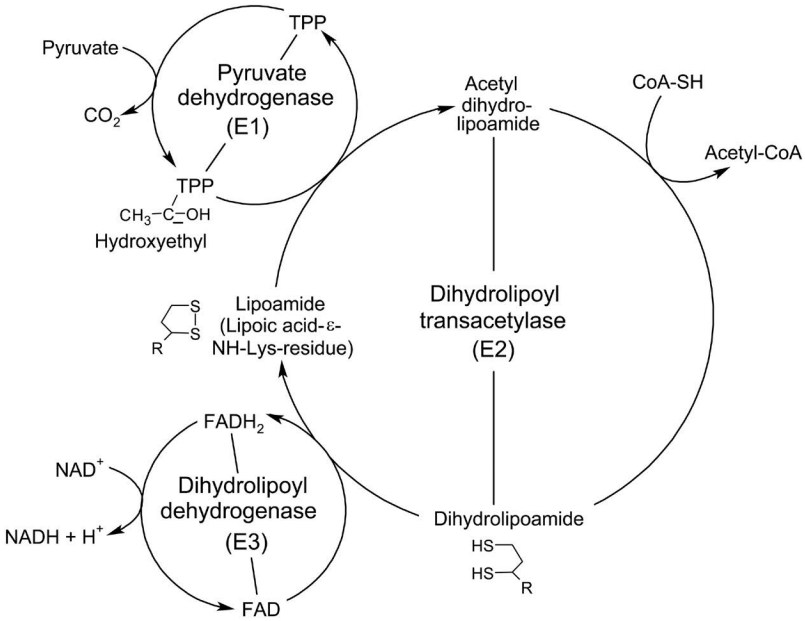


FIGURE 5.9 The pyruvate dehydrogenase (PDH) complex in animal cells. This enzyme complex consists of three different proteins that work together to decarboxylate pyruvate and generate acetyl-CoA in mitochondria. Thiamine pyrophosphate (TPP; thiamine diphosphate), NAD^+, FAD, and CoA are required as cofactors for the indicated enzymes. (Adapted from Murray et al. 2001.)

PDH requires 4 vitamins (thiamine, niacin, riboflavin, and pantothenic acid) as coenzymes. Thiamine is a component of thiamine pyrophosphate (thiamine diphosphate). Riboflavin (vitamin B_2) is a part of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). Niacin (nicotinic acid, nicotinamide) is a B-group vitamin that is required for the formation of NAD^+ and $NADP^+$. Pantothenic acid (formed from pantoic acid and β-alanine) is needed for the synthesis of CoA-SH (coenzyme A). A nonvitamin cofactor for PDH is α-lipoic acid.

Decarboxylation of pyruvate to hydroxyethyl derivative of thiamine diphosphate (TPP):

$$\text{Pyruvate} + \text{TPP} \rightarrow \text{CO}_2 + \text{Hydroxyethyl-TPP}$$
$$(\text{Pyruvate dehydrogenase})$$

Transfer of acetyl group from hydroxyethyl-TDP to CoA-SH to from acetyl-CoA:

$$\text{Hydroxyethyl-TDP} + \text{Oxidized lipoamide} \rightarrow \text{Acetyl lipoamide}$$
$$\text{Acetyl lipoamide} + \text{CoA-SH} \rightarrow \text{Acetyl CoA} + \text{Dihydrolipoamide}\ (\text{reduced lipoamide})$$

The above two steps are catalyzed by dihydrolipoyl transacetylase.

Dihydrolipoamide is converted to oxidized lipoamide by dihydrolipoyl dehydrogenase that requires FAD and NAD^+ as coenzymes:

$$\text{Dihydrolipoamide} + \text{FAD} \rightarrow \text{Oxidized lipoamide} + \text{FADH}_2$$
$$\text{FADH}_2 + \text{NAD}^+ \rightarrow \text{FAD} + \text{NADH} + \text{H}^+$$
$$\text{Net reaction: Pyruvate} + \text{NAD}^+ + \text{CoA} \rightarrow \text{CO}_2 + \text{Acetyl-CoA} + \text{NADH} + \text{H}^+$$

Pyruvate dehydrogenase in cells is regulated by end-product inhibition and covalent protein modification via cAMP-dependent phosphorylation and phosphatase-catalyzed dephosphorylation (Figure 5.10). The conversion of dephosphorylated PDH (active form) to phosphorylated PDH (inactive form) is catalyzed by PDH kinase. PDH phosphatase converts the inactive form to the active form of PDH. PDH is inactive in a phosphorylated form, but is active in its dephosphorylated form. Thus, glucagon inhibits pyruvate oxidation by PDH, thereby promoting the conversion of pyruvate into glucose in hepatocytes.

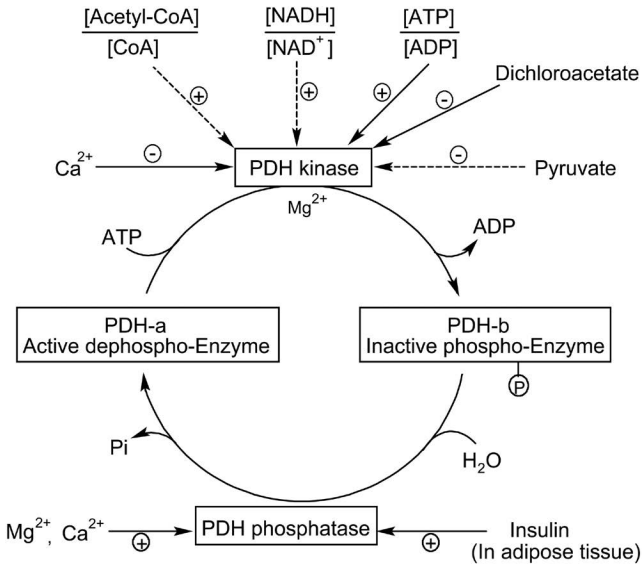


FIGURE 5.10 Regulation of the pyruvate dehydrogenase (PDH) complex by end-product inhibition and covalent protein modification in animal cells. PDH activity is regulated by protein phosphorylation (inactive state, b form) by PDH kinase and dephosphorylation (active state, a form) by PDH phosphatase. Factors (e.g., insulin, Mg^{2+}, and Ca^{2+}) that activate PDH phosphatase, as well as factors (e.g., pyruvate, Ca^{2+}, and dichloroacetate) that inhibit PDH kinase, enhance pyruvate decarboxylation. In contrast, factors (e.g., high ratios of ATP/ADP, NADH/NAD^+, and acetyl-CoA/CoA, which are indicators of high-energy levels in cells) reduce pyruvate decarboxylation by stimulating PDH kinase. The signs (+) and (–) denote activation and inhibition, respectively. (Adapted from Devlin 2011.)

Oxidation of Acetyl-CoA via the Mitochondrial Krebs Cycle and ATP Synthesis Overall Reaction of the Krebs Cycle

As indicated in Chapter 1, a metabolic cycle is defined as the process in which an overall chemical change is brought about by a cyclic reaction sequence. A metabolic cycle is highly efficient because there is no production of "wasteful" byproducts. The Krebs cycle is a great example. This metabolic cycle was discovered by Dr. Hans Krebs in 1937, for which he was awarded the Nobel Prize in Physiology or Medicine in 1953.

The Krebs cycle was worked out with the use of in vitro preparations of pigeon pectoral muscle. All the constituent enzymes (including NAD^+-dependent isocitrate dehydrogenase 3) of the Krebs cycle are located within the matrix of the mitochondria, with the exception of succinate dehydrogenase which is located on the inner surface of the inner mitochondrial membrane. Except for the condensation of OAA with acetyl-CoA to form citrate, all reactions of the Krebs cycle are reversible. In the liver, the Krebs cycle occurs primarily in periportal hepatocytes. The overall reaction of this metabolic pathway is summarized as follows:

$$\text{Acetyl-CoA} + 3\text{NAD}^+ + \text{FAD} + \text{GDP} + \text{Pi} + 2\text{H}_2\text{O} \rightarrow 2\text{CO}_2 + 3\text{NADH} + \text{FADH}_2 + \text{GTP} + \text{CoA} + 3\text{H}^+,$$

where NAD^+ is the oxidized form of nicotinamide adenine dinucleotide, FAD is flavin adenine dinucleotide,

and CoA is coenzyme A. The intermediates of the Krebs cycle are neither formed nor destroyed in the net operation of the cycle. For the net oxidation of glucose, amino acids, and fatty acids, they have to be converted to acetyl-CoA. Note that some of the intermediates can exit the mitochondria into the cytosol for participation in other metabolic pathways, such as glucose and amino acid synthesis. The Krebs cycle is active in tissues that have high metabolic rates, including the liver, brain, heart, kidneys, small intestine, skeletal muscle, and BAT (Tekwe et al. 2013, 2023).

The overall reaction for the complete oxidation of glucose to CO_2 and H_2O is as follows. As indicated previously, the amount of ATP production (30 or 32 mol of ATP/mol of glucose) depends on whether NADH is transported from the cytosol into mitochondria via the glycerophosphate shuttle or the malate shuttle, respectively.

$$C_6H_{12}O_6 + 6O_2 + 30 \text{ or } 32\ ADP + 30 \text{ or } 32\ Pi \rightarrow 6CO_2 + 6H_2O + 30 \text{ or } 32\ ATP$$

Note that isocitrate dehydrogenase 2 (exclusively in the mitochondria), which requires $NADP^+$ as its cofactor, does not contribute to the Krebs cycle but rather may play a role in NADPH provision.

Isozymes catalyzing some reactions that are either the same as, or similar to, those in the Krebs cycle exist in the mitochondria and other cellular compartments. For example, a cytosolic isoform of aconitase (accounting for 65% of total enzymatic activity) occurs in animal tissues (Kennedy et al. 1992). In addition, there are $NADP^+$-dependent isocitrate dehydrogenases 1 and 2 outside the Krebs cycle metabolon. Specifically, isocitrate dehydrogenase 1 is found primarily in the cytosol but is also present within peroxisomes, whereas isocitrate dehydrogenase 2 localizes exclusively to the mitochondria. All these enzymes can contribute to mitochondrial and extramitochondrial α-KG metabolism, as well as mitochondrial NADPH provision and cytosolic iron homeostasis.

Production of ATP and Water in Mitochondria

$NADH$ and $FADH_2$ are produced from acetyl-CoA oxidation in the Krebs cycle. These reducing equivalents are oxidized via the mitochondrial respiratory chain to produce ATP and water, as described in Chapter 1. Briefly, oxidation and phosphorylation are tightly coupled in the respiratory chain, which contains three sites for the synthesis of ATP from ADP plus Pi by ATP synthase. According to Mitchell's chemiosmotic theory on the mechanism of oxidative phosphorylation, oxidation of reducing equivalents in the respiratory chain generates protons (H^+ ions) that are ejected from the mitochondrial matrix into the intermembrane space. The electrochemical potential difference resulting from the asymmetric distribution of H^+ across the mitochondrial membrane is used to drive the formation of ATP from ADP plus Pi. At the terminal step of the electron transport system, two H^+ ions accept ½ O_2^- ($O_2^{\bullet}$) to generate H_2O.

Oxidation of 1 mol of NADH to H_2O results in the production of 2.5 mol of ATP:

$$NADH + H^+ + ½O_2 + 2.5ADP + 2.5Pi \rightarrow NAD^+ + H_2O + 2.5ATP;\ P/O\ ratio = 2.5.$$

Oxidation of 1 mol of $FADH_2$ to H_2O results in the production of 1.5 mol ATP:

$$FADH_2 + ½O_2 + 1.5ADP + 1.5Pi \rightarrow FAD + H_2O + 1.5ATP;\ P/O\ ratio = 1.5$$

Energetics of Acetyl-CoA Oxidation

The reactions for the generation of $NADH$ and $FADH_2$ via the Krebs cycle are as follows:

$$\text{Isocitrate} + NAD^+ \rightleftharpoons \alpha\text{-KG} + CO_2 + NADH + H^+ \quad (2.5\ ATP)$$

$$\alpha\text{-KG} + NAD^+ + CoASH \rightarrow \text{Succinyl-CoA} + CO_2 + NADH + H^+ \quad (2.5\ ATP)$$

$$\text{Succinyl-CoA} + GDP + Pi \rightleftharpoons \text{Succinate} + CoASH + GTP$$

$$GTP + ADP \rightleftharpoons ATP + GDP \quad [\text{nucleoside diphosphate kinase}] \quad (1\ ATP)$$

$$\text{Succinate} + FAD \rightleftharpoons \text{Fumarate} + FADH_2 \quad (1.5\ ATP)$$

$$\text{Malate} + NAD^+ \rightleftharpoons OAA + NADH + H^+ \quad (2.5\ ATP)$$

Thus, net 10 mol of ATP are produced from the oxidation of 1 mol of acetyl-CoA in cells.

Energetics of Glucose Oxidation in Aerobic Respiration

In aerobic respiration, the complete oxidation of 1 mol of glucose to 6 mol of CO_2 and 6 mol of H_2O produces 30 mol of ATP when the cytosolic NADH is transported into mitochondria via the glycerophosphate shuttle. The amount of ATP produced is 32 mol/mol of glucose when the cytosolic NADH is transported into the mitochondria via the malate shuttle. To be more conservative, nutritionists usually use the value of 30 mol of ATP/mol glucose to calculate the energetic efficiency of glucose oxidation in animals (McDonald et al. 2021), as shown below.

$$1\ \text{mol Glucose} \rightarrow 2\ \text{mol Pyruvate via glycolysis} \quad (2\ ATP, \text{net production})$$

$$\text{Oxidation of 2 mol NADH produced from glucose via glycolysis} \quad (3 \text{ or } 5\ ATP)$$

$$2\ \text{mol Pyruvate} \rightarrow 2\ \text{mol Acetyl-CoA} \quad (5\ ATP)$$

Oxidation of 2 mol acetyl-CoA in the Krebs cycle (20 ATP)

Total (30 or 32 ATP, net production)

$$C_6H_{12}O_6\,(\text{glucose}) + 6O_2 \rightarrow 6CO_2 + 6H_2O;$$
$$\Delta H = -670\ \text{kcal/mol glucose} = -3.722\ \text{kcal/g glucose}$$

Nutritional and Physiological Significance of the Krebs Cycle in Animals

Oxidation of Glucose, Fatty Acids and Amino Acids to CO_2

When amounts of dietary carbohydrates, fats, and proteins plus amino acids exceed the needs for synthetic pathways in animals, these nutrients are oxidized to CO_2 by conversion to acetyl-CoA and its subsequent oxidation via the Krebs cycle. CO_2 is then expelled from the body through respiration. This is the major mechanism for the irreversible loss of energy substrates from organisms. Thus, the major function of the Krebs cycle is to oxidize acetyl-CoA.

ATP Production

ATP is the major form of chemical energy in cells. Oxidation of acetyl-CoA via the Krebs cycle produces not only CO_2 but also NADH, $FADH_2$, and GTP. GTP and ATP are interconverted by nucleoside diphosphate kinase (GTP + ADP ⇌ GDP + ATP). The oxidation of NADH and $FADH_2$ via the mitochondrial respiratory chain results in the generation of ATP, with O_2 serving as the final oxidant of reducing equivalents to produce H_2O. This reaction eliminates the hydrogen atoms in dietary carbohydrates, fats, and proteins, plus amino acids. The mitochondrial oxidation of acetyl-CoA is the major source of energy in all animal cells with aerobic respiration. Thus, in aerobic respiration, the Krebs cycle plays an essential role in converting food energy into biological energy (ATP) for animals. Of note, glucose is the exclusive metabolic fuel for the brain in both fed and postabsorptive states. Based on the brain sizes of vertebrates (Table 5.10), the rates of glucose utilization by the brain per kg BW are greater in newborn than adult animals, in humans than nonprimates, and in carnivores than in nonprimate omnivores or ruminants. In addition, the oxidation of glucose via the Krebs cycle is an important source of energy for some metabolically active cells and tissues, including photoreceptor cells (rods and cones) of the eyes, skeletal muscles, and the heart. The absence (anoxia) or partial deficiency (hypoxia) of O_2 causes total or partial inhibition of the Krebs cycle.

AMP is a good indicator of cellular energy deficiency because increased hydrolysis of ATP results in the rapid accumulation of ADP and then AMP in cells (ATP → ADP + Pi → AMP + 2Pi). The energy charge of the adenylate pool is a better measure of energy status in cells than the concentration of a single nucleotide. ADP can be converted by adenylate kinase (also called myokinase) into ATP (2ADP ⇌ ATP + AMP). Thus, based on the metabolism of these nucleotides, the adenylate energy charge (normally between 0.70 and 0.95) in cells can be calculated to indicate their energy status.

$$\text{Adenylate energy charge (AEC)} = (\text{ATP} + 0.5\ \text{ADP}) / (\text{ATP} + \text{ADP} + \text{AMP})$$

A Central Role in Cell Metabolism

Acetyl-CoA is a common metabolite of glucose, amino acids, and fatty acids in intracellular compartmentalized reactions. Thus, the Krebs cycle is at the center of metabolism in animals. Some metabolic pathways end with the formation of constituents of the Krebs cycle, while other pathways originate from the cycle. Examples include gluconeogenesis, fatty acid synthesis, as well as the transamination, deamination, and synthesis of amino acids. Therefore, the Krebs cycle plays an important role in both oxidative and synthetic processes; namely, it is an amphibolic pathway that involves both catabolism and anabolism in the same cell.

TABLE 5.10
Brain Sizes and Plasma Glucose Concentrations in Healthy Newborn and Adult Animals[a]

	Newborn Animals			Adult Animals		
Animal Species	Body Weight (g)	Size of Brain (% of Body Weight)	Concentrations of Glucose in Plasma (mM)	Body Weight (kg)	Size of Brain (% of Body Weight)	Concentrations of Glucose in Plasma (mM)[b]
Pig	1.10–1.75	2.58–2.99	4.0–5.5	130–200	0.08–0.09	5.0–5.5
Human	3200	10–11	4.0–5.5	70	2.0	5.0–5.5
Dog	100–700	2.74	4.0–6.9	7–70	0.85	4.4–6.7
Cat	90–120	3.61	4.0–6.9	3.5–5.5	0.80	4.4–6.7
Chicken	35–40	2.24	10.5	0.58–3.4	0.12–0.45	12–15
Cattle	29–41	0.54	2.5–3.4	500–650	0.08	2.5–3.4
Sheep	3.0–5.5	1.5	2.5–3.4	45–150	0.17	2.5–3.4

Source: Bell and Freeman (1971), Bergman (1983), Hetenyi et al. (1972), Kesar (2014), Lin et al. (2020), Lu et al. (2007), Minervini et al. (2016), Swenson and Reece (1984), Tajonar et al. (2023), and Wolf-Vollenbröker et al. (2024).

[a] The values of variables depend on species.

[b] In the post-absorptive state.

Metabolic Control of the Krebs Cycle

Oxidation of acetyl-CoA via the Krebs cycle is regulated by the availability of OAA and at several other stages, which include citrate synthase, isocitrate dehydrogenase, and α-KG dehydrogenase (Chapter 1). Like PDH, the activity of α-KG dehydrogenase is also regulated by protein phosphorylation (inactive state) and dephosphorylation (active state). The factors that regulate the activity of these enzymes include citrate, Ca^{2+}, ADP, NAD^+/NADH, ATP, and CoA. When the production of acetyl-CoA from glucose exceeds its oxidation, the acetyl-CoA is diverted to the synthesis of fatty acids in the liver and adipose tissue. If the production of acetyl-CoA from fatty acids exceeds its oxidation, the acetyl-CoA is used for the synthesis of ketone bodies in the liver. When the liver produces excessive amounts of ketone bodies, severe ketoacidosis occurs, as in cows during the first month of lactation and in sheep during late (e.g., the last 4 weeks) gestation. In both cases, reduced voluntary feed intake by the mothers, despite an increased demand for energy to support milk production or fetal growth, results in the increased mobilization of maternal fats to generate ketone bodies. The ketoacidosis in ruminants can be treated with the intravenous administration of propionate, OAA or glucose.

Isotopic Tracing of the Krebs Cycle

Labeled tracers are essential to study energy-substrate oxidation via the Krebs cycle. Therefore, it is imperative to understand the patterns of labeling in intermediates and products. When [1-^{14}C]acetyl-CoA (meaning that the C-1 of acetyl-CoA is labeled with ^{14}C) is introduced into the Krebs cycle only once, it is condensed with OAA to form [5-^{14}C]citrate, [5-^{14}C]cis-aconitate, [5-^{14}C]isocitrate, [5-^{14}C]α-ketoglutarate (α-KG), [4-^{14}C]succinyl-CoA, [4-^{14}C]succinate, and [1,4-^{14}C]succinate. [1,4-^{14}C]Succinate is formed due to the "isotope randomization" of this molecule because it is a symmetric compound and succinate dehydrogenase does not differentiate between its two carboxyl groups. [1,4-^{14}C]Succinate leads to [1,4-^{14}C]fumarate, [1,4-^{14}C]malate, and [1,4-^{14}C]OAA formation. Thus, during the first turn of the cycle, there is no $^{14}CO_2$ produced. Note that two carbon atoms are lost as CO_2 in one revolution of the Krebs cycle. However, they are not derived from the acetyl-CoA that has immediately entered the cycle, but from the portion of the citrate molecule that is derived from OAA. After the first turn of the cycle, the OAA that is regenerated is now labeled as [1,4-^{14}C]OAA. [1,4-^{14}C]OAA is condensed with unlabeled acetyl-CoA to form [1,6-^{14}C]citrate, [1,6-^{14}C]oxalosuccinate, and [1-^{14}C]α-KG, with 50% of $^{14}CO_2$ being lost as ^{14}CO. [1-^{14}C]α-KG is decarboxylated to succinyl-CoA with another 50% of ^{14}C being lost as $^{14}CO_2$. Thus, 100% of ^{14}C is lost as $^{14}CO_2$ during the second turn of the Krebs cycle.

When [2-^{14}C]acetyl-CoA (labeled in the methyl group) is introduced into the Krebs cycle only once, it is condensed with OAA to form [4-^{14}C]citrate, [4-^{14}C]cis-aconitate, [4-^{14}C]isocitrate, [4-^{14}C]oxalosuccinate, [4-^{14}C]α-KG, [3-^{14}C]succinyl-CoA, [2,3-^{14}C]succinate, [2,3-^{14}C]fumarate, [2,3-^{14}C]malate, and [2,3-^{14}C]OAA. Thus, there is no loss of ^{14}C as $^{14}CO_2$ during the first turn of the cycle. During the second turn of the cycle, [2,3-^{14}C]OAA is condensed with unlabeled acetyl-CoA to yield [2,3-^{14}C]citrate, [2,3-^{14}C]oxalosuccinate, [2,3-^{14}C]α-KG, [1,2-^{14}C]succinyl-CoA, [1,2-^{14}C]succinate, and [1,2,3,4-^{14}C]succinate (due to isotope randomization). [1,2,3,4-^{14}C]Succinate leads to [1,2,3,4-^{14}C]fumarate, [1,2,3,4-^{14}C]malate, and [1,2,3,4-^{14}C]OAA. Thus, during the second turn of the Krebs cycle, there is still no loss of ^{14}C as $^{14}CO_2$. During the third turn of the cycle, [1,2,3,4-^{14}C]OAA is condensed with unlabeled acetyl-CoA to form [1,2,3,6-^{14}C]citrate, [1,2,3,6-^{14}C]isocitrate, [1,2,3,6-^{14}C]oxalosuccinate, and [1,2,3-^{14}C]α-KG, with 25% of ^{14}C being lost as $^{14}CO_2$. The decarboxylation of [1,2,3-^{14}C]α-KG to [1,2-^{14}C]succinyl-CoA results in the loss of another 25% of ^{14}C as $^{14}CO_2$. This leads to [1,2-^{14}C]succinate, [1,2,3,4-^{14}C]succinate (due to isotope randomization), and [1,2,3,4-^{14}C]OAA. Thus, during the third turn of the cycle, 50% of ^{14}C is lost as $^{14}CO_2$. By the end of the 15th cycle, all ^{14}C in [2-^{14}C]acetate is lost as $^{14}CO_2$ (Table 5.11).

The labeling pattern of intermediates of the Krebs cycle depends on the labeled compounds introduced into the cycle. This complexity has caused problems in the

TABLE 5.11
The Loss of ^{14}C in [2-^{14}C]Acetyl-CoA as $^{14}CO_2$ in the Krebs Cycle

Turn of the Krebs Cycle	Loss of ^{14}C as $^{14}CO_2$	Accumulated Loss of ^{14}C as $^{14}CO_2$
1st	0%	0%
2nd	0%	0%
3rd	50%	50%
4th	25%	75% (50% + 25%)
5th	12.5%	87.5% (75% + 12.5%)
6th	6.25%	93.75% (87.5% + 6.25%)
7th	3.125%	96.88% (93.75% + 3.125%)
8th	1.56%	98.44% (96.88% + 1.56%)
9th	0.78%	99.22% (98.44% + 0.78%)
10th	0.39%	99.61% (99.22% + 0.39%)
11th	0.195%	99.81% (99.61% + 0.195%)
12th	0.10%	99.91% (99.81% + 0.10%)
13th	0.05%	99.96% (99.91% + 0.05%)
14th	0.025%	99.99% (99.96% + 0.025%)
15th	0.0125%	100% (99.99% + 0.0125%)

Source: Weinman et al. (1957).

Note: When [2-^{14}C]acetyl-CoA (labeled in the methyl group) is introduced into the Krebs cycle only once, it is condensed with OAA to form [4-^{14}C]citrate. There is no loss of ^{14}C as $^{14}CO_2$ during the first and second turns of the cycle. Starting at the third turn of the cycle, 50% of the labeled carbon at the end of the previous cycle is lost. By the end of the 15th cycle, all ^{14}C in [2-^{14}C]acetate has been lost as $^{14}CO_2$.

interpretation of data obtained from isotope tracing studies. For example, in dairy cows, the appearance of ^{14}C from [^{14}C]acetate in glucose due to the isotope randomization should not be taken as evidence for the net synthesis of glucose from acetate. In contrast, net synthesis of glucose from acetate is possible in plants and many microbes due to the presence of the glyoxylate cycle. This metabolic cycle, however, is absent in animals.

Mitochondrial Redox State

β-Hydroxybutyrate dehydrogenase (a mitochondrial enzyme) catalyzes the interconversion of β-hydroxybutyrate and acetoacetate. Thus, the ratio of [β-hydroxybutyrate]/[acetoacetate] can be used as an indicator of the mitochondrial redox state. This enzyme is present in nearly all extrahepatic tissues with mitochondria, including the skeletal muscle, heart, liver, intestine, kidneys, and brain. Because K_{eq} is a constant and intracellular pH is constant under normal physiological conditions, an increase in the ratio of [β-hydroxybutyrate]/[acetoacetate] is equivalent to that of [NADH]/[NAD^+]. When intracellular pH fluctuates considerably, [H^+] must be included in the calculation of K_{eq}.

$$\text{Acetoacetate} + \text{NADH} + \text{H}^+ \rightleftharpoons \beta\text{-Hydroxybutyrate} + \text{NAD}^+$$

$$K_{eq} = \left(\left[\beta\text{-Hydroxybutyrate}\right] \times \left[\text{NAD}^+\right]\right) / \left(\left[\text{Acetoacetate}\right] \times \left[\text{NADH}\right] \times \left[\text{H}^+\right]\right)$$

Crabtree Effect in Animal Cells

The Crabtree effect refers to the inhibition of oxygen consumption by the addition of glucose in animal cells. This results from decreased oxidation of acetyl-CoA via the Krebs cycle by increasing aerobic glycolysis (the production of lactate despite the presence of mitochondria and oxygen.) The Crabtree effect was first described in tumor cells by Crabtree in 1929. It has also been demonstrated in noncancer tissues or cells such as pig platelets, coronary epithelium, thymocytes, and guinea pig sperm. A major role of the Crabtree effect has been suggested to spare endogenous fuels and minimize oxidative metabolism and, therefore, ROS production.

Cytosolic Pentose Cycle

Reactions of the Pentose Cycle

The pentose cycle (also known as the pentose phosphate pathway or hexose monophosphate shunt) is an alternative route for the oxidation of glucose in animal cells (Figure 5.11). It occurs in all cell types and is quantitatively the second most important pathway for glucose oxidation to form CO_2 in the body. In the liver, the pentose cycle occurs primarily in perivenous hepatocytes based on the finding that the activities of its key enzymes (glucose-6-P dehydrogenase and 6-phosphogluconate dehydrogenase) are much greater in these cells than in periportal hepatocytes (Jungermann and Katz 1989). Unlike the oxidation of glucose via glycolysis and the Krebs cycle, there is no production of ATP from glucose metabolism via the pentose cycle. Like the pathway of glycolysis, the pentose cycle occurs in the cytosol (Wamelink et al. 2008). However, in contrast to glycolysis, $NADP^+$, but not NAD^+, is used as a hydrogen acceptor in the pentose cycle.

The sequence of reactions of the pentose cycle can be viewed as consisting of two phases: (a) an oxidative nonreversible phase and (b) a nonoxidative reversible phase. In the oxidative nonreversible phase, glucose-6-P undergoes dehydrogenation and decarboxylation to generate pentose, ribulose-5-P, CO_2, and NADPH. Of note, decarboxylation of 6-phosphogluconate to ribulose-5-P is the only reaction for the production of CO_2 in the pentose cycle. The carbon of the CO_2 molecule originates from the carbon-1 of glucose-6-P. As indicated previously, the carbon-1 of glucose-6-P can also be oxidized to CO_2 via glycolysis and the Krebs cycle. In contrast, the carbon-6 of glucose-6-P is oxidized to CO_2 only via the Krebs cycle.

In the nonoxidative reversible phase, ribulose-5-P is converted back to fructose-6-P through a series of reactions involving four main types of enzymes: epimerase, ketoisomerase, transketolase and transaldolase. Although the individual reactions involved in the conversion of ribulose-5-P to hexose-P are straightforward, it is impossible to depict them in a simple linear sequence. When the pentose cycle starts with 3 mol of glucose-6-P, 2 mol of fructose-6-P and 1 mol of glyceraldehyde are generated as end products. Thus, 4 mol of fructose-6-P and 2 mol of glyceraldehyde are generated if the pentose cycle starts with 6 mol of glucose-6-P. Fructose-6-P can be converted to glucose-6-P by phosphohexose isomerase, whereas 2 mol of glyceraldehyde can form 1 mol of glucose-6-P via the reversed reactions of glycolysis, which are present in all animal cells. The overall reaction of the pentose cycle can be written as follows:

$$6\ \text{G-6-P} + 12\text{NADP}^+ + 6\text{H}_2\text{O} \rightarrow 6\text{CO}_2 + 5\ \text{F-6-P} + 12\text{NADPH} + 12\ \text{H}^+ \quad (1)$$

(G-6-P = glucose-6-phosphate; F-6-P = fructose-6-phosphate)

Because F-6-G can be converted to G-6-P, reaction (1) can be rearranged as follows:

$$6\ \text{G-6-P} + 12\text{NADP}^+ + 6\text{H}_2\text{O} \rightarrow 6\text{CO}_2 + 5\ \text{G-6-P} + 12\text{NADPH} + 12\text{H}^+ \quad (2)$$

Thus, the net reaction of the pentose phosphate pathway is as follows:

$$\text{G-6-P} + 12\text{NADP}^+ + 6\text{H}_2\text{O} \rightarrow 6\text{CO}_2 + 12\text{NADPH} + 12\ \text{H}^+ \quad (3)$$

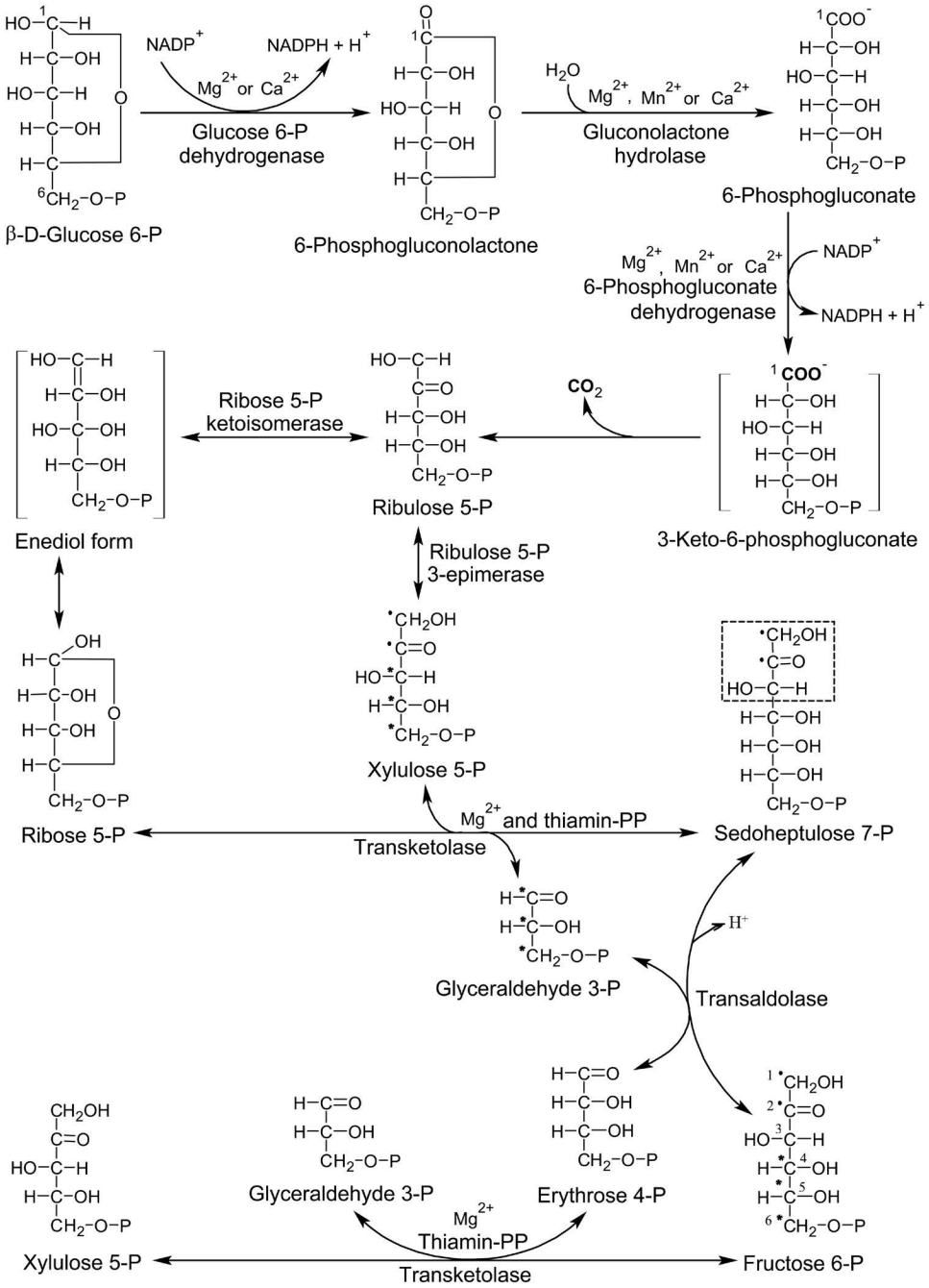


FIGURE 5.11 The pentose cycle in animal cells. The pentose cycle, which is also known as the pentose phosphate pathway or hexose monophosphate shunt, is present in the cytosol of all animal cells. This pathway consists of an oxidative nonreversible phase and a nonoxidative reversible phase. In the oxidative nonreversible phase, glucose-6-phosphate undergoes dehydrogenation and decarboxylation to generate pentose, ribulose-5-phosphate, CO_2, and NADPH. In the nonoxidative reversible phase, ribulose-5-phosphate is converted back to glucose-6-phosphate. When NADPH is not oxidized via the mitochondrial electron system, no ATP is produced from glucose catabolism through the pentose cycle. Erythrose 4-P may be reduced to erythritol by NADPH-dependent erythrose 4-P reductase. The numbers 1, 2, 3, 4, 5, and 6 indicate the position of carbons in molecules. The signs "." and "*" denote specific carbon atoms.

As written, reaction 3 erroneously suggests that 1 mol of glucose is completely oxidized to 6 mol of CO_2 via the pentose cycle. However, this is not the case. Reaction 3 only indicates the high efficiency of NADPH production from glucose via the pentose cycle.

Activity of the Pentose Cycle in Animal Tissues and Cells

The pentose cycle is active in the liver, adipose tissue, adrenal cortex, thyroid, erythrocytes, testis, enterocytes, proximal renal tubules, lactating mammary gland, activated

phagocytes (macrophages and monocytes), and activated neutrophils. For example, in neonatal-pig enterocytes and activated rat macrophages, ~15% and 25–30% of glucose is utilized via the pentose cycle, respectively (Wu 1996; Wu and Marliss 1993). In contrast, the pentose cycle has low activity in nonlactating mammary glands, resting macrophages, other resting mononuclear cells (e.g., lymphocytes), endothelial cells, and skeletal muscle. Thus, in resting macrophages and endothelial cells, only ~ 5% and 2–3% of glucose taken up by the cells is metabolized via the pentose cycle, respectively (Wu and Marliss 1993; Wu et al. 1994). A relatively low activity of the pentose cycle in cells such as endothelial cells does not necessarily indicate a minor role of this pathway in cell function. For example, NADPH is essential for NO synthesis in endothelial cells. Interestingly, the pentose cycle is a major pathway for glucose utilization in decapod crustaceans (e.g., freshwater crayfish) during the ecdysis stage (McWhinnie 1962).

The tissues or cells that possess a relatively high level of pentose cycle activity have one or more of the following characteristics: (a) active synthesis of fatty acids (e.g., liver, adipose tissue, and lactating mammary gland) and steroids (e.g., liver, placenta, and adrenal cortex); (b) active synthesis of superoxide anion (O_2^-) from O_2 via the nonmitochondrial respiratory chain (e.g., phagocytes); and (c) active synthesis of NO from arginine [e.g., activated macrophages (probably with exception of guinea-pig macrophages) and activated hepatocytes]. All of these synthetic processes require NADPH and play important roles in either storing energy (adipose tissue) or host defense (macrophages).

Physiological Significance of the Pentose Cycle

Glucose metabolism via the pentose cycle produces Ribose-5-P, which is converted into PRPP (5-phosphoribosyl-1-pyrophosphate). The latter is required for the synthesis of purine and pyrimidine nucleotides (RNA and DNA; Chapter 4). In addition, the pentose cycle is the major metabolic pathway for the generation of NADPH from $NADP^+$ (Wu et al. 2001). Many biochemical reactions are dependent on NADPH, and some of them are outlined as follows:

1. Fatty acid synthesis (e.g., liver, adipose tissue, and mammary tissue) in the cytosol (Chapter 6).
2. Synthesis of cholesterol and steroid hormones from acetyl-CoA in specific tissues (e.g., the liver, adrenal cortex, testis, and ovary; Chapter 6).
3. Synthesis of dopamine, epinephrine (adrenaline), and norepinephrine (noradrenaline) in the cytosol of adrenal medulla and nervous tissue (Chapter 7).
4. Reduction of GSSG (oxidized glutathione) to 2 GSH (reduced glutathione) in the cytosol, which helps convert H_2O_2 to H_2O in some tissues (e.g., RBCs, red muscle, lens, and cornea; Chapter 7).
5. Participation in the cytosolic sorbitol (polyol) pathway for the synthesis of fructose (see below).
6. Synthesis of proline from pyrroline-5-carboxylate (P5C) in the cytosol. The proline-P5C cycle has been proposed as a mechanism for transferring NADPH from the cytosol to mitochondria (Chapter 7).

$$\text{P5C} + \text{NADPH} + \text{H}^+ \rightarrow \text{Proline} + \text{NADP}^+$$

7. Synthesis of superoxide anion (O_2^-) by NADPH oxidase in phagocytes. This enzyme is located in both the cytosol and the plasma membrane. Consumption of O_2 and production of both O_2^- and H_2O_2 by macrophages and neutrophils are increased in response to immunological stimulation, which is referred to as respiratory burst. Increased respiratory burst has been found to be associated with enhanced killing of microbes by phagocytes.

$$2O_2 + \text{NADPH} + \text{H}^+ \rightarrow 2O_2^- + \text{NADP}^+ + 2\text{H}^+ \quad (\text{NADPH oxidase})$$

$$2O_2^- + 2\text{H}^+ \rightarrow H_2O_2 + O_2 \quad (\text{Superoxide dismutase})$$

$$O_2^- + H_2O_2 \rightarrow O_2 + HO^- + HO^{\cdot} \quad (\text{Haber-Weiss reaction})$$

8. Synthesis of NO from L-arginine by NO synthase in the cytosol, plasma membrane, and mitochondria. NO synthase requires tetrahydro-L-biopterin (BH4), FAD^+, FMN, Ca^{2+} and calmodulin.

$$\text{L-Arginine} + O_2 + 1.5\text{NADPH} + 1.5\text{H}^+ \rightarrow \text{L-Citrulline} + \text{NO} + 1.5\text{NADP}^+$$

9. Synthesis of P5C from glutamate in the mitochondria of enterocytes of most mammals (including pigs, rats, sheep, cattle, and humans). P5C synthase is almost exclusively located in the mitochondria of the small intestine in those mammals. This indicates the importance of the small intestine in citrulline and arginine metabolism in mammals. Note that P5C synthase is absent in the small intestine of chicks and is present at low activity in the feline small intestine.

$$\text{Glutamate} + \text{NADPH} + \text{H}^+ + \text{ATP} \rightarrow \text{P5C} + \text{NADP}^+ + \text{ADP} + \text{Pi} \quad (\text{P5C synthase})$$

10. Synthesis of glucuronic acid, ascorbic acid (vitamin C), and pentoses via the uronic acid pathway, another cytosolic pathway for the metabolism of glucose-6-P (see below).
11. Conversion of NADPH into NADH in mitochondria. NADPH can be converted into NADH by

mitochondrial nicotinamide nucleotide transhydrogenase (Hoek and Rydstrom 1988).

$$NADP^+ + NADH + H^+_{out} \rightleftharpoons NADPH + NAD^+ + H^+_{in}$$

12. Formation of erythritol. Phosphatases convert erythrose-4-phosphate to erythrose, which is subsequently reduced to erythritol by NADPH-dependent erythrose reductase. Erythritol is quantitatively excreted in the urine without undergoing metabolic change in the body.

In mammalian cells, nicotinamide nucleotide transhydrogenase is an integral protein of the mitochondrial inner membrane. The forward reaction above (transfer of electrons from NADH to $NADP^+$) is coupled to the translocation of protons from the cytosolic side to the matrix side of the mitochondrial inner membrane to generate NADPH in mitochondria. Wu (1996) reported that this reaction is not sufficient for the intramitochondrial provision of NADPH from NADH for citrulline synthesis from glutamine in pig enterocytes. In the reverse reaction of the nicotinamide nucleotide transhydrogenase, the transfer of electrons from NADPH to NAD^+ is coupled to the translocation of protons from the matrix side to the cytosolic side of the mitochondrial inner membrane to produce NADH. This allows for the oxidation of the cytosolic NADPH to produce ATP after the nucleotide enters the mitochondria from the cytosol through the malate shuttle or the glycerophosphate shuttle.

Quantification of the Pentose Cycle

The yields of $^{14}CO_2$ from [1-^{14}C]glucose and [6-^{14}C]glucose have been used to estimate the activity of the pentose cycle in animal cells (e.g., hepatocytes and adipocytes). This is based on the assumptions that $^{14}CO_2$ is produced from [1-^{14}C]glucose via both the pentose cycle and the Krebs cycle and that $^{14}CO_2$ is produced from [6-^{14}C]glucose only via the Krebs cycle. Note that glucose has to be converted to pyruvate before its carbons are oxidized via the Krebs cycle.

1. *Katz and Wood's equation*. In 1963, Katz and Wood (1963) developed the following classic equation for estimating the flux of glucose through the pentose cycle:

$$\frac{G1_{CO_2} - G6_{CO_2}}{1 - G6_{CO_2}} = \frac{3\ PC}{1 + 2\ PC}$$

$G1_{CO2}$: $^{14}CO_2$ produced from [1-^{14}C]glucose at steady state;
$G6_{CO2}$: $^{14}CO_2$ produced from [6-^{14}C]glucose at steady state;
PC: Fraction of glucose that is metabolized to CO_2 and glyceraldehyde 3-P via the pentose cycle.

In deriving the above equation, Katz and Wood made the following important assumptions: (a) isotopic steady states for the production of $^{14}CO_2$ from both [1-^{14}C]glucose and [6-^{14}C]glucose; (b) cell homogeneity in a tissue; namely, the tissue can be treated mathematically as if it were a single cell, or all cells are metabolically identical; (c) gluconeogenesis is absent in the tissue or cells; and (d) complete mixing of fructose-6-P produced by the pentose cycle with a pool of hexose-P formed from the entering glucose.

2. *Larrabee's approach*. The assumptions used to derive the Katz and Wood equation may make it not applicable to many tissues or cells, including macrophages, endothelial cells, enterocytes, and chicken embryos. Also, considerable amounts of time are often required for the production of $^{14}CO_2$ from [6-^{14}C]glucose to reach an isotopic steady state. Unfortunately, many investigators have used the Katz and Wood equation to estimate the pentose cycle activity with no statement of the assumptions implicit in its use or even though the implicit assumptions cannot be validated. Because of these concerns about the Katz and Wood equation, Larrabee (1989, 1990) proposed that the difference in $^{14}CO_2$ production from [1-^{14}C]glucose and [6-^{14}C]glucose can be used to estimate the activity of the pentose cycle in metabolically homogenous or nonhomogenous cells (tissues), in an isotopic steady or nonsteady state, and in the presence or absence of gluconeogenesis in tissues or cells. The lower and upper values for the flux of glucose through the PC can be optimized by following the time-course of the difference in $^{14}CO_2$ from [1-^{14}C]glucose and [6-^{14}C]glucose, but useful values can be obtained when the time-course data are not available.

During any time period, the flux of glucose to the pentose cycle is as follows:

Glucose to PC flux $\geq {}^*C1 - {}^*C6$
$^*C1$: $^{14}CO_2$ produced from [1-^{14}C]glucose.
$^*C6$: $^{14}CO_2$ produced from [6-^{14}C]glucose.
Lower limit of glucose to PC flux $\geq {}^*C1 - {}^*C6$;
Upper limit of glucose to PC flux $\leq {}^*C1$ at steady state.

Thus, a lack of difference in $^{14}CO_2$ production from [1-^{14}C]glucose and [6-^{14}C]glucose is not necessarily evidence of the absence of a pentose phosphate pathway in animal cells. Also, when $^*C1$ is much greater than $^*C6$, the true value of glucose to PC flux approaches $^*C1$. Larrabee (1989) has reported that, in the peripheral ganglia of chicken embryos, the lower limit of glucose to PC flux is 34 μmol/g/h and the upper limit is 46 μmol/g/h. Thus, the flux of glucose to PC lies between 34 and 46 μmol/g/h. Based on the rate of glucose uptake (125 μmol/g/h), 27–37% of the glucose utilized by these cells is metabolized via the pentose cycle.

Regulation of the Pentose Cycle

Activity of the pentose cycle in animal cells is regulated by the availability of $NADP^+$, which is a cofactor for glucose-6-P dehydrogenase and 6-phosphogluconate dehydrogenase. Thus, oxidation of NADPH to $NADP^+$ through NADPH-dependent reactions plays an important role in controlling the oxidative phase and, therefore, the entire phase of the pentose cycle. On the other hand, glucose-6-P dehydrogenase is subject to (a) feedback inhibition by NADPH; and (b) allosteric inhibition by LCFA acyl-CoA (e.g., palmitoyl-CoA) and dehydroepiandrosterone, competitive inhibitors (e.g., glucosamine-6-P for glucose-6-P and gallated catechins for $NADP^+$), and uncompetitive inhibitors (thienopyrimidine and quinazolinone derivatives; Figure 5.12). Therefore, the pentose cycle can be stimulated when utilization of NADPH is augmented in cells. Additionally, expression of glucose-6-P dehydrogenase is enhanced by insulin, glucocorticoids, thyroid hormones, a high-carbohydrate diet, hypoxia, and H_2O_2 at transcriptional and/or translational levels (Kletzien et al. 1994). Because both the pentose cycle and glycolysis involve glucose-6-P as a common substrate, these two pathways may compete in the cytosol, depending on metabolic needs for NADPH or NADH. Insulin enhances glucose metabolism through the pentose cycle in cells (e.g., endothelial cells; Wu et al. 1994), likely via mechanisms involving increases in glucose uptake, hexokinase activity, Ca^{2+} influx into cells, and NADPH oxidation. Glucose-6-P dehydrogenase is inhibited by NADPH and fatty acyl-CoA.

Metabolism of Glucose via the Uronic Acid Pathway

In addition to the pentose cycle and glycolysis, glucose-6-P is utilized through the uronic acid pathway in animal cells, which involves the conversion of glucose-6-P to glucuronic acid, ascorbic acid (vitamin C), and pentoses (Figure 5.13). Although the uronic acid pathway is quantitatively a minor pathway for glucose metabolism in animal cells, glucuronic acid is involved in the conjugation with toxic metabolites and foreign chemicals (xenobiotics including polyphenols and environmental toxins) as glucuronides for excretion via urine and feces. In the liver, the uronic acid pathway occurs primarily in perivenous hepatocytes. Uridine diphosphate (UDP)-glucuronate is the active form of glucuronate for reactions involving the incorporation of glucuronic acid into proteoglycans and heteropolysaccharides (e.g., hyaluronic acid) or for reactions in which glucuronate is conjugated to such substrates as steroid hormones, certain drugs, and bilirubin (the metabolite of heme). In humans and other primates, as well as guinea pigs, ascorbic acid cannot be synthesized from glucose because of the absence of L-gulonolactone oxidase. Like the pentose cycle, the uronic acid pathway does not produce ATP.

Gluconeogenesis

Definition of Gluconeogenesis

Gluconeogenesis is defined as the metabolic process by which glucose is formed from noncarbohydrates, including lactate, pyruvate, glycerol, propionate, amino acids, and odd-numbered LCFAs. This is a biosynthetic process because a 3-carbon substance is converted into the 6-carbon glucose. Propionate (a C_3 SCFA) is the major substrate for gluconeogenesis in ruminants, followed by amino acids such as serine, threonine, glutamate, and glutamine. In mammals and birds, gluconeogenesis occurs only in the liver (mainly periportal hepatocytes) and kidneys (almost exclusively in proximal renal tubules) because they possess glucose-6-phosphatase

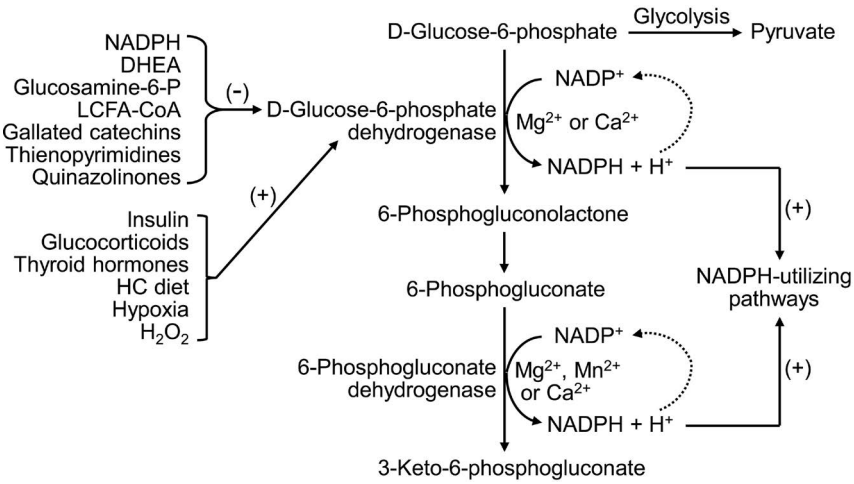


FIGURE 5.12 Regulation of the pentose cycle in animal cells. Glucose-6-phosphate dehydrogenase is a key rate-controlling enzyme in glucose metabolism via the pentose cycle. In short-term regulation, this enzyme is inhibited by NADPH, dehydroepiandrosterone (DHEA), glucosamine-6-phosphate, long-chain fatty acyl-CoA (LCFA-CoA), gallated catechins, as well as synthetic thienopyrimidine and quinazolinone derivatives. In long-term regulation, expression of this enzyme is activated by insulin, glucocorticoids, thyroid hormones, a high carbohydrate diet, hypoxia, and H_2O_2 at transcriptional and/or translational levels. Oxidation of NADPH via NADPH-dependent reactions regenerates $NADP^+$, which allows the dehydrogenation of D-glucose-6-phosphate and 6-phosphogluconate to proceed. The signs (+) and (–) denote activation and inhibition, respectively.

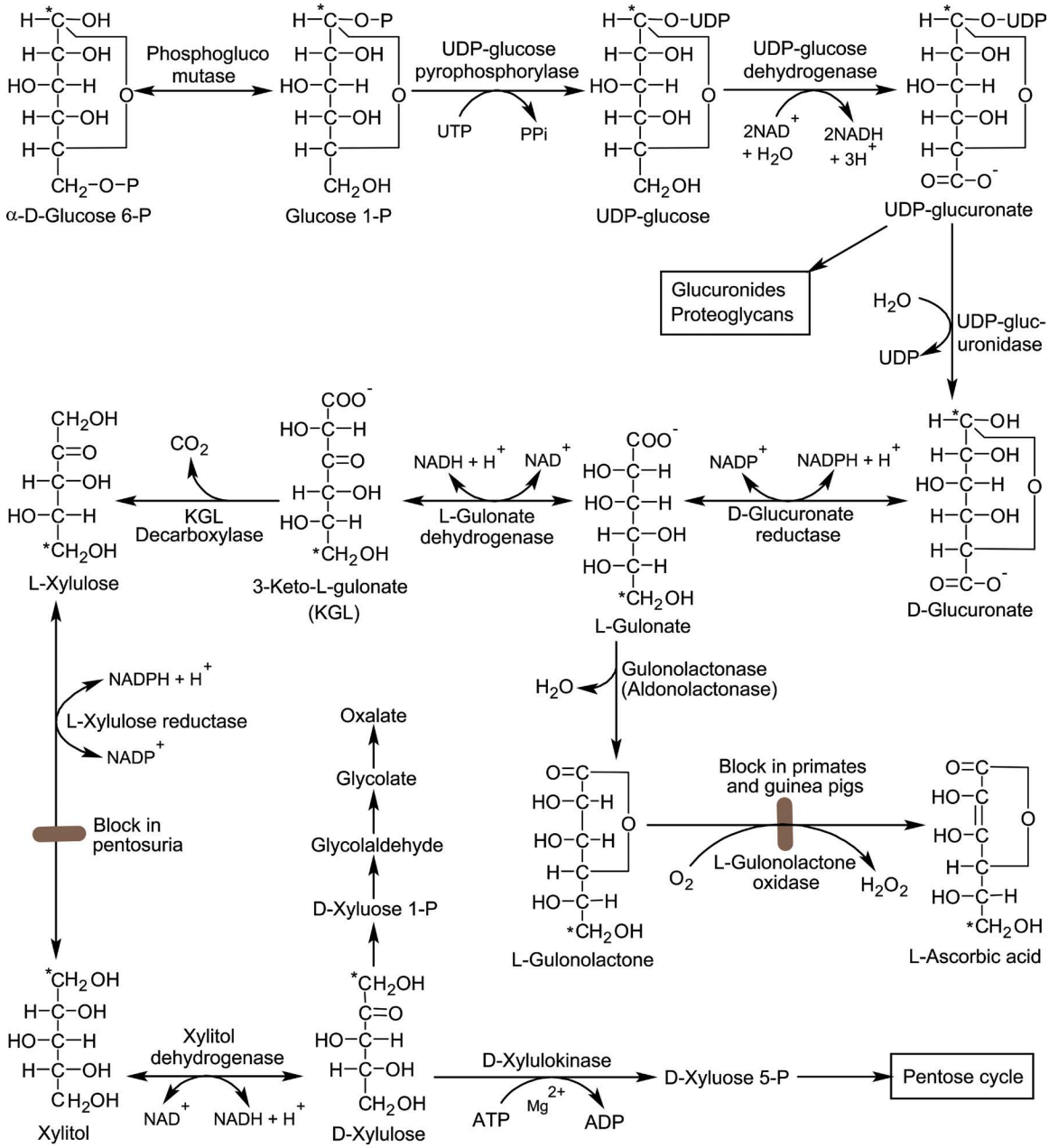


FIGURE 5.13 Glucose metabolism via the uronic acid pathway in the cytosol of animal cells. The initial molecule of this pathway is D-glucose-6-phosphate, which is produced from D-glucose by hexokinases I, II, and III, or glucokinase. The hydroxyl group of the terminal carbon in glucose-6-phosphate is oxidized to a carboxyl group. The uronic acid pathway generates glucuronic acid, pentose, and ascorbic acid (vitamin C). Glucuronic acid plays an important role in (a) the excretion of toxic metabolites and foreign chemicals (xenobiotics) as glucuronides and (b) the synthesis of proteoglycans and heteropolysaccharides (e.g., hyaluronic acid). In primates (e.g., humans and monkeys) and guinea pigs, as well as some species of insects, birds, fish, and invertebrates, vitamin C cannot be synthesized from glucose due to the lack of L-gulonolactone oxidase.

activity (Ross et al. 1986; Watford 1985). The newly synthesized glucose is released and transported via blood circulation to other tissues (Figure 5.14). Specifically, periportal hepatocytes release glucose through GLUT2 in the basolateral membranes into the blood. In the kidneys, the epithelial cells of the early and late proximal tubules reabsorb 98% and 2% of glucose from the tubular lumen via Na^+-dependent SGLT2 and SGLT1 in the apical membranes, respectively, while releasing glucose via Glut2 in the basolateral membranes into the blood (Ghezzi et al. 2018).

Plasma glucose concentrations are determined by the balance between the rates of glucose production and utilization in the body. Partly due to impaired glucose synthesis, hypoglycemia can occur in neonates (e.g., piglets and

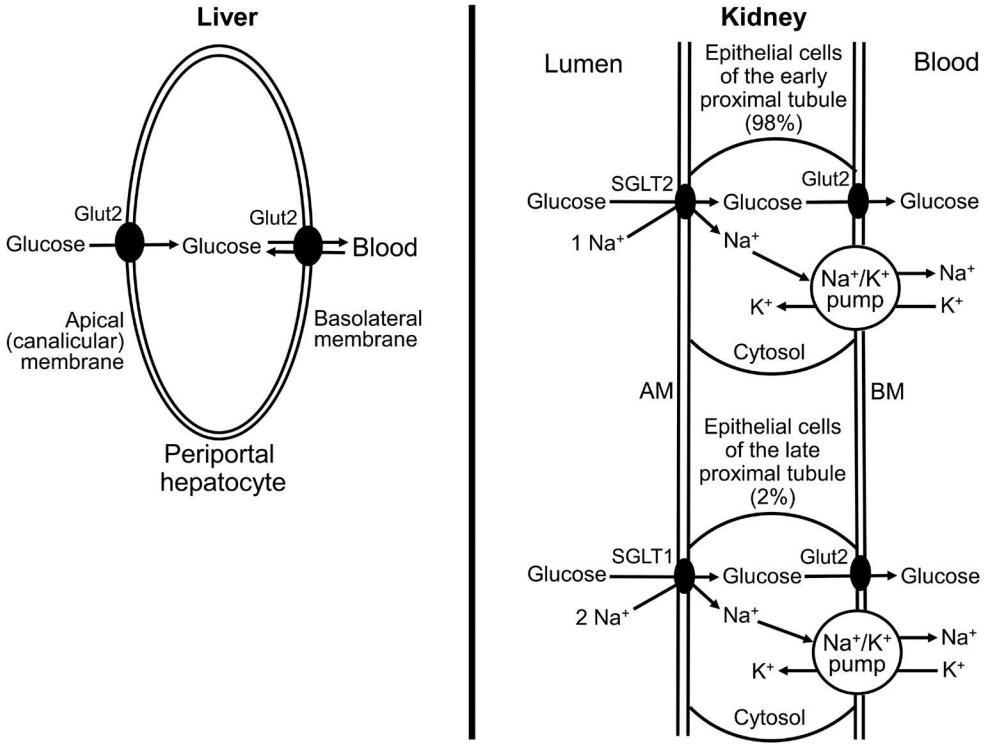


FIGURE 5.14 Release of glucose from the liver and kidneys. In the liver, GLUT 2 mediates the uptake (from the canaliculus and blood) and release (into the blood) of glucose by periportal hepatocytes via their apical and basolateral membranes. In the kidneys, the epithelial cells of the early and late proximal tubules reabsorb 98% and 2% of glucose from the tubular lumen via Na^+-dependent SGLT2 and SGLT1 in the apical membrane (AM), respectively, while releasing glucose via GLUT2 in the basolateral membrane (BM) into the blood. The Na^+/K^+ pump (also known as Na^+/K^+ ATPase) exports 3 mol of Na^+ ions out of the cell and imports 2 mol of K^+ ions into the cell for the hydrolysis of 1 mol of ATP.

preterm infants), late pregnancy in ruminants, small-for-gestational-age infants, and patients with sepsis or tumors. In contrast, intensive exercise enhances hepatic gluconeogenesis and, therefore, the plasma concentration of glucose in healthy animals. Similarly, hyperglycemia occurs in diabetic patients not treated with insulin because of both increased gluconeogenesis and reduced glucose catabolism in the whole body.

Pathway of Gluconeogenesis

Gluconeogenesis is considered as a partial reversal of the glycolytic pathway because many of the reactions of glycolysis participate in gluconeogenesis (Figure 5.15). The reactions common to both glycolysis and gluconeogenesis are near-equilibrium reactions in which a small increase in product concentrations or a decrease in substrate concentrations can reverse the direction of the metabolic flux. However, there are 3 nonequilibrium reactions in glycolysis:

1. between PEP and pyruvate ($\Delta G = -26.4$ kJ.mol^{-1}) [pyruvate kinase]
2. between fructose-6-P and fructose-1,6-bisphosphate ($\Delta G = -24.5$ kJ.mol^{-1}) [PFK-1]
3. between glucose and glucose-6-P ($\Delta G = -32.9$ kJ.mol^{-1}) [Hexokinase]

Reversal of the direction of flux through the nonequilibrium reactions would require very large changes in the concentrations of substrates and products, and such large changes in intracellular metabolites would not be physiologically feasible. Thus, these reactions are described as the "energy barriers" to gluconeogenesis (Krebs 1964) and are circumvented by different reactions catalyzed by the following four enzymes: pyruvate carboxylase, PEPCK, fructose-1,6-bisphosphatase, and glucose-6-phosphatase (Van de Werve et al. 2000). The latter enzyme is expressed almost exclusively in the liver and kidneys.

Conversion of Pyruvate to PEP

$$\text{Pyruvate} + CO_2 + \text{ATP} \rightarrow \text{Oxaloacetate} + \text{ADP} + \text{Pi} \quad (\text{pyruvate carboxylase})$$

Pyruvate carboxylase requires vitamin B, biotin, and Mg^{2+}. In the liver and kidneys, this enzyme is located exclusively in the mitochondrion. Pyruvate carboxylase is also present in the WAT of animals studied, including cattle, pigs, humans, rats, and chickens.

$$\text{OAA} + \text{GTP} \rightarrow \text{PEP} + CO_2 + \text{GDP} \quad (\text{PEPCK})$$

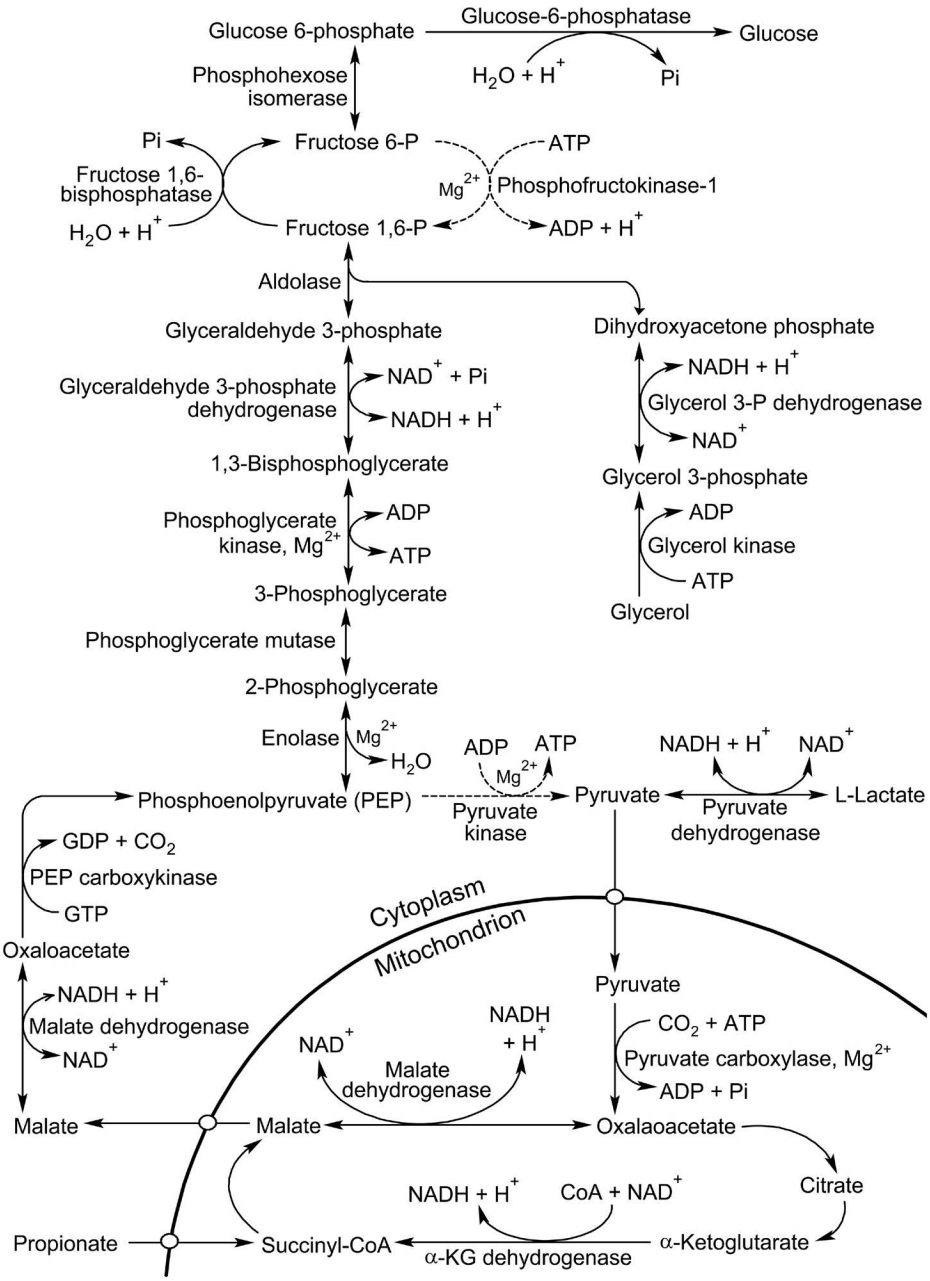


FIGURE 5.15 Gluconeogenesis from pyruvate, lactate, glycerol, and glucogenic amino acids in the liver and kidneys of animals. Synthesis of glucose from pyruvate, lactate, glycerol, and glucogenic amino acids involves reversal reactions of glycolysis. The irreversible reactions of glycolysis between phosphoenolpyruvate (PEP) and pyruvate, between fructose-6-phosphate and fructose-1,6-bisphosphate, and between glucose and glucose-6-phosphate are circumvented by different reactions catalyzed by pyruvate carboxylase, PEP carboxykinase, fructose-1,6-bisphosphatase, and glucose-6-phosphatase. The latter enzyme is expressed almost exclusively in the liver and kidneys. The intracellular location of PEP carboxykinase affects the formation of glucose from amino acids and glycerol, but not from lactate or pyruvate.

In animals, PEPCK uses GTP or inosine 5'-triphosphate (ITP), but not ATP, as a substrate. This enzyme is highly active in the liver and kidneys but also occurs in WAT and BAT and is distributed in mitochondria, the cytosol, or both compartments in a species- and tissue-specific manner. For example, in rats, mice, and golden hamsters, PEPCK is located exclusively in the cytosol. However, PEPCK is almost entirely a mitochondrial enzyme in the liver of pigeons, chickens, and rabbits, and in these species, PEP is transported from the mitochondria to the cytosol for conversion into fructose-1,6-bisphosphate (Table 5.12). The intracellular distribution of PEPCK in the mitochondria and cytosol of chicken kidneys varies with nutritional and physiological status: mitochondria ≫ cytosol (fed), mitochondria > cytosol (48-h starved), mitochondria = cytosol (96-h starved and acidotic). In humans, cattle, sheep, pigs, guinea pigs, and frogs, PEPCK is equally distributed in the cytosol and mitochondria. The location of PEPCK in the

TABLE 5.12
Species Differences in Intracellular Distribution of Phosphoenolpyruvate Carboxykinase (PEPCK) in the Liver and Kidney of Animals

Animal Species	Tissue	Intracellular Distribution
Rat, Hamster	Liver	Primarily cytosolic (90%); PEPCK-M (10%)
	Kidney	PEPCK-M = PEPCK-C
Mouse	Liver	Primarily cytosolic (95%); PEPCK-M (5%)
	Kidney	PEPCK-M = PEPCK-C
Rabbit	Liver	Primarily mitochondrial (90%); PEPCK-C (10%)
	Kidney	PEPCK-M >> PEPCK-C (Fed)
Chicken, pigeon (post-hatching)	Liver	Exclusively mitochondrial (100% PEPCK-M)
	Kidney	Both mitochondrial and cytosolic PEPCK-M (50%) = PEPCK-C (50%) [Fed] PEPCK-M > PEPCK-C (48-h fasted) PEPCK-M = PEPCK-C (96-h fasted or acidotic)
Chicken (embryo)	Liver	PEPCK-M (50%) = PEPCK-C (50%)
	Kidney	PEPCK-M (15%) < PEPCK-C (85%)
Dog, Cat	Liver	PEPCK-M (50%) = PEPCK-C (50%)
	Kidney	PEPCK-M (65%) > PEPCK-C (35%)
Human, Pig	Liver	PEPCK-M (60%) > PEPCK-C (40%)
	Kidney	PEPCK-M = PEPCK-C
Guinea-pig	Liver	PEPCK-M (60%) > PEPCK-C (40%)
	Kidney	PEPCK-M (41%) > PEPCK-C (59%)
Cattle, Sheep	Liver	PEPCK-M (60%) > PEPCK-C (40%)
	Kidney	PEPCK-M = PEPCK-C
Frog	Liver	PEPCK-M (50%) = PEPCK-C (50%)
	Kidney	PEPCK-M = PEPCK-C

Source: Croniger et al. (2006), Watford (1985), Wolf and Mehlman (1972), and Yang et al. (2009). Unless indicated otherwise, the subcellular distribution of PEPCK in the livers is described for fed animals of various species.

Note: PEPCK-M, mitochondrial isoform of PEPCK; PEPCK-C, cytosolic isoform of PEPCK

mitochondria of the chicken liver explains why chicken hepatocytes have a limited ability to synthesize glucose from amino acids (Watford et al. 1981). In WAT and BAT, which lack gluconeogenesis, PEPCK plays an important role in TAG synthesis (Yang et al. 2009).

Conversion of Fructose-1,6-Bisphosphate Into Fructose-6-P

$$\text{Fructose-1,6-bisphosphate} + H_2O + H^+ \rightarrow \text{Fructose-6-P} + \text{Pi} \quad (\text{Fructose-1,6-bisphosphatase})$$

This enzyme is present in the cytosol of the liver and kidney and has been demonstrated in skeletal muscle. Fructose-1,6-bisphosphatase is inhibited by fructose-2,6-bisphosphate, a molecule discovered in 1980 (Van Schaftingen et al. 1980). Fructose-2,6-bisphosphate plays an important role in the regulation of glycolysis and gluconeogenesis.

Conversion of Glucose-6-P Into Glucose

$$\text{Glucose-6-P} + H_2O + H^+ \rightarrow \text{Glucose} + \text{Pi} \quad (\text{Glucose-6-phosphatase})$$

This enzyme is present in the cytosol of the liver and kidney, but is absent from skeletal muscle, heart, and adipose tissue.

Net Reaction of Gluconeogenesis from Pyruvate

$$2\text{Pyruvate} + 4\text{ATP} + 2\text{GTP} + 2\text{NADH} + 2H^+ + 2H_2O \rightarrow \text{Glucose} + 4\text{ADP} + 2\text{GDP} + 6\text{Pi} + 2\text{NAD}^+$$

Thus, 6 mol of ATP are required to synthesize 1 mol of glucose from 2 mol of pyruvate. Gluconeogenesis requires large amounts of ATP, which is supplied by fatty acid oxidation.

Physiological Substrates for Gluconeogenesis

1. *Lactate*. Large amounts of lactate are produced primarily from glucose in animals. It has been estimated that about 120 g of lactate is produced each day by a normally active man. Of this, about 40 g are produced by tissues that are virtually anaerobic in their metabolism (e.g., RBCs, kidney medulla, and retina). The contribution of other tissues and cells (e.g., skeletal muscle, small intestine, brain, skin, cells of the immune system) will vary depending on physiological and pathological conditions. Normally, plasma concentration of lactate is about 1 mM, which represents a steady-state concentration that reflects the balance between rates of lactate production and utilization.

   The principal tissue for lactate utilization is the liver, where lactate is converted mainly to glucose and glycogen and, to a lesser extent, to TAGs. However, other tissues, such as the skeletal muscle and heart, can both oxidize lactate and convert it to glycogen. The conversion of lactate into glucose or glycogen removes the proton (H^+) produced via glycolysis and, therefore, plays a role in maintaining acid-base balance in the body. In fed and 3-day fasted adult sheep, lactate contributes to ~15% and 10% of glucose synthesis in the whole body, respectively (Bergman 1983).

   $$2\ \text{L-Lactate} + 2\text{NAD}^+ \rightleftharpoons 2\text{Pyruvate} + 2\text{NADH} + 2H^+ \rightarrow \text{D-Glucose}$$

2. *Glycerol*. In mammals and birds, glycerol is formed primarily from the hydrolysis of TAGs by lipoprotein lipase, adipose TAG lipase, and hormone-sensitive lipase in WAT and skeletal muscles that do not express glycerol kinase. The mammary gland of mammals is also a source of glycerol because this organ does not contain glycerol kinase. Thus, the concentration of glycerol in plasma is often used as an indicator of lipolysis. During fasting and intensive exercise, when lipolysis is enhanced, glycerol is an important substrate for gluconeogenesis during

prolonged starvation. For example, a fasting, resting man generates 19 g of glycerol each day, and almost all of the glycerol is converted to glucose. In fed and 3-day fasted adult sheep, glycerol contributes to ~5% and 25% of glucose synthesis in the whole body, respectively (Bergman 1983).

$$\text{Glycerol} + \text{ATP} \rightarrow \text{Glycerol-3-P} + \text{ADP}$$
$$(\text{Glycerol kinase; liver and kidney})$$

$$\text{Glycerol-3-P} + \text{NAD}^+ \rightarrow \text{Dihydroxyacetone-P} + \text{NADH} + \text{H}^+ \quad (\text{Glycerol-3-P dehydrogenase})$$

$$\text{Dihydroxyacetone-P} \rightleftharpoons \text{Glyceraldehyde-3-P}$$
$$(\text{Phosphotriose isomerase})$$

$$\text{Dihydroxyacetone-P} + \text{Glyceraldehyde-3-P} \rightleftharpoons \text{Fructose-1,6-P}$$
$$(\text{Aldolase})$$

3. *Amino acids.* Experiments in the 1930s demonstrated that based on their capacity for gluconeogenesis or ketogenesis, amino acids could be divided into three classes: glucogenic, ketogenic, and glucogenic-plus-ketogenic (Table 5.13). Glucogenic amino acids (Ala, Arg, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser, and Val) are those amino acids whose metabolites can be converted to glucose through the production of pyruvate or one of the intermediates in the Krebs cycle. Ketogenic amino acids (Leu and Lys) are those amino acids whose metabolism gives rise solely to acetyl-CoA (the precursor of ketone bodies), which cannot be converted to glucose in animals. Glucogenic-plus-ketogenics amino acids (Ile, Phe, Thr, Trp, and Tyr) are those amino acids whose metabolism generates intermediates that can be converted to both glucose and acetyl-CoA. Alanine and glutamine are major glucogenic amino acids in the mammalian liver. Additionally, serine plays an important role in hepatic glucose production by ruminants, particularly during sepsis and pregnancy. Renal gluconeogenesis is low in fed animals (e.g., sheep and pigs) but is increased in food-deprived animals, with glutamine being the most important substrate. In fed and 3-day fasted adult sheep, amino acids contribute to ~30% and 45% of glucose synthesis in the whole body, respectively (Bergman 1983).

TABLE 5.13
Glucogenic and Ketogenic Amino Acids in Animals

Glucogenic Amino Acid	Ketogenic Amino Acid	Glucogenic Plus Ketogenic Amino Acid
Ala, Cys, Gly, Hyp, Ser ---> Pyruvate	Lys ---> Acetyl-CoA	Ile ----> Propionyl-CoA, Acetyl-CoA
Arg, Cit, Gln, Glu, His, Orn, Pro ---> α-KG	Leu ---> Acetyl-CoA	Phe, Tyr ---> Fumarate, Acetoacetate
Asn, Asp ---> OAA	Taurine ---> Acetyl-CoA	Thr ---> Propionyl-CoA, Acetyl-CoA
GABA, Met, Val ---> Succinyl-CoA		Trp, β-alanine ---> Pyruvate, Acetyl-CoA

Note: Cit, citrulline; GABA, γ-aminobutyrate; Hyp, 4-hydroxyproline; α-KG, α-ketoglutarate; Orn, ornithine

4. *Propionate.* Propionate is the major source of glucose in ruminants (e.g., cattle, sheep, and goats) and enters the main gluconeogenic pathway via the Krebs cycle after conversion to succinyl-CoA (Figure 5.16). In this pathway, propionyl-CoA carboxylase requires vitamin biotin and methylmalonyl-CoA isomerase requires vitamin B_{12}

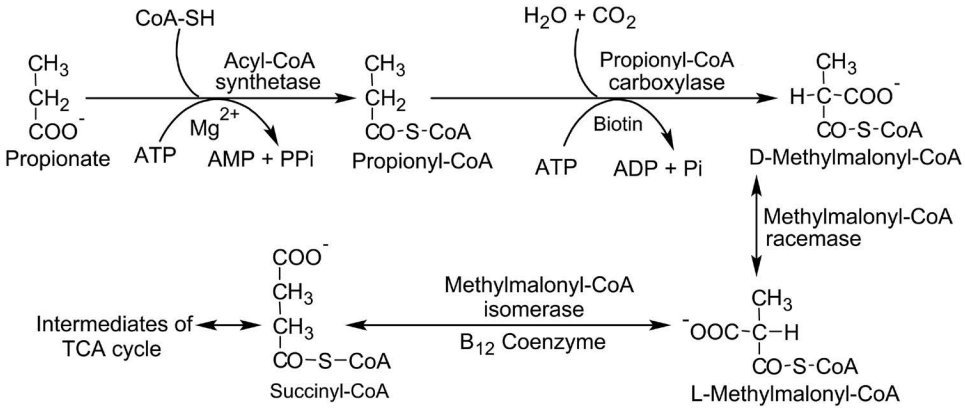


FIGURE 5.16 Conversion of propionate into succinyl-CoA that enters the main pathway of gluconeogenesis in the liver of ruminants. Propionate, which is produced from the fermentation of carbohydrates in the rumen, is quantitatively the single most important substrate for hepatic synthesis in fed ruminants. This metabolic pathway occurs in the mitochondrial matrix of hepatocytes and requires vitamin biotin and B_{12} as cofactors. Methylmalonyl-CoA racemase is an epimerase, and methylmalonyl-CoA isomerase is a mutase. Gluconeogenesis from propionate plays an important role in maintaining glucose homeostasis in ruminants.

(cobalamin). Wilson et al. (1983) reported that, in fed ewes, the contribution of propionate to glucose synthesis increased from 37% in the nonpregnant state and in mid-pregnancy to 55% in late pregnancy and to 60% during lactation. In 3-day fasted sheep, there is no detectable uptake of propionate by the liver and kidneys; thus, there is little synthesis of glucose from this SCFA (Bergman 1983).

5. *Acetate* in organisms other than animals. Conversion of acetate to glucose occurs via the glyoxylate cycle, which is widely found in microbes (including bacteria in the rumen), yeasts, fern spores, and plant seeds (Figure 5.17). In addition to the typical Krebs cycle enzymes (citrate synthase, aconitase and malate dehydrogenase), the glyoxylate cycle includes two unique enzymes, isocitrate lyase and malate synthase. This pathway circumvents the decarboxylation steps of the Krebs cycle and produces 1 mol of succinate from 2 mol of acetyl-CoA, thereby allowing for the conversion of fatty acids to glucose in organisms other than animals. All attempts to demonstrate the glyoxylate cycle in insects have so far failed. Much evidence shows that this metabolic pathway is absent from animal cells (Ward 2000).

Regulation of Gluconeogenesis

Gluconeogenesis is limited in animals fed a high-starch and adequate protein diet (Kristensen and Wu 2012). During fasting, the concentrations of glucagon in the blood increase, whereas those of insulin and glucogenic amino acids decrease despite the increased release of amino acids from skeletal muscle. In addition, the release of glycerol from WAT is enhanced in food-deprived animals. During exercise, both the release of lactate and amino acids from skeletal muscle and the release of glycerol from WAT are elevated, as are the concentrations of glucagon and epinephrine in the blood. All of these metabolic changes during fasting and exercise favor gluconeogenesis.

Intracellular Compartmentation

In the liver, gluconeogenesis occurs in periportal hepatocytes, as does fatty acid oxidation. The presence of both pathways in the same cell confers the following metabolic advantages to (a) efficiently couple ATP produced from fatty acid oxidation with glucose synthesis; and (b) facilitate the metabolic control of gluconeogenesis through the production of acetyl-CoA, citrate, and ATP from fatty acid oxidation. In the kidneys with cytosolic PEPCK, catabolism of Gln or Asp provides OAA and malate in mitochondria, and the malate enters the cytosol for efficient conversion into glucose (Watford 1985).

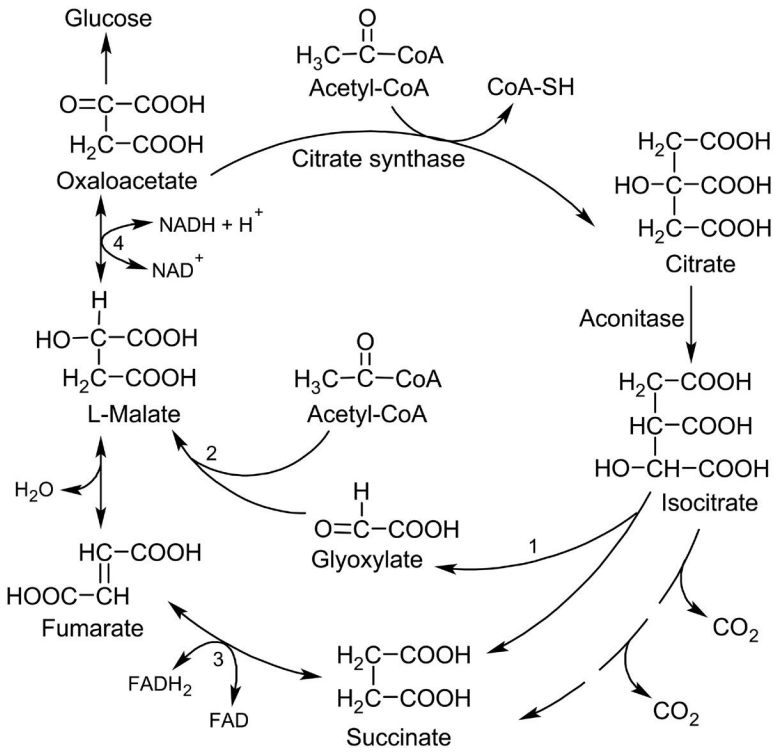


FIGURE 5.17 The glyoxylate cycle in organisms other than animals. In microbes (including bacteria in the rumen), yeasts, fern spores, and plant seeds, acetate is converted into glucose via the glyoxylate cycle. In this pathway, isocitrate is cleaved into glyoxylate and succinate. Glyoxylate combines with acetyl-CoA to form L-malate, whereas succinate undergoes dehydrogenation to yield fumarate. The enzymes that catalyze the indicated reactions are (1) isocitrate lyase, (2) malate synthase, and (3) succinate dehydrogenase. The glyoxylate cycle is absent from animals, which do not contain isocitrate lyase or malate synthase.

Provision of Glucogenic Precursors, ATP, and NADH

Because the flux-generating steps for gluconeogenesis are the concentrations of its substrates, amino acids, glycerol, lactate, and propionate, rates of production of these precursors in animals are one important factor for determining hepatic and renal gluconeogenesis (Neese et al. 1995). For example, an inborn deficiency of pyruvate carboxylase, which converts pyruvate into OAA, causes hypoglycemia, hyperammonemia (due to the accumulation of lactate that leads to impairments in intestinal citrulline synthesis and then the hepatic urea cycle), and even death in humans. Likewise, the availability of ATP (particularly from oxidation of LCFAs and amino acids) and NADH in the liver and kidneys can limit glucose synthesis from potential substrates. Let us use birds to exemplify a key role of NADH in gluconeogenesis (Figure 5.18). In chicken hepatocytes where PEPCK is localized exclusively in the mitochondria, the metabolism of glucogenic amino acids does not generate NADH in the cytosol and, therefore, no glucose even under the conditions of 48-h fasting (Watford et al. 1981). In contrast, in chicken kidney tubules where PEPCK is localized in the cytosol, metabolism of glucogenic amino acids generates NADH in this compartment and, therefore, glucose under both fed and fasting conditions (Watford et al. 1981).

Intracellular Concentrations of Acetyl-CoA, Citrate, and Adenine Nucleotides

Gluconeogenesis is also regulated by concentrations of acetyl-CoA, citrate, and adenine nucleotides in hepatocytes and renal tubules (Figure 5.19). Specifically, acetyl-CoA activates pyruvate carboxylase and therefore increases the conversion of pyruvate to OAA. In addition, acetyl-CoA inhibits pyruvate dehydrogenase, thereby enhancing the availability of pyruvate for glucose production. Citrate (derived from acetyl-CoA) and ATP also inhibit PFK-1. These changes explain why the oxidation of fatty acids, which produces acetyl-CoA, inhibits glycolysis and stimulates gluconeogenesis. Furthermore, the oxidation of fatty acids provides ATP that is required for gluconeogenesis, as noted previously. Both gluconeogenesis and fatty acid oxidation occur in periportal hepatocytes. Thus, the impairment of fatty acid oxidation causes hypoglycemia in mammals. Likewise, under conditions where mitochondrial ATP production is reduced and cytosolic ADP or AMP concentration is elevated, gluconeogenesis in hepatocytes and the kidneys is decreased. This is because ADP and AMP inhibit pyruvate carboxylase and fructose-1,6-bisphosphatase, respectively.

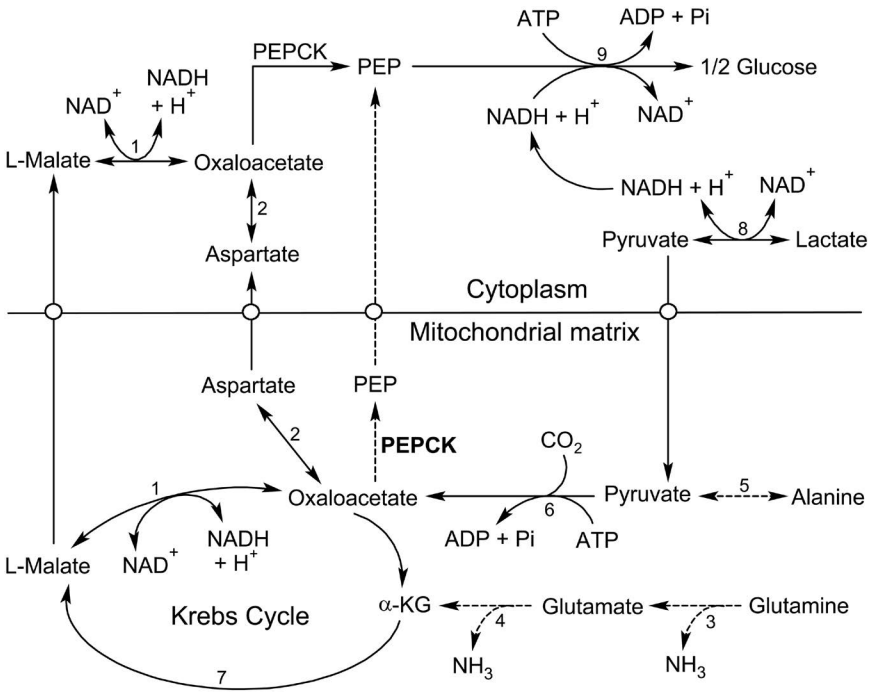


FIGURE 5.18 An important role for the availability of NADH and the intracellular localization of phosphoenolpyruvate (PEP) carboxykinase (PEPCK) in gluconeogenesis. For cells where PEPCK is localized exclusively in mitochondria, the metabolism of glucogenic amino acids does not generate NADH in the cytosol and, therefore, produces no glucose. In contrast, in cells where PEPCK is localized in the cytosol, the metabolism of glucogenic amino acids (e.g., alanine, glutamine, and glutamate) produces NADH in the cytosol and, therefore, glucose. Stoichiometrically, 0.5 mol of glucose is synthesized from 1 mol of alanine. The enzymes that catalyze the indicated reactions are (1) malate dehydrogenase, (2) aspartate transaminase, (3) phosphate-activated glutaminase, (4) glutamate dehydrogenase, (5) alanine transaminase, (6) pyruvate carboxylase, (7) Krebs-cycle enzymes converting α-ketoglutarate (α-KG) into L-malate, (8) lactate dehydrogenase, and (9) glucogenic enzymes converting PEP into glucose. (Adapted from Watford 1985.)

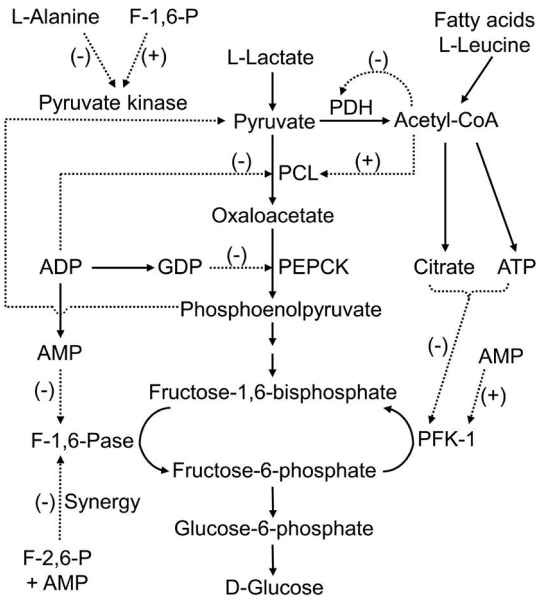


FIGURE 5.19 Regulation of hepatic and renal gluconeogenesis by intracellular concentrations of acetyl-CoA, citrate, alanine, and adenine nucleotides. The factors that increase intracellular fructose-6-phosphate concentrations favor gluconeogenesis and include (a) enhanced activities of fructose-1,6-bisphosphatase (F-1,6-Pase), pyruvate carboxylase (PCL), and phosphoenolpyruvate carboxykinase (PEPCK); and (b) reduced activities of phosphofructokinase 1 (PFK-1), pyruvate kinase, and pyruvate dehydrogenase (PDH). Conversion of fructose-1,6-bisphosphate (F-1,6-P) to fructose-6-phosphate provides the substrate for glucose synthesis and relieves an allosteric effect of F-1,6-P on activating pyruvate kinase. Citrate, ATP, acetyl-CoA, and alanine promote glucose synthesis, whereas fructose-2,6-bisphosphate (F-2,6-P) and AMP suppress this pathway through the synergic inhibition of fructose-1,6-bisphosphatase (F-1,6-Pase). The signs (+) and (–) denote activation and inhibition, respectively.

Glucagon and Fructose-2,6-Bisphosphate (F-2,6-P)

Glucagon inhibits glycolysis and stimulates gluconeogenesis, whereas the opposite is true for F-2,6-P, which is the most potent activator of PFK-1 and inhibitor of F-1-6-Pase (Hers and Van Schaftingen 1982). By increasing the intracellular concentration of cAMP, glucagon promotes the phosphorylation of pyruvate kinase, thereby reducing its enzymatic activity and favoring the conversion of pyruvate into glucose. Additionally, cAMP-dependent kinase phosphorylates phosphofructokinase-2/fructose-2,6-bisphosphatase (a bifunctional enzyme; i.e., one protein having two different enzymatic activities), resulting in an increase of fructose-2,6-bisphosphatase activity but a decrease in phosphofructokinase-2 (Figure 5.20). This leads to a reduction in the intracellular concentration of F-2,6-P, which favors gluconeogenesis. Such a mechanism also explains why an excess or deficiency of glucose in blood inhibits or promotes gluconeogenesis, respectively (Figure 5.21). Specifically, the concentration of F-2,6-P in hepatocytes and renal tubules is elevated in response to an abundant supply of glucose, thereby enhancing glycolysis to form pyruvate. Conversely, the concentration of F-2,6-P in hepatocytes and kidneys is lowered in response to an inadequate provision of glucose, thereby stimulating the conversion of OAA and pyruvate into glucose.

Insulin and Gluconeogenesis

In healthy animals, high concentrations of blood glucose after consumption of a carbohydrate or protein meal stimulate the release of insulin from pancreatic ß-cells, whereas the blood concentration of insulin is decreased during

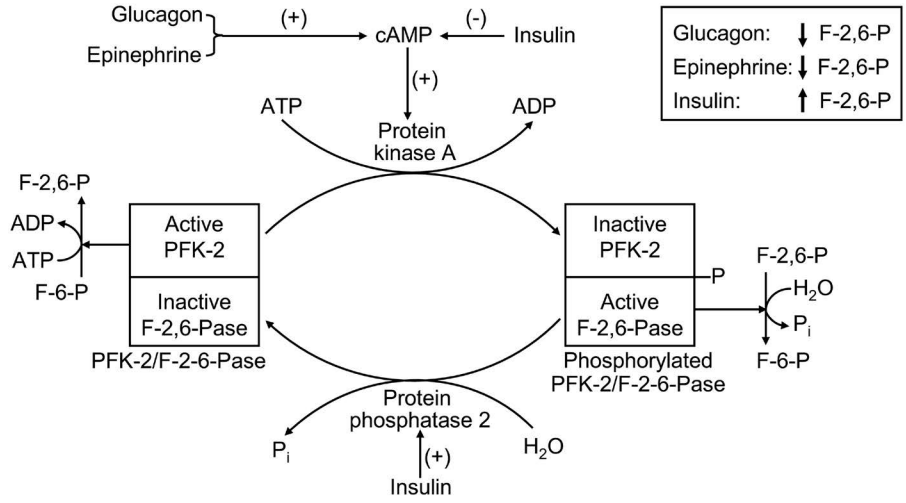


FIGURE 5.20 Regulation of phosphofructokinase 2/fructose-2,6-bisphosphatase by fructose-2,6-bisphosphate. Phosphofructokinase 2/Fructose-2,6-bisphosphatase is a bifunctional enzyme (one protein having two different enzymatic activities). Phosphorylation of this enzyme by cAMP-dependent protein kinase increases fructose-2,6-bisphosphatase (F-2,6-Pase) activity while decreasing phosphofructokinase-2 (PFK-2) activity. Glucagon and epinephrine increase cAMP production, thereby reducing the conversion of fructose-6-phosphate (F-6-P) into fructose-2,6-bisphosphate [F-2,6-P, an inhibitor of fructose-1,6-bisphosphatase (F-1,6-Pase)] to favor glucose synthesis. In contrast, insulin has an opposite effect and plays an important role in suppressing hepatic gluconeogenesis. The signs (+) and (–) denote activation and inhibition, respectively.

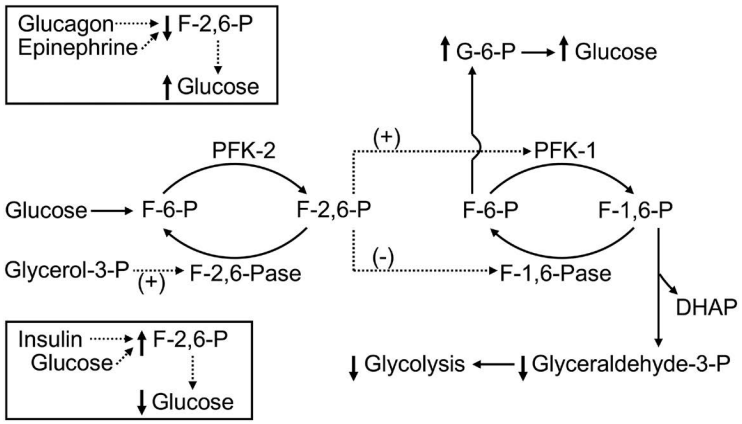


FIGURE 5.21 Regulation of hepatic and renal gluconeogenesis by glucose availability and hormones through a change in the intracellular concentration of fructose-2,6-bisphosphate (F-2,6-P). Elevations in the circulating levels of glucose and insulin result in an increase in concentrations of fructose-2,6-bisphosphate [F-2,6-P, an activator of phosphofructokinase 1 (PFK-1) and an inhibitor of fructose-1,6-bisphosphatase (F-1,6-Pase)] in the liver and kidneys, thereby inhibiting glucose synthesis. In contrast, when the circulating levels of glucagon and epinephrine are elevated, concentrations of F-2,6-P in the liver and kidneys are decreased, thereby promoting glucose synthesis. *Note:* DHAP, dihydroxyacetone phosphate; G-6-P, glucose-6-phosphate. The signs (+) and (–) denote activation and inhibition, respectively.

fasting. High circulating levels of insulin antagonize the action of glucagon and inhibit hepatic gluconeogenesis by decreasing (a) intracellular concentration of cAMP due to the activation of 3'-5'-cyclic nucleotide phosphodiesterase in hepatocytes; (b) uptake of lactate by the liver; (c) the concentrations of glucogenic amino acids in the blood; and (d) expression of glucogenic enzymes in the liver and kidneys. Thus, glucose synthesis from amino acids and other substrates is limited in the liver of growing pigs fed a high-starch and 16%-CP diet (Figure 5.22). Partly through its effect on inhibiting hepatic gluconeogenesis, insulin plays a role in stimulating food intake by animals (Nizielski et al. 1986).

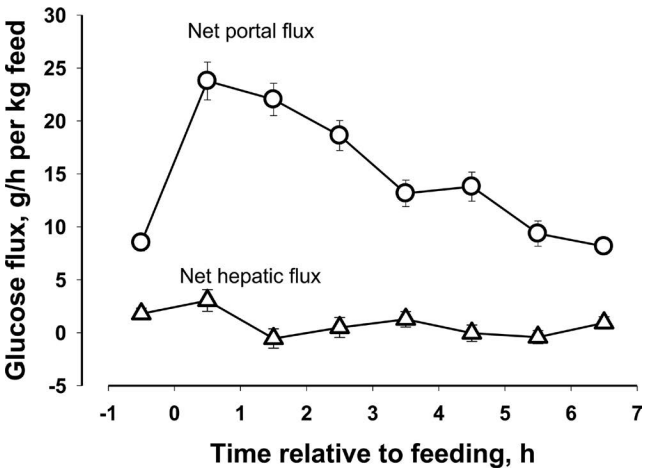


FIGURE 5.22 Net portal flux (open bar) and net hepatic flux (filled bar) of glucose in 60-kg growing pigs fed a high-starch and 16% crude-protein diet at the rate of 3.6% of body weight per day. Feed was divided into 3 equally sized meals per day at 8-h intervals. A positive flux denotes net release, and a negative flux denotes net uptake across the tissues. Each data point represents mean ± SEM, n = 16. [Reproduced from a figure freely available in the public domain (Kristensen and Wu (2012).]

$$\text{ATP} \rightarrow \text{cAMP} + \text{PPi} \quad (\text{Adenylate cyclase})$$

$$\text{cAMP} + \text{H}_2\text{O} \rightarrow \text{AMP} \quad (\text{Class 3 cyclic nucleotide phosphodiesterase; PDE-3})$$

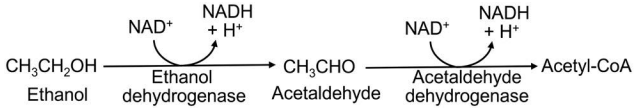
Glucocorticoids and Gluconeogenesis

Glucocorticoids are steroid hormones that promote gluconeogenesis in the liver via increasing protein degradation in skeletal muscle to provide glucogenic amino acids, intracellular cAMP concentration, and expression of glucogenic genes (Devlin 2011). Notably, through activating cytosine-cytosine-adenosine-adenosine-thymidine (CCAAT)/enhancer-binding protein-β and cAMP regulatory element-binding protein, glucocorticoids enhance the transcription of the PEPCK gene for promoting hepatic gluconeogenesis. In a concerted response, glucocorticoids reduce glucose uptake and utilization by skeletal muscle and WAT through antagonizing insulin's action, thereby providing glucose for hepatic glucose synthesis. Therefore, excess glucocorticoids result in hyperglycemia and insulin resistance in animals.

Ethanol and Gluconeogenesis

High concentrations of ethanol (e.g., 10 mM) inhibit hepatic gluconeogenesis from lactate, glycerol, dihydroxyacetone, proline, serine, alanine, fructose, and galactose (Krebs et al. 1969). In contrast, ethanol does not affect glucose synthesis from pyruvate in the liver. When ethanol is oxidized by alcohol dehydrogenase to form acetaldehyde, NAD^+ is reduced to NADH + H^+, thereby lowering the ratio of [free NAD^+]/[free NADH] in hepatocytes. The disturbance of the intracellular [free NAD^+]/[free NADH] ratio is exacerbated when acetaldehyde is oxidized by NAD^+-dependent

acetaldehyde dehydrogenase to acetic acid. This leads to decreases in the formation of pyruvate and therefore glucose from lactate. Because conversion of pyruvate into glucose does not require NAD^+, this pathway is not suppressed by alcohol. Ethanol inhibits gluconeogenesis from glycerol, dihydroxyacetone, glucogenic amino acids, fructose, and galactose, likely through reducing their uptake by the liver.



Quantification of Gluconeogenesis

In Vitro Incubation of Cells

Hepatocytes are incubated with physiological concentrations of glucogenic substrates (labeled or unlabeled) to determine the production of glucose (labeled or unlabeled). Specifically, an appropriately labeled substrate is used to measure the formation of glucose from the particular substrate. For example, if hepatocytes are incubated in 1 ml of buffer containing 1 mM lactate plus [U-^{14}C]lactate (3×10^6 dpm) and [^{14}C]glucose (6000 dpm) is formed, the amount of glucose synthesized by the cells can be calculated based on the assumption that the intracellular specific radioactivity of [U-^{14}C]lactate is similar to its extracellular specific radioactivity. First, we need to know the concept of the specific radioactivity (SA) of a radiolabeled compound (e.g., ^{14}C-labeled lactate), or isotope enrichment (IE) of a stable isotope (e.g., ^{13}C-labeled lactate).

$$\text{SA} = \frac{\text{Amount of a radioactive isotope (dpm)}}{\text{Mass of labeled plus unlabeled isotopes (e.g., nmol)}} = \frac{^{14}\text{C (dpm)}}{^{14}\text{C (nmol)} + ^{12}\text{C (nmol)}}$$

$$\text{IE} = \frac{\text{Mass of a stable isotope (e.g., µmol)}}{\text{Mass of labeled plus unlabeled isotopes (e.g., µmol)}} = \frac{^{13}\text{C (µmol)}}{^{13}\text{C (µmol)} + ^{12}\text{C (µmol)}}$$

Examples of calculations of SA and product production:

$$\text{Specific activity (SA) of lactate} = 3 \times 10^6 \text{ dpm}/1000 \text{ nmol} = 3000 \text{ dpm/nmol lactate} = 3000/3 = 1000 \text{ dpm/nmol C}$$

$$\text{Glucose synthesis from lactate} = \frac{\text{Labeled glucose produced (dpm)}}{\text{SA of [U-}^{14}\text{C] lactate (dpm/nmol of C)}} \times \frac{1}{6} = \frac{6000 \text{ dpm}}{1000 \text{ dpm/nmol C}} \times \frac{1}{6} = 1 \text{ nmol glucose}$$

Perfused Organs

An isolated, perfused liver is often used to study gluconeogenesis (Ekberg et al. 1995). The liver is perfused with physiological concentrations of glucogenic substrates (labeled or unlabeled), and effluent perfusate is collected for the determination of the production of glucose (labeled or unlabeled). The calculation of glucose synthesis is the same as that for in vitro cell incubation.

In Vivo Study

Labeled isotopes must be used to study gluconeogenesis in vivo to distinguish the sources of potential substrates (Chung et al. 2015). Animals or humans are often intravenously infused with a suitable radioactive or stable isotope, and labeled glucose is measured by a liquid scintillation counter (for radioactive isotopes) or mass spectroscopy (for stable isotopes). Of course, in vivo experiments are much more complex than in vitro incubation and organ perfusion studies in terms of calculation and data interpretation.

Nutritional and Physiological Significance of Gluconeogenesis

Maintenance of Glucose Homeostasis in Animals

It must be recognized that a constant supply of glucose is necessary as the dominant source of energy for the nervous system when the circulating levels of ketone bodies are low (e.g., <0.15 mM) and as the sole source of energy for RBCs under all conditions. This is particularly important for strict carnivores [e.g., cats, tigers, carnivorous fish, and certain marine mammals (sea lion, seal, and white whale)], who ingest a limited amount of carbohydrate from diets. Gluconeogenesis is also essential for ruminants, who absorb little or no glucose in the small intestine under normal feeding conditions. In adult sheep fed Lucerne hay at 0.5-h intervals, the rates of glucose production by the liver and kidney are 4.5 and 0.7 g/h, respectively. The rate of gluconeogenesis matches the rate of glucose utilization in the sheep, which is 1.4 and 3.8 g/h in the splanchnic bed (the portal-drained viscera + liver) and other tissues (including skeletal muscle, brain, heart, blood, kidneys, and adipose tissue), respectively (Bergman 1983). Thus, a failure of gluconeogenesis is usually fatal for animals (Yang et al. 2009).

Normal concentrations of glucose in plasma are ~ 5.0–5.5 mM in postabsorptive dogs, cats, humans, pigs, and rats, ~ 2.5–3.4 mM in ruminants, and ~ 12–15 mM in chickens. Below a critical glucose concentration in plasma (e.g., 3.5 mM in pigs and 1 mM in sheep), animals exhibit brain dysfunction, which can lead to coma and death. In this regard, it is noteworthy that a 34-year-old obese man (96.8 kg; body mass index, 30.2 kg/m^2) can survive a complete and voluntary 50-day fast (with the consumption of adequate water and vitamins; Elliott et al. 2016). Interestingly, Yorkshire pigs (with an initial BW of 65 kg) can survive a 28-day fast, with serum concentrations of some amino acids (arginine, cystine, proline, tryptophan, and tyrosine) being unchanged; histidine and methionine decreasing by 54%; and all other proteinogenic

amino acids increasing by 45–125%, and serum concentrations of free fatty acids increasing by 413% (Klaine et al. 1977).

Because nonruminants are capable of gluconeogenesis, they do not have specific dietary requirements for carbohydrates. This is consistent with experimental results from studies involving pigs, rats, poultry, fish, and marine mammals. However, it should be noted that (a) dietary fiber is beneficial for maintaining the intestinal health of nonruminants (particularly gestating and lactating sows), and (b) total dependence on glucose provision by endogenous synthesis from amino acids will not only increase the cost of animal production but will also negatively impact the function of the liver, kidneys, and possibly other organs.

Low-carbohydrate Diets or High Demands for Glucose

In nonruminants, whenever the carbohydrate content of diets is low or demands for carbohydrate by those tissues that depend exclusively on glucose for energy are high, gluconeogenesis becomes particularly important. If gluconeogenesis is insufficient, hypoglycemia occurs in animals. Although most diets are rich in carbohydrates, some (e.g., the traditional diet of the Eskimos and carnivores) are low in carbohydrates. Under these feeding conditions, animals require a high level of gluconeogenesis to maintain normal glucose concentrations in plasma.

Fasting

During fasting, carbohydrate intake is absent, and after endogenous glycogen reserves have been utilized within 12 hours, gluconeogenesis plays an important role in maintaining glucose homeostasis in animals (Brosnan 1999). Before plasma concentrations of ketone bodies are sufficiently increased, the neurons in the brain depend on glucose for energy provision. This necessitates gluconeogenesis in response to fasting. Of note, rates of whole-body glucose production in fasted animals depend on the availability of glucogenic substrates and, therefore, the stage of food deprivation.

Neonates

Hypoglycemia usually occurs in neonates (e.g., piglets with limited WAT) and particularly in the newborn infants of diabetic mothers. High levels of glucose in maternal blood lead to hyperglycemia in the fetus, which activates the release of insulin by fetal pancreatic β-cells. At birth, the newborn continues to increase insulin production but no longer receives an increased transfer of glucose from the maternal blood. Thus, within the first days after birth, hypoglycemia usually develops in neonates with prenatal exposure to hyperglycemia.

Lactation

During lactation, large amounts of glucose are used for the synthesis of both lactose and TAGs by mammary glands. Thus, gluconeogenesis is markedly enhanced in a mother nursing her offspring. This is particularly important for lactating ruminants that face an additional nutritional problem, namely, the extensive fermentation of carbohydrates by ruminal microbes and little absorption of glucose by the small intestine. Propionate is the principal glucogenic substrate for ruminants, as noted previously.

Acid-base Balance

Conversion of some substrates (e.g., lactate) to glucose consumes H^+, thus playing a role in regulating the acid-base balance. This is of particular importance during intensive exercise, when the high rates of glycolysis in skeletal muscle produce large amounts of lactate. Synthesis of glucose from lactate can conserve the glucose carbons (particularly in ruminants), in contrast to the irreversible oxidation of lactate to CO_2 and water. Because lactate is derived from glucose, gluconeogenesis from lactate does not result in a net production of glucose in animals. Similarly, conversion of glutamate and aspartate into glucose in the liver and kidneys is also associated with the removal of H^+. Likewise, the use of glutamine as a glucogenic precursor in the kidneys during starvation generates ammonia to aid in buffering the H^+ that is produced through hepatic ketogenesis.

$$\text{Lactate oxidation to } CO_2\text{: } C_3H_5O_3^- + H^+ + 3O_2 \rightarrow 3CO_2 + 3H_2O$$

$$\text{Lactate} \rightarrow \text{Glucose: } 2C_3H_5O_3^- + 2H^+ \rightarrow C_6H_{12}O_6$$

Species Differences in the Development of Gluconeogenesis

There are species differences in the development of hepatic and renal gluconeogenesis. For example, because the avian egg has only a trace amount of carbohydrate, the chick embryo develops all enzymes for this metabolic pathway in the liver by day 13 of incubation (Evans and Scholz 1973). The activities of key gluconeogenic enzymes in the liver, i.e., pyruvate carboxylase, PEP carboxykinase (PEPCK), fructose-1,6-bisphosphatase (also known as hexosediphosphatase), and glucose-6-phosphatase, increase to maxima on days 16 and 17 of incubation (Pearce 1971). For comparison, there is no gluconeogenesis from glycerol, pyruvate, or amino acids in avian embryonic kidneys (Evans and Scholz 1973). On hatching, as the diet is shifted to a high-starch diet, the activities of the hepatic gluconeogenic pathway decrease, with lower activities in adult fowl than in late embryonic or newly hatched birds, whereas renal glucose synthesis develops after hatching with amino acids being major substrates than glycerol and pyruvate. In contrast, hepatic and renal gluconeogenesis is very limited in the mammalian fetus (which receives a supply of glucose from the maternal blood) but develops rapidly after birth to be fed on a high-fat but low-carbohydrate diet (i.e., milk).

Glycogen Metabolism

Glycogen as a Hydrophilic Macromolecule

Excess glucose is stored as glycogen in animals and as starch in plants. Glycogen is a highly branched polysaccharide molecule, with α-glucose residues linked by

α-1,4 glycosidic bonds (main chain) and by α-1,6 glycosidic bonds. Glycogen can have a molecular weight of 10^8, a mass equivalent to 6×10^5 glucose residues. Although almost all animal cells contain glycogen, the liver, skeletal muscle, and heart are the major sites of glycogen synthesis and deposition in animals (Chapter 2). Glycogen is a hydrophilic macromolecule associated with water in a ratio of 1:3. Thus, deposition of 1 g glycogen in skeletal muscle retains 3 g water (Brooks and Fahey 1984). Muscle (skeletal muscle plus heart) and liver are the major sites of glycogen storage in animals.

Pathway of Glycogen Synthesis (Glycogenesis)

As noted previously, the liver, skeletal muscle, and heart are major sites for glycogen synthesis (glycogenesis) in animals. There are both direct and indirect pathways for this metabolic pathway. The direct pathway utilizes glucose as a substrate, whereas the indirect pathway depends on C-3 units, such as alanine and lactate. Glycogenesis occurs in the cytosol, and its pathway is outlined in Figure 5.23. Glucose is first phosphorylated to glucose-6-P, catalyzed by hexokinase in muscles and glucokinase in the liver. [Note that glucose-6-P is also the initial substrate of the pentose cycle.] Glucose-6-P is converted to glucose-1-P by phosphoglucomutase. In this reaction, phosphoglucomutase itself is phosphorylated, and the phospho-group participates in a reversible reaction in which glucose-1,6-bisphosphate is an intermediate.

$$\text{Enzyme-P} + \text{Glucose-6-P} \rightleftharpoons \text{Enzyme} + \text{Glucose-1,6-P} \rightleftharpoons \text{Enzyme-P} + \text{Glucose-1-P}$$

Subsequently, glucose-1-P reacts with uridine triphosphate (UTP) to form uridine diphosphate glucose (UDPGlc), which is catalyzed by UDPGlc pyrophosphorylase. The subsequent hydrolysis of inorganic pyrophosphate (PPi) by inorganic pyrophosphatase pulls the reaction to the right side of the equation.

$$\text{UTP} + \text{Glucose-1-P} \rightleftharpoons \text{UDP-Glucose} + \text{PPi}$$

Glycogenin (a protein with molecular weight of 37–38 kDa) serves as the physiological primer for glycogen synthesis (Figure 5.23). About 8 glucose residues (in the form of UDPGlc) are added to the tyrosine-194 of glycogenin to form fully glucosylated glycogenin (protein primer for synthesis of proglycogen). The fully glucosylated glycogenin serves as the protein primer for the synthesis of proglycogen (400 kDa) in the presence of proglycogen synthase, branching enzyme, and UDPGlc. Glucose residues (in the form of UDPGlc) are further added to proglycogen to form macroglycogen (10^4 Kda) in the presence of macroglycogen synthase and branching enzyme.

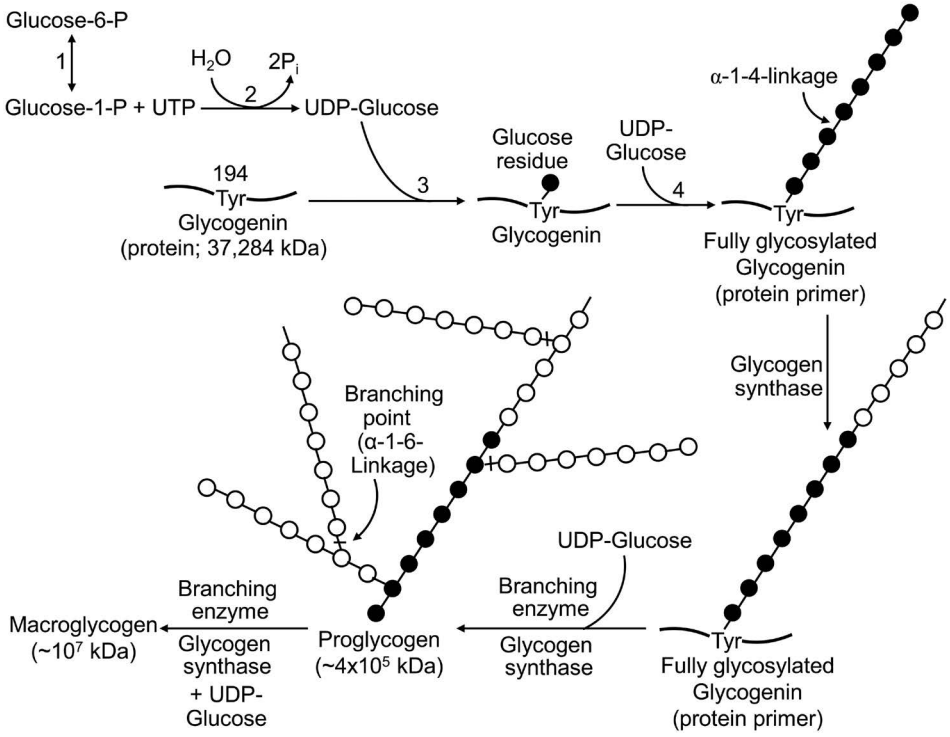


FIGURE 5.23 Synthesis of glycogen (glycogenesis) in animals. Glycogenin is the primer for glycogen synthesis in animal cells. The glucose moiety from UDP-Glucose is attached to the tyrosine-194 of glycogen, followed by the formation of glucose polymers in the α-1,4 linkage (main chain). The branching enzyme forms α-1,6 linkages of glucose molecules at the branch. The enzymes that catalyze the indicated reactions are (1) phosphoglucomutase, (2) UDP-glucose pyrophosphorylase, (3) glycogenin's tyrosine glucosyltransferase, and (4) spontaneous autocatalysis, adding seven glucose residues to the existing glucose residue linked to Tyr-194 of glycogenin.

As noted previously, glycogen is a highly branched molecule with α-(1,4) and α-(1,6) linkages. The branching results from the action of the glycogen branching enzyme [amylo α-(1,4)→(1,6)-transglucosidase]. This enzyme transfers a part of the α-1,4-chain (minimum length of 6 glucose residues) to a neighboring chain to form an α-1,6-linkage, thus establishing a branch point in the molecule. The branches in glycogen grow by further additions of α-1,4-glucosyl units and further branching.

The discovery of glycogenin in 1985 as a self-glucosylating protein that primes glycogen synthesis has significantly increased our understanding of the mechanism of glycogen synthesis. This discovery was based on the original finding that a liver pellet could synthesize from UDPGlc, a glycogen-like product that can be precipitated by trichloroacetic acid (Krisman and Barengo 1975). This finding changed the traditional view that preexisting glycogen was required as the primer for glycogen synthesis in animals (Alonso et al. 1995).

Pathway of Glycogen Degradation (Glycogenolysis)

Glycogen degradation (also known as glycogenolysis) occurs in the cytosol. This metabolic pathway is not the reverse of glycogenesis but involves separate enzymes and a debranching mechanism (Figure 5.24). The first and rate-controlling step of glycogenolysis is catalyzed by glycogen phosphorylase.

$$\text{Glycogen}\left(C_6\right)_n + \text{Pi} \rightarrow \text{Glycogen}\left(C_6\right)_{n-1} + \text{Glucose-1-P}\ \left(\text{Glycogen phosphorylase}\right)$$

Glycogen phosphorylase is specific for breaking the 1→4-linkages of glycogen to form glucose-1-P. The terminal glucosyl residues from the outermost chains of the glycogen molecule are removed sequentially until approximately 4 glucose residues remain on either side of α-1,6 branches. Glucan transferase transfers a trisaccharide unit (3 glucose residues) from one branch of a glycogen molecule to the other branch, thus exposing the α-1,6 branch point. The hydrolytic splitting of the α-1,6 linkage requires a specific debranching enzyme [amylo(1,6)-glucosidase]. Note that the eukaryotic glycogen debranching enzyme, a bifunctional protein, possesses two different catalytic activities (oligo-1,4→1,4-glucantransferase/amylo-1,6-glucosidase) on a single polypeptide chain (Nakayama et al. 2001). With the removal of the branch, further action by glycogen phosphorylase can proceed to release glucose-1-P. Thus, the combined actions of glycogen phosphorylase and glycogen debranching enzyme lead to the complete breakdown of glycogen. As noted previously, glucose-1-P can be converted to glucose-6-P. The presence of glucose-6-phosphatase in the liver and kidneys (but not in skeletal muscle or heart) enables the formation of glucose from glucose-6-P and glycogen.

Regulation of Glycogenesis

The principal enzyme regulating glycogen synthesis is glycogen synthase. This enzyme exists in either a dephosphorylated state (a form, which is active) or a phosphorylated state (b form, which is inactive). Glycogen synthase is phosphorylated by a protein kinase (mainly protein kinase A),

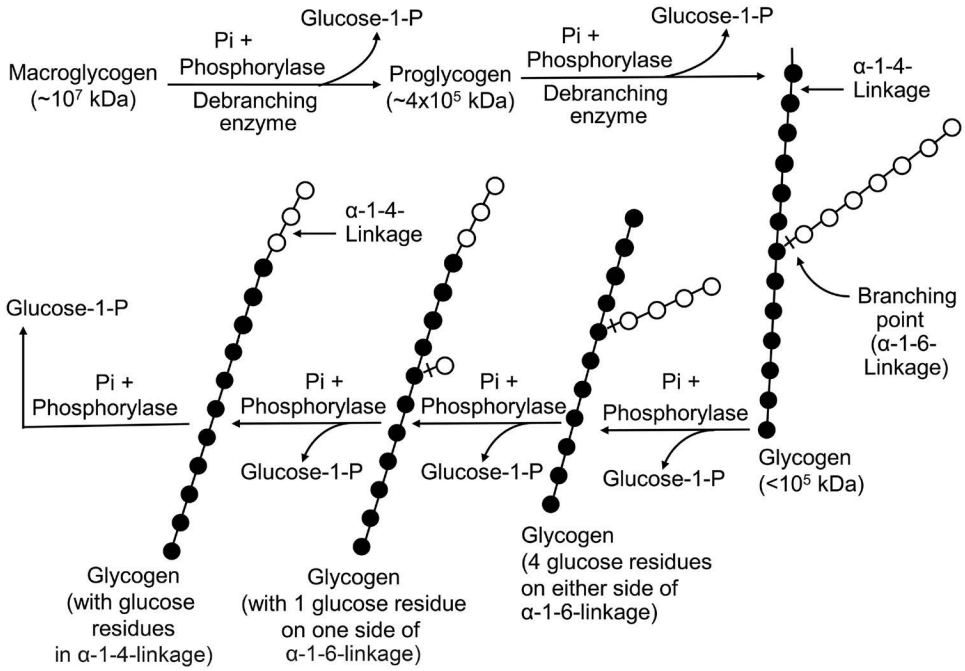


FIGURE 5.24 Degradation of glycogen (glycogenolysis) in animals. Activated glycogen phosphorylase initiates glycogen degradation to form glucose-1-phosphate. The branching α-1,6 linkages in the side chains are hydrolyzed by the debranching enzyme to produce chains of glucose residues in α-1,4 linkages, followed by their cleavage through the action of glycogen phosphorylase. The latter requires Pi for enzymatic activity.

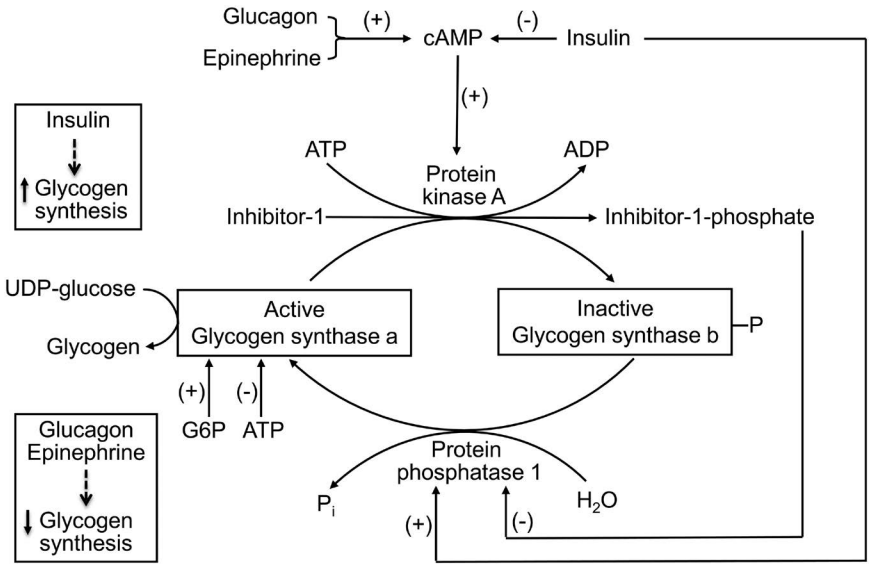


FIGURE 5.25 Regulation of glycogen synthase through protein phosphorylation and dephosphorylation, as well as allosteric control, in animals. Glycogen synthase exists in a dephosphorylated state (active form) or phosphorylated state (inactive form). This enzyme is phosphorylated by protein kinase A, whereas protein phosphatase-1 catalyzes its dephosphorylation. Protein kinase A also phosphorylates inhibitor-1, which is an inhibitor of protein phosphatase 1. Protein kinase A itself is activated by cAMP. By increasing cAMP concentration in cells, glucagon and epinephrine suppress glycogenesis. The opposite effect is true for insulin. Glycogen synthase is allosterically activated by glucose-6-phosphate (G6P) and inhibited by ATP. The signs (+) and (–) denote activation and inhibition, respectively.

whereas glycogen synthase phosphatase (also known as protein phosphatase-1) catalyzes its dephosphorylation (Figure 5.25). Several protein kinases can phosphorylate glycogen synthase, thereby inhibiting its activity. These kinases are known as cAMP-dependent protein kinase, Ca^{2+}/Calmodulin-dependent protein kinase, glycogen synthase kinases 3, 4, and 5. Glycogen synthase phosphatase is inhibited by inhibitor-1-P upon its activation by cAMP-dependent protein kinase. Some hormones and metabolites also affect glycogenesis. For example, insulin increases glycogenesis by promoting dephosphorylation of glycogen synthase b (inactive) to form glycogen synthase a (active), whereas fasting, epinephrine, norepinephrine, and glucagon decrease glycogenesis by promoting phosphorylation of glycogen synthase a (active) to glycogen synthase b (inactive) by stimulating cAMP production. Note that concentrations of these 3 hormones in plasma are elevated during intensive exercise, contributing to elevated concentrations of glucose in plasma.

Glucocorticoids modulate glucose homeostasis in animals partly through control of glycogenesis in a tissue-specific manner. Specifically, glucocorticoids increase glycogen synthesis and storage in the liver by enhancing the availability of glucose, as well as the expression of glucogenic enzymes and glycogen synthase (Devlin 2011). In contrast, glucocorticoids inhibit insulin-stimulated glycogen synthesis in skeletal muscle by antagonizing insulin signaling. Moreover, glucocorticoids inhibit the secretion of insulin from pancreatic β cells and also antagonize the action of insulin in the liver, skeletal muscle, heart, and WAT.

Glucose-6-P (an allosteric activator of glycogen synthase) and glucose (a precursor of glucose-6-P) promote, but ATP (an allosteric inhibitor of glycogen synthase) reduces glycogenesis in eukaryotic cells (e.g., myocytes and hepatocytes; Palm et al. 2013). There is evidence that arginine stimulates glycogen formation in white adipocytes (Egan et al. 1995). In contrast, a high concentration of glycogen reduces glycogenesis through a feedback inhibition mechanism, particularly in hepatocytes. Glycogen synthase is also allosterically inhibited by ATP. The discovery of cAMP as a second messenger in the cell led to the award of the Nobel Prize in Physiology or Medicine to Earl W. Sutherland in 1971.

Intracellular compartmentalization of glycogenesis is another factor regulating this pathway in the liver. Specifically, glycogen synthesis from lactate and amino acids, as well as gluconeogenesis occurs mainly in periportal hepatocytes, whereas glycogen is synthesized from glucose predominantly in perivenous hepatocytes, just like the degradation of glycogen into pyruvate. The separation of these opposing pathways in two different hepatocyte populations makes it possible for the net production of glycogen in the liver.

Regulation of Glycogenolysis

Regulation of Glycogenolysis in Skeletal Muscle and the Heart

Glycogen phosphorylase is the major target for the control of glycogenolysis in skeletal muscle and the heart. This enzyme exists in either the phosphorylated form (a form,

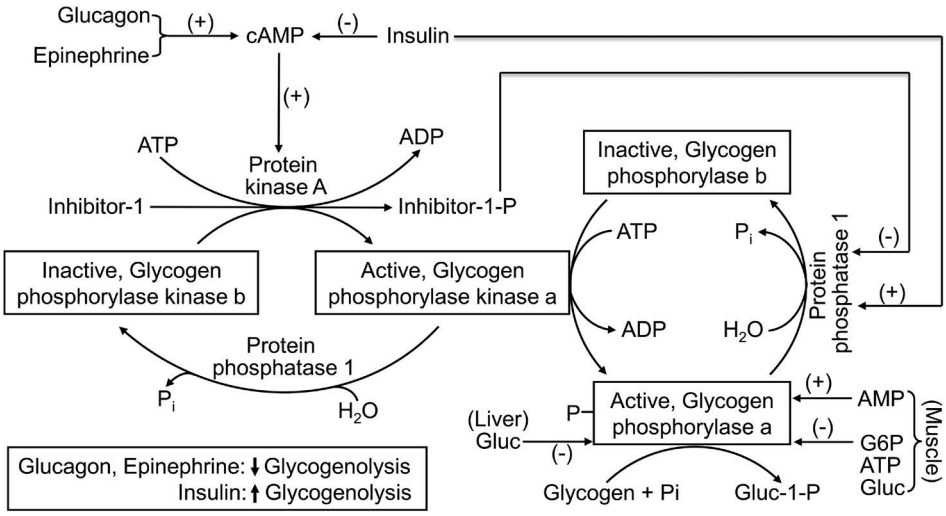


FIGURE 5.26 Regulation of glycogen phosphorylase by protein phosphorylation and dephosphorylation in animals. Glycogen phosphorylase exists in a dephosphorylated state (inactive form) or phosphorylated state (active form). This enzyme is phosphorylated by protein kinase A, whereas protein phosphatase-1 catalyzes its dephosphorylation. Protein kinase A also phosphorylates inhibitor-1, which is an inhibitor of protein phosphatase 1. Protein kinase A itself is activated by cAMP. By increasing cAMP concentration in cells, glucagon and epinephrine stimulate glycogenolysis. The opposite effect is true for insulin. Note that allosteric regulation by metabolites [e.g., glucose (Gluc)] differs between skeletal muscle and liver. The signs (+) and (–) denote activation and inhibition, respectively.

active) or the dephosphorylated form (b form, inactive). Phosphorylase kinase converts phosphorylase b (inactive) to phosphorylase a (active), thereby increasing the activity of glycogen phosphorylase (Figure 5.26). Elucidation of this mechanism resulted in the award of the Nobel Prize in Physiology or Medicine to Edwin G. Krebs and Edmond H. Fischer in 1992. Like glycogen synthase, phosphorylase kinase is also phosphorylated by cAMP-dependent protein kinase. However, unlike glycogen synthase, phosphorylation increases glycogen phosphorylase activity, thereby promoting glycogenolysis. Ca^{2+} synchronizes the activation of glycogen phosphorylase with muscle contraction by stimulating a calmodulin-dependent phosphorylase kinase. It is noteworthy that protein phosphorylation and calcium binding each partially activate phosphorylase kinase independent of the other, and the full activation of glycogen phosphorylase requires both events. Protein phosphatase-1 is inhibited by inhibitor-1-P upon its activation by the cAMP-dependent protein kinase. Fasting, epinephrine, and norepinephrine enhance glycogenolysis in skeletal and cardiac muscles by elevating intracellular cAMP concentrations. Of note, glucocorticoids play a permissive role for catecholamine-induced glycogenolysis in skeletal muscle (Devlin 2011). In contrast, insulin suppresses intramuscular glycogenolysis by inhibiting glycogen phosphorylase activity. In skeletal muscle, this enzyme is allosterically stimulated by AMP (the intramuscular concentration of which increases during strenuous exercise) and inhibited by glucose-6-P and ATP (the intramuscular concentration of which increases after feeding).

When animals (including humans) are exposed to elevated ambient temperatures (e.g., 40°C vs. 20°C), plasma concentrations of catecholamines (e.g., adrenaline and noradrenaline) and glucocorticoids (e.g., cortisol in mammals and corticosterone in birds) are augmented. This results in the activation of the autonomic nervous system and associated physiological responses, including (a) increases in body temperatures and heart rates and (b) the redistribution of blood flow from the viscera and, in the case of pregnant dams, conceptuses and uterus to the skin for thermoregulation via radiant heat loss, resulting in reductions in the flows of blood, oxygen, and nutrients into internal organs and fetuses. Both catecholamines and glucocorticoids stimulate the production of cAMP and, therefore, cAMP-dependent glycogenolysis (via glycogen phosphorylase) and glycolysis (via PFK-1) in skeletal muscle and the heart, as well as other tissues (Febbraio et al. 1994; Gonzalez-Rivas et al. 2020). Such metabolic changes adversely affect animal (including human) health in medicine and meat quality in animal agriculture. For example, acute heat stress immediately before the slaughter of livestock (e.g., pigs and cattle), poultry, and fish stimulates glycogenolysis and glycolysis in skeletal muscle, resulting in a rapid decline in pH, decreases in cell membrane integrity and water-holding capacity, and eventually pale, soft, and exudative (PSE) meat (Gonzalez-Rivas et al. 2020). Likewise, chronic heat stress depletes muscle glycogen stores before the slaughter of the farm animals, leading to increases in postmortem intramuscular pH and water-holding capacity, and eventually dark, firm, and dry (DFD) meat (Gonzalez-Rivas et al. 2020).

Regulation of Glycogenolysis in the Liver

Hepatic glycogen phosphorylase also exists in either the phosphorylated form (a form, active) or the

dephosphorylated form (b form, inactive), like its isozyme in muscles (Figure 5.26). However, liver glycogen phosphorylase is immunologically and genetically distinct from muscle phosphorylase. Insulin inhibits glycogenolysis by decreasing intracellular cAMP concentration, whereas fasting, glucagon, epinephrine, and norepinephrine promote glycogenolysis by increasing intracellular cAMP concentration (Devlin 2011). Epinephrine and norepinephrine can also increase hepatic glycogenolysis by mobilizing Ca^{2+} from mitochondria to the cytosol, followed by the stimulation of a Ca^{2+}/calmodulin-sensitive phosphorylase kinase. Thus, cAMP integrates the regulation of glycogenesis and glycogenolysis in the liver through cAMP-dependent protein kinase. Hepatic glycogen phosphorylase activity is allosterically inhibited by glucose but is not sensitive to allosteric control by glucose-6-P, AMP, or ATP. The difference in the allosteric regulation of glycogen phosphorylase between the liver and skeletal muscle reflects their different roles producers and utilizers of glucose, respectively, under various physiological conditions.

Determination of Glycogenesis and Glycogenolysis

Glycogenesis and glycogenolysis can be measured in incubated cells and perfused organs (Ekberg et al. 1995). For example, glycogenesis can be measured in the presence of D-[U-^{14}C]glucose. On the other hand, glycogenolysis can be measured from the production of $^{14}CO_2$ plus [^{14}C]lactate from prelabeled [^{14}C]glycogen. In vivo measurements of glycogenesis and glycogenolysis are technically difficult due to the multiple pathways for the metabolism of their intermediates in animals (Chung et al. 2015).

Nutritional and Physiological Significance of Glycogen Metabolism

The major function of glycogenesis is to store glucose as glycogen immediately after the consumption of a starch-containing meal or when the dietary supply of glucose is in excess. This helps prevent a marked increase in extracellular and intracellular osmolarity that would result from a high concentration of free glucose. In producing ducks, forced-feeding with excess starch for a couple of weeks before marketing helps produce the liver with a large amount of glycogen. However, too much glycogen can impair the function of both the liver and muscles. Hepatic glycogenolysis can immediately provide glucose to maintain the blood glucose concentration during fasting, short periods of starvation (e.g., the diurnal fast), exercise, as well as in other conditions that might result in hypoglycemia (e.g., transient overproduction of insulin or injury). Likewise, muscle glycogenolysis supplies energy during exercise and fasting. Assuming that energy expenditure for a physically active 70-kg man is 3000 kcal/day, the complete oxidation of all the glycogen and glucose in the body can provide at most 1341 kcal or less than half of the energy needed for a day. However, at times when blood glucose is reduced (e.g., starvation) or energy needs are increased (e.g. exercise), muscle and/or liver glycogen can be mobilized to provide glucose or glucose-6-P for metabolic utilization. The importance of glycogen metabolism is epitomized by two diseases: equine exertional myopathy (also known as equine exertional rhabdomyolysis, azoturia, or Monday morning disease) and glycogenosis.

Equine Exertional Myopathy

This is likely a hereditary disorder resulting from excessive lactate production from glycogen or low pH in the skeletal muscles of an exercising horse and inadequate flow of blood to the muscles, leading to hypoxia-induced muscle cramps and damage (King and Mansmann 1997). Equine exertional myopathy is triggered by multiple factors, including the overfeeding of NSCs (e.g., starch-rich grain); poor conditioning or fitness; a sudden increase in workload; imbalances of electrolytes; deficiencies of antioxidant minerals and vitamins; and undesirable weather conditions. As a preventative means, reducing the dietary intake of starch (e.g., limiting grain consumption) is essential to manage horses susceptible to equine exertional myopathy. Treatments may include the restoration of electrolyte balances and removal of grain rations; dietary supplementation with arginine (the precursor of NO as the major vasodilator) and vegetable oil (a major metabolic fuel for skeletal muscle); and limited exercise plus a low-carbohydrate, high-fat diet to reduce lactate production and ensure adequate ATP production in skeletal muscle (Wu 2020).

Glycogenosis

This is an inherited or acquired disorder resulting from an excessive accumulation of glycogen in the liver and skeletal muscle, adversely affecting the physiological functions of these organs. The pathology of glycogenosis involves deficiencies of enzymes in glucose oxidation (including glycolysis), glycogen synthesis (e.g., glycogen synthase), and/or glycogen breakdown (e.g., glycogen phosphorylase). In livestock, this disease may result from intoxication with the alkaloid called castanospermine (an inhibitor of α-glucosidase) (Saul et al. 1985). In some carnivorous fish, such as largemouth bass, hepatic glycogenosis occurs when they are fed a diet containing more than 10% starch (DM basis; Li et al. 2020). The treatment of glycogen storage disease is dependent on its type. Animals with the inherited disorder are generally fed frequently with small meals of carbohydrates (e.g., cornstarch) to prevent low blood glucose levels. Animals with acquired disease may be fed diets containing low starch but high levels of amino acids/protein and vegetable oil as energy sources for the liver and skeletal muscle (Wu 2020).

Glyceroneogenesis in Animals

Glyceroneogenesis refers to the formation of glycerol from substrates (e.g., from glucogenic amino acids, pyruvate, lactate, malate, and OAA) via nonlipolysis pathways. This metabolic pathway occurs in the liver, kidneys, WAT, BAT, and many other tissues (e.g., testes, placentae, and skeletal

muscle) in mammals (e.g., pigs, humans, and rats), birds, and fish (Yang et al. 2009). PEPCK plays an important role in converting OAA (derived from amino acids, pyruvate, lactate, and malate) into PEP, which is then metabolized to 3-phosphoglycerate, dihydroxyacetone phosphate (DHAP), and glycerol-3-P. Glycerol-3-phosphatase (G3Pase) hydrolyzes glycerol-3-P to glycerol plus Pi.

$$\text{OAA} \xrightarrow{\text{PEPCK}} \text{PEP} \rightarrow\rightarrow \text{3-Phosphoglycerate} \rightarrow \text{DHAP} \rightarrow \text{Glycerol-3-P} \xrightarrow{\text{G3Pase}} \text{Glycerol} + \text{Pi}$$

Glycerol-3-P serves as a substrate for the the synthesis of TAGs or the re-esterification of LCFAs, whereas glycerol is activated by glycerol kinase in the liver for gluconeogenesis. The concentrations of glycerol in terrestrial animals (e.g., pigs and humans) usually do not exceed 0.25 mM. There is evidence that glyceroneogenesis contributes more glycerol-3-P than glucose to very-low-density lipoprotein (VLDL) in overnight-fasted humans (~55% vs. 45%, respectively; Yang et al. 2009). An increase in glyceroneogenesis promotes TAG formation in the WAT of animals exposed to elevated ambient temperatures (Qu and Ajuwon 2018). In addition, glycerol is a vital osmolyte under certain conditions. For example, the rainbow smelt (*Osmerus mordax*), which lives in the waters of the North Atlantic, actively forms glycerol and has an exceedingly high concentration of glycerol (as antifreeze) in plasma (0.4 M) during the winter when the ambient temperature is below 1°C (Driedzic et al. 2006).

Unique Features of Regulation of Glucose Metabolism in Crustaceans

Crustaceans share many features of the regulation of glucose metabolism as reported for vertebrates, including inhibition of gluconeogenesis by dietary glucose intake. For example, hepatic PEPCK activity in Pacific white shrimp is increased when fed a low-starch and low-protein diet but is reduced when fed a high-starch diet (Rosas et al. 2001). In addition to their livers, the gill tissues of crustaceans (e.g., such as the common shore crab) contain all enzymes for gluconeogenesis (Thabrew et al. 1971). Furthermore, in crustaceans, glycogen is stored mainly in skeletal muscles and the hepatopancreas. However, there are major differences in the hormonal regulation of glucose metabolism between crustaceans and vertebrates. Specifically, in contrast to vertebrates (such as mammals, birds, and fish) where insulin and glucagon play major roles in modulating glucose synthesis and utilization, three main hormones used by crustaceans for regulating these biochemical processes are crustacean hyperglycemic hormone (produced by the X-organ–sinus-gland complex in the eyestalk), insulin-like peptides, and insulin-like growth factor (Lee et al. 2014; Wang et al. 2016b). Crustacean hyperglycemic hormone acts like glucagon, whereas insulin-like peptides and insulin-like growth factor function like insulin. Thus, in response to hypoglycemia, crustacean hyperglycemic hormone is released from the sinus gland into the hemolymph, which increases glycogen degradation and glucose concentration in the hemolymph (Chung et al. 2010). This process occurs within 30 min and lasts for 2–3 h. In contrast, when the concentration of glucose in the hemolymph is elevated, the secretion of crustacean hyperglycemic hormone is suppressed, but that of insulin-like peptides and insulin-like growth factor is enhanced to stimulate the synthesis of glycogen and to reduce glucose content in the blood (Gutiérrez et al. 2007; Lee et al. 2014).

Crustaceans have a limited ability to use glucose, which restricts the inclusion level of starch or simple carbohydrates in diets (Table 5.14). Accordingly, in normal crustaceans, the concentrations of glucose in the hemolymph are much lower than those in vertebrates. For example, hemolymph glucose concentration is only 0.9 mM in red swamp crayfish, 0.03–0.19 mM in spinycheek crayfish, 0.8 mM in rock crabs, and 0.77–1.39 mM in black tiger shrimp (Rodríguez-Viera et al. 2014). As in some carnivorous fish such as largemouth bass (Li et al. 2022), excessive dietary starch intake results in abnormal hepatic glycogen accumulation, which in turn impairs their growth performance and health (Wang et al. 2016b).

TABLE 5.14
Inclusion Levels of Carbohydrates in the Diets of Crustaceans

Crustacean Species	Carbohydrate	Dietary Level (% of Diet; DM basis)
Asian tiger shrimp (*Penaeus monodon*)	Trehalose	20
Chinese white shrimp (*Fenneropenaeus chinensis*)	Starch	26
Chinese mitten crab (*Eriocheir sinensis*)	Cellulose	≤ 3
Asian tiger shrimp (*Penaeus monodon*)	Gelatinized flour	≤ 35
Pacific white shrimp (*Litopenaeus vannamei*)	Chitin	≥ 0.54
Pacific white shrimp (*Litopenaeus vannamei*)	Starch	20
Pacific white shrimp (*Litopenaeus vannamei*)	Cellulose	10
Brown shrimp (*Penaeus aztecus*)	Glucose	< 10
Northern pink shrimp (*Penaeus duorarum*)	Glucose	< 10
Kuruma shrimp (*Penaeus japonicus*)	Glucose	<10
Swimming crab (*Portunus trituberculatus*)	Corn starch, wheat starch, and dextrin	20
Swimming crab (*Portunus trituberculatus*)	Potato starch	< 20

Source: Li et al. (2022), Wang et al. (2016b), and Zhang et al. (2021).

FRUCTOSE METABOLISM IN ANIMAL TISSUES

Synthesis of Fructose from Glucose in a Cell-Specific Manner

In animals, fructose is synthesized from glucose via aldose reductase and sorbitol dehydrogenase in a tissue-dependent manner (Figure 5.27). These enzymes are localized in the cytosol. The liver, ovaries, placenta (trophoblast cells), seminal vesicles, kidneys, RBCs, and eyes (lens, retina, and Schwann cells) all express aldose reductase. This enzyme requires NADPH as the essential cofactor. Thus, the pentose cycle plays an important role in the conversion of glucose to sorbitol and, therefore, into fructose. Among these tissues and cells, only the liver, ovaries, placenta, and seminal vesicles also have sorbitol dehydrogenase activity and can therefore synthesize fructose from glucose. Based on the high abundance of fructose in the semen of nearly all mammals studied and in fetal fluids (including fetal blood, allantoic fluid, and amniotic fluid), the net rates of fructose synthesis are likely high in the seminal vesicles and placentae.

The synthesis of fructose from glucose is up-regulated by reproductive hormones. There is evidence that 17-β-estradiol and progesterone enhance the expression of aldose reductase in animal tissues. This is consistent with the increased abundance of fructose in fetal fluids during pregnancy (Chapter 2). The concentrations of fructose in fetal blood, allantoic fluid, and amniotic fluid are high, partly due to the lack of transport of fructose from the fetus to the mother. In males, several lines of evidence show that testosterone plays a key role in inducing fructose production (Gonzales 2001; Schirren 1963). First, no fructose is detected in the seminal glands of various male domestic animals while they are infantile. Second, fructose is present in pubescent animals with the significant production of testosterone. Third, the castration of pubescent animals results in reduced concentrations of fructose in the seminal vesicles and semen, whereas the administration of testosterone to these males restores fructose concentrations in both seminal vesicles and semen.

Pathways for Fructose Catabolism

The overall pathways for fructose catabolism to produce glycerol, 3-phophoglycerate, and glucose in the cytosol of animal cells are shown in Figure 5.28. It should be borne in mind that the metabolic fate of fructose is highly cell-specific. For example, the liver is the principal organ to degrade fructose in mammals, birds, and fish. Much of the fructose metabolized in the liver of mammals and birds is recovered as glucose (Heald 1962). To date, little is known about fructose utilization by fish or crustaceans (Li et al. 2022).

The transport of fructose into cells is mediated primarily by GLUT2 in hepatocytes but by GLUT5 in extrahepatic cells. This is the first step for fructose utilization in animals. The GLUT5 protein is barely detectable in hepatocytes of nonruminant mammals (e.g., humans, rats, and pigs; Aschenbach et al. 2009), but significant GLUT5 mRNA levels are present in avian and bovine hepatocytes (Gilbert et al. 2007). During pregnancy, progesterone and estrogen upregulate the uptake of fructose by cells in conceptuses (Moses et al. 2022a). In postnatal mammals or post-hatching birds, insulin stimulates fructose uptake by skeletal muscle but has no effect in the liver. Thus, the whole-body clearance of this sugar is impaired in diabetic patients or subjects with insulin resistance.

Upon entering the cell, the catabolism of fructose can be initiated by three enzymes: fructokinase (also known as ketohexokinase), hexokinase, and sorbitol dehydrogenase (Figure 5.27). The expression of these enzymes is cell-specific, and the direction of the net reaction is dependent on the intracellular concentrations of glucose and related metabolites. For example, fructose is converted into glucose solely in the liver and kidneys. In addition, fructose is degraded into lactate and CO_2 as a source of energy in both hepatocytes and spermatozoa (Jones 1998), as well as mammalian conceptuses (Moses et al. 2022a). Furthermore, there is a significant activity of sorbitol dehydrogenase in the liver, kidneys, and eyes, but the conversion of sorbitol into glucose in other tissues is very limited. Overall, fructose-1-P is generated by fructokinase, whereas fructose-6-P is produced by hexokinases. Fructose-1-P and fructose-6-P may be interconverted in animal tissues by phosphofructomutase. This would be analogous to the interconversion between glucose-6-P and glucose-1-P by phosphoglucomutase, which is widely expressed in animal tissues. Although phosphofructomutase is not known to be present in animals, this enzyme has been reported in bacteria, and

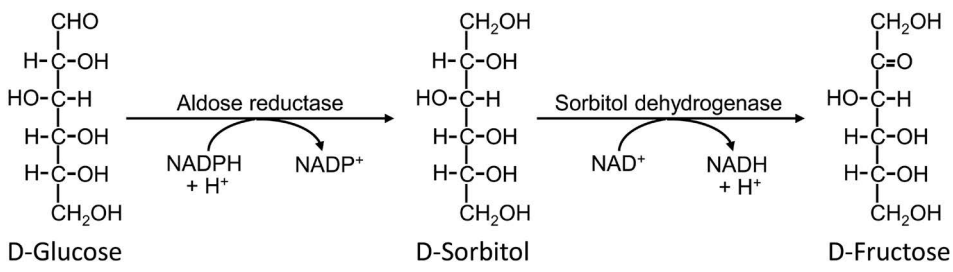


FIGURE 5.27 Synthesis of D-sorbitol and D-fructose from D-glucose in certain animal tissues. The liver, ovaries, placenta, and male seminal vesicles can convert D-glucose into D-fructose in the cytosol via aldose reductase and sorbitol dehydrogenase (also known as iditol dehydrogenase and ketose reductase). This sorbitol (polyol) pathway requires NADPH and NAD^+ as cofactors.

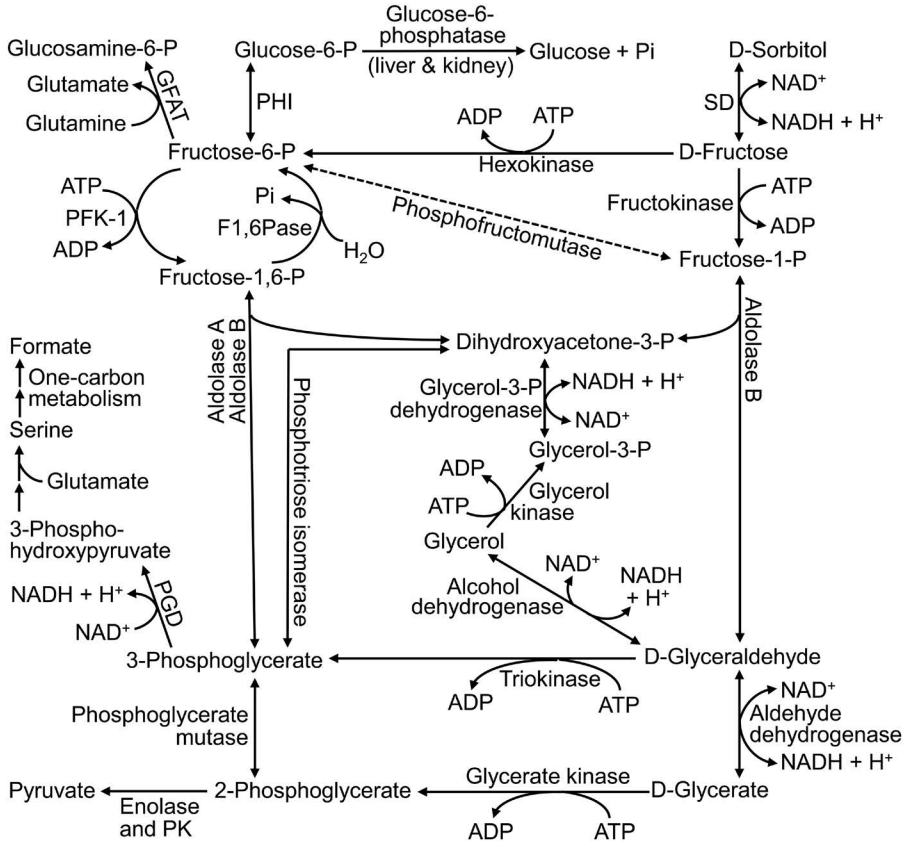


FIGURE 5.28 Catabolism of D-fructose in animals. Catabolism of fructose is initiated by fructokinase, hexokinase, and sorbitol dehydrogenase (SD) in a cell-specific manner. The liver is the principal organ for degrading fructose in all animals, whereas glucosamine-6-P is formed from fructose-6-phosphate and glutamine by glutamine:fructose-6-phosphate transaminase (GFAT) in tissues (including liver and placentae) and cells (e.g., erythrocytes and vascular endothelial cells). Fructokinase, hexokinase, and sorbitol dehydrogenase convert fructose into fructose-1-phosphate, fructose-6-phosphate, and sorbitol, respectively. Fructose-1-phosphate and fructose-6-phosphate may be interconverted by phosphofructomutase or phosphohexokinase. Fructose-1-phosphate is cleaved by aldolase B into dihydroxyacetone-3-phosphate and D-glyceraldehyde, whereas fructose-6-phosphate is degraded by aldolases A and B to dihydroxyacetone-3-phosphate and 3-phosphoglycerate. Fructose-6-phosphate can also be converted into glucose-6-phosphate or fructose-1-6-bisphosphate and can therefore participate in the synthesis of glycosaminoglycans (including hyaluronan and chondroitins) via GFAT. In the liver and kidneys, the 3-carbon intermediates of fructose (e.g., glyceraldehyde, glycerol, glyceraldehyde-3-phosphate, and serine) can be converted into glucose. In both the liver and conceptuses, formate is generated from fructose via the formation of serine and one-carbon metabolism. *Note:* F1,6Pase, fructose-1,6-bisphosphatase; PGD, 3-phosphoglycerate dehydrogenase; PFK1, phosphofructokinase 1; PHI, phosphohexose isomerase; and PK, pyruvate kinase.

its expression is induced by elevated levels of fructose or mannose (Binet et al. 1998). In animal tissues, fructose-1-P is cleaved by aldolase B into dihydroxyacetone-3-P and D-glyceraldehyde, whereas fructose-6-P is degraded by aldolases A and B to dihydroxyacetone-3-P and 3-phosphoglycerate. Fructose-6-P is (a) either converted into glucose-6-P or fructose-1-6-bisphosphate and therefore enters the pentose cycle; or (b) participates in the synthesis of amino sugars and glycosaminoglycans (including hyaluronan and chondroitins) via glutamine:fructose-6-P transaminase (Kramer et al. 2020; Moses et al. 2022b). The later pathway (hexosamine-synthetic pathway) is activated by fructose, glucose, and glutamine. Like fructose, glutamine is also highly abundant in the fetuses of sheep, cattle, and pigs [e.g., 25 mM in ovine allantoic fluid on Day 60 of gestation (Kwon et al. 2003)]. Thus, fructokinase and hexokinase initiate the catabolism of fructose into 3-carbon intermediates (e.g., possibly glyceraldehyde, glycerol, glyceraldehyde-3-P, and serine). Note that the cleavage of fructose-1-P into dihydroxyacetone-3-P and D-glyceraldehyde bypasses the rate-controlling step of glycolysis catalyzed by PFK1 that is subject to inhibition by ATP, citrate, low pH, and hypoxia.

Because fructose plays an important role in stimulating the growth and development of conceptuses of ungulates (including ruminants and pigs), there is increasing interest in the underlying biochemical mechanisms (Bazer et al. 2024). In the presence of physiological concentrations, more fructose is used than glucose via the pentose cycle, the Krebs cycle, and one-carbon metabolism (formate formation). Additionally, glucosamine-6-P, but not glucose, is

formed from fructose in trophectoderm cells (Kim et al. 2012) and conceptuses (Johnson et al. 2023; Wang et al. 2016a) of ruminants and pigs. In contrast to glucogenic organs (liver and kidneys), tissues (e.g., porcine and ovine conceptuses) that lack gluconeogenesis use fructose mainly via fructolysis (analogous to glycolysis for the production of pyruvate and lactate from glucose), the pentose cycle, and the Krebs cycle (Johnson et al. 2023; Moses et al. 2022a, 2024a,b). Both fructolysis and the pentose cycle help to conserve fructose carbons without inducing insulin insensitivity in conceptuses. Interestingly, there is no conversion of fructose into lipids in ovine or porcine conceptuses, but a large amount of formate (a precursor of purines) is formed from fructose (Figure 5.28) in both ovine and porcine conceptuses (Halloran et al. 2023; Moses et al. 2022a,b).

In postnatal mammals, fructose is cleared rapidly from blood circulation. However, a high intake of dietary fructose can increase the concentrations of fructose in their plasma, resulting in insulin resistance due, in part, to elevated production of glucosamine that reduces the pentose cycle and NO production in the vasculature (Wu and Meininger 2002). In addition, as noted previously, sorbitol dehydrogenase can initiate fructose metabolism. Interestingly, this reaction appears to be age-specific. Before birth, little fructose is converted into sorbitol in mammalian fetuses or avian embryos. However, postnatally, sorbitol dehydrogenase traps fructose as sorbitol inside cells such as hepatocytes, renal tubules, and the retina, as the equilibrium of this enzyme favors the formation of sorbitol from glucose. The accumulation of sorbitol in the eyes is a major cause of blindness in animals and humans with long-term diabetes. Thus, in contrast to fetuses, high circulating levels of fructose are toxic in infants, adolescents, and adults.

Nutritional, Physiological, and Pathological Significance of Fructose

Beneficial Effects of Fructose in Reproduction

The unusually high abundance of fructose in male semen and in fetal fluids raises an intriguing question about its physiological significance in reproduction. The conversion of glucose into fructose can minimize the irreversible loss of glucose carbons, while fructose, like glucose, can help sustain protein synthesis. An important role for fructose in female and male fertility is consistent with the following lines of evidence. First, Kim et al. (2012) have demonstrated that, like 4 mM glucose, 4 mM fructose stimulates the MTOR signaling pathway to promote protein synthesis in ovine and porcine trophectoderm cells, as well as their proliferation and remodeling. Second, there are reports that the presence of 2 mM fructose in the culture medium improves the development of bovine embryos, but 2 mM glucose is toxic to the bovine embryos (Barceló-Fimbres and Seidel 2008). Third, in humans and farm mammals, a low concentration of fructose in semen is associated with infertility and serves as an indicator of poor-quality spermatozoa (Schirren 1963). Fourth, adding 10 mM fructose to semen enhances the fertility of artificially inseminated buffalo from 25% to 40% (Akhter et al. 2014). Collectively, these results indicate that fructose is essential for mammalian fertility.

Pathological Effects of Excess Fructose

High intake of fructose or sucrose may result in the development of insulin resistance and obesity in postnatal animals (Gaby 2005). The underlying mechanisms are multifactorial and include the impaired utilization of glucose by the skeletal muscle, heart, and WAT due to defects in cell signaling. In diabetic subjects, sorbitol accumulation increases intracellular osmolality, and therefore this sugar is a significant factor that contributes to retinal damage and renal abnormalities. It is possible that fructose itself is not toxic to animal cells. However, by inducing insulin resistance and interfering with glucose oxidation, excess fructose adversely affects the health of postnatal mammals. Interestingly, this problem does not occur in the fetus, likely because (a) fetal glucose, but not fructose, returns to the mother for disposal; (b) the production of ROS in the fetus is minimized because glucose and amino acids, rather than fatty acids, are the main metabolic fuels; and (c) high concentrations of glutathione and glycine in the conceptus protect the fetus from oxidative injury.

GALACTOSE METABOLISM IN ANIMAL TISSUES

Pathway of UDP-Galactose Synthesis from Glucose in Animal Tissues

Galactose is a C-4 epimer of glucose. Although the spontaneous epimerization of glucose yields galactose, this reaction occurs at a very slow rate. In animal tissues, including the lactating mammary gland, UDP-galactose is formed from glucose by several cytosolic enzymes. Specifically, glucose is phosphorylated by hexokinase into glucose-6-P, which is converted into glucose-1-P by phosphoglucomutase. In addition, UDP-Pyrophosphorylase catalyzes the formation of UDP-glucose from glucose-1-P and UTP. UDP-glucose-4-epimerase (also known as UDP-galactose 4-epimerase) converts UDP-glucose into UDP-galactose (Figure 5.29). UDP-galactose in the cytosol is transported into the Golgi lumen by an active transporter for the synthesis of glycolipids, phospholipids, proteoglycans, and glycoproteins in all cells.

The epithelial cells of nearly all lactating mammary glands contain lactose synthase in the Golgi lumen to synthesize D-lactose from UDP-galactose and β-D-glucose, but this enzyme is absent from other tissues (Rezaei et al., 2016). Thus, there is no biosynthesis of D-lactose or galactose from glucose in cells other than mammary epithelial cells. Lactose synthase is a complex of galactosyltransferase and α-lactalbumin (the regulatory subunit of the enzyme complex). α-Lactalbumin is only synthesized by epithelial cells of the lactating mammary gland, indicating

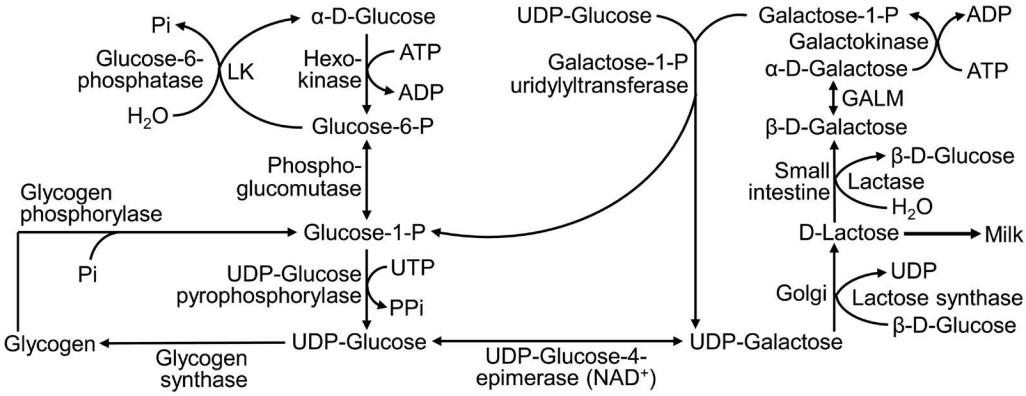


FIGURE 5.29 Conversion of D-glucose into D-lactose and D-galactose in lactating mammals. This series of cytosolic reactions is called the Leloir pathway. The epithelial cells of lactating mammary glands contain lactose synthase in the Golgi lumen to synthesize D-lactose from UDP-galactose and β-D-glucose. However, this enzyme is absent from other tissues. UDP-glucose-4-epimerase (also called UDP-galactose-4-epimerase) requires NAD^+ as the cofactor. *Note:* GALM, galactose mutarotase; LK, liver and kidneys.

an important role for amino acids (as the precursors of proteins) in lactose production by lactating mammals. In non-mammary epithelial cells, the lack of α-lactalbumin does not allow for the formation of a functional lactose synthase complex from galactosyltransferase, and therefore no lactose is produced (Permyakov et al. 2016). Lactose synthesis is triggered by prolactin and is enhanced by growth hormone and insulin-like growth factor-I (Rezaei et al. 2016).

There is a species difference in lactose synthesis among lactating mammals. For example, the mammary glands of the California sea lion contained galactosyl transferase activity and all the enzymatic activities required for the formation of UDP-galactose from glucose (Johnson et al. 1972). However, galactokinase activity and α-lactalbumin were not detectable, the activity of galactose-1-P uridyl transferase was extremely low, and lactose synthetase activity was negligible in the mammary tissue of the lactating sea lion. This accounts for the absence of lactose from the milk of this animal (Chapter 2).

Pathway of Galactose Catabolism

Galactose exists in both α- and β-configurations in nature. This sugar is present in milk and other dairy products, as well as plant-source materials (e.g., sugar beets and mucilages). In the small intestine, lactase (bound to the surface of enterocytes) hydrolyzes D-lactose into β-D-galactose and β-D-glucose. The initial step for galactose catabolism is the conversion of β-D-galactose to α-D-galactose by galactose mutarotase. The metabolism of α-D-galactose to UDP-glucose is catalyzed sequentially by galactokinase, galactose-1-P uridyl transferase, and UDP-galactose-4-epimerase (Figure 5.29). This series of reactions, which take place in the cytosol, is called the Leloir pathway. Specifically, galactokinase phosphorylates α-D-galactose into galactose-1-P. Subsequently, galactose-1-P uridyltransferase transfers a UMP group from UDP-glucose to galactose-1-P to form UDP-galactose (Yager et al. 2004). Finally, UDP galactose-4-epimerase interconverts UDP-galactose and UDP-glucose. UDP-Glucose is the substrate for glycogen synthesis in all cell types. The degradation of glycogen by glycogen phosphorylase generates glucose-1-P, which is converted into glucose-6-P by phosphoglucomutase. In the liver and kidneys, glucose-6-phosphatase catalyzes the formation of glucose from glucose-6-P. In all cells, glucose-6-P is metabolized into pyruvate and lactate. In mitochondria, pyruvate can be further oxidized through the Krebs cycle and the respiratory chain to CO_2 and H_2O.

In addition to the reactions described above, galactose can be degraded by additional pathways. For example, galactose is reduced by aldose reductase into galactitol in the cytosol of most animal cells (Figure 5.30). Galacitol can be accumulated in tissues when the extracellular concentration of galactose is high because animals lack enzymes to degrade galacitol. Furthermore, the livers of animals express enzymes to sequentially convert galactose into D-galactonolactone, D-galactonate, β-keto-D-galactonate, and xylulose (Cuatrecasas and Segal 1966). Xylulose is then phosphorylated by xylulose kinase into xylulose-6-P, which enters the pentose cycle for metabolism into ribose-5-P and NADPH (Figure 5.29). These reactions also take place in bacteria and fungi for galactose utilization. These microbes also contain aldose reductase to convert galactose into tagatose-6-P.

Physiological and Pathological Significance of Galactose

Galactose is required for lactose synthesis and milk production by the lactating mammary glands of nearly all terrestrial mammals (Rezaei et al. 2016). Thus, this monosaccharide plays an important role in the nutrition and growth of suckling neonates. Such a notion is true for some, but not all, sea mammals, depending on lactose concentrations in their milk. In addition to lactose synthesis, galactose participates in the galactosylation of proteins and

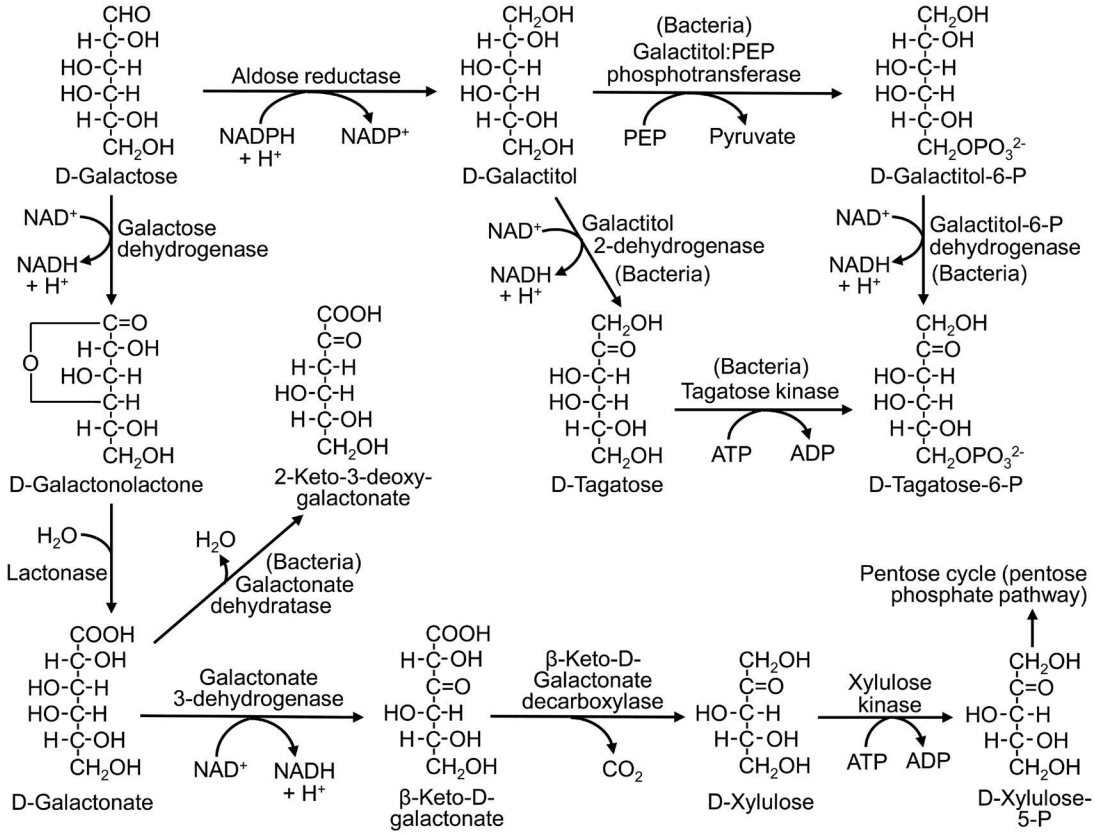


FIGURE 5.30 Catabolism of D-galactose in animals and microbes. The catabolism of D-galactose is initiated by both galactokinase (Figure 5.29) and galactose dehydrogenase and aldose reductase in animal tissues. However, animals lack enzymes to degrade D-galacitol (the product of aldose reductase). In contrast, bacteria and fungi possess all the enzymes to degrade D-galactose. *Note:* PEP, phosphoenolpyruvate.

lipids in the Golgi (Coelho et al. 2015). For example, galactosyl transferase catalyzes the conversion of UDP-galactose and *N*-acetyl-D-glucosamine into galactosyl-*N*-acetyl-D-glucosamine, which is used for glycoprotein production. Additionally, within the endoplasmic reticulum, ceramides are galactosylated by UDP-galactose:ceramide galactosyltransferase into galactosylated ceramides. These compounds play important roles in glycosphingolipid synthesis, the regulation of axon conductance in the nervous system, and cell signaling. In animals with an inherited deficiency of a key enzyme (e.g., galactokinase or galactose-1-P uridylyltransferase) in the pathway of galactose degradation, a metabolic disorder known as galactosemia occurs. This disease is characterized by such syndromes as an enlarged liver, cirrhosis, renal failure, cataracts, vomiting, seizure, and brain damage (McAuley et al. 2016). Evidence shows that excessive galactitol induces cataract formation in the lens and contributes to cell damage in the central nervous system, whereas excessive galactose-1-P impairs hepatic metabolism and causes liver dysfunction. Interestingly, chickens, which have a low activity of hepatic galactokinase (Gordon et al. 1971), are very susceptible to the galactose toxicity syndrome. Thus, either 10% or 15% of galactose in a diet causes high mortality in broiler chicks (Douglas et al. 2003). This illustrates another difference in carbohydrate metabolism between mammals and birds. Poultry diets should not include moderate or high levels of galactose (e.g., >4%, as-fed basis). Note that chicks can tolerate some galactose in diets (e.g., ≤4%, on an as-fed basis) without affecting their growth performance because the microflora in their large intestine contains enzymes for degrading this sugar (Wu 2020).

NUTRITIONAL AND PHYSIOLOGICAL EFFECTS OF DIETARY NSPs IN ANIMALS: NONRUMINANTS

Effects of NSPs on Feed Intake by Animals

High intake of dietary water-soluble or insoluble NSPs reduces feed intake by nonruminants (e.g., pigs and poultry) via different mechanisms (Bedford and Morgan 1996; Lindberg 2014). Soluble NSPs (e.g., pectin, guar gum, arabinoxylans, β-glucans, and sodium alginate) readily dissolve in water to become gelatinous and viscous substances, which increase viscosity (fluid resistance to flow)

in the lumen of the stomach and small intestine. This leads to decreases in gastric emptying, small-intestinal contractions, and digesta transit through the small intestine. Note that some readily fermentable fibers are not gel formers, and some viscous fibers are not all readily fermented. There is evidence that via sensory mechanisms in the mucosae of the gastrointestinal smooth muscle and the gut-brain signaling pathway, dietary soluble NSPs stimulate the release of peptide YY and glucagon-like peptide-1 (GLP-1) but inhibit the release of ghrelin by the GIT (Greenway et al. 2007, Zijlstra et al. 2007). Because peptide YY and GLP-1 suppress, but ghrelin promotes, food consumption, the effects of soluble NSPs on the release of these bioactive peptides help explain how these NSPs reduce appetite and feed intake by animals.

In contrast to soluble NSPs, insoluble NSPs (e.g., cellulose and hemicellulose) are nonviscous, have negligible effects on gastric emptying, and increase the rate of transit of food through the small intestine. There is evidence that insoluble NSPs decrease appetite and feed intake at the initial level of the small intestine. Specifically, insoluble NSPs reduce the absorption of glucose by the small intestine, leading to an increase in its concentration at the terminal ileum and, therefore, the secretion of glucagon-like peptide-1. In addition, insoluble NSPs stimulate the secretion of CCK (Holt et al. 1992). Both GLP-1 and CCK are known to contribute to satiety and food intake suppression (Flint et al. 1998).

Effects of NSPs on Nutrient Digestibility, Growth, and Feed Efficiency

High intake of either water-soluble or insoluble NSPs reduces nutrient digestibility, growth rate, and feed efficiency in nonruminants (e.g., pigs and poultry) through common and also different mechanisms (Bedford and Morgan 1996; Lindberg 2014). Both soluble and insoluble NSPs bind to, and entrap through encapsulation, nutrients (e.g., lipids, protein, starch, vitamins, and minerals) found within plant cells, forming fibrous complexes. These effects of NSPs result in (a) dilution of digestive enzymes and their substrates, (b) interference of enzyme-substrate interaction, (c) reduced digestion of nutrients, and (d) reduced and delayed absorption of nutrients (including glucose, lipids, and amino acids) in the small intestine. In addition, the soluble NSP-induced increase in viscosity within the lumen of the small intestine, as noted previously, further impairs nutrient digestion and absorption through (a) decreasing small-intestinal contractions and (b) increasing the thickness of the unstirred water layer. Furthermore, through mechanical stimulation, insoluble NSPs shorten the time of the transit of digesta through the small intestine, thereby reducing nutrient digestion and absorption. For example, feed intake, DM digestibility, and daily weight gain were 4.8%, 3.2%, and 16% lower in postweaning young pigs fed a 15.2% NDF diet (containing 5% bamboo meal) than those fed an 11.9%-NDF diet (Yu et al. 2016). Similarly, high inclusion levels of soluble or insoluble NSPs suppress feed intake, nutrient digestibility, and weight gains in growing pigs (Longland et al. 1994), as well as rainbow trout, tilapia, and Atlantic salmon (Refstie et al. 1999; Shiau et al. 1988). Of note, in obese animals, dietary supplementation with soluble or insoluble NSPs increases the fecal excretion of N, ash, and fat, while reducing postprandial glycemic response, blood lipid concentrations, and risk for cardiovascular disease (Blaak et al. 2012).

Effects of NSPs on Intestinal Health

Although a high percentage of NSPs in the diet is an antinutritional factor, their adequate intake is very important for the intestinal health of animals. All NSPs undergo little degradation in the small intestine, but they are either fully or partially fermented in the large intestine to generate SCFAs and also affect the microbial population in the gut (Maciorowski et al. 1997). Thus, the effects of dietary NSPs on intestinal health are affected by their physical and chemical properties, including the degree of their solubility in water (Bach Knudsen et al. 2017). Soluble NSPs are also highly fermentable in the large bowel to produce SCFAs, which are major metabolic fuels for colonocytes and regulators of their gene expression. The hydrolysis of soluble NSPs and proteins in plant ingredients provides both carbon and N sources for bacteria to grow. Therefore, bacteria, lipids, fermentation products (including SCFAs, lactate, NH_4^+, and gases), and other biomass (e.g., endogenous secretions and undigested material) are the major contributors to the increase in stool bulk with the intake of rapidly fermented soluble NSPs (e.g., those in oat bran), while decreasing the risk for colon cancer in animals (Turner and Lupton. 2021). There is evidence that dietary supplementation with β-glucan and mannan-oligosaccharides or feed additives (e.g., dried yeast cell walls) that are rich in these NSPs improves intestinal microbial composition and intestinal health in animals, including dogs (Van den Abbeele et al. 2020).

Insoluble NSPs (e.g., wheat bran) have no appreciable water holding capacity. They are only partially digestible in the colon and cecum and thus have a lesser effect on SCFA production than soluble NSPs. However, insoluble NSPs provide a bulking effect, increase fecal mass, shorten the time of the transit of luminal contents through the large intestine, and alleviate constipation (Bach Knudsen et al. 2017). Insoluble NSPs act as effective laxatives by binding to luminal undigested material and stimulating the gut mucosa through mechanoreceptors, thereby inducing secretion and peristalsis. Large, coarse particles provide a greater laxative efficacy than fine, smooth particles (no or little effect; Blaak et al. 2012). Stool weight is generally increased by adding insoluble NSPs to the diet. There is evidence that a diet rich in wheat bran can reduce the risk of the incidence of colitis and colon cancer in rats (Turner and Lupton 2021; Zoran et al. 1997). Such an effect is particularly important for both humans and companion animals (such as dogs and cats).

The proportions of water-soluble NSPs in sugar beet pulp, oat bran, barley, rye, and beans are higher than those in alfalfa hay, wheat straw, wheat bran, palm kernel expeller, bamboo meal, as well as the hulls of wheat, oats, corn, and rice. In all these products, insoluble NSPs are more abundant than soluble NSPs. Of interest, comparable amounts of NSPs from wheat and oat brans have the same effect on daily fecal output, even though > 90% and only 50–60% of their NSPs are water-insoluble, respectively. However, wheat bran is more effective than oat bran in the prevention and treatment of constipation, whereas oat bran can lower blood concentrations of glucose, TAGs, and cholesterol in animals to a greater extent than wheat bran. A low level of either soluble or insoluble NSPs in the diet can cause constipation, colonic inflammation, and the occurrence of hemorrhoids in nonruminants (Bach Knudsen et al. 2017).

As herbivores, horses have evolved to consume diets that are low in starch but rich in NSPs. These diets usually consist of forages (e.g., hay, haylage, and grass), which are vital to the health of the digestive system. There are reports that inclusion of soluble fiber in diets increased the concentrations of total cecal SCFAs and propionic acid, which resulted in stable and constant plasma glycemic and insulinemic responses in horses (Brøkner et al. 2016). NSPs also play an important role in maintaining the intestinal and metabolic health of other nonruminant herbivorous mammals, such as rabbits (Davies and Davies 2003).

NUTRITIONAL AND PHYSIOLOGICAL EFFECTS OF DIETARY NSPs IN ANIMALS: RUMINANTS

Effects of NDF on Ruminal pH and Environment

A term commonly used in ruminant nutrition is NDF, which primarily refers to the amount of cellulose, hemicellulose, and lignin in the feedstuff. Thus, NDF is equivalent to water-insoluble NSPs in nonruminant nutrition but is an essential nutrient for postweaning ruminants. In both grazing and feedlot ruminants (e.g., cattle), NDF plays a vital role in maintaining their ruminal health by preventing the occurrence of ruminal acidosis through the following mechanisms: (a) promoting rumination to allow for a greater amount of buffer to reach the rumen environment via saliva and digestion; (b) slowing down the fermentation of starches and NSPs to form SCFAs to maintain ruminal pH; (c) decreasing the concentration of acetic acid in the rumen; and (d) facilitating the fermentation of dietary protein and NPN substances to yield NH_3, which takes up H^+ as NH_4^+. Individual roughage sources can influence the frequency of rumination, but ruminal NDF content rarely has a negative effect on beef cattle consuming an appropriate amount of a high-concentrate diet (Galyean and Defoor 2003).

Effects of NDF on Intestinal Health

SCFAs have the same physiological functions in the hindgut of ruminants as in nonruminants, as noted previously. Because not all dietary fibers are fermented in the rumen, those that escape the rumen enter the large intestine, where they are extensively fermented by bacteria to affect the metabolism and health of colonocytes, as well as fecal formation, bulking, and excretion. These effects of NDF vary with its types, the ratio of cellulose to hemicellulose, and lignification of the plant cell wall. The fiber carbohydrate portion is positively correlated with ruminal or physical fill and is generally associated with good intestinal health in feedlot beef cattle (Galyean and Defoor 2003). Conversely, an inadequate intake of NSPs adversely affects gastrointestinal function in ruminants.

Effects of NDF on Lactation and Growth Performance

NDF must be fermented in the rumen to exert its major nutritional effect on the lactation and growth performance of ruminants. Thus, the digestibility of NDF is an important indicator of forage quality, particularly because the content and ruminal degradability of NDF in forages vary widely with their species, maturity, growing environment, and lignification. A greater amount of rumen-fermentable NDF results in a greater amount of metabolizable energy available to ruminants, ultimately enhancing feed efficiency and performance in both dairy and beef cattle production systems. For example, because of reduced acetate synthesis by ruminal bacteria, a diet containing <25% NDF or <16% NDF from forage, despite an adequate amount of total carbohydrate, reduces milk fat content in lactating cows (Clark and Armentano 1993). A one-unit increase in NDF digestibility, in vitro or in situ (within the range of 54.5% to 62.9%), was found to be associated with a 0.17-kg increase in DMI and a 0.25-kg increase in 4% fat-corrected milk (Oba and Allen 1999). Rations for dairy cows must generally contain at least 25% dietary NDF and a large proportion (at least 76%) of dietary NDF, should come from forages (i.e., minimum forage NDF being 19%). As noted previously, the concentration of NDF from forage in the diet, which is closely related to chewing activity, is a determinant of the ruminal fluid pH of ruminants (including dairy cows). Thus, these animals require sufficient NDF in diets to maximize growth rate and milk yield (NRC 2001).

Effects of NDF on Feed Intake

Feed intake positively influences the production performance of ruminants. Adequate dietary NDF is necessary for maintaining proper ruminal function. However, excess dietary NDF can limit voluntary feed intake because of physical fill in the rumen. Much evidence shows that the relationship between dietary NDF content and feed intake is complex and nonlinear (Arelovich et al. 2008). Depending on the amount and quality of dietary NDF, it can either enhance or reduce feed intake by ruminants. For example, DM intake by beef cattle can increase with increasing dietary NDF content from 7.5% to 22% but can decrease sharply when dietary NDF content increases from 22% to

46%. Digestibility of dietary NDF also affects DM intake. In dairy cows fed silages with similar NDF and CP contents but a higher NDF digestibility, DM intake and milk yield are increased in comparison with the control group of cows fed a diet with a lower NDF digestibility (Oba and Allen 1999). The underlying mechanisms likely involve a series of cell signaling cascades in the GIT-brain axis.

SUMMARY

The GIT is the first site for initiating the utilization of dietary carbohydrates by animals in a species- and age-dependent manner. At birth and during the suckling period, neonatal mammals (both preruminants and nonruminants) have a high activity of intestinal lactase to convert milk lactose into β-D-galactose and β-D-glucose but have very low or no activity of intestinal α-amylase (derived from microbes), sucrase-isomaltase, and maltase-glucoamylase for utilizing starch, glycogen, sucrose, and maltose. In contrast, birds cannot tolerate a large amount of lactose in their diets but can consume and utilize cereal grains immediately after hatching. In postweaning mammals, the intestinal activity of lactase is sharply reduced, but the amounts of salivary and pancreatic α-amylases as well as intestinal α-amylase (derived from microbes), sucrase-isomaltase and maltase-glucoamylase are progressively increased until the small intestine becomes fully mature. Thus, after weaning, mammals can effectively utilize diets containing (a) starch and glycogen, which are hydrolyzed into α-D-glucose in the small intestine under the combined actions of α-amylase, sucrase-isomaltase, and maltase-glucoamylase; and (b) disaccharides (e.g., sucrose, which is hydrolyzed into α-D-glucose and β-D-fructose, as well as maltose, which is hydrolyzed into two units of α-D-glucose). In a noncarnivorous fish, whose pancreas produces and secretes a significant quantity of pancreatic α-amylase, dietary starch or glycogen can serve as a low-cost energy source. In all animals, monosaccharides are absorbed into enterocytes of the small intestine by SGLT1 (glucose and galactose) and GLUT5 (fructose), and the simple sugars enter the portal circulation. Although the enterocytes of all postnatal animals can effectively absorb glucose and galactose and the enterocytes of post-hatching birds can also absorb fructose well, the enterocytes of mammals can significantly take up fructose only after weaning. Thus, sucrose and fructose in diets are toxic to neonatal mammals, and large amounts of lactose in diets cause morbidity in poultry.

In nonruminants (e.g., pigs, poultry, fish, and humans), nearly all indigestible dietary fibers enter the large intestine for fermentation into SCFAs. In contrast, both water-soluble and insoluble complex carbohydrates (e.g., starch, cellulose, and hemicellulose) are extensively fermented into SCFAs, and to a lesser extent, CO_2 and methane, in the rumen of ruminants, such that little glucose is absorbed into enterocytes in cattle, sheep, goats, and deer. The electrochemical Na^+ or proton gradient across the cell membrane provides energy to drive methane production from CO_2 and H_2 (metabolites of bacteria, fungi, and protozoa) by methanogenic archaea in the rumen and GIT. As butyrate is the major metabolic fuel for colonocytes, dietary fiber is crucial for intestinal health in both ruminants and nonruminants. Dietary fiber (e.g., NDF) has an additional role in maintaining proper ruminal pH and functions and is considered a nutritionally essential carbohydrate for ruminants.

After glucose is absorbed by enterocytes, it is metabolized via multiple pathways. In the cytosol of all animal cells, glucose is utilized through glycolysis to yield pyruvate and NADH. Pyruvate is converted into lactate in cells without mitochondria or in cells with mitochondria when glycolysis produces NADH faster than the electron transport chain can oxidize it, such as under hypoxic conditions or during intense exercise. When oxygen supply is adequate, pyruvate and NADH are transported, via specific transporter systems, from the cytosol into mitochondria, where pyruvate is oxidized by PDH into acetyl-CoA and then, through the Krebs cycle, into CO_2, $FADH_2$, and NADH. The reducing equivalents ($FADH_2$ and NADH) are oxidized, through the mitochondrial respiratory chain, into H_2O with the production of 1.5 and 2.5 mol of ATP per 1 mol of $FADH_2$ and NADH, respectively. Of note, glucose is converted into fructose in the liver, ovaries, placenta, and male seminal vesicles. In the reproductive tract, fructose activates mTOR cell signaling, promotes conceptus development, and maintains sperm vitality. In all animals, excess glucose is stored as glycogen primarily in the liver and skeletal muscle through glycogenesis, and glycogen is mobilized rapidly to eventually generate glucose when glycogen phosphorylase is activated by cAMP-dependent protein kinase (e.g., fasting and intensive exercise). Glucose is also metabolized through the pentose cycle to produce NADPH and ribose-5-P and through the uronic acid pathway to generate glucuronic acid, ascorbic acid (vitamin C in some species), and pentoses.

In addition to its catabolism, glucose is synthesized from glucogenic substrates (e.g., alanine, glutamine, serine, glycerol, lactate, pyruvate, and propionate) by the liver and kidneys of animals in a species- and nutritional status-dependent manner. When dietary intake of glucose or glucose-containing carbohydrates is limited or there is no feed intake, glucose synthesis from glucogenic amino acids and glycerol is highly active to provide glucose for tissues and cells of both ruminants and nonruminants, particularly the brain and RBCs. Furthermore, dietary carbohydrates (e.g., starch, glycogen, and oligosaccharides) are extensively fermented to form SCFAs in the rumen and gluconeogenesis from propionate is particularly important in fed ruminants. Thus, these animals must synthesize glucose to survive and grow. In the epithelial cells of lactating mammary glands, glucose is used for the synthesis of galactose and lactose. Dietary galactose is utilized through its initial conversion into glucose. The pathways of glucose metabolism are regulated by hormones (e.g., insulin, glucagon, catecholamines, and prolactin), cellular energy status, and cellular concentrations of metabolites to maintain glucose homeostasis in the blood. Of particular note, glycolysis and gluconeogenesis

TABLE 5.15
Glucose-Related Metabolic Disorders (Including Diseases) in Ruminants and Nonruminants

Metabolic Disorder	Cause	Major Symptoms
Diabetes (type-I)	Lack of insulin secretion	Hyperglycemia, dyslipidemia, ketosis, tissue damage, and death
Diabetes (type-II)	Insulin resistance in tissues	Hyperglycemia, dyslipidemia, ketosis, and tissue damage
EEM	Excess lactate in muscle	Skeletal muscle cramps and damage, and recumbency
Fructose toxicity (mammalian neonate)	Deficiency of GLUT5 in the SI for fructose absorption	Intestinal dysfunction, malabsorption and diarrhea in mammalian neonates (e.g., 0- to 14-day-old pigs)
Galactosemia	Deficiency of GLK or GPUT	Enlargement and cirrhosis of liver; high susceptibility in chicks
Glycogenosis*	Excess glycogen in liver	Enlarged and watery liver, and hepatic dysfunction (e.g., LMB)
Hypoglycemia	Inadequate glucose supply	Brain damage, coma, and death
LaI-Diarrhea (chick)	Feeding lactose to chicks	Diarrhea in 0- to 7-day-old chicks (naturally without lactase)
Ruminal bloat	Excess gasses and foam formation in rumen	Multi-organ (e.g., digestive, circulatory, and respiratory dysfunction) in ruminants (primarily grazing cattle)
Ruminal acidosis	Ruminal pH <5.5 (ruminants)	Reduces roughage digestion and acetate production
Systemic metabolic acidosis	Blood pH < 7.35	Increased concentrations of lactate, decreased concentrations of bicarbonate, and reduced pH in blood
SuI-Diarrhea (pig)	Feeding sucrose to piglets	Diarrhea in 0- to 7-day-old pigs (naturally without sucrase)

Note: EEM, equine exertional myopathy (also known as Monday morning sickness); GLK, galactokinase; GLUT5, glucose transporter 5; GPUT, galactose-1-phosphate uridylyltransferase; LaI, lactose-induced; LMB, large-mouth bass (a carnivorous fish); SI, small intestine; and SuI, sucrose-induced. *glycogen storage disease

are reciprocally controlled by fructose-2,6-bisphosphate in the liver. Such well-coordinated metabolic control is essential for the efficient utilization of dietary carbohydrates and glucose homeostasis, as well as growth, development, and health for all animals. Otherwise, hypoglycemia results in nervous dysfunction and eventually coma and death in all animals. Likewise, impaired utilization of glucose by skeletal muscle results in diabetes, whereas reduced catabolism of galactose causes mortality, particularly in chickens that are highly susceptible to the onset of galactosemia. In all animals, adequate intakes of dietary NSPs are crucial for intestinal health. Major glucose-related metabolic disorders in ruminants and nonruminants are summarized in Table 5.15. Exquisite regulation of carbohydrate metabolism is vital to the growth, development, survival, and health of animals.

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6 Nutrition and Metabolism of Lipids

Lipids are highly reduced molecules, including triacylglycerols (TAGs; also called triglycerides or fats), fatty acids, phospholipids, cholesterol, and related metabolites (Chapter 3). Lipids are soluble in organic solvents (e.g., benzene, ether, or chloroform) and, except for short-chain fatty acids (SCFAs) and medium-chain fatty acids (MCFAs), are only sparingly soluble in water. Thus, complex lipids (e.g., TAGs) and free long-chain fatty acids (LCFAs) or eicosanoids circulate in the blood bound to plasma lipoproteins and albumin, respectively (Goldberg et al. 2009). Within cells, LCFAs and their hydrophobic derivatives bind to fatty acid-binding proteins (FABPs; Pepino et al. 2014). Thus, "free" fatty acids in an animal are actually not free, so as to prevent their toxic effects. In proximate feed analysis, all kinds of lipids are determined together as the ether extract (Li et al. 1990). Because of their major role as energy substrates, fats, and fatty acids are the focus of this chapter regarding lipid nutrition and metabolism.

Lipids are dietary macronutrients and comprise important signaling molecules in nutrient metabolism (Field et al. 1989; Hou et al. 2016). In the lumen of the small intestine, TAGs are emulsified by bile salts, undergo enzymatic hydrolysis to free fatty acids and monoacylglycerols (MAGs), and are then assembled into mixed micelles. Lipid digestion products are transported from the mixed micelles into the enterocyte, where they are re-assembled with apolipoproteins (Apo) into chylomicrons and other lipoproteins for export into lymphatic vessels and then into the blood circulation (Besnard et al. 1996). During their transport in the blood, the lipoproteins are hydrolyzed extracellularly by lipoprotein lipases (LPLs) localized on the endothelial surface of the vasculature within extrahepatic tissues [e.g., skeletal muscle and white adipose tissue (WAT)] (Kersten 2014; Thomson and Dietschy 1981). When the dietary intake of fat is greater than the amount of fat broken down between meals, de novo synthesis of fatty acids will be attenuated, but the excess amount of dietary energy is stored as TAGs in the body (Jobgen et al. 2006). In contrast, when an animal consumes a low-fat but high-starch diet, excess carbohydrates are used for the synthesis of fatty acids and TAGs in a tissue-specific manner, which may contribute to dyslipidemia and fat storage in some individuals. When deficiencies of nutritionally essential ω3 and ω6 unsaturated fatty acids occur, animals exhibit numerous syndromes, such as skin lesions, growth restriction, and reproductive failure (Spector and Kim 2015).

Lipases play important roles in the digestion and metabolism of lipids by hydrolyzing simple and compound lipids, such as TAGs, phospholipids, and galactolipids. In animals, these enzymes include pancreatic lipase, pancreatic lipase-related proteins 1 and 2, lingual lipase, gastric lipase, hormone-sensitive lipase (HSL), adipose triglyceride lipase (ATGL), hepatic lipase, LPL, endothelial lipase, phosphatidylserine phospholipase A1, and carboxyl ester lipase, with their preferred and overlapping substrates. Under the conditions of no or inadequate energy intake (e.g., fasting or early lactation), TAGs are mobilized from tissues (especially WAT but also including the liver) through the action of HSL to generate metabolic fuels such as LCFAs, ketone bodies, and glycerol (Yeaman 1990). In the WAT, ATGL also catalyzes the first step of lipolysis (Zimmermann et al. 2004). Ketone bodies are crucial for the function of the brain when the concentration of glucose in the plasma is reduced because the brain does not take up saturated or monounsaturated LCFAs from the blood due to the blood-brain barrier.

Quantitatively, LCFAs are the major energy substrates for the liver, skeletal muscle, heart, and kidneys in both fed and fasting states, and therefore play important roles in the function of these tissues (Jobgen et al. 2006). Fatty acids are oxidized to CO_2 and H_2O primarily in the mitochondria of cells via the pathway of ß-oxidation. Chain shortening of very long-chain fatty acids (VLCFAs) can also occur in peroxisomes, and the resulting acyl-CoA subsequently enters the mitochondria for ß-oxidation (Reddy and Hashimoto 2001). As in fatty acid synthesis, fatty acid oxidation is regulated by hormones (e.g., insulin, glucocorticoids), metabolites (e.g., acetyl-CoA and malonyl-CoA), peroxisome proliferator-activated receptors (PPARs; members of the nuclear hormone receptor superfamily), and AMP-activated protein kinase (AMPK), and nitric oxide (NO; Dai et al. 2013). Although fats fulfill essential physiological function, their excessive intake contributes to a variety of metabolic disorders and chronic diseases, including obesity, diabetes, and cardiovascular disease in animals (Beitz 1993). However, in livestock (e.g., beef cattle, sheep, pigs) and poultry, a certain level of intramuscular fats [especially monounsaturated fatty acids (MUFAs)] is necessary to improve meat quality (Smith 2013, 2017). Thus, studies of lipid nutrition and metabolism are of great importance for both animal agriculture and medicine.

DIGESTION AND ABSORPTION OF LIPIDS IN NONRUMINANT MAMMALS AND BIRDS

Overview

TAGs account for 98–99% of total lipids in the diets of most nonruminant animals (e.g., pigs, dogs, cats, rodents, birds, fish, and crustaceans) with different mature body weights (BWs). However, herbivorous animals (e.g., horses and rabbits) consume large amounts of galactolipids from vegetative material (Drackley 2000). Swine diets usually contain 5% lipids, but pigs can digest at least 20% lipids (including TAGs) in diets (on an as-fed basis). Lipid content (on an as-fed basis)

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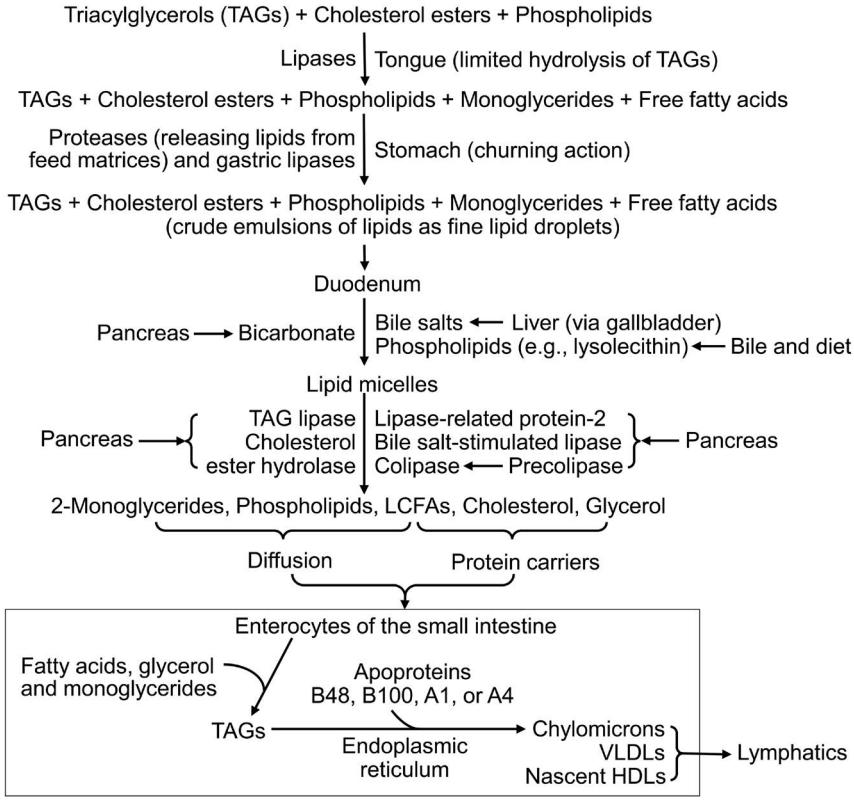


FIGURE 6.1 An overview of lipid digestion and absorption in the gastrointestinal tract of nonruminant animals. The digestion of lipids begins within the mouth and then the stomach and is completed primarily in the small intestine. The products of lipid digestion are absorbed into the enterocytes. The jejunum is the major site for the digestion and absorption of lipids. HDLs, high-density lipoproteins; LCFAs, long-chain fatty acids; VLDLs, very low-density lipoproteins.

in chicken diets is usually 5%, 6%, and 8%, respectively, for starters (1–21 days of age), growers (22–35 days of age), and finishers (36–49 days of age). Equine diets usually contain 4–5% lipids, but horses can digest up to 20% lipids (including TAGs) in diets [on a dry matter (DM) basis] without the risk of developing diarrhea. Fish diets usually contain 5–15% lipids, but some fish (e.g., salmon) can digest 30% lipids (including TAGs) in diets (on an as-fed basis). In nonruminant mammals and birds, some digestion of lipids begins in the mouth (lingual lipase) and stomach (gastric lipase), but most digestion occurs in the small intestine. The products of lipid digestion are absorbed into the enterocytes of the small intestine (Phan and Tso 2001). Events of lipid digestion and absorption in all nonruminant mammals include: (a) the hydrolysis of lipids by lipases (primarily from the pancreas); (b) the micellar solubilization of lipid digestion products in the small intestine; (c) the uptake of the solubilized products by enterocytes through the apical membrane; (d) the resynthesis of TAGs, as well as the assembling of chylomicrons, nascent very low-density lipoproteins (VLDLs), and premature high-density lipoproteins (HDLs) in enterocytes; and (e) the secretion of the lipoproteins into the lymphatic circulation (most nonruminants) or the portal vein (poultry). Among pancreatic, gastric, and lingual lipases, the pancreatic lipase is quantitatively most important for the digestion of dietary TAGs in all vertebrates studied, including mammals, birds, and fish (DeNigris et al. 1988; Lim et al. 2022; Moreau et al. 1988; Nolasco-Soria 2023). An overview of lipid digestion and absorption is provided in Figure 6.1.

Digestion of Lipids in the Mouth and Stomach

Omnivorous Nonruminant Mammals and Birds

In nonruminant mammals and birds, the digestion of lipids starts in the oral cavity through the action of lingual lipases (Table 6.1), which are secreted by the acinar cells of the sublingual glands in the tongue to hydrolyze TAGs (Doreau and Chilliard 1997). Lipid digestion continues in the stomach through: (a) proteases in the stomach that release lipids from feed matrices; (b) the formation of a coarse lipid emulsion due to gastric acidic medium and motility (i.e., peristalsis or churning action); and (c) the actions of lingual and gastric lipases (Thomson and Dietschy 1981). The gastric lipases are secreted by chief cells located in the fundic region of the stomach (Table 6.1). Both lingual and gastric lipases attack

TABLE 6.1
Lingual and Gastric Lipase Activities in Animals

Substrate	Animal Species	Lingual Lipase[a]	Gastric Lipase[a]	Preduodenal Lipase		Pancreatic Lipase	
				U/organ[b]	U/kg BW[b]	U/organ[b]	U/kg BW[b]
Triolein ($C_{18:1}$)[c]	Baboon	0.04	109	---	---	---	---
	Guinea pig	0	27	---	---	---	---
	Human	0.3	118	---	---	---	---
	Mouse	250	4.5	---	---	---	---
	Rabbit	5	78	---	---	---	---
	Rat	824	0.04	---	---	---	---
Tributyrin ($C_{4:0}$)[d]	Baboon	3.5	136	1.32×10^3	88	---	---
	Calf	121	---	13.1×10^3	73	7.10×10^5	3.9×10^3
	Cat	0[e]	109[f]	---	---	---	---
	Dog	3.0	239	13.6×10^3	230	---	---
	Guinea pig	3.5	30	432	864	---	---
	Horse	3.5	87	30.9×10^3	62	1.91×10^6	3.8×10^3
	Macaque	---	115	2.11×10^3	210	---	---
	Mouse	67	4.0	7	100	---	---
	Pig	3.5	71	5.19×10^3	52	1.31×10^6	13.0×10^3
	Rabbit	1.7	431	7.13×10^3	3.24×10^3	47.0×10^3	36.1×10^3
	Rat	248	1.0	73	243	5.2×10^3	17.3×10^3
	Sheep	102	2.0	3.0×10^3	55	1.0×10^5	1.8×10^3
FTA	Chicken[g]	360	372	---	---	530	525

[a] Enzymatic activity is expressed as μmol of free fatty acids released per min per gram of wet tissue (lingual serous glands or gastric mucosa).
[b] 1 U (unit of enzymatic activity) = μmol of free fatty acids released per min.
[c] DeNigris et al. (1988) for overnight-fasted young adult males.
[d] Moreau et al. (1988). Values refer to the circumvallate papillae area of the tongue in all animals except for calves and sheep, as well as the fundic mucosae of the stomach in nonruminants except for pigs or the omasum region in ruminants. In calves and sheep, lingual lipase activities refer to the root of the tongue and pharynx-glosso-epiglottic area. In pigs, gastric lipase activity refers to the cardiac mucosae of the stomach.
[e] Knospe and Plendl (1997).
[f] Descroix-Vagne et al. (1993).
[g] Kawabata et al. (2018) for 1- to-2-week-old chickens. Lingual lipase activity refers to the tongue tip. Lipase activities are 645 and 1326 U/g wet tissue in the rostrum (beak) and pancreas of chickens, respectively. Pancreatic lipase activity per organ or per kilogram of BW is calculated on the basis of the assumption that the pancreas weights 0.4 g and represents 0.04% of the BW in 1- to-2-week-old chickens.
Note: BW, body weight; FTA, fluorogenic triglyceride analog; ---, data are not available.

primarily SCFA and MCFA linkages on the *sn*-3 position of TAGs to generate diacylglycerols (DAGs; Velazquez et al. 1996). Gastric lipase activity is greater in suckling neonates than that in adults and is greater towards milk TAGs than pancreatic lipase (Drackley 2000). In both the young and adults, the crude emulsions of lipids formed in the stomach enter the duodenum as fine lipid droplets. The relative contribution of lingual and gastric lipases to TAG hydrolysis varies with animal species and with age within a species. For example, adult rodents and ruminants generally have a relatively high activity of lingual lipase but a negligible activity of gastric lipase, but the opposite is true for adult primates and carnivores (Table 6.1). Overall, the hydrolysis of TAGs into fatty acids, MAGs, and glycerol is quantitatively limited in the mouth and stomach of nonruminants when compared to the small intestine.

Carnivorous Mammals

There is no detectable or very low lingual lipase in cats and dogs (Knospe and Plendl 1997; Moreau et al. 1988; Nelson et al. 1977). In these carnivorous mammals, lipid digestion begins in the stomach through the action of gastric lipase (Lim et al. 2022). Gastric peristalsis breaks dietary lipid into small droplets and mixes it with other macronutrients and gastric lipase. In cats and dogs, approximately 25% and

75% of ingested TAGs are hydrolyzed by gastric and pancreatic lipases, respectively (Lim et al. 2022).

The biochemical and physiological properties of gastric lipase in cats and dogs are similar to those in other mammals (He et al. 2024a). In cats, gastric lipase is primarily localized to the epithelial cells of the gastric mucosa before weaning (Knospe and Plendl 1997) but is present mainly in gastric fluid after weaning (Pansu et al. 1987). In dogs, gastric lipase is inactive at pH 1.5 and 7.0, and its enzymatic activity is reduced substantially at pH 6. The canine gastric lipase has low activity toward long-chain TAGs, but effectively hydrolyzes medium-chain TAGs. For example, at pH 4, gastric lipase is 13 times more active with long-chain than short-chain TAGs as substrates (Carrière et al. 1991). In dogs with pancreatic insufficiency, the ability to digest medium-chain TAGs and long-chain TAGs is reduced by about 25% and 95%, respectively (Rutz et al. 2004). This may also be true for cats.

As in noncarnivorous mammals, such as adult humans weighing 59–93 kg (Lavigne et al. 1978), there is a strong inverse relationship between BW and gastric emptying time in dogs. For example, when adult dogs consume a meal that meets 50% of the daily energy requirements, large breeds of dogs (e.g., ~60 kg BW) retain food in the stomach for a longer period and have a lower rate of gastric emptying, compared with small breeds of dogs (e.g., 3.5 kg BW; Bourreau et al. 2004). In dogs, gastric emptying is slowed by dietary fat, increased by gastrin (a peptide hormone that stimulates the secretion of HCl by the parietal cells of the stomach and aids in gastric motility), and influenced by meal size. In both cats and dogs, as in other mammals, gastric secretion is stimulated by dietary protein intake and meal size.

Digestion of Lipids in the Small Intestine

General Process

The digestion of lipids in nonruminants occurs predominantly in the small intestine. This process requires secretions from the liver (bile via the gallbladder) and the pancreas, the formation of lipid micelles, as well as the enzymatic hydrolysis of TAGs to fatty acids, MAGs, phospholipids, and cholesteryl esters. The primary components of the bile are bile salts and phospholipids, whereas TAG lipases, procolipase, phospholipases (including pro-phospholipase A_2), and $NaHCO_3$ (for pH adjustment in the small intestine) are constituents of pancreatic juice necessary for lipid digestion (Drackley 2000). The pancreatic lipases are released by the acinar cells of the pancreas, and the bicarbonate is secreted from the centroacinar and ductal cells of the pancreas. Pro-colipase and pro-phospholipase A_2 are activated in the lumen of the duodenum by trypsin through limited proteolysis to form the active colipase and pro-phospholipase A_2. Pro-colipase may also be activated by a pancreatic pro-colipase activation peptide, which is a pentapeptide. Thus, interorgan cooperation is required for complete lipid digestion. Impaired digestion and absorption of fats can result from (a) deficiencies of taurine, glycine, protein, and phospholipids, leading to the impaired formation of micelles; (b) diseases that impair the secretion of bile salts, such as biliary obstruction or liver diseases; and (c) diseases that influence the secretion of TAG lipase, colipase and bicarbonate from the pancreas, such as pancreatic cancer and cystic fibrosis (Goldstein and Brown 2015). In these cases, medium-chain TAGs can be better tolerated as a source of dietary energy (He et al. 2024a). For both mammals and birds, lipid digestion takes place mainly in the jejunum and, to a lesser extent, in the duodenum and the ileum (Iqbal and Hussain 2009).

Formation of Lipid Micelles

Gastric lipid emulsions that enter the duodenum mix well with bile salts and pancreatic juice (including lipase) to undergo marked changes in chemical and physical forms. Specifically, TAGs, MAGs, fatty acids, cholesterol, and phospholipids aggregate with bile salts to form small emulsion particles (Figure 6.2). The emulsion particles are

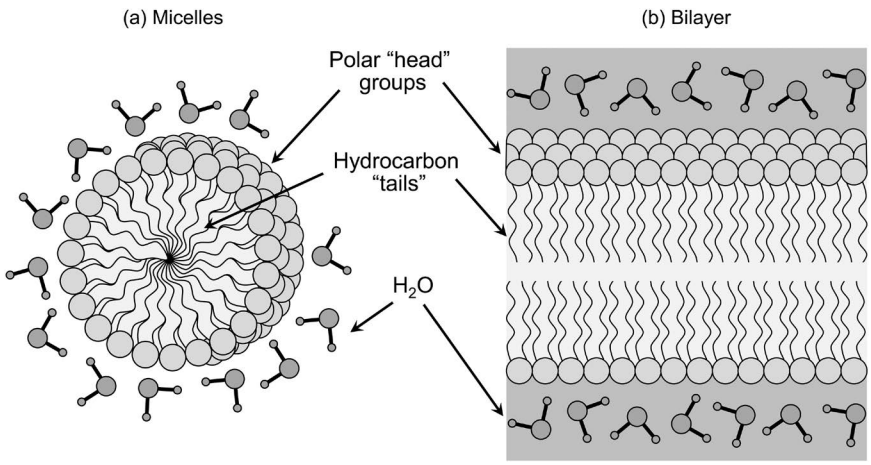


FIGURE 6.2 Formation of micelles from lipids and bile salts in the lumen of the small intestine. The lipid micelles are coated with bile salts. Hydrophilic "head" groups and hydrophobic "tails" are on the surface and the core of micelles, respectively. The drawing of a lipid bilayer on a cell membrane is shown for comparison.

coated with bile salts. Emulsion increases the surface area of the fat and allows for greater surface exposure to pancreatic lipases, colipase, and esterases. The lipases, with the aid of colipase, hydrolyze TAGs to MAGs, phospholipids to lysophospholipids, and cholesteryl esters to free cholesterol; all of these reactions also produce LCFAs.

Lysophosphatidylcholine (produced from biliary and dietary phosphatidyl choline) and MAGs play a key role in the formation and stabilization of the micelles, which are very small globules that are formed spontaneously in the lumen of the duodenum (Phan and Tso 2001). The major phospholipid in the intestinal lumen is phosphatidylcholine, which is derived mostly from bile (80%) but also from diet (10%). The micelles are globules with polar heads (groups) at the globule surface to interact with water and nonpolar tails (hydrocarbon chains) being sequestered inside the globule to exclude water. Bile salts and MAGs contain portions of molecules that can interact with water and other portions that can interact with lipids. The formation of micelles increases the solubility of the lipid complex in an aqueous solution and reduces the cytotoxicity of free fatty acids and bile salts. Micelles serve to transport their lipid components to the endothelial cells of blood vessels. As noted in Chapter 3, bile salts, which are the conjugates of bile acids with taurine and/or glycine, are synthesized from cholesterol in the liver and secreted into the duodenum. Before secretion from the liver, there are both glycine (predominantly) and taurine salts of hyocholic acid (primarily cholic acid) and chenodeoxycholic acid (CDCA) in pigs, but only taurine salts of cholic acid (primarily cholic acid) and CDCA in poultry. The bile conjugates (primarily cholic acid) of adult cattle and sheep contain mainly glycine and taurine, respectively.

Digestion of TAGs, Phospholipids, and Cholesterol Esters

The pancreatic enzymes primarily responsible for lipid digestion are TAG lipase, lipase-related protein-2, bile salt-stimulated lipase (also known as carboxyl ester lipase), precolipase, and cholesteryl esterase (Lowe 2002). Pancreatic TAG lipase hydrolyzes (a) the *sn*-1 and *sn*-3 linkages of a long-chain TAG to produce one 2-MAG and two LCFA molecules and (b) the *sn*-1, *sn*-2, and *sn*-3 linkages of medium-chain TAGs to produce three MCFA molecules and one glycerol molecule (Figure 6.3). This enzyme does not hydrolyze the *sn*-2 linkage of long-chain TAGs and, thus, lipolysis stops at the 2-MAG stage. Only a small amount of DAGs is produced from the degradation of TAGs in the nonruminant small intestine. The action of pancreatic lipases generates a liquid-crystalline interface at the surface of the emulsion particles.

Pancreatic lipase-related protein-2 has broad substrate specificity and acts preferentially on MAGs, phospholipids, and galactolipids. Similarly, bile salt-stimulated lipase has broad substrate specificity and is capable of hydrolyzing

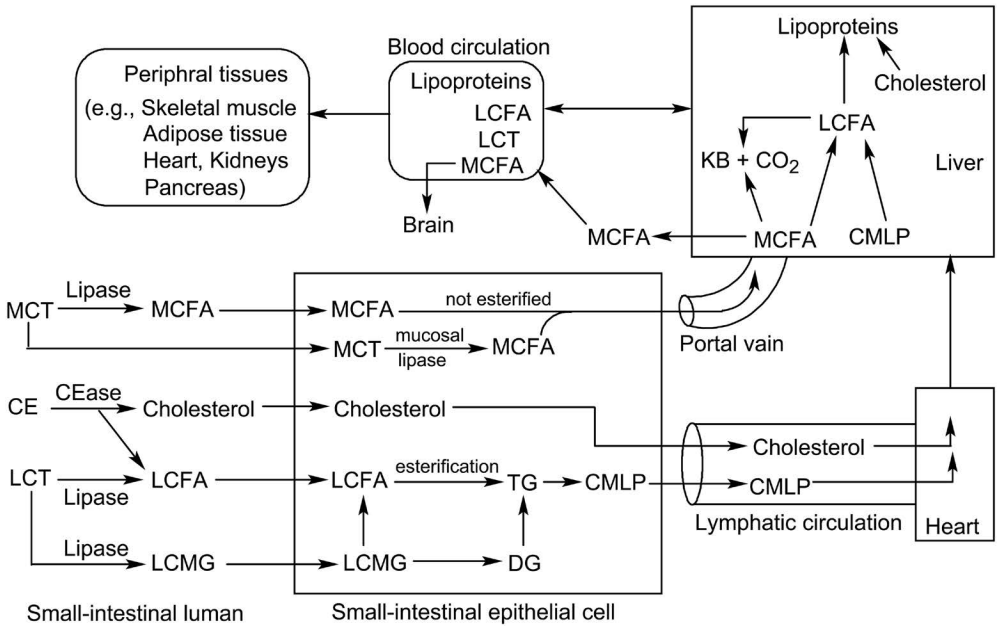


FIGURE 6.3 Digestion and absorption of lipids in the small intestine of nonruminants. Hydrolysis of medium-chain triacylglycerols (MCT) and long-chain triacylglycerols (LCT) by pancreatic lipases and of cholesterol esters (CE) by cholesterol esterase (Cease) occurs in the lumen of the nonruminant small intestine. Enterocytes take up medium-chain fatty acids (MCFA), long-chain fatty acids (LCFA), cholesterol, long-chain monoacylglycerol (LCMG), and cholesterol. Medium-chain fatty acids are not actively esterified into triacylglycerols (TG) in the cells and are absorbed directly into the portal vein. In the endoplasmic reticulum of enterocytes, LCFA and LCMG are re-esterified to TG through the formation of diacylglycerols (DG). TG is incorporated into chylomicrons plus other lipoproteins (CMLP) for export into lacteals. CMLP and MCFA are catabolized by the liver and peripheral tissues.

TAGs, phospholipids and cholesteryl esters, as well as the esters of ω3 and ω6 polyunsaturated fatty acids (PUFAs; Thomson and Dietschy 1981). In addition, phospholipids are hydrolyzed by phospholipase A_2 to produce mainly lysophospholipids and LCFAs. For example, phosphatidylcholine is hydrolyzed in the lumen to form lysophosphatidylcholine. Cholesteryl esters are also hydrolyzed by pancreatic cholesteryl esterase to yield cholesterol and LCFAs (Lowe 2002). Colipase is a small protein required for the optimal enzyme activity of pancreatic lipase. By binding to the C-terminal, noncatalytic domain of pancreatic lipase, colipase prevents the inhibitory effect of bile salts on the lipase-catalyzed intra-duodenal hydrolysis of dietary long-chain TAGs.

Emulsification of the hydrophobic products of lipids continues actively in the small intestine in preparation for their efficient absorption across the enterocyte via micelles. The micelles are needed to move the water-insoluble lipids (e.g., fatty acids, MAGs, and cholesterol) across the unstirred water layer outside the brush-border membrane of enterocytes to the surface of these cells for absorption.

Digestibility of Dietary Lipids

In addition to the pancreas, the lactating mammary glands of mammals such as humans, rats, and mice synthesize and secrete bile salt-stimulated lipase and pancreatic lipase-related protein 2 (Li et al. 2007). Thus, neonatal mammals efficiently digest milk fats, as do postweaning mammals consuming solid foods. For example, the digestibility of sow's milk fat by 2-day-old pigs is 95% and is even higher in older pigs (Manners 1976). The digestibility of TAGs in grain cereals or added lipids is high in postweaning nonruminant mammals (e.g., often 90–95% in pigs) and in post-hatching poultry (e.g., 82% at Day 2, 89% at Day 21, and 90–95% after 3 weeks of age) (Doreau and Chilliard 1997; Li et al. 1990). The digestibility of dietary TAGs is 90–95% in horses and 85–95% in fish. Efficient absorption of lipids by enterocytes ensures that dietary fat is maximally available to be used as a major source of energy for supporting cellular functions. TAGs serve as reservoirs for energy storage, lipoprotein trafficking, bile acid synthesis, and steroidogenesis in animals.

The rates of digestion and absorption of dietary lipids are enhanced by the presence of unsaturated fatty acids in diets. Much evidence shows that intestinal TAG digestibility increases with increasing unsaturation and decreases with increasing chain length in saturated or unsaturated LCFAs. Three reasons can explain this nutritional phenomenon. First, unsaturated fatty acids (e.g., oleic, linoleic, and α-linolenic acids) readily form mixed micelles with bile salts and have a higher solubility in bile acid micelles than long-chain saturated fatty acids. Second, MAGs and unsaturated fatty acids act synergistically to promote the incorporation of saturated fatty acids in diets into micelles in the small intestine. Third, the solubility of saturated or unsaturated LCFAs in micelles decreases as the hydrocarbon chain length increases. These principles can guide the practice of animal feeding. For example, the digestibility of saturated fats is poor in young chicks (e.g., 40% and 79% for tallow at Days 1 and 7 of age), due to the limited production of bile salts (Carew et al. 1972). However, the presence of unsaturated fatty acids (e.g., vegetable oil) in the diet enhances the digestibility of saturated fats in the birds (Carew et al. 1972).

Absorption of Lipids by the Small Intestine

General Process

The process of lipid absorption in the small intestine involves several steps: (a) the diffusion of micelles through the unstirred water layer; (b) the passage of lipid digestion products through the plasma membrane of enterocytes via simple diffusion (protein carrier-independent), passive transport (protein carrier-independent; e.g., several fatty acid transporters), or active transport (protein carrier-dependent); (c) the cytosolic activation and esterification of fatty acids; (d) the formation of chylomicrons and lipoproteins; and (e) the exocytosis of chylomicrons, VLDLs, and HDLs from enterocytes into lymphatic circulation via the lamina propria. For nonruminant mammals and birds, the absorption of lipids occurs primarily in the jejunum and, to a lesser extent, in the duodenum and ileum (Iqbal and Hussain 2009). SCFAs and MCFAs (which are all water-soluble) in the lumen of the small intestine enter the portal circulation (Table 6.2). Absorption of lipids from the small

TABLE 6.2
Digestion and Absorption of Long- and Medium-Chain Triacylglycerols in the Small Intestine of Animals

Variable	Long-Chain TAG	Medium-Chain TAG
Paracellular absorption in the stomach and SI	No	Some
Hydrolysis by gastric lipase in the stomach	Limited	High
Requirement for bile salt for micellarization in SI	Yes	No
Hydrolysis by pancreatic lipase in SI	High	Very high
Solubility of fatty acids in water	Insoluble	Very high
Absorption of digestion products from the intestinal lumen into the intestinal epithelium	Mainly via diffusion but some via FA transporters (e.g., CD36)	Mainly via transmembrane transporters
Re-esterification and chylomicron formation in Ent	Yes	No
Primary transport of digestion products from the intestinal epithelium into blood	Lymphatic vessels in mammals and fish; portal vein in birds	Portal vein in mammals, birds, and fish

Source: He et al. (2024).

CD36 = cluster of differentiation 36 (also known as fatty acid translocase; transporting long-chain fatty acids in an energy-independent manner); Ent = enterocytes; FA = fatty acid; SI = the small intestine; TAG = triacylglycerols

intestine can be reduced by a high intake of dietary fibers. Any damage to the pancreas or small intestine can result in lipid malabsorption.

Absorption of Lipids into Enterocytes

MAGs (e.g., primarily 2-MAG), phospholipids, and a small amount of TAGs enter enterocytes primarily by simple diffusion through the lipid membrane (Figure 6.3). The absorption of LCFAs from the small-intestinal lumen occurs via both simple diffusion and protein carriers (e.g., CD36) in an energy-independent manner (Pepino et al. 2014). Genetic deletion of CD36 (a multiligand receptor) reduces the uptake of LCFAs by enterocytes (Goldberg et al. 2009). Expression of fatty acid transporters is upregulated by the presence of dietary fat or some forms of genetic obesity. In contrast, the absorption of cholesterol requires a transporter, possibly in an energy-dependent process. A protein carrier, named Niemann-Pick C1-like 1 (NPC1L1) protein, has been identified at the apical membrane of enterocytes as a cholesterol uptake transporter, which plays a crucial role in the ezetimibe-sensitive cholesterol absorption pathway (Kawase et al. 2015). Expression of *Npc1l1* is enhanced in the cholesterol-depleted porcine intestine and suppressed in mice fed a cholesterol-rich diet. Of note, ATP-binding cassette (ABC) transporters, ABCG5 and ABCG8, which are also localized at the apical membrane of enterocytes as cholesterol efflux transporters, promote the active efflux of cholesterol and plant sterols from enterocytes back into the intestinal lumen for excretion (Kawase et al. 2015). Thus, the net absorption of cholesterol is determined by the balance between the influx and efflux of intraluminal cholesterol molecules crossing the brush border membrane of the enterocyte. In animals, there is net absorption of dietary cholesterol (~50%; with the remainder being excreted in feces), but virtually no absorption of plant sterols under physiological conditions. Fatty acids with less than 14 carbons are not actively esterified into TAGs in enterocytes and are absorbed directly into the portal vein (Figure 6.4).

Bile salts are released from the disruption of the micelles at the duodenum, jejunum, and ileum after the products of

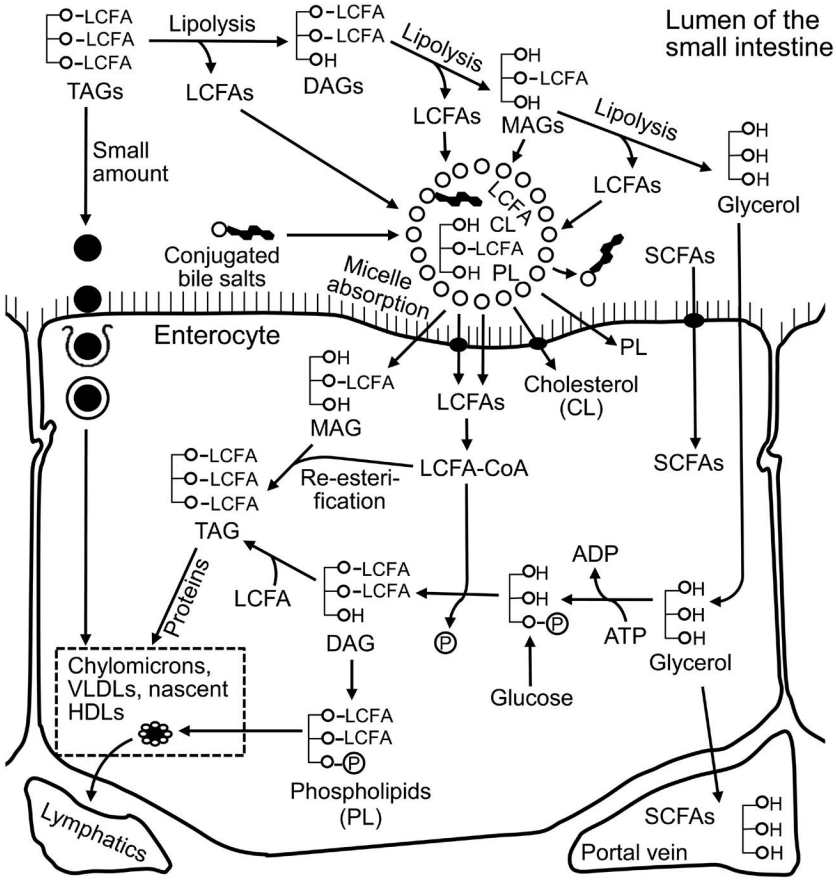


FIGURE 6.4 Uptake of lipids in micelles by enterocytes of nonruminants. In the lumen of the small intestine, triacylglycerols (TAGs) are enzymatically hydrolyzed to diacylglycerols (DAGs) and monoacylglycerols (MAGs). Enterocytes take up (a) MAGs (primarily 2-MAG) and phospholipids (PL) through simple diffusion, (b) cholesterol (CL) and short-chain fatty acids (SCFAs) through specific transmembrane protein carriers, and (c) long-chain fatty acids (LCFAs) through both simple diffusion and transmembrane protein carriers. Chylomicrons, very low-density lipoproteins (VLDLs), and nascent high-density lipoproteins (HDLs) are assembled from TAGs, proteins, phospholipids, and cholesterol. Through the lamina propria of the small-intestinal mucosa, SCFAs and glycerol enter the portal vein, whereas lipoproteins move into the lymphatics (mammals, birds, and fish), the portal vein (birds), or the hemolymph (crustaceans).

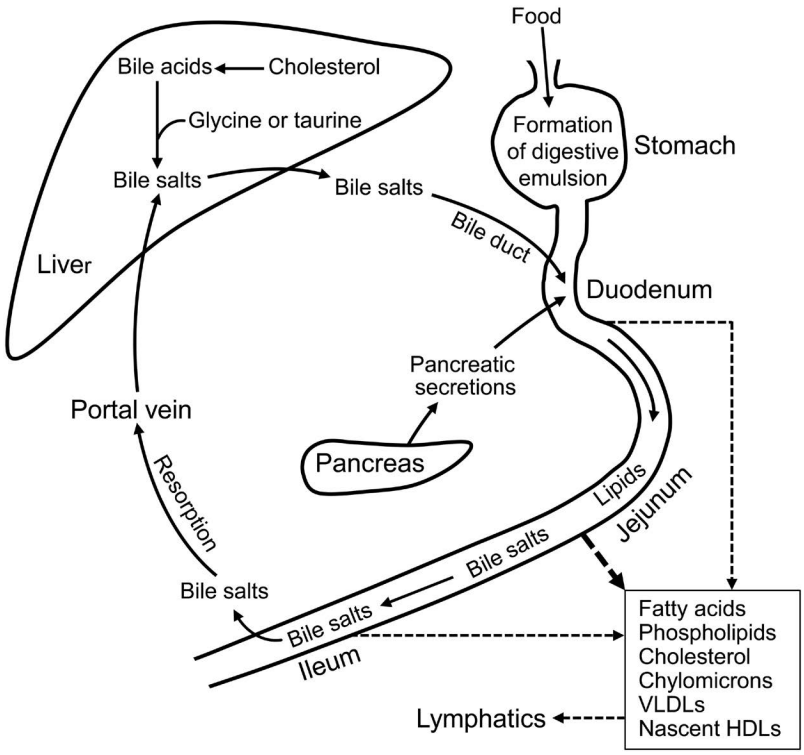


FIGURE 6.5 The enterohepatic circulation. Bile salts play an important role in the absorption of lipid digestion products into enterocytes of the small intestine (primarily the jejunum). In the terminal ileum, most (95%) bile salts are absorbed by an active transport system into the hepatic portal blood and then enter the liver for re-secretion into the lumen of the duodenum.

lipid digestion are absorbed by the enterocytes. In the terminal ileum, most (95%) bile salts are absorbed by an active transport system into the hepatic portal blood and enter the liver for re-secretion into the lumen of the duodenum. This is called "enterohepatic circulation" (Figure 6.5), which is active in animals (including pigs, fowls, and humans). The small amount (5%) of bile salts in the ileum that is not absorbed from the terminal ileum flows to the large intestine, where they are converted into products called "secondary bile salts" by microbes and excreted in the feces. The enterohepatic circulation helps conserve bile salts in the body.

Luminal bile salts are taken up by the small intestine via passive transport absorption, active transport in the distal ileum, and passive absorption in the colon. The general consensus from numerous studies is that ileal active transport is the major route for the uptake of bile salts, whereas the passive or facilitative absorption present along the length of the small intestine may be significant for unconjugated and some glycine-conjugated bile acids (Foley et al. 2019; Meier and Stieger 2002).

Resynthesis of TAGs in Enterocytes

In the smooth endoplasmic reticulum (ER) of enterocytes, lysophosphatidyl choline undergoes reacylation to form phosphatidylcholine, whereas LCFAs are reversibly and noncovalently bound to cytosolic FABPs and activated by long-chain acyl-CoA synthetase (ACS_L) to yield acyl-CoAs (Phan and Tso 2001). The acyl-CoA is either metabolized in the smooth ER, mitochondria, and peroxisomes or bound to acyl-CoA-binding proteins to constitute an intracellular acyl-CoA pool (Figure 6.3). Most acyl-CoA esters are used for esterification with MAGs for the resynthesis of TAGs. The MAG pathway is primarily responsible for the resynthesis of TAGs from fatty acids, but the glycerol-3-P pathway also plays a role, especially in ruminants (see the section on TAG synthesis in this chapter). Oxidation of fatty acids to CO_2 and water is limited in the small intestine (Jobgen et al. 2006).

Assimilation of Wax Esters

Wax esters (long-chain fatty alcohols esterified to LCFAs) are significant dietary neutral lipids for birds, including seabirds and some passerines (Chapter 3). These animals can assimilate the wax esters in enterocytes with higher efficiencies (greater than 90%) than those attainable by mammals (<50%). This feature of lipid digestion can be explained by the following reasons, as proposed by Place (1992). First, the concentrations of bile salts in the small-intestinal lumen (~50 mM) and gallbladder (>600 mM) in birds are much greater than those in mammals. Second, birds possess the ability of regular retrograde movement of duodenal contents to the gizzard, which aids in further digestion of wax esters. Third, birds have a high capacity to hydrolyze wax esters as effectively as TAGs. Therefore, despite the similar processes of lipid digestion between

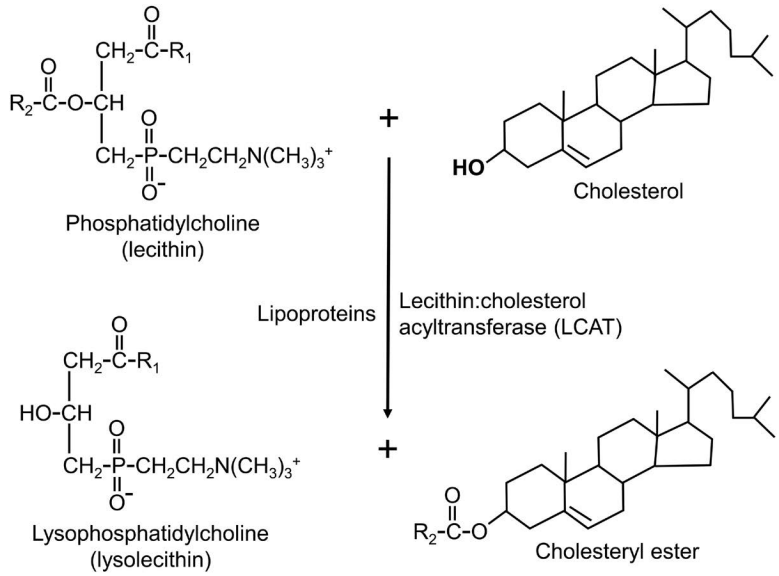


FIGURE 6.6 Formation of cholesteryl esters from cholesterol and phosphatidylcholine in lipoproteins by lecithin:cholesterol acyltransferase (LCAT). This enzyme is expressed in enterocytes and other cell types to catalyze the esterification of cholesterol.

birds and mammals, birds can utilize dietary wax esters more efficiently than mammals.

Assembly of Chylomicrons, VLDLs, and Nascent HDLs in Enterocytes

Apoproteins B (predominantly ApoB48 and a trace amount of ApoB100) and microsomal triglyceride transfer protein (MTP) participate in the efficient assembly and secretion of lipoproteins in enterocytes (Iqbal and Hussain 2009). ApoB48, which is a truncated form of Apo100, is produced by the post-transcriptional editing of the mRNA for apoB100 (Davidson and Shelness 2000). In the ER of these cells, TAGs, cholesterol, and phospholipids are assembled, along with apoproteins, primarily into chylomicrons (containing ApoB48 and ApoA4) and, to a much lesser extent, VLDLs (also containing ApoB48) and nascent HDLs (containing ApoA1). Esterification of cholesterol as cholesteryl esters is crucial for the assembly of these lipoproteins. About 70% of the cholesterol in lipoproteins exists in the form of cholesteryl esters, and 30% in the free form. In enterocytes, the formation of cholesteryl esters in lipoproteins is catalyzed by: (a) lecithin:cholesterol acyltransferase [LCAT, also known as phosphatidylcholine acyltransferase (PCAT); Figure 6.6]; and (b) acyl-CoA:cholesterol acyltransferase (ACAT; Figure 6.7). LCAT and ACAT convert free cholesterol into cholesteryl ester (a more hydrophobic

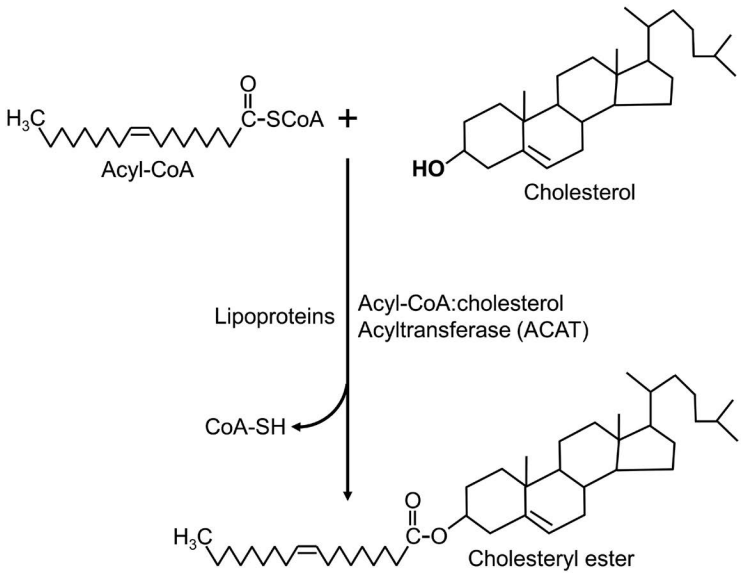


FIGURE 6.7 Formation of cholesteryl esters from cholesterol and acetyl-CoA in lipoproteins by acyl-CoA:cholesterol acyltransferase (ACAT). This enzyme is expressed in enterocytes and other cell types to catalyze the esterification of cholesterol.

form of cholesterol), which is then sequestered into the core of lipoprotein particles (Figure 6.3). The transfer of cholesteryl esters generated by LCAT and ACAT to the core of the lipoprotein particles and the subsequent maturation of the lipoproteins requires the action of a phospholipid transfer protein, which provides phospholipids to allow for surface expansion of the particles. LCAT is present in multiple tissues (including the small intestine and liver) and is released from the liver into the blood circulation. On the other hand, ACAT (a membrane-bound protein) utilizes long-chain fatty acyl-CoA and cholesterol as substrates to form a cholesteryl ester in the cytosol. In mammals, two isoenzymes, ACAT1 and ACAT2, which are encoded by two different genes, regulate cholesterol homeostasis in tissues. Defects in LCAT and ACAT result in the abnormal packaging of lipoproteins by enterocytes.

Processing and Release of Lipoproteins by Enterocytes of the Small Intestine

In animals, chylomicrons are formed exclusively in the enterocytes of the small intestine. Chylomicrons are very heterogeneous in size. They consist of a core of TAGs and cholesteryl esters, and a surface monolayer of phospholipids, cholesterol, and apolipoproteins (Wang et al. 2015). The nascent intestinal lipoproteins are processed in the Golgi apparatus before being released by the enterocytes through exocytosis via the secretory pathway at the basolateral surface of the enterocyte (Kohan et al. 2010).

In nonruminant mammals, chylomicrons, VLDLs, and HDLs are too large to pass directly into the venous blood stream draining the small intestine and are instead secreted into the intestinal lymphatics, which drain eventually into the venous system (Chapter 1). In reptiles, chylomicrons and VLDLs also enter the lymphatics and then the bloodstream (Price 2017). By contrast, in birds (e.g., poultry) that have a poorly developed lymphatic system, very-large lipoprotein particles (called portomicrons) are assembled inside enterocytes, have a much lower proportion of TAGs than mammalian chylomicrons, and are transported through the basolateral membranes of the enterocytes into the portal vein (Fraser et al. 1986). The avian small intestine does not appear to release VLDLs or HDLs. Thus, the intestine-derived lipoproteins carry dietary TAGs, cholesterol, and phospholipids to extraintestinal tissues.

Although the output of lipoproteins from the enterocytes is known to be required for the complete absorption of dietary lipids, the movement of these lipid-protein complexes from the intercellular space through the basement membrane to the lamina propria is not fully understood. It is possible that the movement of chylomicrons through the lamina propria occurs via simple diffusion that is facilitated by interstitial hydration and the one-way directional flow of lymph in the intestinal lacteals (Phan and Tso 2001).

Diurnal Changes in Intestinal Lipid Absorption

Results from studies with rodents indicate diurnal changes in intestinal lipid absorption (Iqbal and Hussain 2009). In postabsorptive rodents, plasma lipid concentrations peak at 1–2 h after feeding and are maintained within a narrow physiological range thereafter. Also in rats, the rate of lipid absorption is greater at 12:00 midnight than at 12:00 noon because of their nocturnal feeding behavior. Using in situ loops and isolated enterocytes, circadian variations in the rate of lipid digestion have been reported to result from changes in intestinal expression of MTP (e.g., greater expression of MTP at 12:00 midnight than at 12:00 noon) and, therefore, the intestinal assembly of lipoproteins. This pattern of MTP expression maximizes lipid absorption at the time of feeding and may also be responsible for the postprandial and diurnal changes in the plasma concentrations of chylomicrons and VLDLs.

DIGESTION AND ABSORPTION OF LIPIDS IN PRERUMINANTS

Digestion of Lipids in the Mouth and Small Intestine: Limited Digestion by Salivary Lipase in Mouth, Forestomaches, and Abomasum

The content (expressed on a DM basis) of lipids in milk varies greatly among species, such as 16% for horses, 37% for ruminants (cattle and sheep), 40% for pigs, 47% for rats, and 49% for rabbits. The saliva of preruminants contains lipase (known as pregastric esterase) to allow for limited hydrolysis of dietary TAGs. This enzyme also acts in their abomasum in a manner similar to that of pancreatic lipase in the small intestine to cleave butyrate and other fatty acids from milk TAGs. The activity of pregastric esterase decreases with age and normally disappears by three months of age in calves fed whole milk (Drackley 2000). The decrease in the secretion of the pregastric esterase is more rapid in calves consuming liquid skim milk or high-roughage diets than in cow-nursed neonates. Because the rumen of preruminants is underdeveloped in its function and microbiota, unsaturated fatty acids in the diet can escape hydrogenation during their passage from the mouth to the small intestine. Thus, the tissues of preruminants contain more unsaturated fatty acids than adult ruminants. Overall, the rate of lipid digestion is low in the mouth, forestomaches, and abomasum of preruminants, and the immature reticulorumen can absorb a small amount of SCFAs from the milk and its limited microbial fermentation.

Digestion of Lipids in the Mouth and Small Intestine: Extensive Digestion of Lipids in the Small Intestine

The small intestine is the primary site for lipid digestion in preruminants. The processes of intestinal lipid digestion in these animals (e.g., suckling calves and lambs) with a nonfunctional rumen are the same as those in preweaning monogastric mammals. Specifically, the pancreas of preruminants secretes pancreatic lipase and colipase into the lumen of the duodenum. The activity of these enzymes is high at birth and may double during the first weeks of

postnatal life (Porter 1969). Thus, young ruminants can efficiently digest milk TAGs, with a true digestibility of 95–99% (Gerrits et al. 1997), as well as other animal and vegetable fats that are emulsified to particle sizes of <4 μm in diameter. The digestibility of TAGs in common nonmilk foods is generally also high in preruminants (e.g., 90–95%; Bauchart 1993). However, these young animals have a lower ability to hydrolyze fats, predominantly tristearin, than the natural milk fats.

Absorption of Lipids by the Small Intestine

Dietary lipids and their digestion products are absorbed by the small intestine in preruminants, as in preweaning nonruminant mammals. As noted previously, there is little esterification of MCFAs within enterocytes. Thus, when preruminants are either nursed by their mothers or fed replacer liquid milk, MCFAs (water-soluble) present in the lumen of the small intestine are absorbed through the apical and basolateral membrane MCFA transporters of enterocytes into the interstitial space of the lamina propria. MCFAs then enter the intestinal venule and, through the small vein, the portal vein. LCFAs (lipid-soluble) and MAGs are absorbed through the apical and basolateral membrane of specific transporters of enterocytes, or simple diffusion, into the cells. Preruminants secrete relatively large amounts of bile salts and lysophospholipids into the lumen of the duodenum for solubilizing dietary lipids and, therefore, exhibit high rates of absorption of saturated fatty acids in milk (95–99%). Inside the enterocytes, the absorbed lipids are assembled into chylomicrons (predominantly apo B48), VLDLs and nascent HDLs, which are exported into the lamina propria and then enter either the intestinal lymphatic vessel or the portal vein in preruminants (Bauchart et al. 1989). In preruminants, 80% of lipids in the intestinal lymph are present in the form of TAGs (Laplaud et al. 1990).

DIGESTION AND ABSORPTION OF LIPIDS IN RUMINANTS

There are marked differences in lipid digestion and absorption between ruminant and nonruminant animals (Nafikov and Beitz 2007). Specifically, ruminants exhibit (a) negligible salivary lipase activity; (b) active hydrolysis of TAGs in the rumen; (c) a limited ability of ruminal microbes to degrade dietary fatty acids in comparison with carbohydrates; (d) a relatively high capacity for the ruminal biohydrogenation of unsaturated fatty acids; and (e) relatively low pancreatic lipase activity in the small intestine (Palmquist 1988). A high rate of TAG hydrolysis and a low rate of further metabolism of free fatty acids in the rumen creates a deterrent effect that is toxic to its microbes. This limits the inclusion of fats in ruminant diets (a maximum of 7% on a DM basis). In ruminants, there is virtually no absorption of MCFAs and LCFAs in the rumen, omasum, or abomasum, and no modification of these fatty acids in the omasum or abomasum (Bauchart 1993). In contrast, large amounts of SCFAs are absorbed from the rumen through its epithelium into the blood circulation (Figure 6.8). Thus, the absorbed fatty acids in the small intestine are more saturated than dietary fatty acids. This explains why, although the diets of ruminants naturally contain high proportions of unsaturated fatty acids (particularly linoleic and α-linolenic acids), their fat deposits are very rich in stearic acid. Dietary lipids that escape the rumen and microbial lipids are further

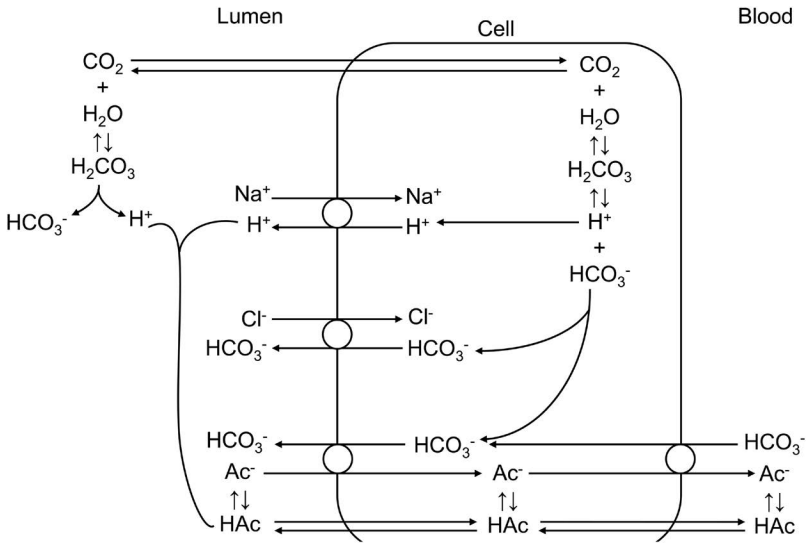


FIGURE 6.8 Absorption of short-chain fatty acids (SCFAs) from the lumen of the rumen into the blood circulation. The epithelial cells of the rumen express protein transporters to take up SCFAs (e.g., acetic, propionic, and butyric acids) from the ruminal lumen. This process is associated with the secretion of H^+ from the cells, as well as of HCO_3^- from both the cells and the blood. Acetic acid is used as an example of SCFAs. AC^-, acetic acetate; HAc, acetic acid.

hydrolyzed in the small intestine. Overall, the true digestibility of dietary esterified lipids (including TAGs) in the entire digestive tract of ruminants is 70–75%.

Digestion of Lipids in the Rumen

Overview of Roles of Ruminal Microbes

Bacteria are primarily responsible for lipid digestion in the rumen. There is little evidence for a nutritionally significant role of ruminal protozoa and fungi, or salivary and plant lipases, in this process (Palmquist 1988). However, protozoa play a role in the hydrolysis and isomerization of dietary phospholipids (Doreau and Ferlay 1994). Of note, the hydrolysis and isomerization of lipids are extracellular processes. Ruminal microbes synthesize MCFAs and LCFAs from SCFAs that are derived from their metabolism of dietary carbohydrates, amino acids, and TAGs, while producing branched-chain fatty acids from branched-chain amino acids. These newly formed fatty acids are incorporated primarily into microbial cell membrane phospholipids and TAGs for digestion in the abomasum and small intestine. Fatty acids attached to feed particles enter the abomasum and then the small intestine. The flow of LCFAs leaving the rumen is higher than their dietary intake in ruminants fed diets that generally contain a low amount of lipids (2–5% of dietary DM) due to bacterial synthesis in the rumen (Doreau and Ferlay 1994; Wu and Palmquist 1991).

Products of Lipid Hydrolysis

The lipids in the diets of ruminants exist mainly in the esterified forms of mono- and di-galactoglycerides in forages, as TAGs in concentrates and, to a lesser extent, as phospholipids in all plant-based feedstuffs (Drackley 2000). In these galactoglycerides, fatty acids are mainly linoleic acid ($C_{18:2}$ ω6) and α-linolenic acid ($C_{18:3}$ ω3). The hydrolysis of the ester linkages in TAGs, phospholipids, and glycolipids is the initial step of lipid digestion by bacterial enzymes in the rumen. Specifically, digalactodiglycerides are hydrolyzed by bacterial α-galactosidase to yield galactose and monogalacto-diglycerides. The latter are further hydrolyzed by bacterial β-galactosidase to generate galactose, fatty acids, and glycerol. There are no detectable intermediate compounds (e.g., mono- or diglycerides) in ruminal contents (Doreau and Ferlay 1994). Glycerol and galactose are further metabolized to SCFAs (propionate and butyrate) in the rumen.

Extensive hydrolysis of esterified dietary lipids is carried out by bacterial lipases (Bauman and Griinari 2003). For example, *Anaerovibrio lipolytica* is responsible for the hydrolysis of TAGs, as *Butyrivibrio fibrisolvens* is for the hydrolysis of phospholipids and glycolipids. Bacterial lipases attack *sn*-1, *sn*-2, and *sn*-3 linkages of TAGs, and thus there is little production of MAGs in the rumen, in contrast to lipid digestion in nonruminants. Glycerol and galactose, which are released from lipid hydrolysis, are fermented to primarily yield SCFAs. Approximately 85–95% of esterified lipids are hydrolyzed to their constituent components (e.g., LCFAs, glycerol, and galactose) in the rumen of ruminants.

Negligible Degradation of Medium- and Long-Chain Fatty Acids in the Rumen

Although bacteria possess enzymes for α- and β-oxidation, these biochemical reactions are not favored under the anaerobic conditions in the rumen. Thus, the degradation of palmitic acid to CO_2 and acetate by mixed ruminal microbes is negligible, as determined by the recovery of ^{14}C from [1-^{14}C]palmitic acid in $^{14}CO_2$ (0.03%; an indicator of both α-oxidation and Krebs cycle activity) and ^{14}C-acetate (1.09%; an indicator of β-oxidation) after a 4-h period of incubation with ruminal contents (Wu and Palmquist 1991). Likewise, mixed ruminal microbes have a very limited ability to degrade MCFAs (Bayourthe et al. 1994; Yamano et al. 2023). Consequently, the rumen produces little SCFAs from MCFAs or LCFAs.

Because glycerol and galactose moieties account for ~10% and 20% of TAGs (g/g), respectively, and the assumption that 75% of the diet-derived glycerol and galactose is converted into SCFAs, it can be estimated that dietary lipids provide only a small amount (0.4–1%) of the SCFAs produced in the rumen under normal feeding (i.e., dietary lipid content = 2–5% of DM). Therefore, in the rumen, unesterified fatty acids are hydrogenated and incorporated into microbial lipids.

Biohydrogenation of Unsaturated Fatty Acids

Because the rumen lacks molecular oxygen, the oxidation of fatty acids to CO_2 and water by ruminal microbes is absent, as noted previously. Thus, the disposal of hydrogen atoms occurs through the reduction of double bonds in unsaturated fatty acids to yield saturated fatty acids. This biochemical reaction serves as a defense mechanism for the survival and activity of the microbes (Jenkins 1993). These unsaturated fatty acids, which are released from TAG hydrolysis, are first isomerized and then hydrogenated extensively by bacteria and protozoa to yield saturated fatty acids. The forages of grazing ruminants typically contain 2–3% fatty acids (on a DM basis), with a higher ratio of unsaturated fatty acids to saturated fatty acids than the diets of nonruminants. For example, α-linolenic acid ($C_{18:3}$; *cis*-9, *cis*-12, *cis*-15) accounts for 61% of total fatty acids in pasture grass (Palmquist 1988). In contrast, linoleic acid ($C_{18:2}$; *cis*-9, *cis*-12) is the predominant fatty acid in cereal grains and seeds (e.g., 47% in corn grain). Indeed, the major substrates for ruminal biohydrogenation are linoleic and linolenic acids. The rate of rumen biohydrogenation of unsaturated fatty acids is typically faster with increased unsaturation. For most diets, linoleic acid and α-linolenic acid are hydrogenated to the extent of 70–95% and 85–100%, respectively, with stearic acid ($C_{18:0}$) as a major product (Beam et al. 2000). On average, ~90% of dietary lipids reach the duodenum as saturated fatty acids in ruminants (Doreau and Ferlay 1994).

The process of biohydrogenation also involves isomerization as the initial reaction, resulting in the formation of many intermediate compounds with *trans*- or conjugated double bonds (e.g., the production of conjugated linoleic acid and vaccenic acid from linoleic acid) and saturated fatty acids (Figure 6.9). For example, an isomerization of the *cis*-12 double bond to a *trans*-11 configuration generates a conjugated di- or trienoic fatty acid. Then, a reduction of the *cis*-9 double bond yields a *trans*-11 fatty acid. Finally, the hydrogenation of the *trans*-11 double bond produces stearic acid (linoleic and linolenic acid pathways) or *trans*-15 $C_{18:1}$ (linolenic acid pathway). Of note, two key biohydrogenation intermediates are (a) *trans*-11 $C_{18:1}$ (vaccenic acid), which is formed from linoleic acid and α-linolenic acid, and (b) *cis*-9, *trans*-11 conjugated linoleic acid, which is formed from linoleic acid. Eight positional isomers of CLA are possible, but the predominant product in the rumen is the *cis*-9, *trans*-11 isomer (Azain 2003). In the rumen, >90% of PUFAs are hydrogenated, and <10% of them escape the rumen (Drackley 2000). Because unsaturated LCFAs are toxic to many species of bacteria, particularly those involved in fiber digestion, and because there is excess hydrogen in the rumen, biohydrogenation is favorable for the formation of methane from CO_2 and $[2H^+]$, as well as the production of propionate. However, large amounts of unsaturated LCFAs can exceed the capacity of biohydrogenation to bring about undesirable effects on the ruminal microbial population and dietary fiber digestion. Thus, because lipolysis is a prerequisite for biohydrogenation to proceed in the rumen and because the isomerases for converting a *cis*- to a *trans*-double bond act only on unesterified fatty acids with a free carboxyl group, the feeding of rumen-protected lipids helps to minimize lipolysis in the rumen and maximize their passage to the small intestine.

Digestion of Lipids in the Abomasum

As noted previously, LCFAs in the rumen are not absorbed into its epithelial cells, but rather will enter the abomasum. The lipids that leave the rumen (pH 5.5–6) consist predominantly of (a) free fatty acids (85–90%) as their potassium, sodium, or calcium salts; and (b) phospholipids (10–15%) as part of microbial cell membranes. In the abomasum (~ pH 2), the fatty acid salts are dissociated to release free fatty acids, which then flow into the small intestine, the primary site for their absorption. The profile of fatty acids that reach the small intestine is very different from that in the diet consumed by the animals (Drackley 2000).

Digestion of Lipids in the Small Intestine

Approximately 85% (80–90%) of the lipids (excluding SCFAs) that enter the small intestine are free LCFAs attached to dietary feed particles, with the remaining lipids being microbial phospholipids, as well as a small amount of TAGs and glycolipids from residual feed material (Palmquist 1988). These esterified fatty acids are hydrolyzed by pancreatic lipases in the ruminant small intestine, as described for the nonruminant small intestine. Compared with nonruminants, lipid digestion in the small

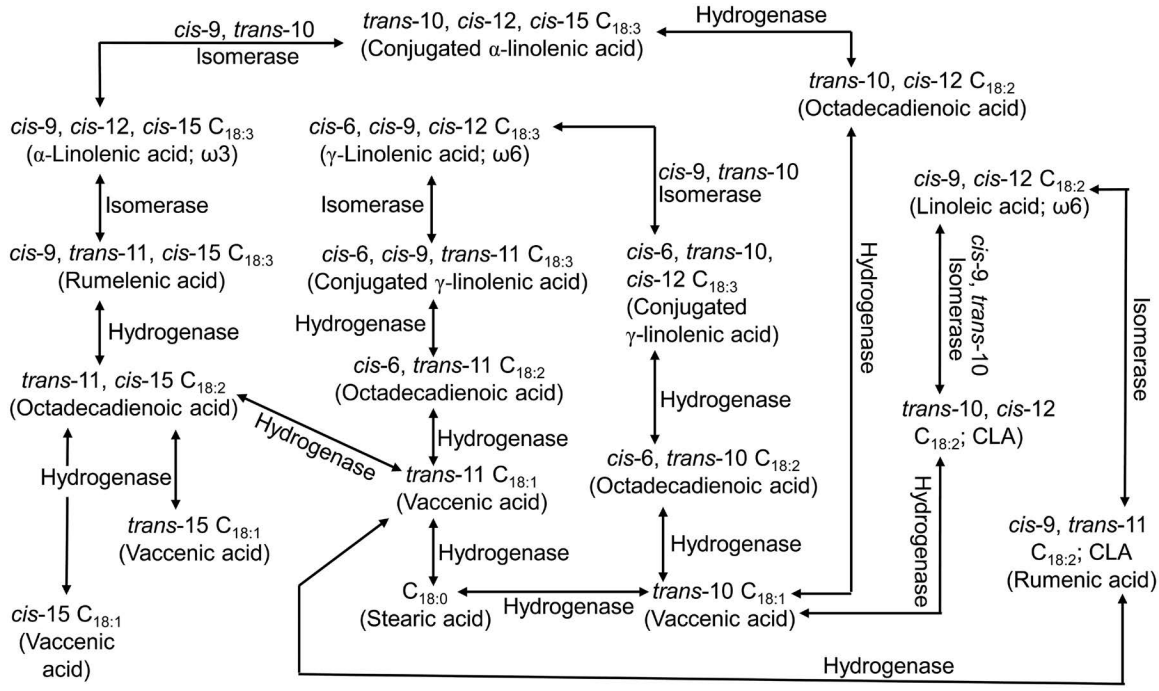


FIGURE 6.9 Formation of conjugated fatty acids by ruminal bacteria. Through the isomerization and hydrogenation of *cis*- and *trans*-polyunsaturated fatty acids, a variety of conjugated fatty acids are produced in ruminal bacteria. Examples include the formation of conjugated linoleic acid (CLA) from linoleic acid and of conjugated α-linolenic acid from α-linolenic acid.

intestine of ruminants is only moderate due primarily to the limited amounts of bile salts and pancreatic lipases released into the small intestine of ruminants (Bergen and Mersmann 2005).

The ruminal microbial lipases that enter the small intestine and the pancreatic lipases that are secreted into the small intestine have relatively low activities to hydrolyze highly saturated (or hydrogenated) TAGs in ruminants. Thus, the digestibility of fats (6–8% of dry digesta) entering the small intestine of these animals is ~80%, which is lower than that usually seen in nonruminants (Doreau and Chilliard 1997). The intestinal digestibility of fats is similar between 16- and 18-carbon fatty acids (~75–80%) and is slightly greater for fats with unsaturated fatty acids than for those with saturated fatty acids (Avila et al. 2000). The extent of intestinal TAG hydrolysis is reduced as the dietary level of fat is increased or when factors such as low ruminal fluid pH and ionophores inhibit the activity and growth of bacteria. For example, in dairy cows, true fat digestibility in the small intestine decreases progressively from 95% to 78% when dietary fat intake increases from 0.2 kg/day (1% fat on a DM basis) to 1.4 kg/day (8% fat on the DM basis) (Bauchart 1993; Drackley 2000). This is due to a relatively low activity of ruminal lipase. There is evidence that when dietary fat content exceeds 7% (on a DM basis), the number and activity of ruminal bacteria are reduced substantially to impair the hydrolysis of dietary fats, proteins, and fibers, as well as amino acid and SCFA synthesis, in the rumen (Nafikov and Beitz 2007). Thus, although fat supplementation has become a common practice to increase the energy density of diets for high-producing dairy cows, a total fat content greater than 7% in diets (on a DM basis) is not recommended for ruminants, such as cattle, goats, and sheep (Drackley 2000).

Absorption of Lipids by the Small Intestine

Absorption of Lipids into Enterocytes

The absorption of fatty acids, phospholipids, and cholesterol by the small intestine (primarily the jejunum) of ruminants is the same as that in nonruminants, except that lysophosphatidylcholine replaces MAGs (which is not an end product of lipid hydrolysis in the rumen) as an emulsifying agent (Doreau and Chilliard 1997). In the upper small intestine of ruminants, lysophosphatidylcholine is produced from lecithin (the main phospholipid in ruminal microbes, pancreatic juice, and bile) by phospholipase secreted from the pancreas. Pancreatic juice and bile salts, which enter the duodenum through the common bile duct, are essential for lipid absorption. Bile salts are also essential for dissociating fatty acids from feed particles. In ruminants, there are more taurine- than glycine-conjugated bile salts, because the taurine-conjugates are more soluble at the low pH found in their small intestine. In ruminants fed conventional diets, 15–25% of total fatty acids in the digesta are absorbed in the upper jejunum, and 55–65% are absorbed in the middle and the lower jejunum (Bauchart 1993).

The fatty acids absorbed by the small intestine are transported to various tissues, including mammary glands, skeletal muscle, heart, and adipose tissue (Bergen and Mersmann 2005). Of note, some fatty acids with a *trans*-double bond between the 10th and 11th carbons, which are produced in relatively large amounts by the rumen of lactating cows that are fed an excessive amount of grain or a low-fiber diet, may inhibit milk fat synthesis and, therefore, may result in milk fat depression (Bauman and Griinari 2003).

Resynthesis of TAGs in Enterocytes

The synthesis of TAGs from free fatty acids and glycerol in the ER of ruminant enterocytes occurs via the glycerol-3-P pathway (see the section on TAG synthesis in this chapter). The 2-MAG pathway for TAG synthesis is absent because little 2-MAG enters the small intestine from the abomasum. Oxidation of fatty acids to CO_2 and water is limited in the small intestine, as compared to that of glutamate, glutamine, and aspartate (Wu et al. 2024).

Assembly and Release of Chylomicrons and VLDLs by Enterocytes

Apo B and MTP are essential for the assembly and secretion of chylomicrons and VLDLs in the ER of enterocytes in ruminants, as described previously for nonruminant mammals. The chylomicrons in ruminants are analogous to those in nonruminant mammals but have a lower proportion of TAGs (75% vs. 85–90%) and a higher proportion of phospholipids (20–25% vs. <10%) as compared to nonruminant mammals (Harrison and Leat 1975). This reflects a regularly lower content of TAGs in ruminant feedstuffs than in most nonruminant diets. In ruminants, as in nonruminant mammals, both chylomicrons and VLDLs are released by the enterocytes of the small intestine into lymphatic vessels, and the proportion of chylomicrons to VLDLs greatly depends on dietary lipid intake. For example, in sheep fed a regular low-lipid diet (containing <10 g TAGs/day), the ratio of VLDLs to chylomicron in the lymph is 73:27 (g/g; Harrison and Leat 1975). However, following a 24-h period of duodenal infusions of corn oil (containing 52.6% linoleic acid; 48 g TAGs/day) to sheep, lymph lipoproteins consist of 38.5% VLDLs and 61.5% chylomicrons (Harrison et al. 1974). In ruminants, increasing the dietary intake of fats or PUFAs stimulates the secretion of chylomicron from the small intestine but reduces the proportion of output of VLDLs among lipoproteins.

DIGESTION AND ABSORPTION OF LIPIDS IN FISH AND CRUSTACEANS

Digestion of Lipids by the Intestine in Fish

The diets of fish may contain 6–40% lipids (on a DM basis) as TAGs, phospholipids, waxes, and free fatty acids, all of which generally have a high percentage of unsaturated fatty acids. As in nonruminant mammals, the digestion of lipids in fish requires emulsifiers (proteins, phospholipids,

and bile salts) and pancreatic juices (including sodium bicarbonate, as well as pancreatic lipase, colipase, and cholesteryl ester hydrolase) in the proximal part of the digestive tract. Lipase is present in fish, but its activity varies markedly among different species (e.g., Atlantic salmon, rainbow trout, hybrid bass, and Zebrafish; Bakke et al. 2011; Tocher and Sargent 1984). For example, colipase-dependent pancreatic lipase (which has a high specificity and digestive efficiency for TAGs) and bile salt-dependent carboxyl ester lipase (which has a broad specificity for lipids including wax esters) are the main lipases in freshwater fishes and marine fishes, respectively. Fish synthesize phospholipases to efficiently hydrolyze phospholipids. In these aquatic animals, dietary content and types of lipids can affect the secretion of pancreatic lipase, colipase, and cholesteryl ester hydrolase from the pancreas to adapt to the different sources of foods. The digestibility of lipids in fish is 85–99%, depending on various factors (Bakke et al. 2011). These factors include species, breeds, developmental stage, feeding rate, water temperature, lipid source and type, and dietary composition of all other nutrients (particularly protein and phosphatidylcholine). For example, lipid digestibility decreases as the chain length of fatty acids in TAGs increases but is enhanced as the degree of unsaturation of fatty acids in TAGs increases. Also, given the important roles of many different proteins in the hydrolysis of TAGs and phospholipids, protein malnutrition impairs dietary lipid digestibility in fish, as in mammals.

Absorption of Lipids by the Intestine in Fish

In fish with pyloric ceca (the blind appendages attached to the proximal intestine), the primary site of lipid absorption is the ceca, rather than the proximal intestine (Sire et al. 1981). Specifically, the products of lipid digestion (including MAGs, free fatty acids, and glycerol) are mixed as micelles with bile salts, lysophosphatidylcholine, cholesterols, and fat-soluble vitamins. As the intestine contracts, the mixed micelles move through the luminal unstirred water layer into the surface of the intestinal enterocytes for absorption through specific transporters and diffusion. For example, intestinal-membrane SCFA exchangers have been recognized for herbivorous teleost (Titus and Ahearn 1991), whereas intestinal FABP has been identified in both zebrafish (Danio rerio) and common carp (Bakke et al. 2011). Water-soluble SCFAs and MCFAs exit the basolateral membrane of the enterocyte into the lamina propria and then enter the intestinal venule and the portal vein (Table 6.2). Within the enterocytes, the absorbed LCFAs are esterified with MAGs or glycerol to form TAGs, which are packaged primarily as chylomicrons and VLDLs (Sire et al. 1981; Tocher and Glencross 2015).

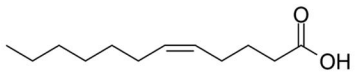
Although dietary lipids (including LCFAs) are efficiently absorbed ultimately into the blood of fish (Tocher and Glencross 2015), the processes of the trafficking of dietary fats and LCFAs from the enterocytes into the systemic circulation are poorly understood. The lymphatic system in fish is not as well-developed as in mammals (Steffensen and Lomholt 1992). Thus, there have been suggestions that the intestine-derived chylomicrons and VLDLs enter both the lymphatics and portal circulation (Rust 2002). In their studies with rainbow trout, Sire et al. (1981) found only a trace amount of gastrically administered ^{14}C-labeled linoleic and palmitic acids in the liver and blood within 6 h after administration despite the appearance of a large amount of the tracers at the intestine (44% and 56% of the administered dose, respectively, in the caeca). These authors concluded that postprandially, lipids are absorbed slowly into the lymph rather than the portal vein. In agreement with these results, Eliason et al. (2010) reported that the concentrations of total lipids and TAGs in the portal vein of rainbow trout fed a lipid-containing meal (1% of BW) did not differ at 0, 3, 6, and 12 h after feeding. Collectively, available evidence shows that the intestine-derived chylomicrons and VLDLs exit the basolateral membrane of the enterocytes into the intestinal lymphatics.

The factors that affect lipid digestion also influence lipid absorption in fish. For example, in these animals, SCFAs and MCFAs, which are soluble in water, are absorbed faster in the intestine than saturated LCFAs, which have a high degree of hydrophobicity and a low micellar solubility and which must pass through the luminal unstirred water layer to reach the enterocytes (Bakke et al. 2011). In addition, free (nonesterified) fatty acids and phosphatidylcholine are absorbed at higher rates than TAGs because the former have a smaller size and a lower hydrophobicity than the latter. Furthermore, as in mammals, high levels of dietary neutral TAGs lead to their accumulation in the enterocytes of fish, therefore reducing their export across the basolateral membrane of the enterocytes and, consequently, the absorption of lipids into the cells across their apical membrane. This may explain why the bioavailability of dietary lipids to the extraintestinal tissues of fish decreases as the dietary content of TAGs increases.

Digestion and Absorption of Lipids in Crustaceans

Mechanisms for the digestion and absorption of dietary lipids in crustaceans are similar to those in nonruminant mammals except for the following (Vogt 2021). First, in crustaceans, the digestion of dietary lipids occurs in the pyloric region of the stomach, where lipases (released as inactive enzymes primarily by the F-cells of the hepatopancreas) are activated via limited proteolysis and stored as the major enzymes hydrolyzing TAGs and other lipids. Second, in crustaceans, lipid emulsifiers are fatty acyl-dipeptides and fatty acyl-taurine rather than bile salts (that are not synthesized). Interestingly, the main conjugating fatty acid is *cis*-5-dodecenoic acid. Third, in crustaceans, the products of gastric lipid digestion move to the hepatopancreas for absorption into its R-cells, where they are resembled as phospholipid-rich HDLs for export into the hemolymph. Fourth, in crustaceans, the midgut tube and hindgut play a minor role in food processing and

lipid absorption. Interestingly, lipids constitute about 30% of DM in the hepatopancreas to support metabolic needs during critical events such as molting, starvation, reproduction, and limb regeneration.



cis-5-Dodecenoic acid ($C_{12}H_{22}O_2$; MW = 198.30)

LIPOPROTEIN TRANSPORT AND METABOLISM IN ANIMALS

The chemical composition and secretion of intestinal lipoproteins affect dietary lipid utilization by animals and, therefore, their growth and production performance, as well as the quality of their meats, eggs, and milk. Efficient use of dietary lipids for lean tissue gains and the formation of high-quality products depends on the knowledge of lipoprotein transport and metabolism among key organs, such as the small intestine, liver, skeletal muscle, and WAT. These processes are qualitatively similar among various animal species, although quantitative differences are noted.

Release of Lipoproteins from the Small Intestine and Liver

Overview

TAGs are the predominant lipids in chylomicrons and VLDLs, whereas cholesterol and phospholipids are the main lipids in low-density lipoproteins (LDLs) and HDLs, respectively (Puri 2011). LDLs contain about 10–15% TAGs. Within the blood stream, these lipoproteins are so large that they cannot pass through the endothelial cells of the capillaries without prior extracellular hydrolysis by LPL. There is tremendous interest in lipoprotein metabolism in animals because its abnormality causes fatty livers and chronic diseases (e.g., diabetes, hypercholesterolemia, hypertriacylglycerolemia, and atherosclerosis) and contributes to obesity in animals. The pathways of lipid transport or lipoprotein metabolism are similar between ruminants and nonruminant mammals, but there is a difference in the entry of the small intestine (diet)-derived lipoproteins (including chylomicrons and VLDLs) into the intestinal mucosa between mammals and birds, as noted previously. Under normal feeding conditions, chylomicrons and VLDLs are the major lipid fractions entering lymphatic vessels in nonruminant mammals and ruminants, respectively. The release of chylomicrons and VLDLs from the mammalian small intestine (or portomicrons from the avian small intestine) and the release of VLDLs and mature HDLs from the liver of vertebrates (including birds) increase with the intestinal load of absorbed TAGs.

In mammals, through the lymphatic circulation, chylomicrons and VLDLs enter the systemic blood circulation, and their remnants are taken up primarily by the liver through the Apo E receptor and the LDL receptor, respectively (Figure 6.10). The liver synthesizes and releases nascent VLDLs and mature HDLs. Of note, hepatic LPL is expressed during the perinatal period but is absent from adult animals (including humans; Table 6.3). In healthy animals, fatty acids and glycerol are produced from the hydrolysis of lipoprotein TAGs by LPL for utilization in a tissue-dependent manner. For example, fatty acids hydrolyzed from TAGs in the WAT may enter the blood stream or be taken up by adipocytes for re-esterification into TAGs in lipid droplets, whereas glycerol is not used by adipocytes but is taken up by other tissues (including the liver and skeletal muscle) for gluconeogenesis, oxidation, or incorporation of LCFAs into TAGs. The liver is the final organ for the catabolism of lipoprotein cholesterol to form bile acids, whereas the kidneys play a major role in the degradation of the protein components of the lipoproteins by peripheral tissues.

Metabolism of Chylomicrons, VLDLs, and LDLs

Synthesis and Release of Chylomicrons and VLDLs by the Small Intestine and of VLDLs by the Liver

After a meal containing lipids, chylomicrons (containing Apo B48) and nascent VLDLs (containing Apo B100 and Apo A4) are synthesized from fatty acids, glycerol, 2-MAGs, cholesteryl esters, free cholesterol, phospholipids, and many different proteins (including apoproteins) in the ER of enterocytes (Tso et al. 2001). Thereafter, the chylomicrons and VLDLs are transported to the Golgi apparatus for final processing and assembly, such as the addition and glycosylation of many proteins (including apoproteins). The Golgi vesicles containing chylomicrons and VLDLs migrate toward the basolateral membrane of enterocytes, and these two lipoproteins are discharged into the intercellular space of the lamina propria through exocytosis (reverse pinocytosis; Besnard et al. 1996). The composition of fatty acids in the TAGs of chylomicrons closely resembles that of the dietary lipids consumed by animals. Chylomicrons are the major lipoproteins secreted by the small intestine in the fed state, whereas VLDLs are the only lipoproteins released by the gut during fasting (Yen et al. 2015). The release of chylomicrons and VLDLs from the small intestine is enhanced by high fat feeding, and their ratios in the intestinal lymph are increased by the amount of PUFAs (e.g., linoleic acid) entering the duodenum. Apo A4 in chylomicrons may mediate lipid-induced satiety in animals through cell signaling in the hypothalamus of the brain (Tso and Liu 2004). Phosphatidylcholine is required for the assembly (occurring in the smooth ER) and excretion of chylomicrons and VLDLs in the small intestine and liver, respectively.

In fowl, the small intestine releases portomicrons (equivalent to mammalian chylomicrons) into the portal vein. These particles are likely too large to pass through the endothelium of the hepatic capillary bed of birds or to be ultimately taken up by their hepatocytes (Hermier 1997).

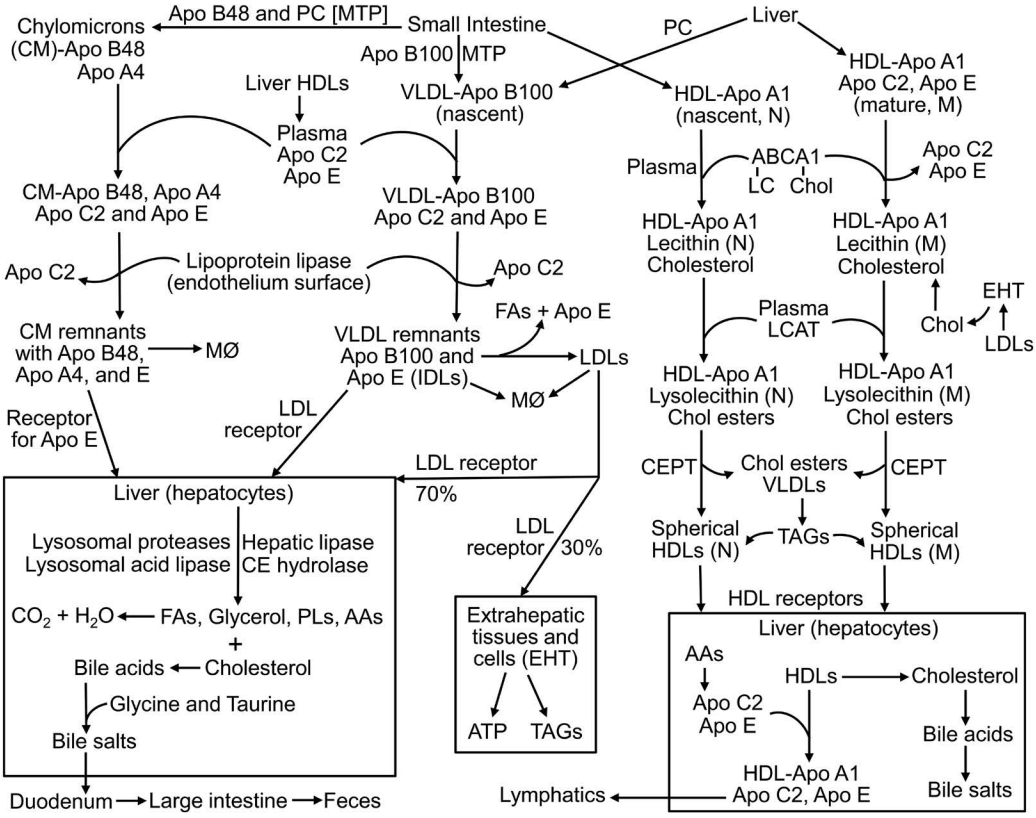


FIGURE 6.10 Interorgan metabolism of lipoproteins in animals. The small intestine releases chylomicrons, very low-density lipoproteins (VLDLs), and nascent high-density lipoproteins (HDLs), whereas the liver releases VLDLs and mature HDLs. During their transport through blood circulation, chylomicrons and VLDLs acquire apolipoprotein (Apo) C2 and E from HDLs and lose TAGs to become chylomicron remnants and VLDL remnants [intermediate-density lipoproteins (IDLs) and low-density lipoproteins (LDLs)]. Chylomicron remnants are taken up mainly by the liver through the Apo E receptor, whereas the VLDL remnants are taken up primarily by the liver and, to a less extent, peripheral tissues, through the LDL receptor. During their transport in the blood circulation, nascent HDLs (N) from the small intestine and mature HDLs (M) from the liver receive lecithin (LC) and free cholesterol (Chol) from a plasma lipid transporter [known as ATP binding cassette transporter A1 (ABCA1); released from the liver and intestine]. Thereafter, the plasma lecithin:cholesterol acyltransferase (LCAT), which is activated by Apo A1, converts lecithin and free cholesterol on the HDLs into lysolecithin and cholesteryl esters, respectively, yielding spherical HDLs. Through the action of the plasma CETP, one third of the LCAT-derived cholesteryl esters are transferred from the spherical HDLs to VLDLs to promote VLDL catabolism. The spherical HDLs are taken up by the liver. Free fatty acids (FAs) from lipoproteins are oxidized to CO_2 and water by the liver and peripheral tissues, whereas cholesterol is converted into bile acids in hepatocytes for ultimate excretion into the feces. Phosphatidylcholine (PC) is required for the assembly and secretion of VLDLs and chylomicrons in the ER of the liver and small intestine, respectively; these proteins are synthesized from amino acids (AAs). The VLDLs and HDLs move through the fenestrae between endothelial cells to enter the blood in the hepatic sinusoids. EHT, extrahepatic tissues; MØ, macrophages.

TABLE 6.3
Tissue- and Cell-Specific Distribution of Enzymes that Degrade Lipoproteins

Enzyme	Hepatocytes	WAT	Skeletal Muscle	Heart	Kidneys Muscle	Smooth	Macrophages
HSL	Negligible	High	High	High	Medium	Low	Low
LPL	Absent	High	High	High	Medium	Low	Low
LAL	High	Low	Low	Low	High	Low	Medium
HL	High	Absent	Absent	Absent	Absent	Absent	Low
Proteases	High	Low	Medium	Medium	High	Low	Medium
LCEH	High	Low	Low	Low	Medium	Low	Medium

Source: Fried et al. (1993), Jobgen et al. (2006), and Yeaman (1990).

Note: High, high activity; Low, low activity; Medium, medium activity; HL, hepatic lipase (extracellular); HSL, hormone sensitive lipase (intracellular); LAL, lysosomal acid lipase; LCEH, lysosomal cholesteryl ester hydrolase (cholesteryl esterase); LPL, lipoprotein lipase (extracellularly localized on the endothelium surface of the microvasculature within extrahepatic tissues to hydrolyze both lipoproteins and cholesteryl esters to produce fatty acids and glycerol); WAT, white adipose tissue.

Therefore, the intestine-derived lipoproteins are not catabolized by the avian liver (Hermier 1997). The portomicrons are absent from the plasma of fasted birds and present only at a very low concentration in the plasma of fed neonatal birds due to the relatively low amounts of fats in most poultry diets and the rapid catabolism of portomicrons in extrahepatic tissues.

In preruminants, most of the intestine-derived VLDLs are exported into the intestinal lymphatics, but some are taken into the portal vein, whereas the liver releases an appreciable amount of HDLs and a small amount of VLDLs (Laplaud et al. 1990). In lymph samples obtained 10 h after a meal (i.e., at peak lipid absorption), the molar ratio of chylomicrons to VLDLs is ~4:l. Of note, VLDLs represent only 5% of the total lipoproteins in the plasma of young calves and are absent from fetal calves (Bauchart 1993). In preruminants with limited ruminal fermentation and biohydrogenation, the compositions of fatty acids in chylomicrons and VLDLs are greatly influenced by dietary lipid intake.

In all animals, the liver does not produce or release chylomicrons. However, hepatocytes of the liver synthesize VLDLs from TAGs, cholesterol, phospholipids, Apo B100 and other proteins, and then release the VLDLs. The VLDLs released from the liver travel through the lymphatics to the blood circulation (Goldstein and Brown 2015). In the fed state, the liver is the major source of plasma VLDLs. Under physiological conditions, hepatic assembly and secretion of VLDLs, as well as hepatic release of free fatty acids, are suppressed by insulin. The underlying mechanisms are as follows: (a) insulin reduces the availability of free fatty acids in the plasma for hepatic uptake due to an increase in their use by the WAT for TAG synthesis and a decrease in whole-body lipolysis, and (b) insulin activates the (phosphatidylinositol 3)-kinase signaling, which inhibits the addition of TAGs to the precursor apoproteins by the ADP-ribosylation factor. This is consistent with the role of insulin acting as an anabolic hormone to enhance energy storage as TAGs in both the liver and the WAT. In contrast, in obesity and type II diabetes mellitus with insulin resistance, a major feature of metabolic syndrome is hepatic hypersecretion of VLDLs, leading to hypertriglyceridemia.

Metabolism of Chylomicrons and VLDLs during Their Transport in the Blood

After the intestine-derived chylomicrons, as well as the intestine- and liver-derived VLDLs, exit the lymphatics, they sequentially enter the microvasculature, venous blood circulation, right heart, lungs, left heart, aorta, and arterial blood vessels (Chapter 1). During their transport in the blood, chylomicrons and VLDLs acquire Apo C2 and Apo E from the mature HDLs in the plasma that are released by the liver (Figure 6.10). Thereafter, most of the TAGs on the chylomicrons and VLDLs are hydrolyzed extracellularly by the LPL localized on the luminal surface of the capillary endothelium within extrahepatic tissues (particularly skeletal muscle and WAT; Fried et al. 1993). The LPL reaction removes ~80% of TAGs from chylomicrons to form their remnants; subsequently, Apo C2 (the activator of the LPL) is released from the chylomicrons to terminate the hydrolysis of their TAGs by the endothelium surface-bound LPL. The LPL reaction also removes ~60% of TAGs from VLDLs, and this hydrolysis, along with the terminal release of Apo C2 from the VLDLs, yields intermediate-density lipoproteins (IDLs). The removal of ~60% TAGs from IDLs by the endothelium surface-bound LPL, along with the release of Apo E from the IDLs, generates LDLs (Goldstein and Brown 2015). Thus, the VLDL remnants are IDLs and LDLs. Of note, the hydrolysis of chylomicrons and VLDLs by the LPL also produces fatty acids, glycerol, phospholipids, and free cholesterol. Approximately 7.5%, 2.5% and 90% of Apo B100 in the plasma is found in VLDLs, IDLs and LDLs, respectively. LDLs are the major carrier of cholesterol in humans, pigs, rabbits, sheep, and guinea pigs. The chylomicron and VLDL remnants are ~50% smaller in diameter than their parent lipoproteins, and can pass, along with fatty acids, phospholipids, cholesterol and glycerol, through the endothelial cells of the capillaries into interstitial space for uptake by the liver and/or peripheral (nonhepatic) tissues.

Uptake and Metabolism of Circulating Chylomicron and VLDL Remnants in the Liver and Peripheral Tissues

VLDLs in the blood move through the fenestrae between endothelial cells to enter hepatocytes in hepatic sinusoids. The liver and, to a much lesser extent, macrophages take up, from the blood circulation, the chylomicron remnants (containing both Apo B48 and Apo E) through a receptor specific to Apo E (Figure 6.10). This organ and, to a lesser extent, certain extrahepatic tissues and cells (e.g., skeletal muscle, heart, adipose tissue, kidneys, and macrophages) also take up LDLs (containing Apo B100) through the LDL receptor for catabolism (Goldstein and Brown 2015). Furthermore, the liver takes up a small amount of IDLs (containing Apo B100) through the LDL receptor. The rate of internalization of chylomicron remnants, IDLs and LDLs through receptor-mediated endocytosis is determined by the amount and activity of their respective receptors, rather than by the concentrations of the lipoproteins (Goldstein and Brown 2015). Hepatic lipase, which is synthesized and released by hepatocytes, is crucial for extracellular lipoprotein metabolism in the liver. This is because hepatic lipase is strategically localized at the surface of liver sinusoid capillaries and the surface of hepatocytes to hydrolyze extracellular TAGs, cholesteryl esters, and phospholipids in the chylomicron remnants, IDLs, and LDLs that enter the liver.

In both the liver and extrahepatic tissues, lysosomal acid lipase (LAL) can hydrolyze intracellular TAGs and cholesteryl esters in the endocytosed lipoproteins, and lysosomal cholesteryl ester hydrolase (cholesteryl esterase) can degrade intracellular cholesteryl esters (Table 6.3). HSL hydrolyzes intracellular TAGs in extrahepatic tissues (particularly skeletal muscle and adipose tissue; Yeaman 1990). In the WAT of mammals (e.g., humans, pigs, and rats) and birds (e.g., chickens), the first step in lipolysis is also catalyzed by ATGL (Cerk et al. 2018; Deiuliis et al. 2008; Lee et al. 2009). The products of the catabolism of chylomicron

remnants, IDLs, and LDLs are fatty acids, glycerol, phospholipids, free cholesterol, and amino acids. These metabolites have different metabolic fates depending on the type of tissue. For example, fatty acids are oxidized to CO_2 and H_2O in hepatocytes and skeletal muscle. In contrast, free cholesterol is converted into bile acids in hepatocytes but is transferred to HDL particles in extrahepatic tissues.

The physiological significance of the LDL receptor for LDL metabolism is epitomized by the finding that a genetic defect in the receptor seen in familial hypercholesterolemia greatly contributes to dyslipidemia in affected subjects (Goldstein and Brown 2015). The liver and kidneys are the major extrahepatic sites to degrade lipoproteins in chylomicron remnants and LDLs, respectively. Approximately 70% and 30% of LDLs are degraded in the liver and extrahepatic tissues, respectively (Puri 2011). Thus, the liver plays a central role in the catabolism of chylomicrons, VLDLs and LDLs.

Uptake and Metabolism of LCFAs by the Liver and Peripheral (Nonhepatic) Tissues

The LCFAs released from the partial degradation of chylomicrons and VLDLs in the plasma by the endothelium surface-bound LPL are taken up into the liver, as well as peripheral (nonhepatic) tissues and cells, such as skeletal muscle, heart, WAT, kidneys, and macrophages through simple diffusion and membrane-bound transporters (Figure 6.3). Deletion of a major LCFA transporter, CD36, greatly impairs the transport of LCFAs by these tissues and cells, as well as the clearance of lipoproteins from the blood (Goldberg et al. 2009). In skeletal muscle, the abundance of the CD36 protein is chronically upregulated by insulin, muscle contraction, and PPAR-δ to increase the uptake of LCFAs (Nahle et al. 2008). Fatty acids are the major metabolic fuels in the liver, skeletal muscle, and heart; contribute substantially to ATP production in the kidneys; and are components of all cell membranes. PUFAs are also signaling molecules in cells. All fatty acids can be used for the synthesis of TAGs in tissues, particularly the liver and WAT in a species-dependent manner (Jobgen et al. 2006), and participate in cell metabolism including the assembly of membrane lipid bilayers.

Turnover and Functions of Chylomicrons and VLDLs

Chylomicrons released from the small intestine, as well as nascent VLDLs released from the small intestine and liver, are rapidly metabolized in animals. For example, the labeled fatty acids in the TAGs of chylomicrons administered intravenously have a half-life of 8–9 min in small animals (e.g., rats) and <60 min in large animals (e.g., 14 min in healthy humans and 51 min in hypertriglyceridemic subjects; Cortner et al. 1987). About 80% of the fatty acid label is found in the adipose tissue, heart, and skeletal muscle, whereas 20% of the label is in the liver. In contrast, the half-life of Apo B100 in IDLs or LDLs (~2 days) is much longer than that of chylomicrons or VLDLs. Chylomicrons and VLDLs mainly transport TAGs to the liver and peripheral (nonhepatic) tissues to either be used for energy or stored. In contrast, LDLs primarily deliver cholesterol to various tissues (e.g., liver and adrenal cortex) for the synthesis of bile acids and steroid hormones in a cell-specific manner.

Regulation of LPL Activity

Different isoforms of LPL are synthesized and secreted onto the endothelial surface of the capillaries by the parenchymal (the main part) cells of different tissues (e.g., adipocytes in the WAT, myofibers in skeletal muscle, and cardiomyocytes in heart; Lee et al. 2014; Macfarlane et al. 2008). LPL is activated by Apo C2 (a component of HDLs) but inhibited by Apo C3 (a component of VLDLs) and Angptl4 (protein angiopoietin-like 4) in animals (Goldberg et al. 2009). Regulation of LPL expression and activity by hormones, dietary lipid intake, and other factors (e.g., cytokines and exercise) is tissue-specific (Table 6.4), depending on the nutritional state and physiological needs of animals. For example, LPL activity is enhanced in the WAT but reduced in skeletal muscle and heart in response to physiological levels of insulin, but the opposite is true during fasting. This reflects the different functions of these organs in TAG metabolism. Specifically, LPL in the WAT facilitates the extracellular hydrolysis of blood TAGs to LCFAs and glycerol for uptake by adipocytes, where LCFAs and glycerol are re-esterified into TAGs for storage (Kersten 2014). On the other hand, LPL in skeletal muscle and heart promotes the intravascular conversion of extracellular (blood) TAGs into LCFAs for use as major metabolic fuels. In animals (e.g., pigs, chickens, rats, and humans), high-fat diets (e.g., 40% of energy from fat) decrease LPL activity in the WAT to modulate the deposition of TAGs in adipocytes, while increasing LPL activities in skeletal muscle and heart to provide LCFAs for uptake (Chilliard 1993; Delorme and Harris 1975; Takahashi and Ide 1999). Interestingly, very high fat intake (e.g., 80% of energy from fat) increases LPL activity in the WAT of rats to facilitate the accretion of TAGs in adipocytes (Deshaies et al. 1988). Overall, insulin stimulates the deposition of TAGs in the WAT in the fed state, whereas TAGs in the blood are utilized by skeletal muscle for ATP production to spare glucose during fasting. Thus, LPL controls the partitioning of dietary and endogenous TAGs into storage or oxidation in animals, thereby playing an important role in whole-body fat homeostasis.

In mammary glands, LPL expression is enhanced during late pregnancy and lactation through the effects of prolactin to provide fatty acids for milk TAG synthesis (Rezaei et al. 2016). This enzyme converts blood-borne TAGs into LCFAs and glycerol for uptake by mammary epithelial cells. Although LPL is not directly activated by a cAMP-dependent kinase, the expression and abundance of this enzyme are increased in the brown adipose tissue (BAT), WAT, heart, skeletal muscle, and adrenal glands during cold exposure (Goldberg et al. 2009). Likely mediators for the enzyme induction are elevated levels of thyroid hormones and catecholamines in the plasma to promote the transcription of target genes. This is physiologically important to provide fatty acids for nonshivering heat production by the various tissues.

TABLE 6.4
Effects of Hormones, Nutritional Status, and Other Factors on Expression and Activities of Lipoprotein Lipase and Hormone-Sensitive Lipase (HSL) in White Adipose Tissue (WAT) and Skeletal Muscle (SKM)

Hormones, Nutritional Status, and Other Factors	Lipoprotein Lipase			HSL		
	WAT	SKM	Heart	WAT	SKM	Heart
Hormones						
Catecholamines	↓	↑	↑	↑	↑	↑
Glucagon	↓	↑	↑	↑	↑	↑
Glucagon-like peptide-1	↓	↑	↑	↑	↑	↑
Glucocorticoids						
Short-term	↑	↑	↑	↑	↑	↑
Long-term	↑	↑	↑	↓	↓	↓
Growth hormone	↓	↑	↑	↑	↑	↑
Insulin	↑	↓	↓	↓	↓	↓
Parathyroid hormones	↓	↑	↑	↑	↑	↑
Thyroid hormones	↓	↑	↑	↑	↑	↑
Nutritional status						
Fasting (vs. fed state)	↓	↑	↑	↑	↑	↑
Feeding (vs. fasting)	↑	↓	↓	↓	↓	↓
High-fat diet (vs. low-fat diet)	↓[a] or ↑[b]	↑	↑	↓	↓	↓
High-starch diet (vs. low-starch diet)	↑	↓	↓	↓	↓	↓
High-protein diet (vs. normal-protein diet)	NC	↑	↑	↑	↑	↑
ω3-Polyunsaturated fatty acids	↓	↑	↑	↑	↑	↑
Other factors						
Insulin-like growth factor I	↓	NC	NC	NC	NC	NC
AMP-activated protein kinase	↓	↑	↑	↑	↑	↑
Exercise	↓	↑	↑	↑	↑	↑
Interferon-α	↓	↓	↓	↑	↑	---
Interferon-γ	↓	↓	↓	↑	↑	↓
Interleukin-1	↓	↓	↓	↑	↑	↓
Lipopolysaccharide	↓	↓	↓	↑	↑	↓
Peroxisome proliferator-activated receptor-α	↑	↑	↑	↑	↑	---
Peroxisome proliferator-activated receptor-γ	↑	NC	↑	↓	NC	---
Peroxisome proliferator-activated receptor-δ/β	↓	↑	↑	↑	↑	---
Tumor necrosis factor-α	↓	↓	↓	NC	↑	↓

Source: Bijland et al. (2013), Donsmark et al. (2004), Goldberg et al. (2009), Huang et al. (2016), Lee et al. (2014), Macfarlane et al. (2008), Oscarsson et al. (1999), Picard et al. (2002), and Pulinilkunnil and Rodrigues (2006).

[a] Takahashi and Ide (1999). Fat provides approximately 40% of the dietary energy.

[b] Deshaies et al. (1988). Fat provides 80% of the dietary energy.

Note: ↓, decrease; ↑, increase; NC, no change; "---", data are not available.

In the liver, LPL is expressed during fetal and early postnatal life, but thereafter is suppressed by a novel transcription factor, termed RF-1-LPL that binds to an NF-1-like site in the region of the glucocorticoid response element (Semenkovich et al. 1989). The hepatic expression of LPL during the perinatal period is also induced by thyroid hormones and glucocorticoids. The loss of LPL in the liver of adult animals maximizes the delivery of both dietary and endogenous lipids (e.g., TAGs and cholesterol) to extrahepatic tissues. The replacement of LPL in the liver is hepatic lipase, as noted previously.

Regulation of Hepatic Lipase Activity

Hepatic lipase is expressed almost exclusively in hepatocytes, and its mRNA is not detected in other tissues, including the pancreas, WAT, lung, heart, skeletal muscle, mammary gland, adrenal gland, and ovary (Semenkovich et al. 1989). The expression of hepatic lipase is enhanced by growth hormone, androgens and glucocorticoids, but inhibited by estrogens, ω3 PUFAs, and cholesterol to control TAG uptake by hepatocytes and the concentrations of free fatty acids in the plasma (Perret et al. 2002). Sphingomyelin is also an inhibitor

of hepatic lipase activity, such that this enzyme is most active when sphingomyelin is depleted from lipoprotein substrates (Yang and Subbaiah 2015). This stimulation of hepatic lipase is greater with LDLs and VLDLs than with HDLs.

Metabolism of HDLs

Release of Nascent HDLs from the Small Intestine and of Mature HDLs from the Liver

HDL metabolism begins in both the small intestine and the liver (Figure 6.10). HDLs, which have different proportions of apolipoproteins, cholesterol and phospholipids than chylomicrons and VLDLs, take part in the metabolism of lipoprotein TAGs and cholesterol. Enterocytes of the small intestine synthesize and release, into the lymphatic circulation, a small amount of newly formed HDLs (called nascent HDLs), which contain Apo A1 but not Apo C2 or Apo E (Goldstein and Brown 2015). In contrast, hepatocytes of the liver synthesize and release a large amount of mature HDLs with Apo A1, Apo C2 and Apo E. The HDLs released from the liver travel through the lymphatics to the blood circulation. In mammals, birds, and fish, the liver is the predominant source of plasma HDLs. To exit the hepatocytes, HDLs move through the fenestrae between endothelial cells to enter the blood in hepatic sinusoids.

Metabolism of Intestine-Derived HDLs during Transport in the Blood

After entering the blood, the Apo A1 of the intestine-derived HDLs interacts extracellularly with a lipid transporter [known as ATP binding cassette transporter A1 (ABCA1)], which is released primarily from the liver (70%) and, to a lesser extent, from the small intestine (30%; Lee et al. 2012). This interaction promotes the transfer of phospholipids and free cholesterol from ABCA1 to Apo A1, generating discoidal nascent HDLs. Thus, ABCA1 mediates the assembly of free cholesterol and phospholipids with Apo A1. Thereafter, the plasma LCAT, which is activated by Apo A1, converts phosphatidylcholine and free cholesterol on the HDLs into lysophosphatidylcholine and cholesteryl esters, respectively, yielding spherical HDLs (Puri 2011). Through the action of the plasma cholesteryl ester transfer protein (CETP), one third of the LCAT-derived cholesteryl esters are transferred from the nascent HDLs to VLDLs to form spherical HDLs, thereby promoting VLDL catabolism. This reaction also results in the incorporation of TAGs into the HDL particles. The spherical HDLs, which are recognized by the liver, have a surface layer of polar lipids and apolipoproteins.

Metabolism of Liver-derived HDLs during Transport in the Blood

As noted previously, the liver releases mature HDLs containing Apo A1, C2, and E into the blood. These mature HDLs transfer Apo C2 and E to chylomicrons and VLDLs, yielding smaller HDL particles (Semenkovich et al. 1989). The metabolism of the liver-derived HDLs during transport in the blood resembles that of the intestine-derived nascent HDLs, except that the liver-derived HDLs collect more free cholesterol from peripheral (nonhepatic) tissues and cells than the intestine-derived nascent HDLs. Ultimately, spherical HDLs containing high proportions of lysophosphatidylcholine and cholesteryl esters are generated and return to the liver. In the plasma, these HDLs comprise the majority (90%) of all the HDLs, which are heterogeneous in size, shape, and protein/lipid content. The process of cholesterol transport from peripheral tissues and cells to the liver is known as "reverse cholesterol transport" (Krieger 1998).

Through their release of Apo C2 and E, liver-derived mature HDLs are required for the metabolism of chylomicrons and VLDLs. This is because Apo C2 is an activator of LPL in tissues, and the Apo E-containing chylomicron remnants are recognized by their Apo E-specific receptors in hepatocytes. Thus, mature HDLs released by the liver act as a repository for Apo C2 and Apo E. The liver is also the ultimate organ for the utilization of HDL cholesteryl esters via the synthesis of bile acids, which are ultimately secreted into the small intestine as bile salts.

Uptake and Metabolism of Plasma Spherical HDLs by the Liver

The plasma spherical HDLs, which are generated from the intestine- and liver-derived HDLs, are taken up by the liver through HDL receptors (e.g., scavenger receptor class B type I). Once inside the hepatocytes, the spherical HDLs have two metabolic fates: (a) either binding to Apo C2 and Apo E to form mature, fully active HDLs for release from the liver, or (b) being hydrolyzed by hepatic lipase, LAL, lysosomal cholesteryl ester hydrolases, and lysosomal proteases to produce fatty acids, glycerol, cholesterol, lysophosphatidylcholine, and amino acids.

Turnover and Functions of HDLs

Proteomic studies have identified the presence of more than 50 different proteins that are associated with the mature HDLs, some of which are proteins that are implicated in the anti-inflammatory functions of HDLs (Iqbal and Hussain 2009). The average half-life of HDLs is 5 days (Puri 2011). Lipids in HDLs are also heterogeneous and include a wide variety of bioactive lipids, including phosphosphingolipids (e.g., sphingosine-1-P), oxysterols, and lysolipids. HDLs serve as: (a) a reservoir of apoproteins that can be transferred to other lipoproteins; (b) acceptors of unesterified cholesterol (because the high content of phospholipids in HDLs can solubilize cholesterol); (c) the site for esterification of cholesterol (through the action of LCAT); and (d) reverse transport of cholesterol from peripheral tissues and cells to the liver for the final disposal of cholesterol as bile acids. Thus, because of their antioxidative and cholesterol efflux roles, HDLs inhibit the development of atherosclerosis, which is the primary underlying cause of cardiovascular disease (Goldstein and Brown 2015).

Important Role for HDLs in Cholesterol Metabolism

Cholesterol metabolism requires esterification reactions, which occur in both cells and the plasma (Puri 2011). In nearly all animal cells, ACAT promotes the formation of cholesteryl esters (e.g., cholesteryl oleate) from cholesterol and fatty acids in the smooth ER. This enzyme is activated by free cholesterol and plays a key role in hepatic bile acid biosynthesis.

Thus, ACAT regulates the intracellular homeostasis of free cholesterol and its release from cells. In the blood, cholesterol is esterified by LCAT, which is released from the liver. The cholesteryl esters are substrates for CETP, which is attached to HDLs in the plasma. CETP accepts TAGs from VLDLs or LDLs and exchanges TAGs for cholesteryl esters from HDLs, and vice versa. In other words, CETP facilitates the transfer of cholesteryl esters and TAGs between lipoproteins in the blood. Therefore, HDLs play an important role in the reverse transport of cholesterol from peripheral tissues to the liver.

Species Differences in Lipoprotein Metabolism

There are species differences in plasma concentrations of lipoproteins. For example, pigs and humans have high levels of LDLs in the plasma, and LDLs are the major lipoproteins in the plasma (Puri 2011). In horses and ruminants, HDLs are the major lipoproteins and there is considerable overlap in the density range of LDLs and HDLs (Drackley 2000). Thus, it is more difficult to separate LDLs and HDLs using conventional ultracentrifugation methods. In animals with familial hypercholesterolemia, IDLs cannot be taken up into the liver and are converted into LDLs. Therefore, plasma levels of LDLs are high in these patients. A defect in LPL may account for high plasma levels of TAGs in some patients.

FATTY ACID SYNTHESIS IN TISSUES

Synthesis of Saturated Fatty Acids from Acetyl-CoA

Acetyl-CoA is the principal building block of fatty acids in the cytosol of nearly all animal cells. In the liver, this metabolic pathway occurs predominantly in perivenous hepatocytes (Cunningham and Porat-Shliom 2021). If acetyl-CoA is formed from the oxidation of pyruvate, amino acids, or fatty acids in the mitochondria, acetyl-CoA is transported into the cytosol via the formation of citrate from oxaloacetate (OAA). The use of mitochondria-generated acetyl-CoA for fatty acid synthesis is a complex biochemical process. First, acetyl-CoA is converted into citrate in the mitochondria, which enters the cytosol through the citrate transporter (Figure 6.11). In the cytosol, citrate is cleaved to form OAA and acetyl-CoA by ATP-citrate lyase (also known as ATP citrate synthase) in the presence of ATP and CoA. If a short-chain fatty acyl-CoA is formed from SCFAs in the cytosol, the acyl-CoA is directly used for local fatty acid synthesis. Of note, it was previously thought that the low activity of ATP-citrate lyase limited the use of glucose for fatty acid biosynthesis in ruminant adipose tissue. However, it has been established that, like many other tissues, the uptake and utilization of glucose for fatty acid synthesis is limited by the activity of 6-phosphofructokinase (Smith and Prior 1981). The rates of synthesis of LCFAs generally increase with postnatal age until adulthood in the livers and WAT of animals and with post-partum days in the mammary glands of lactating mammals (Pearce 1983).

Formation of Malonyl-CoA from Acetyl-CoA by Acetyl-CoA Carboxylase (ACC)

The conversion of acetyl-CoA into malonyl-CoA by acetyl-CoA carboxylase (ACC) is the initial and rate-controlling step in fatty acid synthesis from acetyl-CoA (Figure 6.12). This multi-subunit enzyme is localized in the ER and requires the reduced form of nicotinamide adenine dinucleotide

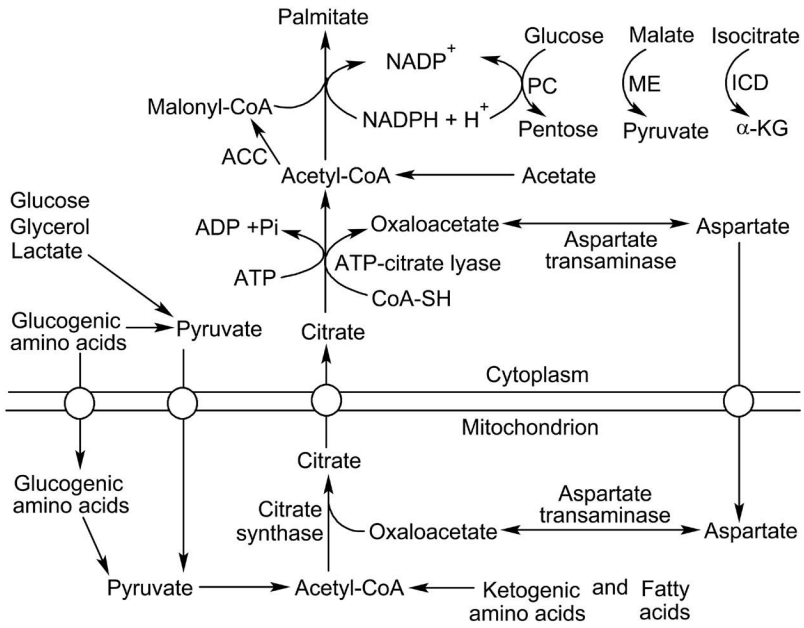


FIGURE 6.11 Translocation of acetyl-CoA from mitochondria into the cytosol. Acetyl-CoA and OAA are converted into citrate by citrate synthase in mitochondria. The citrate leaves the mitochondria through the citrate transporter into the cytosol, where citrate is cleaved by ATP-citrate lyase into acetyl-CoA and OAA. ACC, acetyl-CoA carboxylase; ICD, isocitrate dehydrogenase; ME, malic enzyme; PC, pentose cycle.

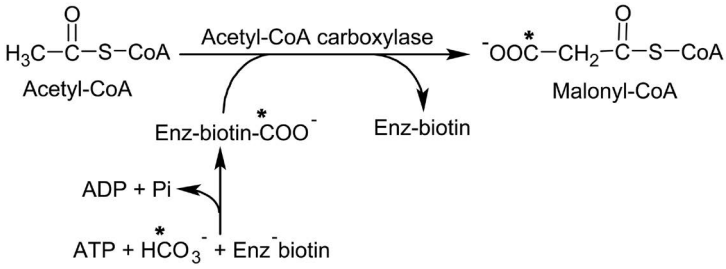


FIGURE 6.12 The conversion of acetyl-CoA into malonyl-CoA by acetyl-CoA carboxylase in the cytosol. This is the initial and rate-controlling step in fatty acid synthesis from acetyl-CoA.

phosphate (NADPH), ATP, Mn^{2+}, biotin, and HCO_3^- as cofactors. The ACC-catalyzed reaction involves two steps: the carboxylation of biotin and the transfer of the carboxyl biotin to acetyl-CoA to form malonyl-CoA. Thus, ACC has two catalytic activities (both biotin carboxylase and carboxyltransferase) and, therefore, is a bifunctional enzyme.

Animals have two isoforms of ACC (ACC1 and ACC2), which differ in tissue distribution and function. ACC1 (a cytosolic enzyme) is expressed primarily in lipogenic tissues (e.g., liver, WAT, and lactating mammary glands), where the de novo synthesis of fatty acids is nutritionally and physiologically important. In contrast, ACC2 (located in the outer membrane of the mitochondria) is present mainly in oxidative tissues (e.g., skeletal muscle, heart, and liver), where the β-oxidation of fatty acids provides the bulk of ATP for metabolic utilization. Thus, both ACC1 and ACC2 are highly expressed in the liver, where both the synthesis and oxidation of fatty acids are active in a species-specific manner and are influenced by nutritional and physiological states.

Formation of C_4 Fatty Acid Chain from Acetyl-CoA and Malonyl-CoA by Fatty Acid Synthase

In mammals, birds, fish and yeast, fatty acid synthase (FAS) is a multienzyme complex (Figure 6.13). The FAS

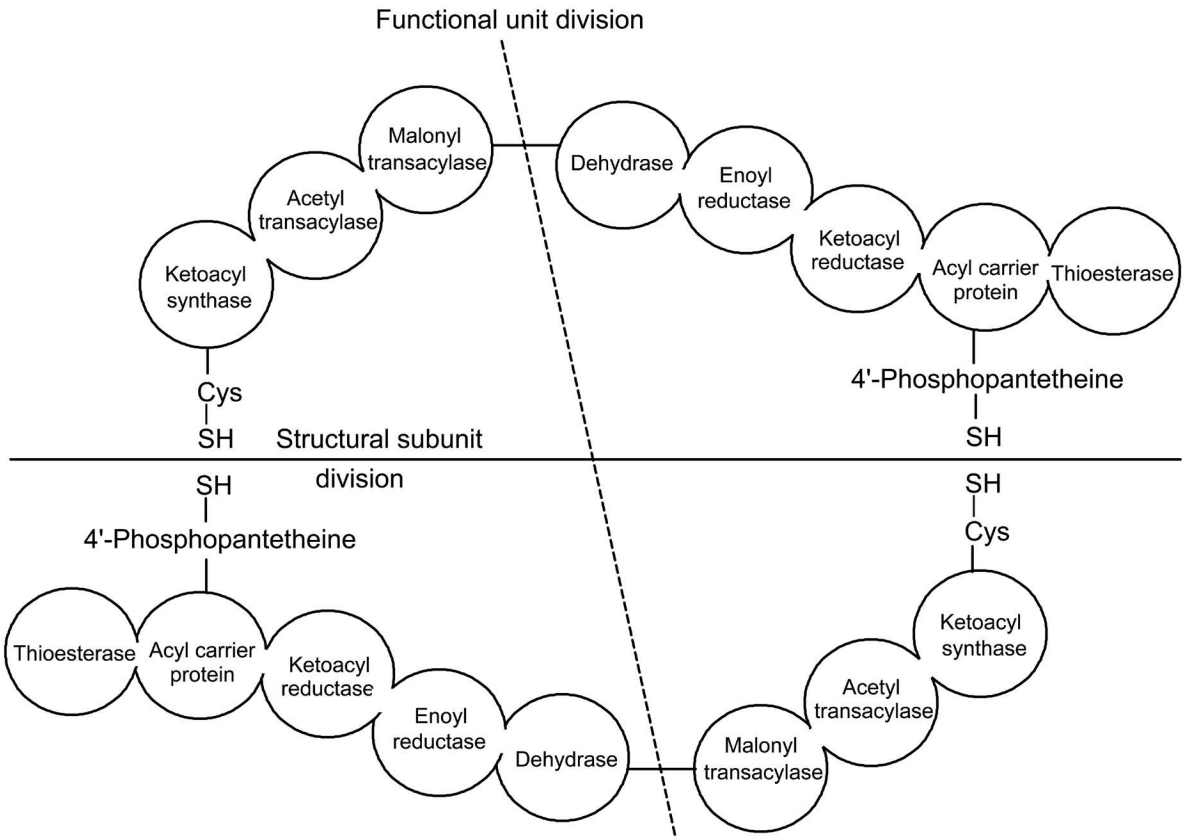


FIGURE 6.13 The fatty acid synthase in mammals, birds, fish, and yeast. This enzyme is a dimer, consisting of two identical monomers in a "head-tail" arrangement. Each monomer (polypeptide) contains 7 different enzymatic activities (ketoacyl synthase, acetyl transacylase, malonyl transacylase, dehydrase, enoyl reductase, ketoacyl reductase, and thioesterase) and the acyl carrier protein. The functional unit consists of one-half of a monomer interacting with the complementary half of the other. (Adapted from Mayes 1996a.)

complex is a dimer, consisting of two identical monomers in a "head-tail" arrangement. Each monomer (polypeptide) contains 7 different enzyme activities (ketoacyl synthase, acetyl transacylase, malonyl transacylase, hydratase, enoyl reductase, ketoacyl reductase, and thioesterase) and the acyl carrier protein (ACP). The functional unit consists of one-half of a monomer interacting with the complementary half of the other. In bacteria and plants, the individual enzymes of the FAS system are separate proteins. The FAS in eukaryotes is an efficient metabolon to synthesize FA from acetyl-CoA.

The formation of the C_4 fatty acid chain from acetyl-CoA and malonyl-CoA is illustrated in Figure 6.14. This process involves decarboxylation, reduction by NADPH, dehydration, and reduction by NADPH. The two units of the FAS complex are represented by 1 and 2 in Figure 6.13. The major source of NADPH is the pentose cycle (pentose phosphate pathway), which occurs in the cytosol (Bauman 1976). Other sources of NADPH, which are quantitatively minor ones, include the malic enzyme (NADP-linked malate dehydrogenase) and isocitrate dehydrogenase in the cytosol (Figure 6.11). In ruminant adipose tissue, there is low malic enzyme activity (Smith and Prior 1981). However, malic enzyme activity in bovine (but not ovine) WAT exceeds ACC and FAS activity (Smith and Prior 1981). This indicates that there is sufficient malic enzyme activity to contribute significant amounts of NADPH to support fatty acid synthesis in bovine adipose tissue.

Addition of Malonyl-CoA to the C_4 Fatty Acid to form C_{16} Fatty Acids

A new malonyl-CoA molecule binds with the -SH group of the 4'-phosphopantetheine (unit 2) of the FAS, displacing the saturated acyl residue onto the free cysteine -SH group (unit 1) of the FAS complex (Figure 6.14). In tissues that synthesize palmitate, the sequence of the reactions is repeated 6 more times to form a palmityl-enzyme complex. Palmitate is liberated from the palmityl-enzyme complex by a seventh enzyme of the FAS, thioesterase. The overall reaction of synthesis of palmitate from acetyl-CoA and malonyl-CoA is shown as follows:

$$\text{Acetyl-CoA} + 7\ \text{Malonyl-CoA} + 14\ \text{NADPH} + 14\ \text{H}^+ \rightarrow \text{Palmitate} + 7\ \text{CO}_2 + 6\ \text{H}_2\text{O} + 8\ \text{CoA} + 14\ \text{NADP}^+$$

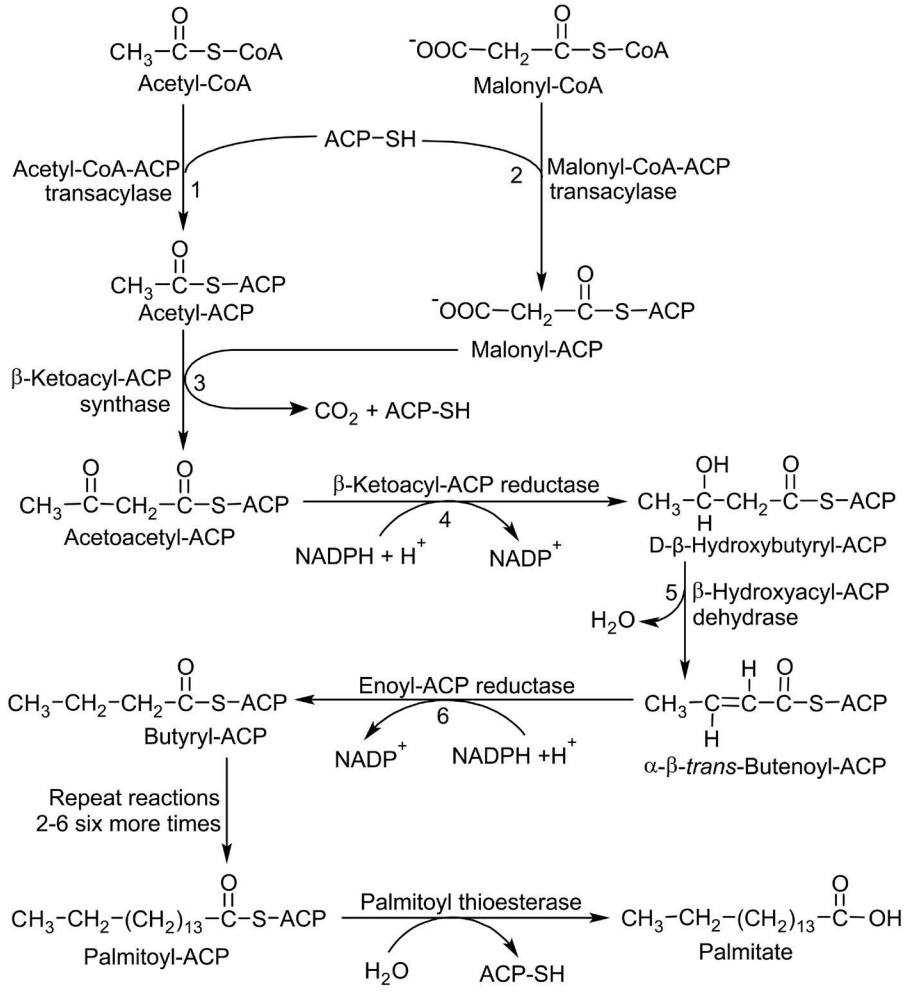
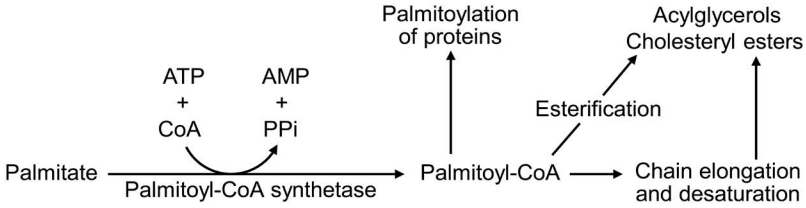


FIGURE 6.14 The synthesis pathway of long-chain fatty acid (e.g., palmitate) from acetyl-CoA in animal cells. These reactions take place in the smooth endoplasmic reticulum. ACP, acyl carrier protein.

Metabolic Fate of Palmitate

Under physiological and nutritional conditions that favor lipogenesis, free palmitate is activated to palmitoyl-CoA by palmitoyl-CoA synthetase in the cytosol. The resulting palmitoyl-CoA is esterified to TAGs and cholesteryl esters, and undergoes chain elongation and desaturation in the smooth ER. Palmitoyl-CoA may also participate in the palmitoylation of cysteine residues of proteins by protein palmitoyltransferases (transmembrane proteins) to regulate their biological activities. Elongation of the fatty acid chain beyond C_{16} occurs in the smooth ER, and this metabolic pathway requires the addition of acetyl-CoA as malonyl-CoA to the hydrocarbon chain, as well as NADPH (Figure 6.15).



Synthesis of Saturated Fatty Acids from Propionyl-CoA or Butyryl-CoA Plus Acetyl-CoA

In addition to acetyl-CoA, the primer for fatty acid synthesis can also be propionyl-CoA or butyryl-CoA. The addition of all the subsequent C_2 units is also via malonyl-CoA incorporation, and this malonyl-CoA is synthesized from acetyl-CoA. The pathway for the synthesis of saturated fatty acids from propionyl-CoA or butyryl-CoA plus acetyl-CoA is the same as described previously for the synthesis of fatty acids from acetyl-CoA. In ruminants, large amounts of propionate are formed in the rumen, which contributes to the formation of fatty acids with an odd carbon number.

Synthesis of Short-Chain Fatty Acids

SCFAs are produced primarily in the rumen of ruminants and in the intestine (mainly the large intestine) of nonruminants as a result of anaerobic bacterial fermentation of dietary fats, protein, and carbohydrates (Chapter 5). In the hindgut of animals, acetate, propionate, and butyrate account for 83% of SCFAs, and can amount to 100–150 mM. Acetate is an important energy substrate, and propionate is a major substrate for gluconeogenesis in ruminants. Butyrate is the preferred energy substrate for colonocytes and modulates their growth and differentiation. A slowly fermentable fiber, such as wheat bran, is more effective than a completely fermentable fiber, such as oat bran, in producing high levels of butyrate throughout the proximal and distal colon (Turner and Lupton 2011). The fact that different dietary substrates are preferentially fermented to different SCFAs may be important in the relationship between diet and intestinal health. Thus, through increased production of butyrate, enhanced gut motility, and dilution of fecal toxic substances, high intake of dietary fibers may protect against colon cancer in animals. Conversely, inadequate intake of dietary fibers may contribute to intestinal dysfunction and colon cancer.

Synthesis of Monounsaturated Fatty Acids in Animals

Synthesis of Δ^9 Monounsaturated Fatty Acids

All animals have acyl-CoA Δ^9 desaturase in the ER to convert a saturated acyl-CoA to a mono-saturated acyl-CoA. Subsequently, an acyl-CoA thioesterase hydrolyzes the acyl-CoA ester to the corresponding free acid and CoA. Acyl-CoA Δ^9 desaturase requires cytochrome b_5, NADH (P)-cytochrome b_5 reductase, and molecular oxygen, and introduces a double bond at the Δ^9 position into a saturated LCFAs (Ntambi 1999). Therefore, mammals, birds, and fish can synthesize the ω9 (oleic acid; $C_{18:1}$ ω9)

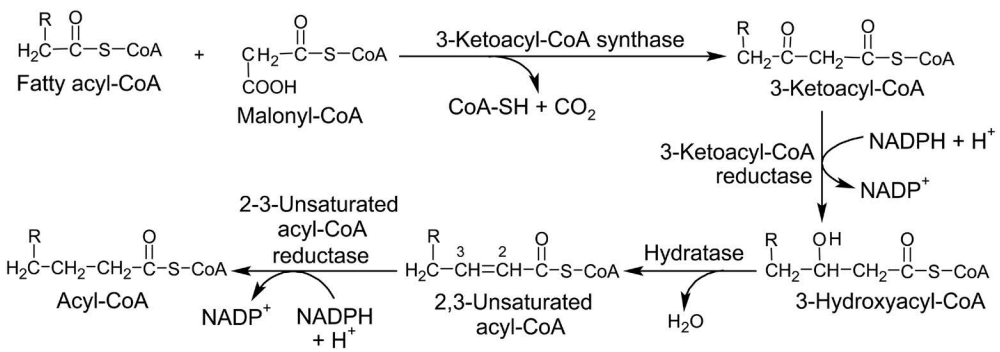


FIGURE 6.15 Elongation of the saturated fatty acid chain beyond C_{16} in the smooth endoplasmic reticulum. This metabolic pathway also requires the addition of (a) acetyl-CoA as malonyl-CoA to the hydrocarbon chain and (b) NADPH + H^+ as the reducing equivalent.

and ω7 (palmitoleic acid; $C_{16:1}$ ω7) families of unsaturated fatty acids from saturated LCFAs [e.g., stearic acid ($C_{18:0}$) and palmitic acid ($C_{16:0}$)], respectively, by a combination of chain elongation and desaturation. Thus, oleic acid and palmitoleic acid are not classified as nutritionally essential for animals, meaning that these fatty acids are not needed in diets.

Saturated fatty acyl-CoA + 2 Ferrocytochrome $b_5 + O_2 + 2\ H^+ \rightarrow$

Unsaturated fatty acyl-CoA + 2 Ferricytochrome $b_5 + 2\ H_2O$ (Acyl-CoA Δ^9desaturase)

Stearoyl-CoA + 2 Ferrocytochrome $b_5 + O_2 + 2\ H^+ \rightarrow$

Oleoyl-CoA + 2 Ferricytochrome $b_5 + 2\ H_2O$ (Stearoyl-CoA desaturase, SCD)

Acyl-CoA + $H_2O \rightarrow$ Free fatty acid + CoA (Acyl-CoA thioesterase)

A nutritionally important acyl-CoA Δ^9 desaturase is stearoyl-CoA desaturase (SCD), which was named for its ability to convert stearic acid into oleic acid (Koeberle et al. 2016). SCD also converts other saturated fatty acids into MUFAs. This enzyme is expressed in many tissues, but its expression varies greatly among animal species. For example, the SCD activity of the rodent liver is two orders of magnitude greater than that of the WAT. In cattle, sheep, and pigs, SCD activity is highest in the WAT, followed by skeletal muscle, intestinal mucosa, and liver in descending order (Kouba et al. 1997; Smith 2013). Indeed, SCD is nearly absent from the bovine liver (Smith 2013). In chickens, SCD activity is much higher in the liver than in the WAT. The tissue distribution of SCD provides a biochemical basis to explain differences in tissue oleic acid concentrations among different species. For example, stearic acid accounts for 12% and 25% of hepatic lipids, respectively, in mice and livestock species (cattle, sheep, and pigs). There is evidence that SCD is the only desaturase whose expression and activity in animal tissues can be substantially modified by diets (Smith 2013).

Introduction of Double Bonds between Δ^9 Carbon and Δ^1 Carbon

In most animals (e.g., cattle, goats, sheep, pigs, rats, dogs, chickens, and many fish species), additional double bonds in unsaturated LCFAs can be introduced between the Δ^9 carbon and the Δ^1 carbon (the carboxyl group), such that the unsaturated LCFAs have double bonds at the Δ^4, Δ^5, and Δ^6 carbon positions in addition to the double bond at the Δ^9 carbon position (Figure 6.16). The double bonds at the Δ^4, Δ^5, and Δ^6 carbon positions are formed by Δ^4, Δ^5, and Δ^6 desaturases, respectively. Mammals, birds, Atlantic salmon, and many other fish species (including freshwater and marine fish) possess separate genes for Δ^5 (*FADS1*) and Δ^6 (*FADS2*) desaturases (Nakamura and Nara 2004). In zebrafish, a bifunctional protein contains both Δ^5 and Δ^6 desaturase activities (Hastings et al. 2001). Δ^6 desaturase catalyzes the first and rate-limiting step for the desaturation and elongation of linoleic acid ($C_{18:2}$ ω6) and α-linolenic acid ($C_{18:3}$ ω3). Thus, ω3, ω6, and ω9 PUFAs are synthesized from their respective precursors by introducing one or more double bonds between the Δ^9 carbon and the Δ^1 carbon, as well as chain elongation, in animal cells (Figure 6.17). There is evidence that the synthesis of PUFAs from linoleic acid and arachidonic acid in the liver and brain of

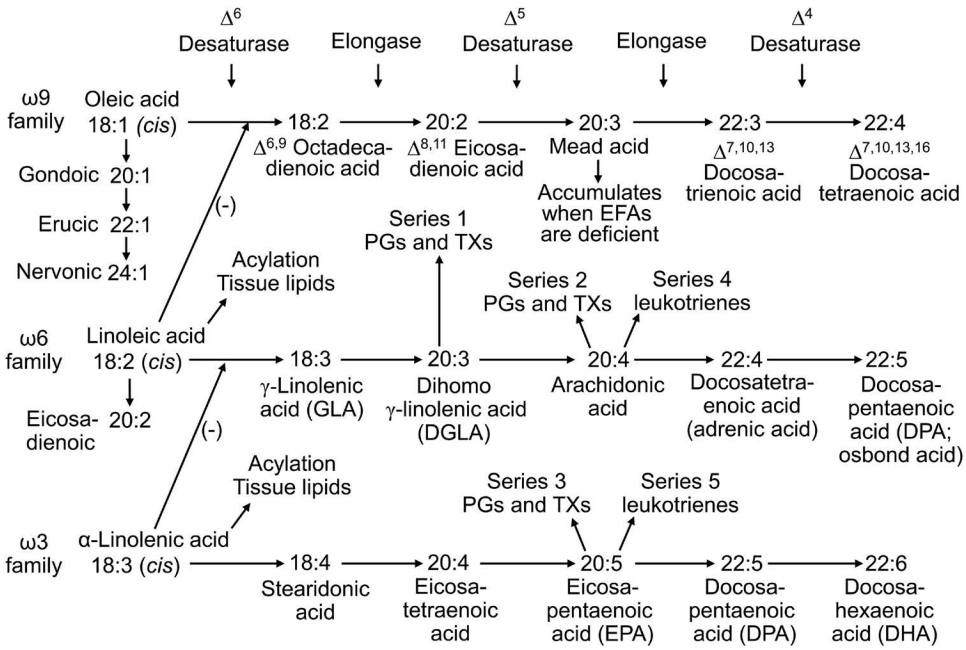


FIGURE 6.16 Formation of eicosanoids of series 1, 2, 3, and 4, as well as leukotrienes of series 4 and 5, from polyunsaturated fatty acids (PUFAs) in animal cells. Linoleic acid (an ω6 PUFA) is the precursor for the synthesis of series 1 and 2 prostaglandins (PGs) and thromboxanes (TXs), as well as series 4 leukotrienes. α-Linolenic acid (an ω3 PUFA) is metabolized to EPA, which is used for the formation of series 3 PGs and TXs, as well as series 5 leukotrienes. Mead acid is formed and accumulates in animals when nutritionally essential fatty acids (EFAs) are deficient.

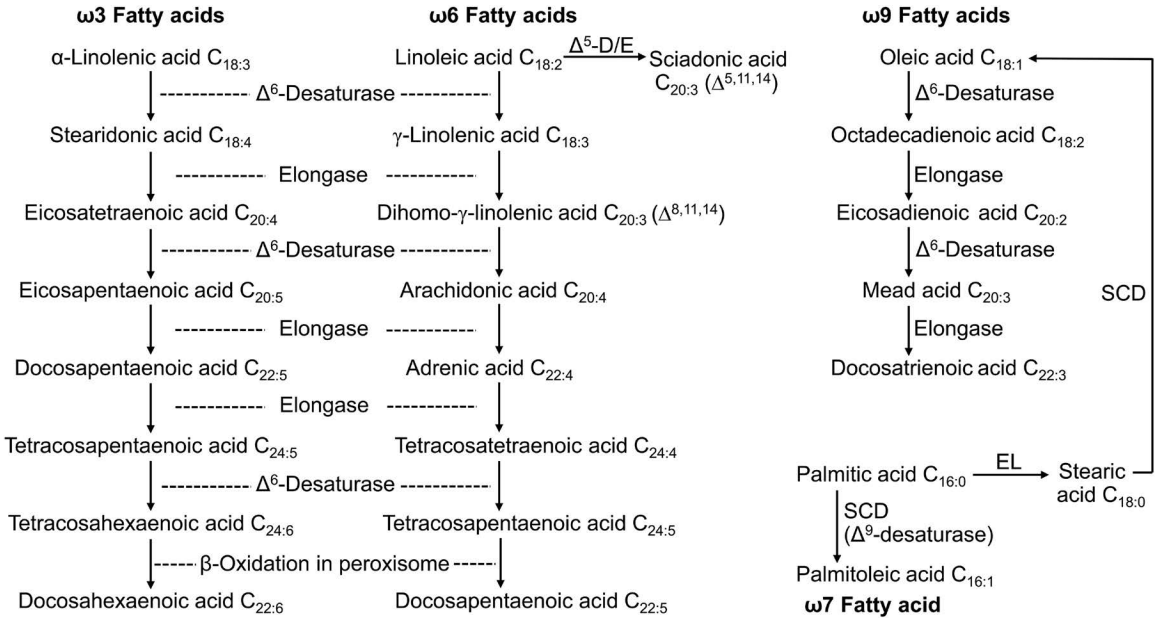


FIGURE 6.17 Elongation of the unsaturated fatty acid chain beyond C_{18}, as well as the formation of monosaturated fatty acids in the smooth endoplasmic reticulum. This metabolic pathway also requires the addition of acetyl-CoA as malonyl-CoA to the hydrocarbon chain, as well as NADPH. The shortening of a polyunsaturated fatty acid chain from C_{24} to C_{22} through β-oxidation in the peroxisome is also shown. In animals, ω7 and ω9 unsaturated fatty acids can be formed from palmitic acid (a saturated long-chain fatty acid) via SCD. All desaturases are localized in the smooth endoplasmic reticulum. Δ^5-D/E = Δ^5-desaturase and elongase; EL = elongase; SCD = stearoyl-CoA desaturase (Δ^9 desaturase).

young pigs is inhibited by dietary CLA in pigs, and this effect is more pronounced in the neonates fed a low fat (3% fat) diet than those fed a high fat (25% fat) diet (Lin et al. 2011).

In contrast to other mammals, the cat family of animals has Δ^5 desaturase activity but little or no Δ^6 desaturase activity in tissues (e.g., the liver), and therefore, cannot convert: (a) linoleic acid into arachidonic acid and ω6 docosapentaenoic acid or (b) α-linolenic acid into EPA and DHA (Sinclair et al. 1979). Because carnivores have very limited ability to synthesize long-chain PUFAs, these animals need a supply of preformed LCFAs (e.g., C_{20} and C_{22} PUFAs) in their diets (Rivers et al. 1975, 1976). Likewise, many insect species have no ability to synthesize linoleic acid, and no mosquito species analyzed to date can convert linoleic acid into arachidonic acid (Dadd and Kleinjan 1979). Interestingly, Hasan et al. (2019) recently reported that Spodoptera exigua larvae (a lepidopteran insect) could form arachidonic acid from linoleic acid in response to bacterial challenge.

A separate gene for Δ^4 desaturase has not been found for mammals or birds. However, in mammalian cells, Δ^6 desaturase has Δ^4 desaturase activity, which allows for a limited conversion of EPA into DHA (Park et al. 2015). Of interest, there is a gene for Δ^4 desaturase in a lower eukaryote, *Thraustochyrium* sp. (Qiu et al. 2001) and in the herbivorous, primitive marine teleost fish (*Siganus canaliculatus*) (Li et al. 2010). Thus, most animals (including dogs, humans, pigs, poultry, rodents, and ruminants) except cats are capable of synthesizing: (a) arachidonic acid ($C_{20:4}$ ω6) and n-6 docosapentaenoic acid ($C_{22:5}$ ω6) from linoleic acid ($C_{18:2}$ ω6); and (b) eicosapentaenoic acid (EPA, $C_{20:5}$ ω3), n-3 docosapentaenoic acid ($C_{22:5}$ ω3), and docosahexaenoic acid (DHA, $C_{22:6}$ ω3) from α-linolenic acid ($C_{18:3}$ ω3; Figure 6.16). Fish and crustaceans also have these synthetic pathways, but their activities are relatively low (Kumar et al. 2018; Rust 2002).

Failure of Animals to Introduce Double Bonds beyond Δ^9 Carbon

All vertebrates (including mammals, birds, and fish) lack enzymes to introduce a double bond in saturated or unsaturated LCFAs beyond the Δ^9 carbon position and, therefore, cannot synthesize linoleic acid or α-linolenic acid from acetyl-CoA or palmitate. These two fatty acids are classified as nutritionally essential for these animals. Of note, PUFAs (e.g., EPA and DHA) are abundant in the liver and adipose tissue of deep-sea fish because the marine animals consume algae that contain these fatty acids. In contrast to vertebrates, plants and bacteria can introduce bonds at the Δ^6, Δ^9, Δ^{12}, and Δ^{15} carbon positions and can therefore synthesize the nutritionally essential fatty acids. Because ruminal bacteria have enzymes for introducing double bonds between the Δ^9 and Δ^1 carbon positions and beyond the Δ^9 carbon position, ruminants that have a functional rumen and consume typical diets do not appear to require supplementation with ω3 (e.g., α-linolenic acid, EPA, or DHA) or ω6 (e.g., linoleic acid and arachidonic acid) PUFAs for normal metabolism.

Differences between Trans Unsaturated Fatty Acids and PUFAs in Animal Nutrition

Trans unsaturated fatty acids are uncommon in nature but are generated mainly as a side product when vegetable oils are hydrogenated in food processing for their conversion from liquids to solids through chemical reactions. Elaidic

acid ($C_{18:1}$ ω9, a *trans* isomer of oleic acid) is the principal *trans* unsaturated fatty acids often found in partially hydrogenated vegetable oils for use in margarine, snack food, packaged baked goods and fried food. Of note, the amount of elaidic acid is negligible (accounting for only ~0.1% of total fatty acids) in the milk and meats of cattle and other ruminants (Alonso et al. 1999). Vaccenic acid is the major MUFAs in ruminant milk and meats. *Trans* unsaturated fatty acids (chemically still unsaturated fatty acids) have a similar straight-chain conformation to saturated fatty acids and are metabolized more like saturated fatty acids than like the *cis* unsaturated fatty acids. Thus, *trans* unsaturated fatty acids can raise LDLs and lowers HDLs in humans (Abbey and Nestel 1994). *Trans* PUFAs do not possess the biological activity of nutritionally essential fatty acids, and can even antagonize the metabolism of nutritionally essential fatty acids and worsen their deficiencies in animals. Thus, *trans* unsaturated fatty acids are of concern in both animal nutrition and food safety.

The composition of dietary unsaturated fatty acids is of nutritional and physiological importance because it (a) affects fatty acid composition and fluidity, as well as the activities of receptors, enzymes, and transporters in cell membranes; and (b) provides ω6 and ω3 fatty acids, which are essential for normal cell function but cannot be synthesized by the body. For example, SCD activity is present in virtually all cell types and is essential for membrane fluidity. Also, arachidonic acid and DHA are deposited in large amounts in the nonmyelin membranes of the developing central nervous system. Inadequate intake of ω6 and ω3 fatty acids may impair neurological development, learning ability, memory, visual function, cardiac and skeletal structures, blood circulation, cell membrane integrity, nutrient absorption and transport, skin integrity, and immune response (Field et al. 2008; Gimenez et al. 2011). In addition, ω3 PUFAs enhance the oxidation of saturated and mono-unsaturated LCFAs to CO_2 and H_2O, thereby reducing the concentrations of fatty acids and TAGs in the plasma. Animal diets must contain ω3 PUFAs for optimal growth and health. A desirable ratio of ω3 to ω6 PUFAs in diets is uncertain, but may be as high as 2:1. Blending different sources of plant oils (e.g., soybean, canola, and flaxseed oils) can partially or mostly replace fish oil in aquafeeds, but adequate animal-sourced foods or both ω3 (α-linolenic acid, EPA, and DHA) and ω6 (linoleic and arachidonic acids) PUFAs must be included in diets for cats and possibly other carnivores.

Measurements of Fatty Acid Synthesis

The de novo synthesis of fatty acids in cells and tissues can be measured with the use of (a) [^{14}C]-labeled acetate, glucose, and amino acids, which can be converted into ^{14}C-labeled LCFAs (e.g., palmitate), or (b) $^{3}H_2O$, which can be incorporated into ^{3}H-labeled LCFAs through labeling of the NADPH + H^+ pool (Smith and Prior 1986). The labeled fatty acid product is then separated from its labeled precursor through extraction by organic solvents. The rate of fatty acid production is calculated as the amount of radioactivity in the labeled fatty acid products divided by the specific radioactivity of the labeled precursor.

Species Differences in the Use of Substrates for De Novo Fatty Acid Synthesis

Substrates (e.g., glucose, lactate, pyruvate, glycerol, and amino acids) that are metabolized to form acetyl-CoA can serve as precursors for fatty acid synthesis (Prior et al. 1981). However, there are species differences in this synthetic pathway (Table 6.5). Glucose is the major substrate

TABLE 6.5
Species-Differences in De Novo Synthesis of Long-Chain Fatty Acids (LCFAs) in the Liver and White Adipose Tissue

	De Novo Synthesis of LCFAs		
Animal Species	**Liver**	**White Adipose Tissue**	**Major Substrates for the De Novo Synthesis of LCFAs**
Cat	Yes	Yes	Amino acids, glucose, lactate, pyruvate, and acetate
Chicken	Yes	Little	Amino acids, glucose, lactate, pyruvate, and acetate
Dog	Yes	Yes	Amino acids, glucose, lactate, pyruvate, and acetate
Guinea pig (adult)	No	Yes	Amino acids, glucose, lactate, pyruvate, and acetate
Guinea pig (young)	Yes	Yes	Amino acids, lactate, pyruvate, and acetate (not glucose)
Human	Yes	No	Amino acids, glucose, lactate, pyruvate, and acetate
Mouse	Yes	Yes	Amino acids, glucose, lactate, pyruvate, and acetate
Pig	Little	Yes	Amino acids, glucose, lactate, pyruvate, and acetate
Rabbit	Yes	Yes	Amino acids, glucose, lactate, pyruvate, and acetate
Rat (adult)[a]	Yes	No	Amino acids, glucose, lactate, pyruvate, and acetate
Rat (young)	Yes	Yes	Amino acids, glucose, lactate, pyruvate, and acetate
Ruminants[b]	No	Yes	Short-chain fatty acids, pyruvate, and lactate (not glucose)

Source: Jobgen et al. (2006), Patel and Hanson (1974), and Shrago and Spennetta (1976).

[a] Hepatic activities of enzymes for the de novo synthesis of LCFAs are greater in adult females than in adult males.

[b] Including cattle, goats, and sheep. Acetate and butyrate are the major precursors of LCFAs in ruminants. Lactate is a much more important substrate for LCFA synthesis in ruminants than in nonruminants.

for fatty acid synthesis in nonruminants. For example, 40% of absorbed glucose is used for fatty acid synthesis in 80-kg pigs. In contrast, glucose is poorly utilized for fatty acid synthesis in ruminants (Smith and Prior 1981) for the following reasons. First, ATP-citrate lyase activity is very low in ruminant adipose tissue, as compared with nonruminant adipose tissue. Second, there is a low rate of production of pyruvate from glucose due to a low activity of hexokinase in ruminant tissues (Table 6.6). In ruminants, SCFAs are the major substrates for fatty acid synthesis. In such cases, acetate, propionate, and butyrate are first activated to form acetyl CoA, propionyl-CoA and butyryl CoA in the cytosol, respectively, thus bypassing the mitochondria and the ATP-citrate lyase step. Because large amounts of propionate are produced in the rumen, there are relatively high amounts of fatty acids with an odd carbon number in ruminant plasma, tissues, and milk (Bergen and Mersmann 2005). In addition, because branched-chain fatty acids are produced from branched-chain amino acids in the rumen, there are relatively large amounts of medium-chain, long-chain, and branched fatty acids in ruminant plasma, tissues, and milk.

Tissue Differences within the Same Animal in the Use of Substrates for De Novo Fatty Acid Synthesis

Although all tissues can synthesize fatty acids from acetyl-CoA, the major site of this metabolic pathway within the same animal is highly dependent on the species (Table 6.5). For example, WAT is the major site for lipid synthesis in non-lactating cattle, sheep, goats, pigs, dogs, and cats (Jobgen et al. 2006). In poultry, humans, and adult rats, the liver is the major site of de novo fatty acid synthesis and lipogenesis (Bergen and Mersmann 2005). In mice, rabbits, and young rats, both the liver and WAT are the major lipogenic sites. The absence of fatty acid synthesis in the WAT of adult rats is due to its lack of ACC activity. In lactating mammals, mammary glands actively synthesize fatty acids and other lipids for milk production. In response to immune activation, macrophages synthesize and release large amounts of fatty acids and their metabolites.

Nutritional and Hormonal Regulation of Fatty Acid Synthesis

Regulation of fatty acid synthesis by nutrients and hormones involves both short-term and long-term

TABLE 6.6
Activities of Lipogenic Enzymes in Animal Tissues[a]

	White Adipose Tissue					Liver				
Enzyme	**Cattle[b]**	**Human**	**Pig**	**Rat**	**Chicken**	**Cattle**	**Human**	**Pig**	**Rat**	**Chicken**
	μmol/min/g wet tissue									
Hexokinase	0.018	0.54	0.56	0.55	0.53	0.31	0.23	5.2	0.27	0.43
Glucose-6-phosphate dehydrogenase	7.25	0.15	13.0	2.93	0.39	0.44	0.83	1.35	4.64	0.59
6-Phosphogluconate dehydrogenase	2.51	1.24	14.7	1.06	0.45	3.08	4.40	1.80	32.3	3.16
Phosphofructokinase-1	0.084	0.32	0.28	0.53	10.3	0.46	…	1.6	1.50	1.35
Pyruvate kinase	0.044	0.48	0.71	1.6	5.85	5.48	21.2	2.8	12.3	6.50
ATP-citrate lyase	0.079	0.05	0.67	1.93	0.09	0.21	0.19	5.0	3.07	7.63
NADP-malate dehydrogenase	0.207	7.0	27.7	0.88	1.61	0.06	…	0.45	8.37	18.4
Malic enzyme	2.52[c]	0.60	22.5	4.36	0.07	0.42	0.89	20.7	5.49	9.6
Pyruvate carboxylase	0.042	0.02	…	1.52	ND[d]	1.38	1.1	2.5	1.58	3.4
Aconitase (aconitate hydratase)	0.049	…	…	…	…	35	…	…	4.62	…
NADP-isocitrate dehydrogenase	1.98	1.93	21.0	4.75	2.5	29.6	49.7	4.0	55.9	59.7
Acetyl-CoA carboxylase	0.054	0.07	0.25	0.15	0.12	2.3	…	0.43	2.03	0.46
Fatty acid synthase	0.089	0.05	2.5	1.5	0.12	1.1	0.40	0.56	2.66	9.21

Source: Bannister (1984), Bee et al. (1999), Cigolini et al. (1988), Gandemer et al. (1985), Goodridge (1968), Heinz et al. (1968), Klaine et al. (1977), Konstantinova and Russanov (1996), Mersmann (1971), Mourot et al. (1995), Mesbah and Baldwin (1983), Murondoti et al. (2004), O'Hea and Leveille (1968, 1969), Qureshi et al. (2023), Shapira et al. (1978); Smith et al. (1982), Smith and Crouse (1984), Smith and Prior (1981), Swierczynski et al. (2000), Wallace and Newsholme (1967), Warren and Tipton (1974), Wieland et al. (1968), and Zelewski and Swierczyński (1990).

[a] Maximal enzymatic activities. It is assumed that fresh liver and white adipose tissue contain 15% and 5% protein, respectively. The sign "---" denotes that data are not available.

[b] Subcutaneous adipose tissue. The rate of incorporation of L-lactate at the extracellular concentration of 10 mM into fatty acids was 48 nmol/min/g wet weight.

[c] Recalculated from the data of Young et al. (1969).

[d] The gene for this enzyme is not expressed in the white adipose tissue of 7-week-old chickens (Wang et al. 2007).

mechanisms (Table 6.7). The major factors affecting the synthesis of saturated straight-chain fatty acid synthesis include the dietary intakes of carbohydrates (e.g., starch, glucose, and fructose) and lipids; the energy and redox statuses of cells; the intracellular concentration of acetyl-CoA (generated from glucose, amino acids, and fatty acid oxidation), malonyl-CoA and NADPH; and the activities of ACC, FAS, and the enzymes of the pentose cycle (Bauman 1976; Herzberg and Rogerson 1988a,b). The activity of ACC is regulated by protein phosphorylation and dephosphorylation, allosteric ligands, and the abundance of protein enzyme. Δ^9 desaturase is the rate-controlling enzyme in the synthesis of MUFAss from saturated fatty acids.

Short-Term Mechanisms

The short-term mechanisms for the regulation of fatty acid synthesis are mainly through the allosteric and/or covalent modifications of ACC, CPT-I, and FAS. The activities of these two enzymes are also regulated by hormones and physiological metabolites. These factors are highlighted in the following paragraphs.

TABLE 6.7
Effects of Hormones, Nutritional Status, and Other Factors on Fatty Acid Synthesis and Oxidation in Adipose Tissue (WAT), Skeletal Muscle (SKM), and Liver

Hormones, Nutritional Status, and Other Factors	Fatty Acid (FA) Synthesis			FA Oxidation		Net Effect in the Body	
	WAT	SKM	Liver	SKM	Liver	TAG Synthesis	TAG Deposition
Hormones							
Catecholamines	↓	↓	↓	↑	↑	↓	↓
Glucagon	↓	↓	↓	↑	↑	↓	↓
Glucocorticoids							
Short-term	↑	↑	↑	↑	↑	↓	↓
Long-term	↑	↑	↑	↓	↓	↑	↑
Growth hormone	↓	↓	↓	↑	↑	↓	↓
Insulin	↑	↑	↑	↓	↓	↑	↑
Parathyroid hormones	↓	↓	↓	↑	↑	↓	↓
Thyroid hormones	↓	↓	↓	↑	↑	↓	↓
Nutritional status							
Fasting (vs. feeding)	↓	↓	↓	↑	↑	↓	↓
Feeding (vs. fasting)	↑	↑	↑	↑	↑	↑	↑
High-fat diet (vs. low-fat diet)	↓	↓	↓	↑	↑	↓	↑
High-starch diet[a]	↑	↑	↑	↓	↓	↑	↑
High-protein diet[b]	↓	↓	↓	↑	↑	↓	↓
ω3-Polyunsaturated fatty acids	↓	↓	↓	↑	↑	↓	↓
Other factors							
AMP-activated protein kinase	↓	↓	↓	↑	↑	↓	↓
Exercise	↓	↓	↓	↑	↑	↓	↓
Interferon-α	↓	↓	↓	↑	↑	↓	↓
Interferon-γ	↓	↓	↓	↑	↑	↓	↓
Interferon-tau	↓	↓	↓	↑	↑	↓	↓
Interleukin-1	↓	↓	↓	↑	↑	↓	↓
Lipopolysaccharide	↓	↓	↓	↓	↓	↓	↓
PPAR-α	---	↓	↓	↑	↑	↓	↓
PPAR-γ	↑	NC	NC	NC	NC	↑	↑
PPAR-δ/β	↓	↓	↓	↑	↑	↓	↓
Tumor necrosis factor-α	↓	↓	↓	↑	↑	↓	↓

Source: Bijland et al. (2013), Donsmark et al. (2004), Goldberg et al. (2009), Huang et al. (2016), Lee et al. (2014), Macfarlane et al. (2008), Minnich et al. (2001), Picard et al. (2002), and Tekwe et al. (2013).

[a] Compared with low-starch diet.

[b] Compared with normal-protein diet.

Note: ↓, decrease; ↑, increase, AMPK, AMP-activated protein kinase; NC, no change; PPAR, Peroxisome proliferator-activated receptor.

Changes in ACC and CPT-I Activities

ACC is inactive in its phosphorylated state (mediated by both cAMP-dependent PKA and AMPK) but is activated via protein dephosphorylation. Eventually, cAMP (increased by glucagon, epinephrine, norepinephrine, and glucocorticoids) activates PKA, which phosphorylates HSL and perilipin to elicit the intracellular hydrolysis of TAGs (lipolysis). In contrast, insulin, which decreases cAMP availability, inhibits lipolysis. Therefore, factors that enhance (e.g., fasting and exercise) or reduce (e.g., feeding and obesity) intracellular cAMP concentrations suppress or promote lipogenesis, respectively (Table 6.7).

ACC is inhibited by long-chain acyl-CoA (and therefore LCFAs) through feed-back inhibition of the enzyme. This indicates that either an abundant provision of dietary fatty acids or an increase in intracellular concentrations of LCFAs reduces the conversion of acetyl-CoA into FA, thereby favoring the oxidation of acetyl-CoA and LCFAs to CO_2 and water. In contrast, malonyl-CoA (formed from acetyl-CoA and HCO_3^- by ACC in the fatty acid-synthetic pathway) enhances fatty acid synthesis and inhibits CPT-I, thereby promoting lipogenesis and reducing LCFA oxidation. Intracellular malonyl-CoA concentrations are elevated in the fed state but reduced during fasting.

Hormones

Insulin is a major hormone regulating fatty acid synthesis in the WAT and liver through: (a) the activation of ACC via protein dephosphorylation as noted above; (b) the enhanced uptake of LCFAs by hepatocytes and adipocytes through increases in the expression of fatty acid transport protein 1 (FATP1; a member of the FATP/Slc27 protein family) and blood flow. In contrast to insulin, ACC is inhibited by glucagon, epinephrine, and norepinephrine (as occurring during fasting and exercise), as well as glucocorticoids (increased in response to stress) via cAMP-dependent phosphorylation and AMPK signaling, as discussed previously. Interestingly, insulin and glucocorticoids act synergistically in the WAT to exert an anabolic effect on fatty acid synthesis and lipogenesis, while promoting the differentiation of adipocytes. However, there is a species difference in the sensitivity of the WAT to insulin. For example, unlike those from nonruminant mammals (e.g., pigs; Smith et al. 1996), bovine adipocytes and adipose tissue explants do not respond to extracellular insulin based on the synthesis of fatty acids from glucose or acetate and the intracellular concentrations of glycolytic intermediates, possibly due to little or no binding of insulin to its plasma membrane receptor (Smith 2017).

Anabolic and catabolic hormones alter intracellular cyclic adenosine monophosphate (cAMP) concentrations through different mechanisms. For example, catecholamines bind to G-protein-coupled β-adrenergic receptors on the plasma membrane to activate adenylate cyclase, such that cAMP is formed from ATP. Thyroid hormones bind to the nuclear thyroid hormone receptors, resulting in enhanced expression of target genes, including adenylate cyclase. Glucagon binds to receptors on the plasma membrane that are coupled to the G protein located on the cytoplasmic side of the plasma membrane. With conformational changes of the G protein, guanosine diphosphate (GDP) in its α-subunit is replaced with guanosine triphosphate (GTP), causing the release of the α-subunit from the β and γ subunits of the G protein. This α-subunit then interacts with adenylate cyclase, resulting in the activation of the enzyme. Growth hormone binds to its plasma membrane receptor, leading to the dimerization of the receptor. Conformational changes in the growth hormone receptor cause the phosphorylation and activation of intracellular Janus kinase (a tyrosine kinase; JAK) and the latent cytoplasmic transcription factors, called signal transducer and activator of transcription (STAT) proteins, STAT1, STAT3, and STAT5. Such a series of signal transduction promotes the expression of target genes, including adenylate cyclase. Glucocorticoids down-regulate the expression of cyclic-nucleotide phosphodiesterase 3B (PDE3B; an enzyme that hydrolyzes cAMP to AMP), thereby elevating cellular cAMP concentration (Xu et al. 2009). In contrast, insulin brings about an opposite effect by promoting protein kinase B (PKB)-mediated phosphorylation after the binding of the hormone to its plasma membrane receptor, leading to the activation of PDE3B to break down cAMP.

Concentrations of glucagon and epinephrine in the plasma are increased during exercise and in response to "fight/flight" stress, cold stress, and high protein intake. The ratio of glucagon to insulin is higher in type I diabetic patients and during fasting. Growth hormone decreases fatty acid synthesis and lipogenesis in tissues by inhibiting expression of the FAS complex and increasing the production of cAMP. Along with its effect on promoting lipolysis, growth hormone decreases fat deposition in animals. Growth hormone is among the most potent hormones for causing an increase in plasma fatty acid concentrations in vivo and is the only pituitary hormone to produce such an effect within 3.5 h after administration (Goodman 1968).

Citrate

As an allosteric effector, citrate activates ACC1. Cytosolic citrate concentrations are increased in the well-fed state. Thus, citrate is an indicator of a plentiful supply of acetyl-CoA from dietary glucose and amino acids as energy substrates in animals. Under these nutritional conditions, enhanced fatty-acid synthesis helps to store dietary energy in TAGs.

Long-chain Acyl-CoA

As allosteric ligands, these derivatives of LCFAs (e.g., palmitic acid and PUFAs) inhibit ACC and FAS. This is a good example of metabolic feedback inhibition by a product of a reaction sequence. In this regard, PUFAs are more inhibitory than saturated LCFAs in rats (Shillabeer et al. 1990) and chickens (Crespo and Estere-Garcia 2002; Wongsuthavas et al. 2007; Yeh et al. 1970). In contrast,

there are reports either that both unsaturated and saturated LCFAs are equally effective in reducing de novo lipogenesis in the WAT of pigs (Allee et al. 1972) or that saturated LCFAs are more inhibitory of this metabolic pathway in pigs than PUFAs (Smith et al. 1996), possibly depending on the dietary composition of lipids and other nutrients, as well as the age and breed of the animals. Thus, LCFAs reduce the expression of FAS in a species- and tissue-specific manner that is consistent with the major site of FA synthesis in animals.

Long-chain acyl-CoA also inhibits pyruvate dehydrogenase (PDH) by inhibiting the ATP-ADP exchange transporter of the inner mitochondrial membrane. Subsequently, an increase in the mitochondrial [ATP]/[ADP] ratio activates PDH kinase, converting PDH from an active form (dephosphorylated) to an inactive form (phosphorylated). This reduces the formation of acetyl-CoA from pyruvate for fatty acid synthesis. In contrast to long-chain acyl-CoA, medium-chain acyl-CoA does not inhibit ACC or FAS in animal cells, indicating that the length of the hydrocarbon chain is essential for an acyl-CoA to serve as an allosteric factor for enzymes.

HCO_3^-

Bicarbonate is required for ACC activity. Carbonic anhydrase converts $CO_2 + H_2O$ into $HCO_3^- + H^+$. Thus, the activity of this enzyme can affect fatty acid synthesis in cells. In addition, when the availability of HCO_3^- is reduced during metabolic acidosis (e.g., ketosis during lactation and pregnancy), fatty acid synthesis in animals is reduced.

Long-Term Mechanisms

Long-term mechanisms for the regulation of fatty acid synthesis mainly involve changes in rates of synthesis and degradation of the related enzymes, as well as insulin sensitivity (Jump et al. 2013). This accompanies the normal daily cycle of feeding-fasting and, therefore, involves complex biochemical processes. Insulin is an important hormone for causing the induction of enzyme biosynthesis, but glucagon and glucocorticoids have the opposite effect. At the gene level, the expression of fatty acid-synthetic enzymes (ACC, FAS, and SCD) and TAG-synthetic enzyme [glycerol-3-P (G3P) acyltransferase (GPAT)] is up-regulated by sterol regulatory element-binding protein-1c (SREBP-1c). SREBP-1c is a transcription factor that up-regulates the transcription of key genes (e.g., both ACC1 and ACC2) in the pathways for the biosynthesis of fatty acids and cholesterol. Notably, feeding a high-starch diet stimulates SREBP-1c expression in the liver and WAT. In contrast, PUFAs inhibit SREBP-1c expression in the lipogenic tissues to reduce fatty acid synthesis by antagonizing the ligand-dependent activation of the liver X receptor (LXR; a member of the nuclear receptor family of transcription factors): $C_{20:5}$ ω3 = $C_{20:4}$ ω6 > $C_{18:2}$ ω6 > $C_{18:1}$ ω9 (Jump et al. 2013).

The tissue-specific expression of ACC1 and ACC2 suggests that they play important roles in regulating the synthesis and oxidation of fatty acids in response to chronic nutritional and hormonal alterations. Finally, the expression of SCD is inhibited by fasting, diabetes, or high dietary intake of PUFAs (e.g., linoleic acid), but enhanced by insulin administration, high dietary intake of starch, saturated fatty acids, or high cholesterol (Ntambi 1999). These long-term mechanisms take several days to fully manifest. Partly because of increased expression of the genes for fatty acid synthesis in the liver and WAT, the activity of this metabolic pathway generally increases with age.

CHOLESTEROL SYNTHESIS AND CELLULAR SOURCES IN ANIMALS

Physiological Significance of Cholesterol

In both vertebrates and invertebrates, cholesterol is an integral component of (a) cell membranes to maintain their integrity, fluidity, and function (e.g., nutrient transport) and (b) lipoproteins for the interorgan transport of TAGs, LCFAs, and lipid-soluble vitamins. In addition, cholesterol is required for the synthesis of (a) steroid hormones (including progesterone, testosterone, and glucocorticoids) necessary for animal reproduction and the regulation of nutrient metabolism; and (b) bile acids crucial for the digestion and absorption of dietary lipids (including TAGs, cholesterol, and LCFAs) and lipid-soluble nutrients (including lipid-soluble vitamins). Furthermore, in most animals (e.g., humans, pigs, and ruminants), cholesterol is metabolized to produce 1,25-dihydroxy-vitamin D to facilitate the regulation of calcium and phosphorus homeostasis. Finally, dietary cholesterol plays an important role in the neurological development of animals and, in the case of crustaceans, molting [e.g., replacement of an old shell with a new one in shrimp; shedding of the rigid outer shell (exoskeleton) to allow the body to grow in crabs]. There is a species difference in cholesterol synthesis because vertebrates (including humans, pigs, poultry, rodents, ruminants, and fish) can form cholesterol de novo but some invertebrates (e.g., crustaceans) cannot do so (Kumar et al. 2018; Van Den Oord 1964). Thus, cholesterol is physiologically essential in all animals and nutritionally essential for some crustaceans (including crabs, shrimp, and crayfish).

Cholesterol Synthesis from Acetyl-CoA in Vertebrates

Ever since cholesterol was first isolated from gallstones in 1784, it has received much attention from scientists in the chemical and biomedical fields. In all mammals, birds and fish, the de novo synthesis of cholesterol occurs in all cell types, but the hepatocytes (predominantly periportal hepatocytes) of the liver contribute 80% of whole-body cholesterol production. Other active tissues for cholesterol synthesis in vertebrates include the intestines, adrenal glands, testes, and ovaries. Specifically, cholesterol is formed from acetyl-CoA through three phases: (a) the synthesis of isopentenyl pyrophosphate, an activated isoprene unit from acetyl-CoA;

(b) the condensation of 6 mol of isopentenyl pyrophosphate to form 1 mol of squalene; and (c) the cyclizing of squalene to form a tetracyclic product, which is subsequently converted into cholesterol (Figure 6.18). In fish such as trout and goldfish, both the liver (the major site) and the intestine are capable of the de novo synthesis of cholesterol.

The first-phase reactions take place in the cytosol, where hydroxymethylglutaryl coenzyme A (HMG-CoA) synthase catalyzes the formation of HMG-CoA from acetyl CoA and acetoacetyl-CoA, followed by the reduction of this intermediate to mevalonate by HMG-CoA reductase and the conversion of mevalonate into 3-isopentenyl pyrophosphate

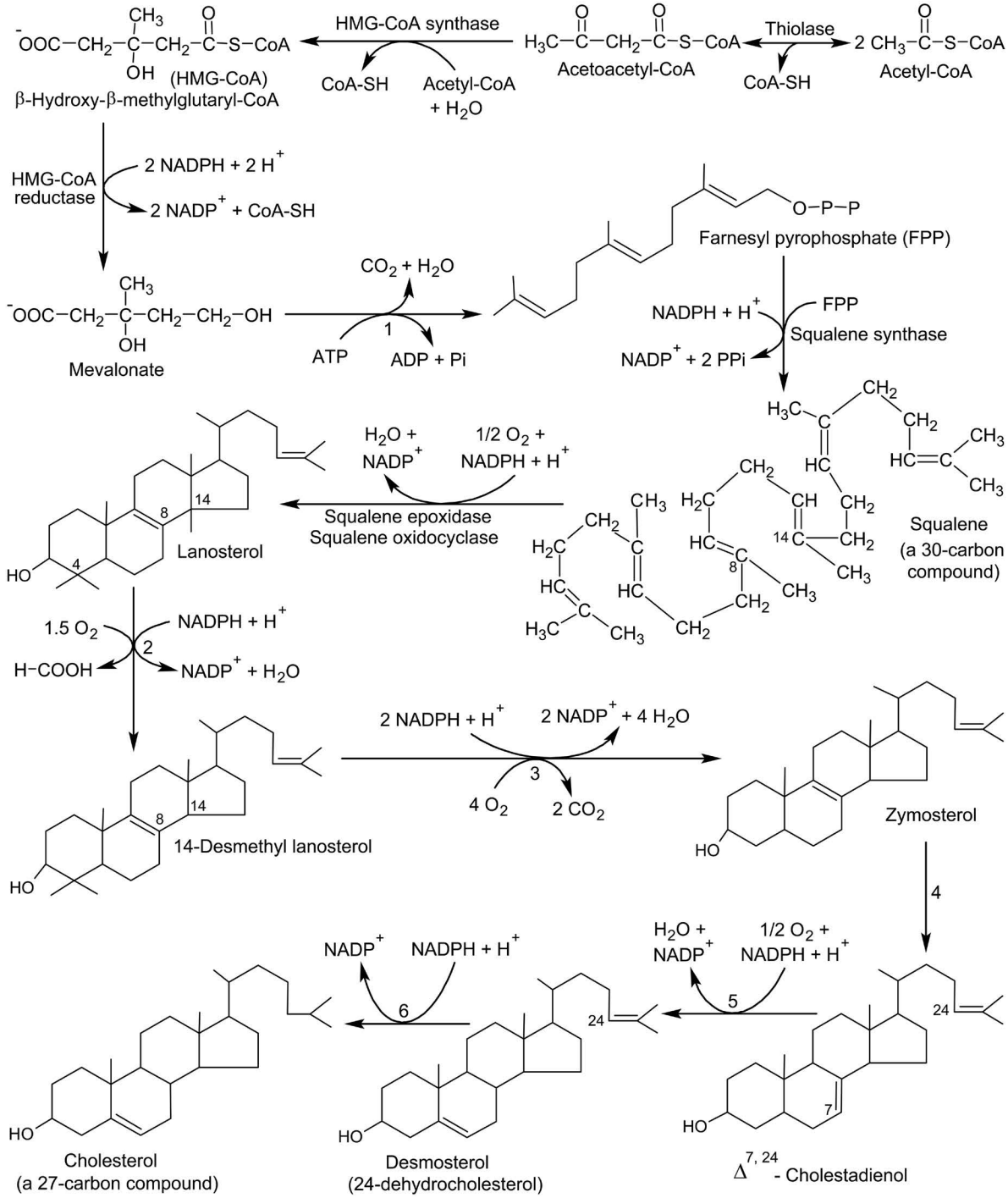


FIGURE 6.18 Synthesis of cholesterol from acetyl-CoA in the liver of animals. This metabolic pathway occurs via three phases: (a) the synthesis of isopentenyl pyrophosphate, an activated isoprene unit from acetyl-CoA; (b) the condensation of 6 mol of isopentenyl pyrophosphate to form 1 mol of squalene; and (c) the cyclizing of squalene to form a tetracyclic product, which is subsequently converted into cholesterol.

by three consecutive ATP-dependent reactions. The HMG-CoA reductase, which is an integral protein of the membrane of the smooth ER, is a rate-controlling enzyme in cholesterol synthesis. The second-phase reactions begin in the smooth ER, where isopentenyl pyrophosphate is isomerized to dimethylallyl pyrophosphate (C_5 unit). The latter condenses with a C_{10} compound (isopentenyl pyrophosphate) in the presence of geranyl transferase to yield a C_{15} compound (farnesyl pyrophosphate), which is reduced by squalene synthase to form squalene.

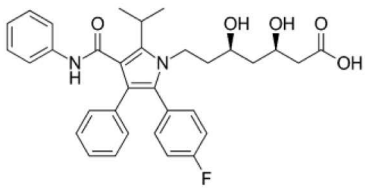
$$2\ \text{Farnesyl pyrophosphate}\ (C_{15}) + \text{NADPH} \rightarrow \text{Squalene}\ (C_{30}) + 2\ \text{PPi} + \text{NADP}^+ + \text{H}^+$$

The third-phase reactions start with the cyclization of squalene through the formation of an intermediate squalene epoxide by O_2^- and NADPH-dependent squalene epoxidase, which is bound to the membrane of the smooth ER. The squalene epoxide is then cyclized to lanosterol by oxidosqualene cyclase. Finally, lanosterol is converted into cholesterol through a series of reactions catalyzed by enzymes associated with the membrane of the smooth ER: the removal of three methyl groups, the reduction of one double bond by NADPH, and the migration of the other double bond.

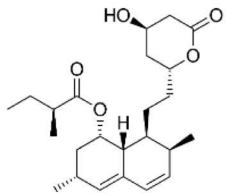
Sources of Cellular Cholesterol and the Regulation of Its Homeostasis in Vertebrates

In vertebrates that are capable of de novo synthesis of cholesterol, there are four sources of cellular cholesterol in hepatocytes: chylomicrons from the diet, extracellular LDLs from small intestine-derived VLDLs, HDLs from the intestine and peripheral tissues, and de novo synthesis (Goldstein and Brown 2015). Dietary cholesterol can be absorbed by enterocytes, but cholesteryl esters in the diet must be hydrolyzed to free cholesterol before intestinal absorption. Much evidence shows that the dietary intake of cholesterol can directly affect its concentration in the liver and plasma (Stellaard 2022). The sources of cholesterol in extrahepatic tissues are chylomicrons from diets, as well as extracellular LDLs from intestine- and liver-derived VLDLs and HDLs. Through the synthesis of cholesterol, dietary intakes of fatty acids, carbohydrates, and amino acids indirectly influence the circulating levels of cholesterol in the blood. In healthy animals, concentrations of cholesterol in the plasma are tightly controlled (Puri 2011).

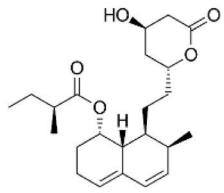
As noted previously, HMG-CoA synthase is the rate-controlling enzyme in hepatic cholesterol synthesis from acetyl-CoA. This pathway is regulated by the amounts of cholesterol that is present in chylomicrons and carried to the liver from peripheral tissues. When intracellular cholesterol levels are increased in hepatocytes and extrahepatic cells, the abundance of their LDL receptors is decreased, thereby reducing the uptake of extracellular LDLs by these tissues (primarily the liver) and increasing LDL concentrations in the plasma. In contrast, statins (polyketide compounds), which include atorvastatin (Lipitor®; synthetic), lovastatin, mevastatin, and other statins, strongly inhibit cytosolic HMG-CoA synthase, thereby reducing plasma cholesterol and LDL levels. The chemical structures of atorvastatin, lovastatin, and mevastatin are shown below. Depending on individuals, the side effects of statins may include muscle pain, liver damage, a reduced synthesis of CoQ_{10}, neuropathy, and an increase in blood glucose concentrations (possibly due to reductions in the uptake and oxidation of glucose by tissues such as skeletal muscle; Khatiwada and Hong 2024). However, the beneficial effect of statins on preventing cardiovascular disease outweighs their adverse effects in animals (including humans).



Atorvastatin (also known as lipitor®, a synthetic statin; $C_{33}H_{35}FN_2O_5$, MW = 558.65 Da)



Lovastatin [a natural product of *Aspergillus terreus and Monascus ruber* (fungi in soil); $C_{24}H_{36}O_5$, MW = 404.55 Da]



Mevastatin [a natural product of *Penicillium citrinum and Penicillium brevicompactum* (fungi in soil); $C_{23}H_{34}O_5$, MW = 390.52 Da]

Lack of De Novo Synthesis of Cholesterol in Invertebrates

In contrast to vertebrates, insects (terrestrial invertebrates) and some marine invertebrates [e.g., whiteleg shrimp (also called Pacific white shrimp, *Litopenaeus vannamei*), lobsters, crabs, oysters, and crayfish] cannot synthesize de novo cholesterol or other sterols (e.g., bile acids or bile alcohols) from acetyl-CoA (Haas et al. 2023; Teshima and Kanazawa 1971). This likely results from the evolutionary losses or mutations of some genes for these synthetic pathways such as those that convert farnesyl pyrophosphate into cholesterol: from farnesyl pyrophosphate to squalene by squalene synthase, from squalene to 2,3-oxisqualene by squalene epoxidase, and the cyclization of 2,3-oxysqualene to lanosterol by lanosterol synthase (Zhang et al. 2019). Therefore, these invertebrates must consume cholesterol from their diets for survival, growth, and development. Dwivedy (1975) reported that housefly larvae required 22 mg cholesterol/100 g diet for optimal growth. Interestingly, optimum dietary levels of cholesterol for crustaceans are generally

greater, ranging from 0.2% and 2.0%, depending on species, developmental stage, size, feed intake and nutrient composition, and aquatic environment (e.g., 0.5–1% for shrimp larvae, 0.2–2% for juvenile shrimp, 0.5–2% for crabs, and 0.5 for crayfish and lobsters; Kumar et al. 2018).

Some crustaceans may be able to convert 24-methyl or 24-ethyl phytosterols into cholesterol in a species-dependent manner: ergosterol, 24-methylcholesterol, and 24-methylcholesta-5,22-dienol in *Artemia salina*; β-sitosterol and desmosterol in *Penaeus japonicus*; desmosterol in *Palaemon serratus*; ergosterol and sitosterol in *Portunus trituberculatus*; and desmosterol in *Sesarma dehaani* (Kumar et al. 2018). Thus, either ergosterol or 24-methylenecholesterol is an effective replacement for cholesterol in the diets of *P. japonicus* larvae, but β-sitosterol is ineffective for juvenile *P. japonicus* or juvenile lobster *Homarus sp.* A combination of phytosterols could partially replace cholesterol in diets for crustaceans such as juvenile *Macrobrachium rosenbergii* (giant freshwater prawn; D'Abramo 1998). Likewise, insects may be able to convert phytosterols into cholesterol or use phytosterols for maintaining survival and growth (Haas et al. 2023).

BILE ACID SYNTHESIS IN ANIMALS

The Classic and Alternative Pathways for Bile Acid Synthesis in the Liver

In the liver of vertebrates (including humans), hepatocytes (predominantly perivenous hepatocytes) synthesize bile acids from cholesterol via both classical and alternative pathways (Figure 6.19), with the classical pathway being the major source (Swell et al. 1980). For example, in the healthy adult human, approximately 500 mg cholesterol is

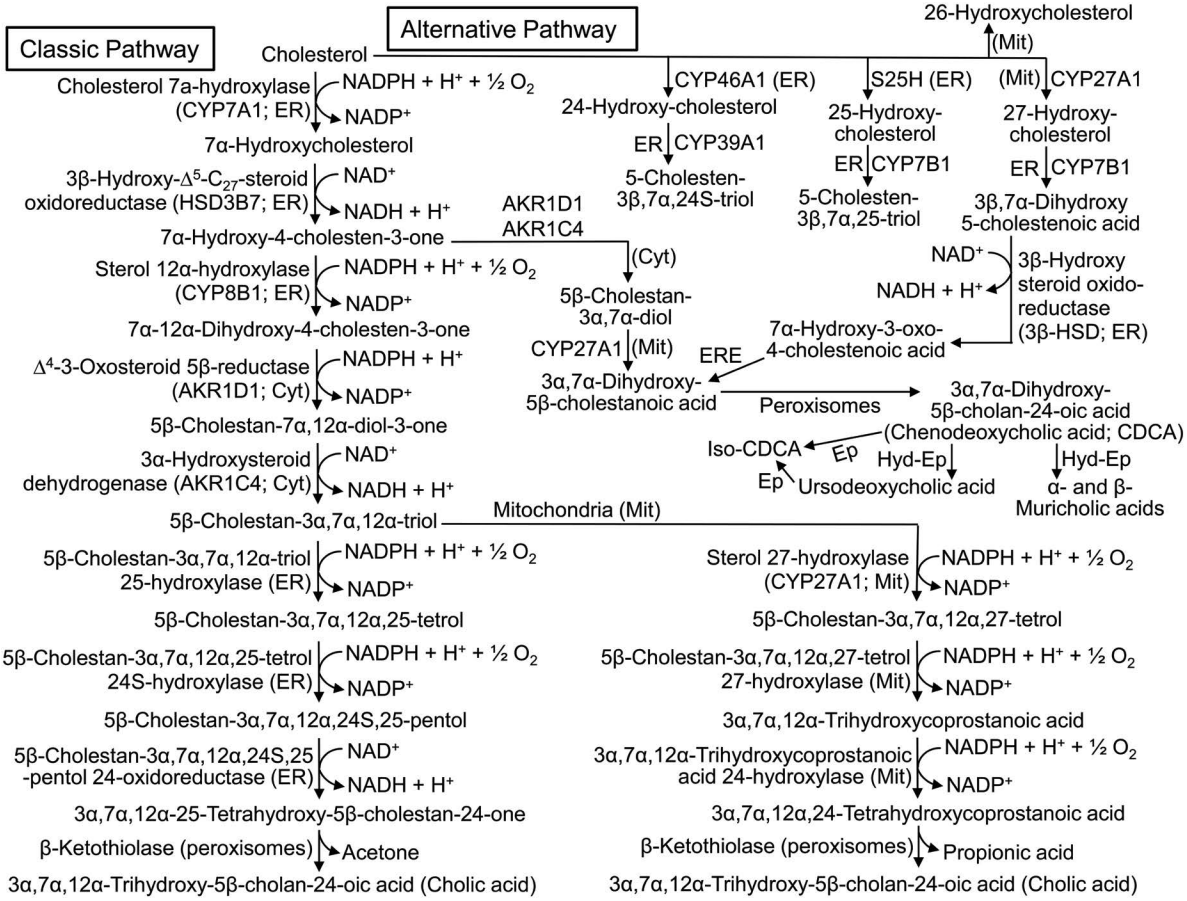


FIGURE 6.19 Synthesis of bile acids from cholesterol in the liver of vertebrates. In the liver of vertebrates (including humans), perivenous hepatocytes synthesize bile acids from cholesterol via both classical and alternative pathways, with the classical pathway being the major source of endogenous cholesterol. The synthetic pathways involve the initial hydroxylation of cholesterol, further modifications to its ring structure, as well as the oxidation and shortening of its side chain. These reactions occur in three different subcellular compartments: the smooth endoplasmic reticulum, cytosol, and peroxisomes. Cholic acid and chenodeoxycholic acid (CDCA) are the major products of the classical and alternative pathways, respectively. 3α,7α-dihydroxy-5β-cholestanoic acid bridges the classic pathway with the alternative pathway in cholesterol metabolism. Note that CYP7A1 and CYP7B1 add an α-7-hydroxyl group to their sterol and oxysterol substrates, respectively. CYP46A1, cholesterol 24-hydroxylase; CYP39A1, 24-hydroxycholesterol 7α-hydroxylase; CYP7B1, oxysterol 7α-hydroxylase; ER, smooth endoplasmic reticulum; ERE, specific hydroxylases and oxidoreductase in the ER; Hyd-Ep, hydroxylation and epimerization; Iso-CDCA, the 3β-hydroxy epimer of CDCA; S25H, sterol 25-hydroxylase.

converted into bile acids per day via the classic pathway, accounting for ~90% of total bile acid synthesis (Russell 2003). Similarly, in healthy mice, about 75% of bile acid synthesis occurs via the classic pathway. The synthetic pathways involve the initial hydroxylation of cholesterol, further modifications to its ring structure, as well as the oxidation and shortening of its side chain (Russell 2003). These reactions occur in four different subcellular compartments: the smooth ER, cytosol, mitochondria, and peroxisomes. Bile acids synthesized by the liver are called primary bile acids. Approximately 50% of the cholesterol synthesized in the liver is converted to bile acids. Other organs do not express all the enzymes required for the de novo synthesis of bile acids. Nearly all the newly synthesized bile acids are covalently conjugated with glycine, taurine, or both to form bile salts in the liver, depending on animal species (Chapter 7). For the secretion of the bile salts from the liver to the duodenum, intrahepatic bile flows from the perivenous to the periportal area vial bile ducts in the opposite direction of the lobular blood flow (Martini et al. 2023). Conversion of cholesterol to bile acids is the primary route to eliminate cholesterol from the body.

In the classical pathway, cholesterol is first oxidized to 7α-hydroxycholesterol by cholesterol 7a-hydroxylase (CYP7A1; an enzyme in the ER). 7α-Hydroxycholesterol is oxidized to 7α-hydroxy-4-cholesten-3-one by 3β-hydroxy-Δ^5-C_{27}-steroid oxidoreductase (HSD3B7; an ER enzyme). 7α-Hydroxy-4-cholesten-3-one undergoes additional modifications to form either 5β-cholestan-3α,7α,12α-triol sequentially in the ER and cytosol or 3α,7α-dihydroxy-5β-cholestanoic acid in the cytosol and mitochondria. 5β-Cholestan-3α,7α,12α-triol is then metabolized to 3α,7α,12α-25-tetrahydroxy-5β-cholestan-24-one and 3α,7α,12α,24-tetrahydroxycoprostanoic acid via ER and mitochondrial enzymes, respectively. In peroxisomes, 3α,7α,12α-25-tetrahydroxy-5β-cholestan-24-one and 3α,7α,12α,24-tetrahydroxycoprostanoic acid are activated to their acyl derivatives and cleaved by β-ketothiolase to form cholic acid, whereas 3α,7α-dihydroxy-5β-cholestanoic acid is oxidized to CDCA and then to muricholic acid and ursodeoxycholic acid (Ferdinandusse and Houten 2006).

In the alternative pathway, cholesterol is first converted into 24-hydroxycholesterol [by sterol 24-hydroxylase (CYP46A1) in ER], 25-hydroxycholesterol (by sterol 25-hydroxylase in ER), 26-hydroxycholesterol (by sterol 26-hydroxylase; a mitochondrial enzyme), and 27-hydroxycholesterol [by sterol 27-hydroxylase (CYP27A1) in mitochondria]. These reactions occur in both the liver and extrahepatic tissues (including the brain and spleen). Oxysterols and C_{27} bile acids formed in extrahepatic cells and tissues are transported to the liver for further metabolism along with 24-, 25-, 26-, and 27-hydroxycholesterols generated by hepatocytes. Specifically, 24-hydroxycholesterol and 25-hydroxycholesterol are oxidized to 5-cholesten-3β,7α,24S-triol and 5-cholesten-3β,7α,25-triol by CYP39A1 and CYP7B1 in ER, respectively. CYP7B1 also acts on 27-hydroxycholesterol to generate 3β,7α-dihydroxy 5-cholestenoic acid, which is then converted by 3β-hydroxysteroid oxidoreductase (3β-HSD; ER) into 7α-hydroxy-3-oxo-4-cholestenoic acid. The latter undergoes ring modifications by hydroxylases and oxidoreductases in the ER to form 3α,7α-dihydroxy-5β-cholestanoic acid (a metabolite of cholesterol oxidation via the classic pathway), which is further metabolized in peroxisomes to CDCA (the common precursor of both muricholic acid and ursodeoxycholic acid; Ferdinandusse and Houten 2006). Thus, 3α,7α-dihydroxy-5β-cholestanoic acid bridges the classic pathway with the alternative pathway in cholesterol metabolism.

Regulation of Bile Acid Synthesis in Liver

Hepatic synthesis of bile acids is regulated by a plethora of factors, including the expression and activities of key enzymes, the availabilities of cholesterol and cofactors (e.g., NADPH and NADH), diets, feed-forward activation by oxysterols through a nuclear receptor called the liver-X-receptor α (LXRα), and feedback repression by bile acids through the farnesoid X receptor (FXR) and fibroblast growth factor 15 (FGF15 in mice or FGF 19 in humans; Choudhuri and Klaassen 2022).

Specifically, dietary intakes and hepatic concentrations of cholesterol (the substrate) drive bile acid synthesis in animals. Second, oxysterols (formed from cholesterol) activate LXRα, which binds to the LXR response element and induces the transcription of the Cyp7α1 gene, thereby promoting bile acid synthesis and cholesterol excretion. In contrast, as the concentrations of bile salts in the intestine are increased, intestinal FXR is activated to stimulate the expression of FGF15 or FGF19 in enterocytes. FGF15 (mouse) or FGF19 (human) enters the portal circulation, reaches the liver, and binds to its hepatocyte membrane receptor complex, leading to decreases in the expression of Cyp7α1 and then bile acid synthesis. In the liver, elevated concentrations of bile acids also activate the FXR to induce the expression of the small heterodimer partner (SHP). SHP binds to the liver receptor homolog 1 (LRH-1) and/or LXRα to form an inhibitory complex that represses the transcription of the Cyp7α1 gene. Furthermore, SHP binds to both the fetoprotein transcription factor and the hepatocyte nuclear factor 4α, and the resulting complex inhibits the transcription of the Cyp8b1 gene. Interestingly, dietary supplementation with lipid-soluble vitamins A and D to mice reduces the hepatic expression of the Cyp7α1 gene and the hepatic synthesis of bile acids by increasing the release of FGF15 from the small intestine (Schmidt et al. 2010). Such effects of lipid-soluble vitamins may serve as a feed-back inhibition of their absorption by the gut when their dietary intakes by animals are augmented. Third, the expression of bile salt and bile acid transporters in the apical and basolateral membranes of the small intestine and liver can also regulate hepatic bile acid synthesis by affecting the enterohepatic circulation, as well as the concentrations of bile salts and bile acids in enterocytes and hepatocytes (Figure 6.20).

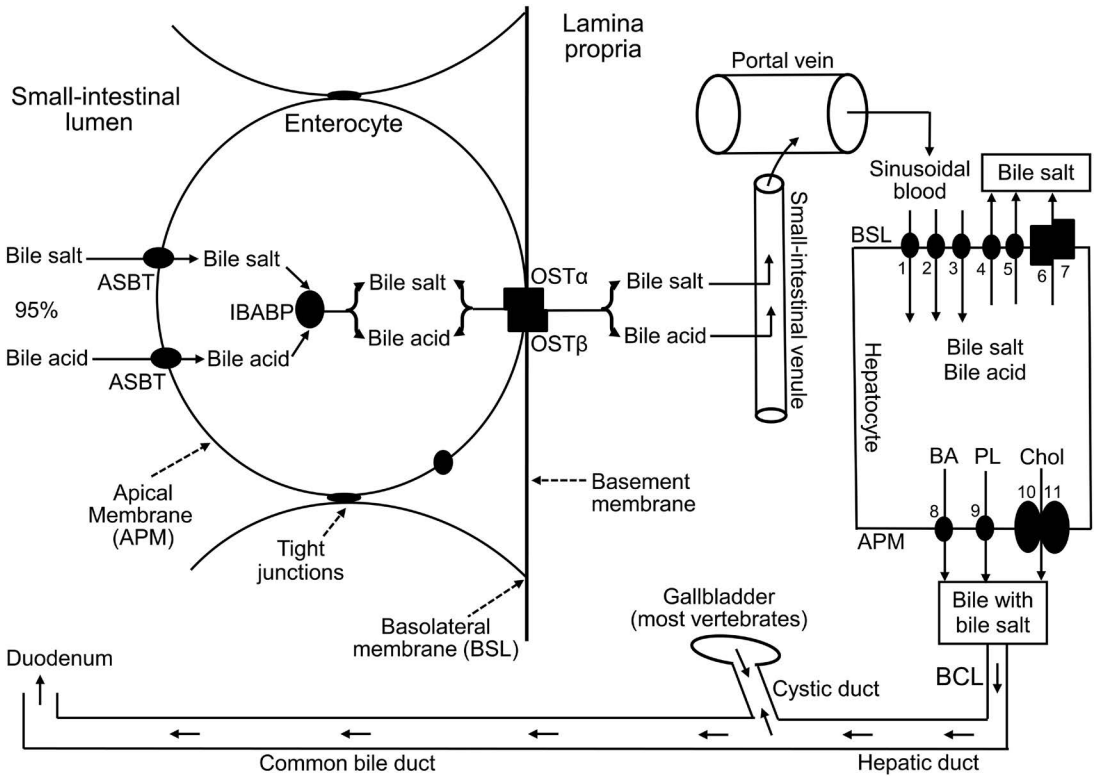


FIGURE 6.20 The interorgan transport of bile salts between the small intestine and the liver. Hepatocytes of the liver release either bile salts into sinusoidal blood via the basolateral membrane transporters or the bile (containing bile salts and virtually no free bile acids) via the apical membrane transporters into the bile canaliculus. The bile enters terminal bile ducts and progressively larger bile ducts and then reaches the hepatic duct. Subsequently, the bile either empties directly into the duodenum via the common bile duct or is diverted into the gallbladder (in most vertebrates) via the cystic duct, depending on physiological or nutritional conditions. In response to feeding, the contraction of the gallbladder releases the bile to the duodenum via the cystic duct and the common bile duct. About 95% of the liver-derived bile salts are absorbed into the portal circulation and transported back to the liver, with the remaining 5% of bile salts entering the large intestine for excretion via the feces. When bile acids are provided in the diet, they are absorbed into the portal circulation and enter the liver for conjugation with glycine, taurine, or both to form bile salts. The indicated bile salt/bile acid transporters are (1) Na^+-taurocholate cotransporting protein (NTCP), (2) organic anion transporting protein family member 1B1 (OATP1B1), (3) organic anion transporting protein family member 1B3 (OATP1B3), (4) multidrug resistance-associated protein 3 [MRP3; also known as ATP-binding cassette subfamily C member 3 (ABCC3)], (5) multidrug resistance-associated protein 4 (MRP4; also known as ABCC4), (6) OSTα, (7) OSTβ, (8) bile salt export pump (BSEP), (9) multidrug resistance protein 3 (MDR3; also known as ABCB4; transporting phospholipids), (10) ATP-binding cassette subfamily G member 5 (ABCG5), and (11) ATP-binding cassette subfamily G member 8 (ABCG8). APM, apical membrane; ASBT, apical Na^+-dependent bile acid transporter; BA, bile salts; BSL, basolateral membrane; BCL, bile canaliculus; Chol, cholesterol; IBABP, ileal bile acid-binding protein [(cytosolic); also known as fatty acid binding protein 6 (FABP6)]; OSTα, organic solute transporter α; OSTβ, organic solute transporter β; and PL, phospholipids.

Finally, dietary supplementation with taurine increases the expression and enzymatic activity of Cyp7α1 in hepatocytes for the synthesis of bile acids in animals that use either taurine or glycine for conjugation, thereby stimulating bile flow and reducing cholesterol concentrations in the plasma and liver (Bellentani et al. 1987; Kibe et al. 1980; Murakami et al. 2016; Russell 2003). Conversely, the hepatic synthesis of bile acid is impaired by a dietary deficiency of taurine (Miyazaki et al. 2022). At present, little is known about the effects of dietary glycine supplementation on bile acid synthesis in animals, despite a previous report that dietary supplementation with taurine (3 × 0.5 g/day) to the healthy adult human increased the proportion of taurine-conjugated bile acids in the bile but dietary supplementation with glycine (3 × 7 g/day) did not affect the pattern of bile acids (Sjövall 1959).

Production of Secondary Bile Acids by Intestinal Microbes

At the terminal ileum, 95% of bile salts return to the liver via the portal vein through enterohepatic circulation, and the remaining 5% enters the large intestine where they undergo modifications by bacterial enzymes (Devlin 2011). In the intestine, microbes transform the primary bile salts

through the deconjugation of glycine or taurine, as well as the dehydroxylation, dehydrogenation, and epimerization of the cholesterol core (Guzior and Quinn 2021). The resultant products are called secondary bile acids. These biochemical reactions increase the diversity and biological functions of bile acids.

Deconjugation of bile salts is an essential function of the gut microbiome. All major bacterial phyla contain enzymes capable of catalyzing the deconjugation reactions. The bacterial phyla include *Bacteroides* spp., Gram-positive genera (e.g., *Bifidobacterium*, *Lactobacillus*, *Clostridium*, *Enterococcus*, and *Listeria*), and Gram-negative genera (e.g., *Stenotrophomonas*, *Bacteroides*, and *Brucella*). In the feces, 98% of bile acids are not conjugated with taurine or glycine, such that these two amino acids can be reabsorbed into the portal vein and conserved. The dehydroxylation of bile acids at C_7 is catalyzed by 7α-dehydratase. Examples of dehydroxylation are the conversion of cholic acid into deoxycholic acid, CDCA into lithocholic acid, hyocholic acid or β-muricholic acid into hyodeoxycholic acid, and ursocholic acid into ursodeoxycholic acid. Epimerization of bile acids occurs via the oxidation of the hydroxyl group by a position-specific hydroxysteroid dehydrogenase (e.g., 7α-HSDH) and the reduction of the resultant product by another position-specific hydroxysteroid dehydrogenase (e.g., 7β-HSDH). For example, the epimerization of cholic acid by intestinal microbes results in the formation of several derivatives, including ursocholic acid (3α, 7β, 12α-trihydroxy-5β-cholan-24-oic acid), 12-epicholic acid (3α, 7α, 12β-trihydroxy-5β-cholan-24-oic acid), and isocholic acid (3β, 7α, 12α-trihydroxy-5β-cholan-24-oic acid). Similarly, through enzymatic reactions involving hydroxylation and epimerization, CDCA can be metabolized to α- and β-muricholic acids or ursodeoxycholic acid, depending on animal species. In addition, CDCA is converted into iso-CDCA (3β,7α-dihydroxy-5β-cholan-24-oic acid) via enzyme-catalyzed epimerization reactions. Alterations in the formation of secondary bile acids may play a role in the development of several diseases, such as cirrhosis, inflammatory bowel disease, and cancer (Guzior and Quinn 2021).

TAG SYNTHESIS AND CATABOLISM IN ANIMALS

TAG Synthesis in Animals

As lipid-soluble oils, high concentrations of free fatty acids are toxic to animals. Although LCFAs are transported in the plasma bound to albumin, there is a limit to the capacity of the protein for carrying a large amount of lipid-soluble material in the bloodstream. In animals, all tissues form TAGs and related esters (e.g., phospholipids) from LCFAs and glycerol-3-P, but the small intestine, liver (predominantly perivenous hepatocytes), WAT and, in the case of lactation, mammary glands play major roles in interorgan TAG metabolism. Specifically, the small intestine digests and absorbs the dietary TAGs, while releasing TAG-rich chylomicrons into the lymph or the portal vein, depending on animal species. In the liver and WAT, the esterification of LCFAs as TAGs is the main means for the transport and storage of fatty acids in the body. In young animals, TAGs are a significant component of growth (Etherton and Walton 1986). In lactating mammals, mammary glands actively synthesize TAGs and release the lipids into the milk. In all postnatal or post-hatching animals, TAGs are essential to sustain life during fasting and starvation. Thus, TAG synthesis is of enormous nutritional and physiological importance. Available evidence shows that two major pathways for TAG synthesis involve either MAGs or G3P as the initial acyl acceptor (Coleman and Mashek 2011). Both pathways use fatty acyl-CoA thioesters as donors of acyl groups and converge on the final acylation step. The quantitative importance of each pathway in maintaining the homeostasis of TAGs depends on animal species and specific tissues. The third, but minor, pathway for TAG synthesis uses dihydroxyacetone-P as the initial acyl acceptor. All three metabolic pathways are illustrated in Figure 6.21.

MAG Pathway for TAG Synthesis

The MAG pathway is primarily responsible for the synthesis of TAGs in enterocytes of nonruminants that take up a large amount of dietary MAGs (Stone 2022), but also occurs in the extraintestinal tissues of all animals (Bell and Coleman 1980). This pathway takes place in the smooth ER. Inside cells, LCFAs are bound to FABPs at each stage of TAG synthesis (Storch and Corsico 2008). In the MAG pathway, 2-MAG and fatty acyl-CoA are covalently joined to form DAGs in a reaction catalyzed by MAG acyltransferase (MGAT). Three isoforms of MGAT have been identified: MGAT1 that is highly expressed in the kidneys, liver, WAT, and stomach; MGAT2 that is predominant in the small intestine and is abundant in the liver; and MGAT3 that has been detected in the intestines, liver, and pancreas (Stone 2022). Further acylation of DAGs by DAG acyltransferase (DGAT) results in TAG formation. Two DGATs have been identified and characterized: DGAT1 and DGAT2. DGAT1 is expressed in many tissues (including the liver, small intestine, adrenal gland, skin, and mammary glands), whereas DGAT2 is expressed mainly in the liver, small intestine, and mammary glands and, to a lesser extent, in testes. MGAT2 and MGAT3 also exhibit DGAT activity (Cao et al. 2007). Finally, DGAT catalyzes the conversion of DAGs to TAGs. In the postprandial state, when fatty acids and MAGs are taken up by enterocytes, the MAG pathway accounts for more than 75% of TAG synthesis in the small intestine (Yen et al. 2015). Through processing the massive influx of substrates after a lipid-containing meal, the MAG pathway plays an important role in the efficient absorption of dietary fats.

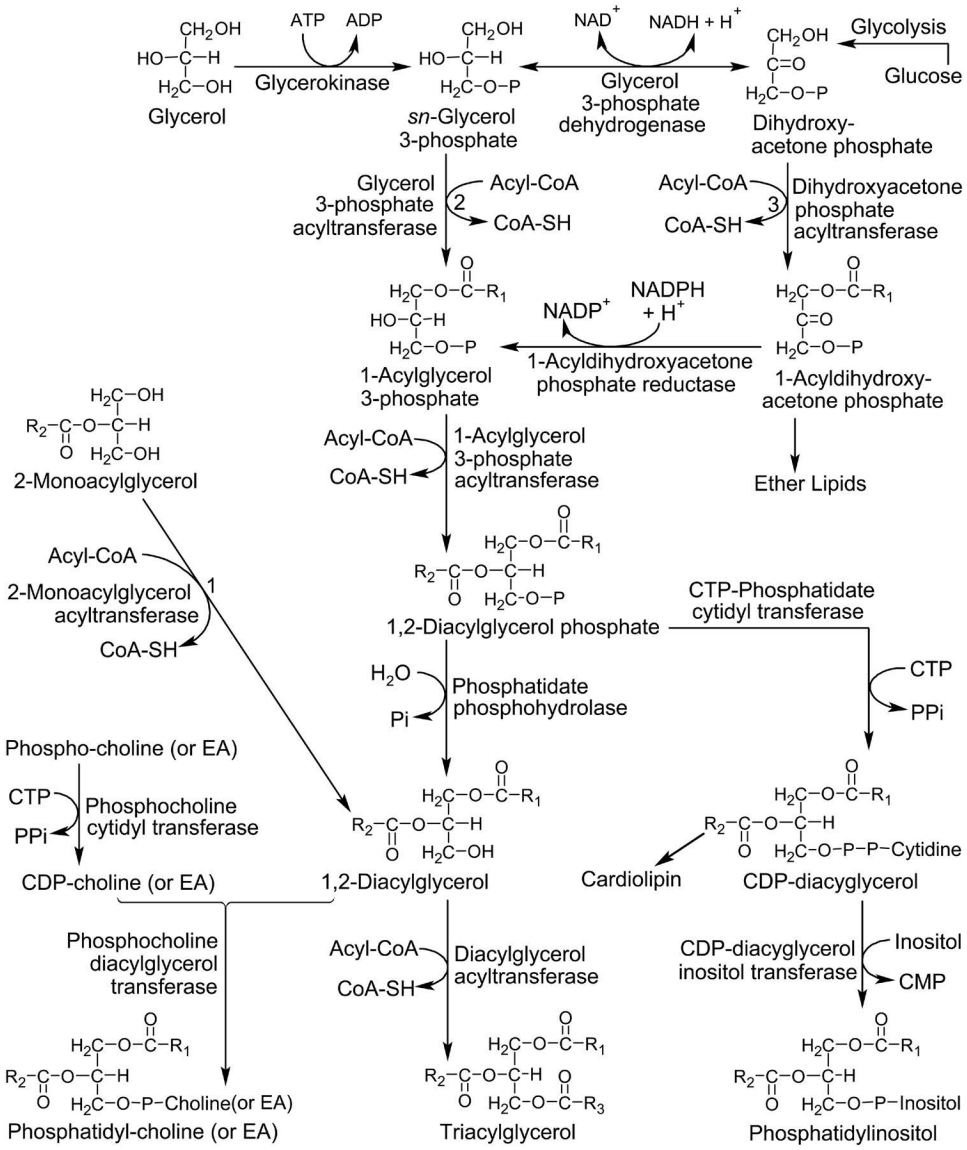


FIGURE 6.21 Synthesis of triacylglycerols in animal tissues. Triacylglycerols are synthesized from LCFAs and glycerol via three pathways involving MAG (Pathway 1), glycerol-3-phosphate (G3P; Pathway 2), or dihydroxyacetone-phosphate (Pathway 3) as the initial acyl acceptor. Nearly all the reactions occur in the smooth endoplasmic reticulum (ER), but G3P acyltransferase is located in both the ER and mitochondria. Pathways 1 and 2 use fatty acyl-CoA thioesters as donors of acyl groups, and they converge on the final acylation step, whereas the product of dihydroxyacetone phosphate acyltransferase is channeled to either Pathway 2 or the formation of each of the indicated lipids. CDP, cytidine diphosphate; CMP, cytidine monophosphate; CTP, cytidine triphosphate; EA, ethanolamine.

G3P Pathway for TAG Synthesis

G3P is formed from both glucose via glycolysis and glyceroneogenic substrates via enzymes including phosphoenolpyruvate carboxykinase (PEPCK; Chapter 5). In extraintestinal tissues, the biosynthesis of TAGs occurs through the G3P pathway (also known as the Kenedy pathway), in which DAGs are produced from G3P after two acylation steps and the removal of the phosphate group. This series of reactions is catalyzed sequentially by three enzymes: GPAT, 1-acylglycerol-3-P acyltransferase (AGPAT; also known as lysophosphatidic acid acyltransferase), and phosphatidic acid phosphatase. As in the MAG pathway, the G3P pathway ends with the conversion of DAGs into TAGs by DGAT. The quantitative significance of the G3P pathway varies with animal species and tissues. For example, in ruminants whose enterocytes absorb only a small amount of dietary MAGs, the G3P pathway is predominant for TAG formation by these cells. Such a pathway is quantitatively more important in the small intestine of nonruminants as compared with ruminants, and occurs in nonruminant enterocytes to a much smaller extent than the MAG pathway. In the liver, ~90% of TAGs are produced via the G3P pathway under

normal physiological conditions (Coleman and Mashek 2011). This metabolic pathway is also predominant in the epithelial cells of the lactating mammary glands (Rezaei et al. 2016). In addition to TAGs, the G3P pathway also generates precursors for the synthesis of major glycerophospholipids that are constituents of all cellular membranes (Bell and Coleman 1980).

GPAT has the lowest activity among all enzymes of the GPAT pathway and is the rate-controlling enzyme for TAG synthesis via the G3P pathway (Coleman and Mashek 2011). GPAT exists in two isoforms: GPAT1 and GPAT2 that localize to the mitochondrial outer membrane, as well as GPAT3 and GPAT4 that occur in the ER (Yu et al. 2018). The lysophosphatidate product of the mitochondrial GPAT1 and GPAT2 is transported to the ER for conversion into TAGs. GPAT1, GPAT2, GPAT3 preferentially use saturated LCFA-CoA, arachidonoyl-CoA, a broad range of LCFAs, and palmitoyl-CoA as substrates, respectively. The expression of these isoforms of GPAT is tissue-specific, namely GPAT1: BAT > WAT > Liver > Skeletal Muscle = Heart > Brain; GPAT2: Testis > Liver > WAT; GPAT3: WAT > Small intestine > Heart > Brain > Liver; and GPAT4: BAT = Testis > Liver, Kidney, Brain, Intestine, and WAT (Yu et al. 2018). GPAT1 accounts for 30-50% and ~30% of the total GPAT activity, respectively, in the liver and heart; GPAT3 for 80% of the total GPAT activity in the WAT; and GPAT4 for 90% of the total GPAT activity in the epithelium of mammary glands.

Additional Pathways for TAG Synthesis

Other pathways for TAG synthesis, which are quantitatively minor, have been proposed. For example, an acyl-CoA-independent DAG transacylase, which catalyzes the conversion of 2 mol of DAGs into 1 mol of TAG and 1 mol of MAG, has been reported in the rat intestine (Lehner and Kuksis 1993). The gene encoding the intestinal DAG transacylase has not yet been identified. The synthesis of some TAGs also occurs through the dephosphorylation of phosphatidic acid and acylation of the resulting DAGs.

Function of DAGs in Protein Kinase C Signaling

Protein kinase C plays an important role in membrane signal transduction to mediate a variety of biological functions, such as cell proliferation, cell differentiation and secretion. The activation of this enzyme requires Ca^{2+} and acidic phospholipid. DAGs enhance Ca^{2+}-sensitivity of protein kinase C to make the enzyme fully active in the presence of physiological concentrations of Ca^{2+}. In animal cells, DAGs are produced by four pathways: the hydrolysis of phospholipids, the deacylation of TAGs, the acylation of MAGs, and the de novo synthesis from glucose-derived glycerol via phosphatidic acid (Puri 2011). Thus, the metabolism of TAGs is closely linked to cell signaling.

Regulation of TAG Synthesis and Accretion in Animals

The synthesis of TAGs depends on the availability of substates (i.e., fatty acids and glycerol), the activities of enzymes in the metabolic pathway, and the endocrine status. The expression of those enzymes is regulated at both transcriptional and translational levels. For example, fasting, glucagon, and exercise decrease, but carbohydrates (including starch and sucrose), insulin, and high-fat feeding increase, the expression and enzymatic activities of GPAT in the ER and mitochondria. In addition, the two isoforms of GPAT are active in a dephosphorylated form (e.g., in the case of insulin to increase phosphatase activity) but inactive in a phosphorylated form (e.g., in the case of AMPK to phosphorylate GPAT).

EPA and DHA (e.g., dietary supplementation with 1 g/day for an adult person) have a potent effect in reducing hepatic TAG synthesis and plasma TAG concentrations in humans and laboratory animals (Weintraub 2014). These essential nutrients act through the following mechanisms: (a) increasing LCFA oxidation, (b) reducing LCFA availability and DGAT activity for TAG synthesis, and (c) enhancing the activity of LPL in the capillary endothelium to hydrolyze TAGs in the circulating VLDLs. Ethyl EPA (E-EPA; a synthetic analog of EPA; Chapter 3) is also as effective as EPA in improving plasma metabolic profiles. Specifically, ingested E-EPA is absorbed into enterocytes where it is de-esterified by carboxylesterase (present in the cytosol and the ER) to EPA. The EPA is reassembled mainly into chylomicrons and, to a much lesser extent, VLDLs, followed by export into the lymph in mammals. Through the metabolism of the circulating chylomicrons and VLDLs, the EPA that is derived from E-EPA is available for the use by the liver and other tissues, as described previously.

Storage of TAGs in WAT and Other Tissues

TAGs are stored primarily in the WAT and, to a lesser extent, other tissues (e.g., the liver and skeletal muscle). In WAT, the stored TAGs are enclosed by a layer of protein called perilipin (Greenberg et al. 1991). This perilipin family of proteins stabilizes lipid droplets and controls lipolysis in adipocytes (Brasaemle 2007). Phosphorylation of perilipin by cAMP-dependent protein kinase is essential for the mobilization of fats from the WAT. In the fatty liver, excess TAGs result in its multiple metabolic dysfunctions. Although an appropriate amount of TAGs between muscle fibers (called intramuscular fats) can enhance meat taste, excess amounts of intermuscular fats reduce meat quality and value.

MOBILIZATION OF TAGs FROM TISSUES TO RELEASE GLYCEROL AND FATTY ACIDS

Intracellular Lipolysis by HSL in Animal Tissues: Tissue Distribution and Function of HSL

When animals have a need for energy beyond that provided from the diet, intracellular TAGs (particularly in skeletal muscle) are hydrolyzed by lipases to yield fatty acids and glycerol (Figure 6.22). This process is called

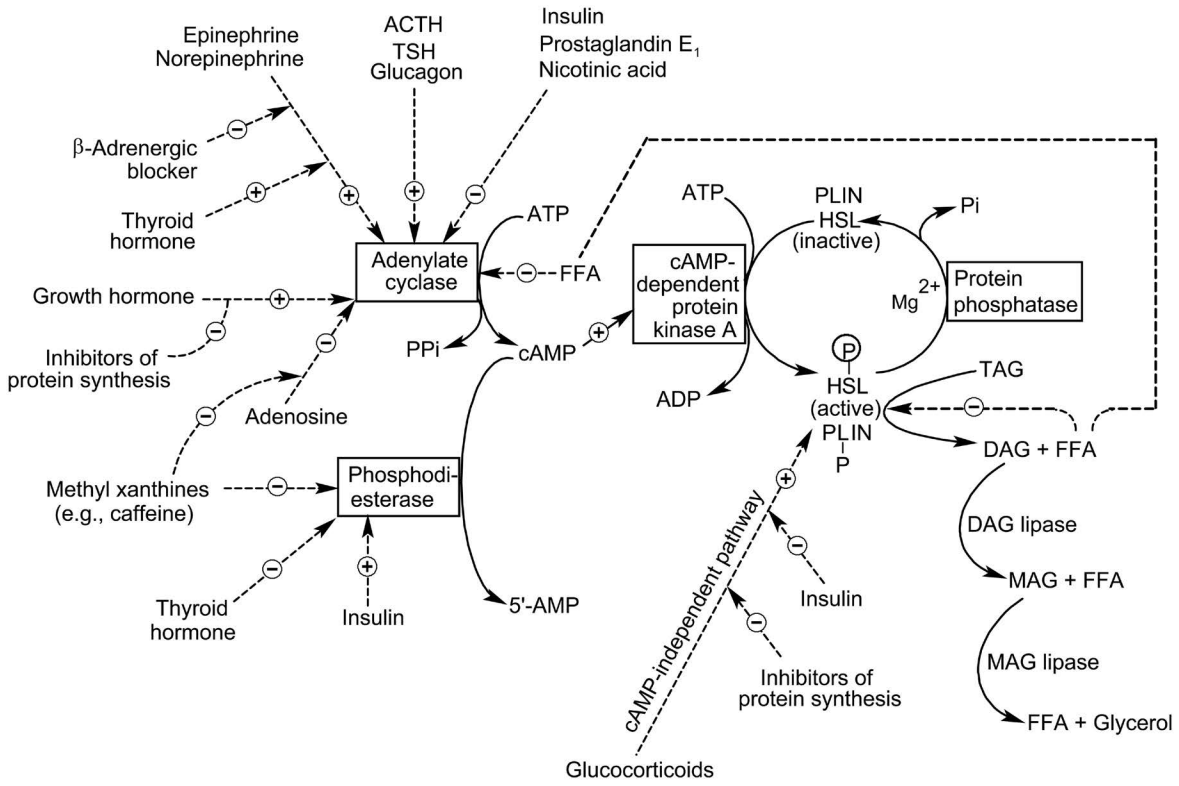
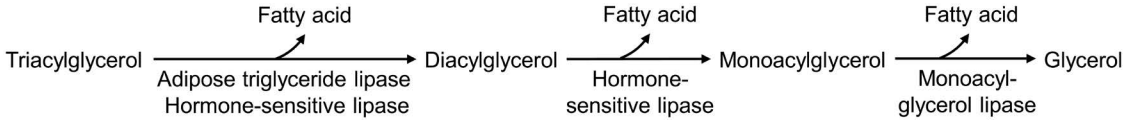


FIGURE 6.22 Mobilization of triacylglycerols from tissues through cAMP-dependent phosphorylation of hormone-sensitive lipase (HSL) and perilipin (PLIN) to release glycerol and fatty acids. In tissues (e.g., skeletal muscle and WAT) that do not have glycerol kinase activity, glycerol is not re-used locally for TAG synthesis. ACTH, adrenocorticotropic hormone; DAG, diacylglycerols; FFA, free fatty acids; MAG, monoacylglycerols; TAG, triacylglycerols; TSH, thyroid-stimulating hormone.

intracellular lipolysis. The WAT has a greater rate of lipolysis than BAT and skeletal muscle (Table 6.8). HSL, acting in concert with ATGL, hydrolyzes the first ester bond in TAGs, yielding one molecule of free fatty acids and one molecule of DAGs. The name HSL was coined to indicate the potent effect of certain hormones (e.g., catecholamines, glucagon, growth hormone, and testosterone) on stimulating HSL activity. As an intracellular neutral lipase, HSL is capable of hydrolyzing not only TAGs, but also DAGs and MAGs, as well as cholesteryl and retinyl esters (Kraemer and Shen 2002). Thus, HSL has broad substrate specificity. Of note, the hydrolytic activity of HSL against DAGs and MAGs is not affected by protein phosphorylation (Yeaman 1990). This feature of HSL differentiates it from other lipases (e.g., LPL and hepatic lipase). In animals, the rates of hydrolysis of TAGs generally decrease with postnatal age. This metabolic pathway is shown for white adipocytes as follows.



Glycerol from TAG hydrolysis is utilized to generate either TAGs through the action of glycerol kinase in tissues other than the WAT and muscle, or glucose in the liver and kidneys (Doreau and Chilliard 1997). Depending on nutritional and physiological conditions, the metabolic fate of fatty acids is either mitochondrial oxidation to produce CO_2, water, and ATP production or hepatic ketogenesis to generate acetoacetate and β-hydroxybutyrate.

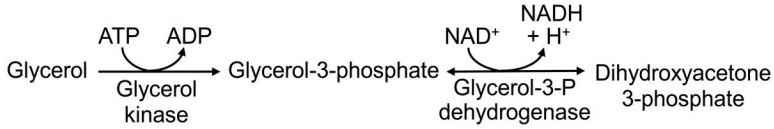


TABLE 6.8
Effects of Oral Administration of Ovine Interferon-tau (IFNT) to Zucker Diabetic Fatty Rats on Substrate Oxidation in Tissues and Metabolite Concentrations in Plasma

		Oral IFNT (μg/kg body weight/day)		
Variable	**Tissue**	**0**	**4**	**8**
Body weight (g)	Initial body weight	96.9 ± 1.6	97.2 ± 1.9	95.9 ± 1.3
	Final body weight	366.4 ± 8.9[a]	357.4 ± 0.47[a]	335.3 ± 7.5[b]
Tissue weight at the end of the study (g)	Liver	23.6 ± 0.67[a]	20.7 ± 0.30[b]	18.3 ± 0.46[c]
	Gastrocnemius muscle	1.32 ± 0.05	1.34 ± 0.04	1.38 ± 0.05
	Brown adipose tissue	1.06 ± 0.09[b]	1.09 ± 0.11[b]	1.55 ± 0.18[a]
	Abdominal adipose tissue	10.8 ± 0.33	10.9 ± 0.72	6.81 ± 0.72
	Inguinal adipose tissue	9.09 ± 0.35[a]	7.43 ± 0.47[b]	5.09 ± 0.49[c]
Oleate oxidation (nmol CO_2/mg tissue/2 h)	Liver	0.17 ± 0.01[b]	0.18 ± 0.01[b]	0.23 ± 0.01[a]
	Gastrocnemius muscle	0.30 ± 0.01[b]	0.31 ± 0.01[b]	0.39 ± 0.01[a]
	Brown adipose tissue	0.20 ± 0.01[b]	0.20 ± 0.01[b]	0.26 ± 0.01[a]
	Abdominal adipose tissue	0.015 ± 0.001	0.016 ± 0.001	0.016 ± 0.001
Glycerol release (nmol CO_2/mg tissue/2 h)	Gastrocnemius muscle	0.26 ± 0.02[b]	0.27 ± 0.02[b]	0.34 ± 0.02[a]
	Brown adipose tissue	1.04 ± 0.05[b]	1.12 ± 0.07[b]	1.36 ± 0.06[a]
	Abdominal adipose tissue	2.63 ± 0.14[b]	2.75 ± 0.15[b]	3.41 ± 0.19[a]
Glucose oxidation (nmol CO_2/mg tissue/2 h)	Liver	0.13 ± 0.01[b]	0.13 ± 0.01[b]	0.18 ± 0.01[a]
	Gastrocnemius muscle	0.15 ± 0.01[b]	0.16 ± 0.01[b]	0.20 ± 0.01[a]
	Brown adipose tissue	0.30 ± 0.01[b]	0.32 ± 0.01[b]	0.62 ± 0.03[a]
	Abdominal adipose tissue	0.14 ± 0.00[b]	0.14 ± 0.01[b]	0.19 ± 0.01[a]
Leucine oxidation[d] (nmol CO_2/mg tissue/2 h)	Liver	0.048 ± 0.004	0.050 ± 0.005	0.051 ± 0.004
	Gastrocnemius muscle	0.36 ± 0.02[b]	0.34 ± 0.01[b]	0.46 ± 0.01[a]
	Brown adipose tissue	0.72 ± 0.04	0.69 ± 0.04	0.68 ± 0.04
	Abdominal adipose tissue	0.27 ± 0.01	0.28 ± 0.02	0.27 ± 0.02
Net TA of leucine[e] (nmol CO_2/mg tissue/2 h)	Liver	0.059 ± 0.004	0.060 ± 0.006	0.062 ± 0.006
	Gastrocnemius muscle	0.46 ± 0.03[b]	0.47 ± 0.02[b]	0.63 ± 0.02[a]
	Brown adipose tissue	1.66 ± 0.09	1.61 ± 0.06	1.63 ± 0.11
	Abdominal adipose tissue	0.38 ± 0.02	0.37 ± 0.02	0.39 ± 0.03
Concentrations of metabolites in plasma	Leucine (μM)	245 ± 10[a]	233 ± 9[a]	196 ± 8[b]
	Glucose (mM)	24.5 ± 0.3[a]	23.8 ± 0.4[a]	21.9 ± 0.4[b]
	Free fatty acids (mM)	1.60 ± 0.06[a]	1.53 ± 0.05[a]	1.34 ± 0.05[b]
	Triacylglycerols (mM)	6.05 ± 0.13[a]	5.90 ± 0.27[a]	5.17 ± 0.11[b]
	Total cholesterol (mM)	5.18 ± 0.23[a]	4.94 ± 0.24[a]	4.23 ± 0.13[b]
	Insulin (pM)	307 ± 10	294 ± 11	301 ± 7
	Leptin (μg/L)	19.6 ± 1.2[a]	19.3 ± 1.0[a]	13.7 ± 0.9[b]

Source: Tekwe et al. (2013). Values are means ± SEM, n = 6. Beginning at 4 weeks of age, Zucker diabetic fatty rats (an animal model of type 2 diabetes) were fed a Purina 5008 diet (containing 23.5% crude protein, 6.0% fat, 34.9% starch, 2.6% sucrose, 0.5% glucose plus fructose, 6.8% minerals, 3.8% fiber, and 17,364 kJ) and received 0, 4, or 8 μg ovine IFNT/kg body weight/day via drinking water. At 12 weeks of age, rats were euthanized to obtain tissues for studies.

[a-c] Within a row, values not sharing the same superscript letter differ ($P < 0.05$).

[d] Oxidative decarboxylation of leucine.

[e] Net transamination (TA) of leucine is the sum of the oxidative decarboxylation of leucine and the net release of α-ketoisocaproate.

HSL is highly expressed in the WAT and steroidogenic tissues, and is found to a lesser extent in a variety of other tissues and cell types, including the heart, skeletal muscle, kidneys, pancreatic islets, testes, ovaries, and macrophages. In contrast, HSL is normally expressed at a very low level in hepatocytes, but its hepatic expression is upregulated by the activation of PPAR-γ (Reid et al. 2008). Under physiological conditions, HSL plays a key role in regulating lipolysis primarily in adipocytes and also contributes to steroidogenesis and spermatogenesis, as well as possibly insulin secretion and action in the whole body (Kraemer and Shen 2002).

Intracellular Lipolysis by HSL in Animal Tissues: Regulation of HSL Activity in Animal Tissues

HSL is the principal target for the regulation of intracellular lipolysis in the WAT, skeletal muscle and heart (Lee et al.

2014). This enzyme is regulated by both short-term (minutes to hours) and long-term (days to weeks) mechanisms. The short-term regulation involves the phosphorylation of proteins, particularly the cAMP-dependent PKA, to convert HSL from an inactive form (dephosphorylated) into an active form (phosphorylated). Without activation by PKA, HSL is inactive in tissues. PKA also phosphorylates perilipin to allow for the access of HSL to TAGs for their hydrolysis. Catecholamines, thyroid hormones, parathyroid hormone, growth hormone, and glucocorticoids activate HSL through increasing the intracellular concentration of cAMP, but insulin has the opposite effect (Table 6.7). In addition, interferon tau (an anti-inflammatory protein produced by ruminant conceptuses) stimulates lipolysis in skeletal muscle, WAT, and BAT (Table 6.8) possibly by activating HSL (Tekwe et al. 2013).

Intracellular Lipolysis by Adipose Triglyceride Lipase: Tissue Distribution and Function of ATGL

ATGL, which is localized in the cytosol and tightly associated with the lipid droplet, is another lipase that hydrolyzes intracellular TAGs to one molecule of DAGs and one molecule of free fatty acids in animal tissues. This TAG hydrolase has little or no activity for DAGs, MAGs, cholesteryl esters, or retinyl esters as substrates. ATGL is mainly expressed in the WAT and BAT but is also present at low levels in the testicles, pancreatic islets, heart, and skeletal muscle (Nagy et al. 2014). ATGL has strong specificity for TAGs containing LCFA esters ($C_{16–20}$) in the *sn*-2 position of the glycerol backbone. Likewise, ATGL acts on the phosphatidylcholine containing a PUFA at the *sn*-2 position. The main product of ATGL is 1,3-DAG, which serves as the preferential substrate of the lipogenic enzyme DGAT-2. Interestingly, after binding to the ATGL's co-activator, which is called CGI-58 (comparative gene identification 58), ATGL starts to hydrolyze the esters of fatty acids present in the *sn*-1 position, releasing 2,3-DAG. The latter is the preferential substrate of HSL. Thus, ATGL and HSL are responsible for the hydrolysis of 95% of TAGs in rodent and human white adipocytes. Interestingly, Fowler et al. (2015) reported that ATGL, not HSL, is the primary lipolytic enzyme in the WAT of fasting elephant seals.

Intracellular Lipolysis by Adipose Triglyceride Lipase: Regulation of ATGL Activity

ATGL is activated by CGI-58, as noted previously. The role of CGI-58 is epitomized by the findings that its genetic deficiency causes Chanarin-Dorfman syndrome, a rare neutral lipid-storage disease which is characterized by an abnormal accumulation of TAGs in nonlipid-storing cell types (e.g., skeletal muscle, heart, and skin), as well as skeletal myopathy and exercise intolerance. The enzymatic activity of ATGL is inhibited by insulin and long-chain fatty acyl-CoA but enhanced by glucocorticoids (Nagy et al. 2014; Perret et al. 2002). ATGL gene expression and protein abundance in the WAT are enhanced by fasting and glucocorticoids but down-regulated by re-feeding. Furthermore, ATGL expression is reduced in animal models (mice and rats) of obesity and type II diabetes mellitus.

Intracellular Lipolysis by Diacylglyceol Lipase (DGL) and Monoacylglycerol Lipase (MGL) in Animal Tissues: Functions of DGL and MGL

Although HSL can hydrolyze DAGs and MAGs, these esters of fatty acids are degraded by DGL and MGL, respectively, in a tissue-dependent manner. In adipocytes, which express little DGL, HSL is the only enzyme catalyzing DAG hydrolysis to form MAGs and fatty acids, and the rate of this reaction is 10- to 30-fold greater than that of TAG hydrolysis. In other cell types, DAGs are degraded by both HSL and DGL to release one molecule of MAGs plus one molecule of free fatty acids. For example, in the brain, DGL hydrolyzes DAGs containing arachidonic acid at the *sn*-2 position to generate one molecule of fatty acids and one molecule of 2-arachidonoylglycerol. The latter is the most abundant ligand for the cannabinoid receptors in the brain to regulate axonal growth and development, as well as the generation and migration of new neurons. 2-Arachidonoylglycerol is also a precursor of arachidonic acid in tissues.

MGL was first isolated from rat adipose tissue and is now known to be present in all other tissues, including the liver, skeletal muscle, heart, brain, lung, stomach, kidney, spleen, adrenal glands, and testes. This enzyme specifically hydrolyzes the *sn*-1 and *sn*-2 ester bonds of MAGs at equal rates but has no catalytic activity against DAGs, TAGs, or cholesteryl esters. In animal tissues (e.g., the brain), MGL is responsible for the hydrolysis of 85% of endocannabinoid 2-arachidonoylglycerol. Such a biochemical reaction regulates the concentration of endogenous cannabinoids (endocannabinoids). The latter are lipid molecules for (a) mediating retrograde signaling at central synapses and other forms of short-range neuronal communication (i.e., neurotransmission) and (b) regulating cell signaling and metabolism. The activities of DGL and MGL for hydrolyzing their respective substrates (DAGs and MAGs) are 10 to 30 times greater than the activities of HSL for TAGs, DAGs, and MAGs in the WAT and other tissues (Yang and Subbaiah 2015).

Intracellular Lipolysis by Diacylglyceol Lipase (DGL) and Monoacylglycerol Lipase (MGL) in Animal Tissues: Regulation of DGL and MGL Activities

DGL is constitutively active, and its enzymatic activity has been reported to be increased by cAMP-dependent protein phosphorylation and palmitoylation in animal tissues (Reisenberg et al. 2012). Palmitoylation, which involves the covalent linkage of a LCFA to a cysteine residue in a protein, can enhance the hydrophobicity of the enzyme, association with the membrane, protein–protein interaction, subcellular trafficking, and substrate access. Available evidence shows that MGL activity is not regulated by protein phosphorylation, but the expression of the MGL gene in the WAT and other tissues is enhanced by glucocorticoids and PPAR-α.

Intracellular Lipolysis by Diacylglyceol Lipase (DGL) and Monoacylglycerol Lipase (MGL) in Animal Tissues: Intracellular Lipolysis by Lysosomal Acid Lipase

LAL is present in the lysosomes of hepatocytes and nonhepatic tissues. This enzyme can hydrolyze intracellular TAGs and cholesteryl esters at an acidic pH and, therefore, is an acidic lipase. LAL is the only lipase that catalyzes intracellular degradation of TAGs in the liver, and is the primary enzyme that hydrolyzes cholesteryl esters derived from LDLs (Dubland and Francis 2015). The resulting cholesterol leaves the lysosome to enter the ER for re-esterification and formation of cytosolic lipid droplets. A deficiency of LAL results in the accumulation of fats in various tissues, such as the liver, small intestine, spleen, adrenal glands, lymph nodes, and the walls of blood vessels. Affected individuals, particularly neonates, exhibit digestive disorders and malabsorption, liver dysfunction or failure, and coronary artery disease.

OXIDATION OF FATTY ACIDS IN ANIMALS

Metabolic Fate of Fatty Acids: CO_2 Production and Ketogenesis

All animals can readily oxidize fatty acids to CO_2 and water in both fed and fasting states. However, not all animals have a high rate of oxidizing fatty acids to ketone bodies (acetoacetate, β-hydroxybutyrate, and acetone), even during a prolonged period of food deprivation. Acetoacetate and β-hydroxybutyrate fulfill physiological functions as metabolic fuels and signals, but acetone is a metabolic waste with an unpleasant odor (Robinson and Williamson 1980). The formation of ketone bodies from acetyl-CoA is known as ketogenesis. In the mitochondria of the liver, whether acetyl-CoA produced from the β-oxidation of LCFAs is oxidized to CO_2 or ketone bodies depends on intracellular concentrations of OAA and malonyl-CoA, CPT-I activity, and HMG-CoA synthase activity. An increase in the hepatic oxidation of fatty acids does not necessarily enhance the production of both CO_2 and ketone bodies from the substrates in the liver. For example, in the liver of rats fed a fat-containing diet, the oxidation of fatty acids to CO_2 and water is augmented but ketogenesis is suppressed, such that the concentrations of acetoacetate and β-hydroxybutyrate are relatively low (<0.2 mM). In contrast, food deprivation results in increased oxidation of fatty acids to CO_2 and the formation of ketone bodies in the liver of most animals (including rats, humans, and ruminants). Furthermore, consumption of a balanced diet containing adequate digestible carbohydrates suppresses ketogenesis while promoting the oxidation of fatty acids to CO_2 and water. These lines of evidence show the complex mechanisms responsible for the regulation of fatty acid oxidation to form CO_2 or ketone bodies. In animals, the rates of oxidation of LCFAs generally decrease with postnatal age.

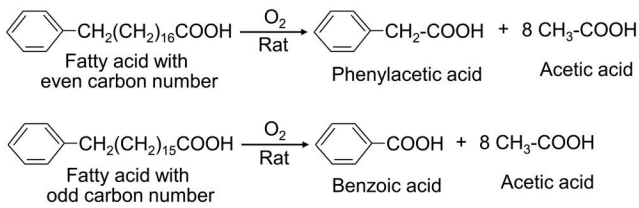
Mitochondrial β-Oxidation of Fatty Acids to CO_2 and Water

Under normal feeding and fasting conditions, fatty acids are a major source of energy for many tissues, including the liver (predominantly periportal hepatocytes), heart, and skeletal muscle. An increase in fatty acid oxidation to generate CO_2 and water occurs when dietary fat intake is increased. Thus, dietary supplementation with fats can improve lactation performance in sows under the conditions of high ambient temperatures (Rosero et al. 2012). During fasting, increased β-oxidation of fatty acids provides acetyl-CoA for the synthesis of large amounts of ketone bodies in most animal species. The ketone bodies serve as important metabolic fuels for the brain, heart, skeletal muscle, intestine, and kidneys when food intake is absent or severely inadequate. Thus, the β-oxidation of fatty acids occurs under conditions of both food deprivation and regular feeding.

Fatty acids with $<C_{20}$ are β-oxidized to CO_2 and water in the mitochondria. LCFAs and VLCFAs ($\geq C_{20}$) are β-oxidized in peroxisomes and the ER to generate shorter-chain fatty acids (e.g., C_{16} or C_{18}), which are then β-oxidized in the mitochondria. Based on tissue mass and the rate of β-oxidation, the liver, heart, skeletal muscle, and kidneys are the major tissues of fatty acid oxidation in animals. In the liver, this metabolic pathway occurs primarily in periportal hepatocytes, as does ketogenesis.

Pathway of Mitochondrial β-Oxidation of Fatty Acids

Early in the 20th century, Knoop (1904) fed rats fatty acid (even carbon number) derivatives with a phenyl group attached to the ω carbon and found that phenylacetic acid was a final product of the oxidation process. However, when fatty acid (odd carbon number) derivatives with a phenyl group attached to the ω carbon were fed to rats, benzoic acid was a final product of the oxidation process. Fifty years later, Lynen and Reichart (1951) discovered that the 2-carbon fragment from ß-oxidation was acetyl-CoA but not acetate.



Fatty acids are oxidized in the mitochondria, which facilitates the oxidation of its product, acetyl-CoA, via the Krebs cycle and the electron transport system. The major pathway of fatty acid oxidation is ß-oxidation, in which 2 carbons are cleaved at a time from acyl-CoA. This cleavage of C_2 units starts at the carboxyl end, with the chain being broken down between the α (C_2) and ß (C_3) carbon atoms, hence the name of ß-oxidation. The pathway of mitochondrial β-oxidation of a fatty acid includes its activation to acyl-CoA, the transfer of acyl-CoA from the cytosol into the mitochondrial matrix, and the ß-oxidation of acyl-CoA to form acetyl-CoA (Bartlett and Eaton 2004).

Activation of a Fatty Acid to Acyl-CoA

The first step in the oxidation of a fatty acid is the formation of its acyl-CoA by ACS (also called thiokinase).

Activation of an LCFA to its acyl-CoA by ACS_L occurs on either the outer mitochondrial membrane or the surface of the smooth ER. This is an ATP-dependent reaction, with two high-energy bonds in one ATP molecule being hydrolyzed to provide energy. The amount of energy needed by ACS is equivalent to the conversion of 2 ATP into 2 ADP + 2 Pi. The long-chain acyl-CoA formed in the cytosol will enter the mitochondrial matrix for ß-oxidation to yield acetyl-CoA.

$$\text{Long-chain fatty acid} + \text{ATP} + \text{CoA} \rightarrow \text{Long-chain acyl-CoA} + \text{AMP} + \text{PPi} \quad (\text{ACS}_\text{L} \text{ in the cytosol})$$

SCFAs and MCFAs are transported from the cytosol into the mitochondria, where these fatty acids are activated to form thiol ester bonds with CoA by mitochondrial ACS for SCFAs and MCFAs, respectively (i.e., ACS_S and ACS_M). For example, acetate and butyrate are converted by acetyl-CoA synthetase to form acetyl-CoA and butyryl-CoA, respectively. Through the mitochondrial β-oxidation, 1 mol of butyryl-CoA is cleaved into 2 mol of acetyl-CoA, with the generation of 1 mol of $FADH_2$ and 1 mol of NADH.

$$\text{Short-chain fatty acid} + \text{ATP} + \text{CoA} \rightarrow \text{Short-chain acyl-CoA} + \text{AMP} + \text{PPi} \quad (\text{ACS}_\text{S} \text{ in mitochondria})$$

$$\text{Medium-chain fatty acid} + \text{ATP} + \text{CoA} \rightarrow \text{Medium-chain acyl-CoA} + \text{AMP} + \text{PPi} \quad (\text{ACS}_\text{M} \text{ in mitochondria})$$

Transfer of Long-chain Acyl-CoA from the Cytosol into the Mitochondrial Matrix

The second step in LCFA oxidation is the transfer of long-chain acyl-CoA as acylcarnitine from the outer mitochondrial membrane into the mitochondrial matrix. This process involves carnitine palmitoyl-transferase I (CPT-I, in the outer membrane of mitochondria), carnitine palmitoyl-transferase II (CPT-II, on the inside of the inner membrane of mitochondria), and carnitine-acylcarnitine translocase (Figure 6.23). Carnitine is synthesized from lysine and methionine in the liver and kidneys. Without carnitine, LCFAs and long-chain acyl-CoA cannot enter the mitochondria. However, SCFAs and MCFAs can penetrate mitochondria without the need for carnitine. CPT-I represents the main site of control for the entry of LCFAs into the mitochondria. CPT-I is the rate-controlling step of the CPT system for hepatic fatty acid oxidation. In rats and pigs, the expression of CPT-I in the liver and extrahepatic tissues (e.g., skeletal muscle) is low at birth but is increased rapidly after birth. In contrast, the expression of CPT-II in the tissues is high during the fetal stage and does not seem to undergo a postnatal change.

The mammalian CPT-1 subfamily of proteins consists of three members: CPT-1A, 1B and 1C (Price et al. 2003). CPT-1C is restricted to the central neural tissues and its function is unknown. CPT-1A (also known as the liver isoform) is expressed in the liver, kidneys, pancreatic islets, and intestine, whereas CPT-1B (also known as the muscle isoform) is present at high activity in the heart, skeletal muscle, testes, and brown adipocytes (Brown et al. 1997).

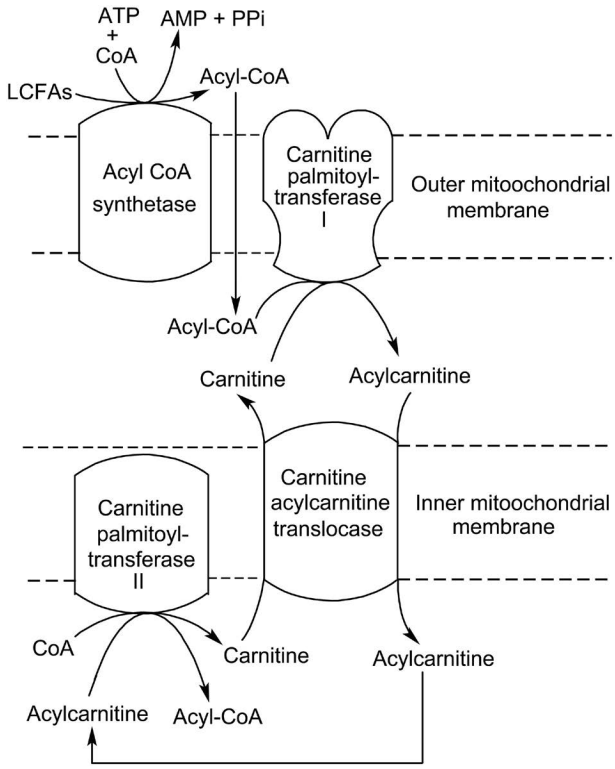


FIGURE 6.23 Transport of long-chain acyl-CoA from the cytosol into the mitochondrial matrix. This process requires carnitine palmitoyl-transferase I (CPT-I, in the outer membrane of mitochondria), carnitine palmitoyltransferase II (CPT-II, on the inside of the inner membrane of mitochondria), and carnitine-acylcarnitine translocase. LCFAs, long-chain fatty acids. (Adapted from Mayes 1996b.)

Compared with the muscle type of CPT-I, the liver isoform of CPT-I has a lower K_m for carnitine and is less sensitive, but still highly sensitive to inhibition by malonyl-CoA.

ß-Oxidation of Acyl-CoA to Form Acetyl-CoA

The third step in fatty acid oxidation is the ß-oxidation of acyl-CoA ($C_{4\text{-}19}$) to form acetyl-CoA (Figure 6.24). This involves four reactions catalyzed by acyl-CoA dehydrogenase (FAD-linked), enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase (NAD^+-linked), and 3-ketoacyl-CoA thiolase. In animals, the activities of enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase, and 3-ketoacyl-CoA thiolase are contained in the same protein, which is called the mitochondrial trifunctional protein. For the cleavage of 1 C_2 unit to form 1 mol of acetyl-CoA, 1 mol of NADH and 1 mol of $FADH_2$ are produced. The overall reaction of ß-oxidation of palmitate is as follows:

$$\text{Palmitate} + 8\text{CoA} + \text{ATP} + 7\text{FAD} + 7\text{NAD}^+ \rightarrow 8\text{Acetyl-CoA} + \text{AMP} + \text{PPi} + 7\text{FADH}_2 + 7\text{NADH} + 7\text{H}^+$$

Fatty acids with an odd carbon number are oxidized in a manner similar to that of those with an even carbon number, until the three-carbon molecule, propionyl-CoA, is generated. This product is further degraded via a series

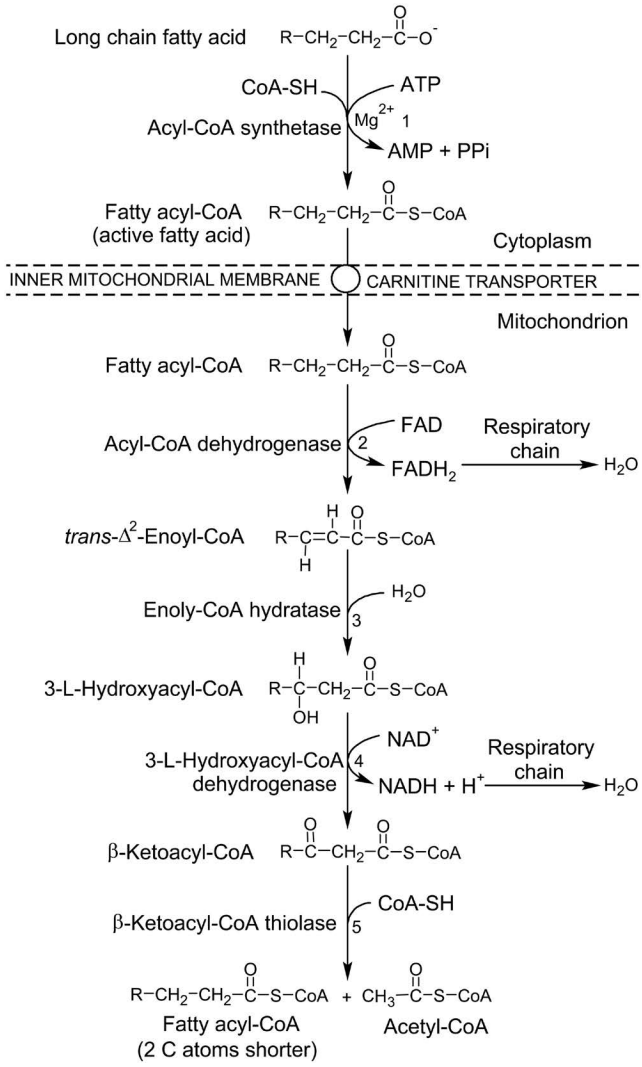


FIGURE 6.24 The ß-oxidation of long-chain acyl-CoA to form acetyl-CoA in the mitochondria of animal cells. In the cytosol, ACS converts a long-chain fatty acid into acyl-CoA, which is transported into the mitochondrial matrix through a carnitine-dependent transport system. During each cycle of the acyl-CoA oxidation, a 2-carbon unit (acetyl-CoA), 1 $FADH_2$, and 1 NADH + H^+ are generated. This biochemical process is called β-oxidation.

of three enzymes—the ATP-dependent propionyl-CoA carboxylase (using biotin as a cofactor), methylmalonyl-CoA racemase, and methylmalonyl-CoA mutase (using vitamin B_{12} as a cofactor)—to form succinyl-CoA. The latter enters the Krebs cycle for oxidation in various tissues, or is utilized for glucose synthesis in the liver. Thus, the propionyl residue from fatty acids with an odd carbon number is the only part of the fatty acids that is glucogenic in animals, particularly ruminants.

Energetics of Fatty Acid β-Oxidation

The major function of mitochondrial β-oxidation of fatty acids is to generate acetyl-CoA. The acetyl-CoA is then oxidized to CO_2 and water via the Krebs cycle and the mitochondrial electron transport system, producing ATP. The amount of ATP produced from the complete oxidation of fatty acids (e.g., 1 mol of palmitate as an example) can be calculated as follows:

$$7\ FADH_2 \rightarrow 10.5\ ATP\ (7 \times 1.5 = 10.5)$$
$$7\ NADH + H^+ \rightarrow 17.5\ ATP\ (7 \times 2.5 = 17.5)$$
$$8\ Acetyl\text{-}CoA \rightarrow 80\ ATP\ (8 \times 10 = 80)$$

The total amount of ATP produced is 108. Because 2 ATP are consumed during the activation of palmitate to palmityl-CoA, the amount of net ATP production for the oxidation of 1 mol of palmitate is 106 mol. The oxidation of fatty acids is the major source of energy for the liver, heart, skeletal muscle and kidneys. In hepatocytes, this metabolic pathway is coupled with gluconeogenesis during fasting.

Oxidation of Long-Chain Unsaturated Fatty Acids

MUFAs (i.e., fatty acids with one double bond) are oxidized by the same set of reactions as saturated fatty acids with even carbon numbers, until the double bond is reached. The enzyme *cis*-Δ^3-enoyl-CoA-isomerase converts the double bond in the *cis* configuration into a *trans* configuration. The newly formed *trans* fatty acid bypasses the hydratase reaction and enters the β-oxidation pathway. Figure 6.25 illustrates the pathway for the oxidation of oleic acid ($C_{18:1}$)

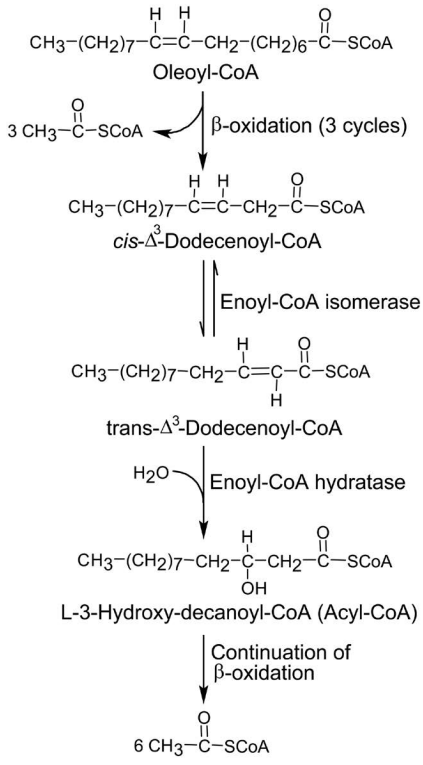


FIGURE 6.25 Oxidation of oleic acid ($C_{18:1}$) as an example of monounsaturated fatty acids that generate acetyl-CoA in the mitochondria of animal cells. Enoyl-CoA isomerase (a mitochondrial enzyme) catalyzes the conversion of a *cis*-or *trans*-double bond of a fatty acid at the γ-carbon (position 3) to a *trans* double bond at the β-carbon (position 2).

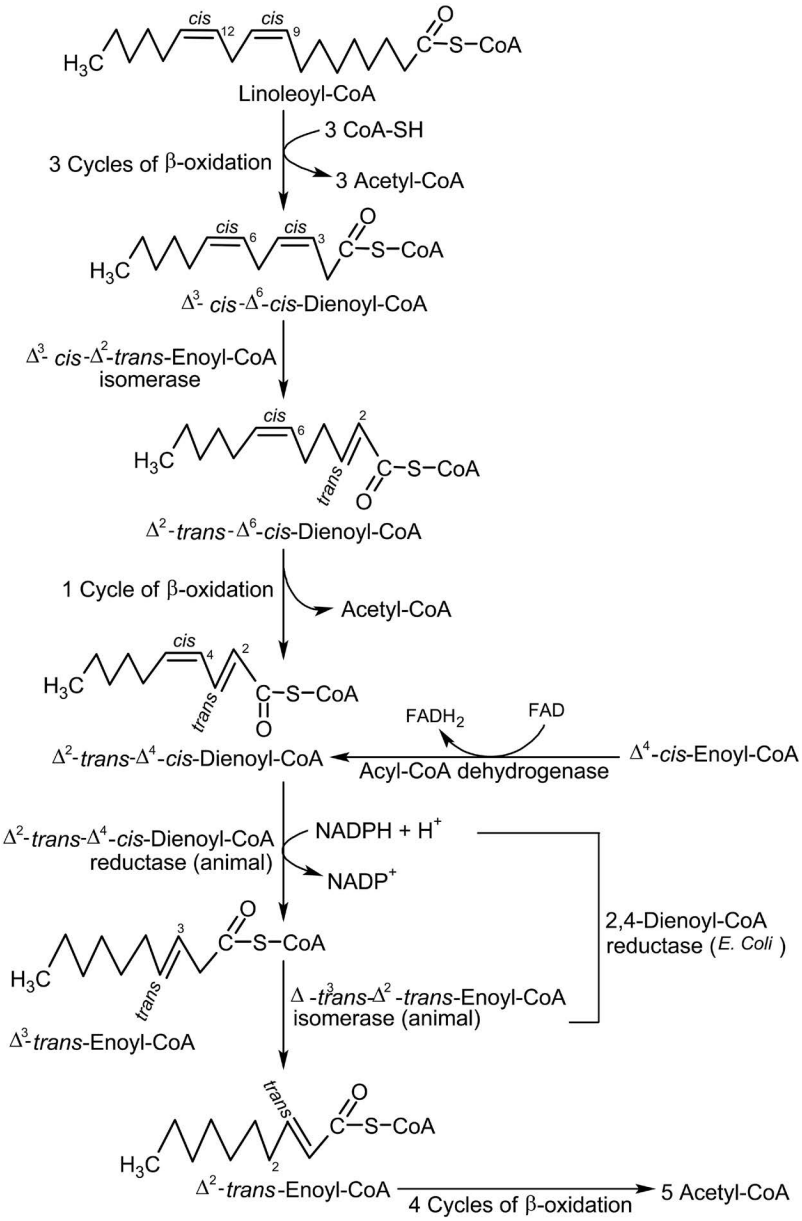


FIGURE 6.26 Oxidation of linoleic acid ($C_{18:2}$) as an example of polyunsaturated fatty acids that generate acetyl-CoA in the mitochondria of animal cells.

as an example for MUFAs to acetyl-CoA. The oxidation of PUFAs follows a similar path to that of MUFAs, except that an additional NADPH-dependent enzyme, 2,4-dienoyl-CoA reductase, is required to reduce one double bond, as shown for the oxidation of linoleic acid ($C_{18:2}$) as an example for PUFAs in Figure 6.26.

Oxidation of Short- and Medium-Chain Fatty Acids

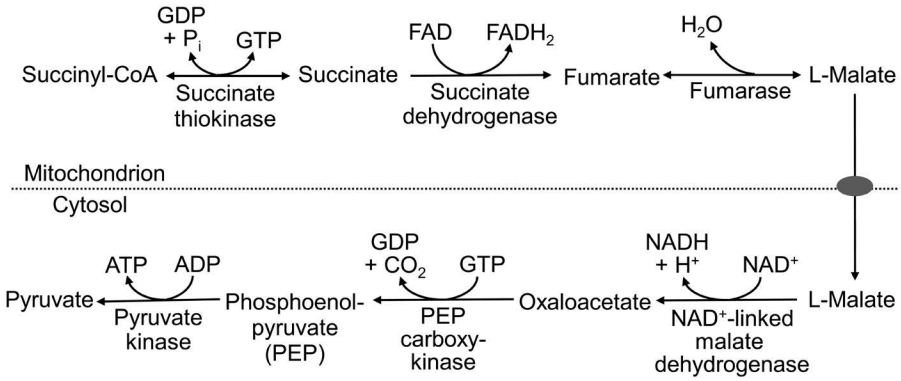
SCFAs and MCFAs are transported from the cytosol into the mitochondria, where these fatty acids are activated to form thiol ester bonds with CoA by mitochondrial ACSs. There are ACS for SCFAs ACS for (ACS_S) and ACS for MCFAs (ACS_M) in animal cells. For example, acetate and butyrate are converted by ACSs to form acetyl-CoA and butyryl-CoA, respectively. Through the mitochondrial β-oxidation, 1 mol of butyryl-CoA is converted into acetoacetyl-CoA (a β-ketoacyl-CoA). The latter is further oxidized to form 2 mol of acetyl-CoA, with the generation of 1 mol of $FADH_2$ and 1 mol of NADH. Alternatively, butyrate can be metabolized to D-β-hydroxybutyrate via the formation of acetoacetyl-CoA and then of HMG-CoA (by HMG-CoA synthase), as described in the following section on ketogenesis.

$$\text{Short-chain fatty acid} + \text{ATP} + \text{CoA} \rightarrow \text{Short-chain acyl-CoA} + \text{AMP} + \text{PPi} \left(\text{ASC}_\text{S} \text{ in mitochondria}\right)$$

$$\text{Medium-chain fatty acid} + \text{ATP} + \text{CoA} \rightarrow \text{Medium-chain acyl-CoA} + \text{AMP} + \text{PPi} \left(\text{ASC}_\text{M} \text{ in mitochondria}\right)$$

Propionate is converted into succinyl-CoA via a series of enzymes, as described in Chapter 5.

Succinyl-CoA is metabolized to pyruvate through the following reactions. Depending on physiological conditions, pyruvate is either oxidized to CO_2 plus H_2O or is used for hepatic glucose production (Chapter 5). Under physiological conditions, propionate is the major substrate for gluconeogenesis in ruminants.



Regulation of Mitochondrial Fatty Acid β-Oxidation

The rates of mitochondrial β-oxidation of fatty acids in multiple tissues are controlled by the availability of extracellular and cytosolic fatty acids, long-chain acyl-CoA transport through the mitochondrial membrane, as well as intramitochondrial enzymes, cofactors and redox state. The factors that influence fatty acid synthesis and lipogenesis also affect the mitochondrial β-oxidation of fatty acids (Table 6.7).

ACC, CPT-I, Hormones, and AMPK

CPT-I is required for the entry of long-chain acyl-CoA from the cytosol into the mitochondrial matrix. Thus, the activity of CPT-I is a rate-controlling factor for mitochondrial β-oxidation (McGarry 1995). In the liver and possibly other insulin-sensitive tissues (e.g., heart and skeletal muscle), CPT-I exerts ~80% of control over the β-oxidation of LCFAs under normal conditions (Bartlett and Eaton 2004). As discussed previously, glucagon, epinephrine, norepinephrine, growth hormone, thyroid hormones, and glucocorticoids inhibit ACC, thereby decreasing the availability of malonyl-CoA (an allosteric inhibitor of CPT-I) and promoting LCFA oxidation in animal tissues. Similarly, AMPK inhibits ACC by phosphorylating this protein, leading to a reduction in malonyl-CoA availability and an increase in LCFA oxidation. In contrast, insulin activates ACC to inhibit the oxidation of fatty acids. Furthermore, cGMP-dependent protein kinase G activates AMPK and inhibits ACC through protein phosphorylation, thereby promoting the oxidation of fatty acids (Jobgen et al. 2006).

Under the conditions of fasting, the concentration of malonyl-CoA in the liver, skeletal muscle, and heart is reduced to promote fatty acid oxidation (Saha et al. 1995). Similar metabolic changes are observed in type I diabetic patients with severe insulin deficiency. In contrast, the concentration of malonyl-CoA in skeletal muscle is elevated in obese and type II diabetic subjects due to increased ACC activity, contributing to impaired oxidation of LCFAs, their intramuscular accumulation, and insulin resistance. Likewise, elevated levels of glucose induce a rise in hepatic and muscular malonyl-CoA, thereby attenuating fatty acid oxidation.

Availabilities of Metabolites

Cofactors and substrates (e.g., OAA, CoA, NADH, NAD^+, FAD, and acyl-CoA), which can affect the activities of dehydrogenases, reductases, citrate synthase, and oxidases, can influence the rate of mitochondrial β-oxidation. For example, if mitochondrial NAD^+ and FAD are limited, and if the re-oxidation of NADH and $FADH_2$ via the mitochondrial electron transport system (e.g., due to reduced coenzyme Q pool, enzyme deficiency, enzyme inhibition, a high ratio of ATP/ADP, or hypoxia) is reduced, β-oxidation itself will be inhibited. Any accumulation of 3-hydroxyacyl-CoA esters following the inhibition of the 3-hydroxyacyl-CoA dehydrogenases will cause feedback inhibition of the 2-enoyl-CoA hydratases and then the acyl-CoA dehydrogenases, leading to impaired production of acetyl-CoA. Furthermore, because mitochondrial CoA concentration is low, intramitochondrial accumulation of acyl-CoA can reduce the availability of free CoA for 3-ketoacyl-CoA thiolase, leading to the accumulation of 3-ketoacyl-CoA, which inhibits 3-hydroxyacyl-CoA dehydrogenase, 2-enoyl-CoA hydratase and acyl-CoA dehydrogenase. Finally, because acetyl-CoA is also an inhibitor of 3-ketoacyl-CoA thiolase, an increase in the ratio of acetyl-CoA/CoA will result in the inhibition of 3-ketoacyl-CoA thiolase, the accumulation of 3-ketoacyl-CoA, and then the inhibition of β-oxidation.

Peroxisomal β-Oxidation Systems I and II

In animals, straight-chain VLCFAs ($\geq C_{20}$), branched-chain VLCFAs ($\geq C_{20}$), dicarboxylic acids, and bile acid

derivatives are β-oxidized through peroxisomal pathways I and II (Van Veldhoven 2010). Peroxisomal ß-oxidation of fatty acids was discovered initially in germinating castor bean seedlings in 1969 and was subsequently found to occur in the rat liver in 1976. It is now known that the peroxisomal ß-oxidation of VLCFAs takes place in the kidneys and other tissues. The products of this metabolic pathway are medium- and long-chain acyl-CoA, as well as H_2O_2 and acetyl-CoA. Peroxisomes also play an important role in the synthesis of bile acids from cholesterol at the steps of side-chain oxidation and cleavage.

Activation of Very Long-Chain Fatty Acids into Very Long-Chain Acyl-CoA

In this metabolic pathway, VLCFAs are first activated by ACS for VLCFAs to form VLCFA acyl-CoA in the cytosol. Peroxisomal system I (also known as the classical peroxisomal ß-oxidation system) is responsible for the ß-oxidation of straight-chain VLCFAs, whereas peroxisomal system II is responsible for the oxidation of branched-chain VLCFAs (Reddy and Hashimoto 2001). Peroxisomal β-oxidation of fatty acids is of minor importance for ATP production, but its related disorders can result in diseases, such as neurological abnormalities.

Transport of Very Long-Chain Acyl-CoA from the Cytosol into the Peroxisome

VLCFA acyl-CoA enters the peroxisome from the cytosol via an ABC transporter protein. This transport system does not require carnitine but is facilitated by ATP, which changes the conformation of the protein transporter. In mammalian peroxisomes, the ABC transport protein is called adrenoleukodystrophy (ALD) protein. The significance of the ALD protein is epitomized by the finding that its inborn deficiency causes damage to the myelin sheath (an insulating membrane that surrounds nerve cells in the brain) and death.

Shortening of Very Long-Chain Fatty Acyl-CoA

The subsequent peroxisomal reactions for the peroxisomal β-oxidation of VLCFA acyl-CoA include dehydrogenation, hydration, a second dehydrogenation, and the thiolytic cleavage (Figure 6.27), which are equivalent to the mitochondrial β-oxidation of fatty acids. However, the first step of the peroxisomal pathway is catalyzed by acyl-CoA oxidase (not by acyl-CoA dehydrogenase like the mitochondrial β-oxidation pathway). In addition, L-bifunctional protein (enoyl-CoA hydratase and L-3-hydroxyacyl-CoA dehydrogenase) catalyzes the conversion of enoyl-CoA into L-3-ketoacyl-CoA in system I, whereas D-bifunctional protein (enoyl-CoA hydratase and D-3-hydroxyacyl-CoA dehydrogenase) catalyzes the conversion of enoyl-CoA into D-3-ketoacyl-CoA in system II. In both systems I and II, the peroxisomal ß-oxidation of VLCFAs generates acetyl-CoA, as well as medium- and long-chain fatty acyl-CoA, which are subsequently transported into the mitochondria for further β-oxidation.

Regulation of Peroxisomal β-Oxidation

Peroxisomal ß-oxidation is regulated by a variety of PPAR ligands through their binding to various PPARs, such as PPAR-α, PPAR-β (also called PPAR-δ), and PPAR-γ (1, 2

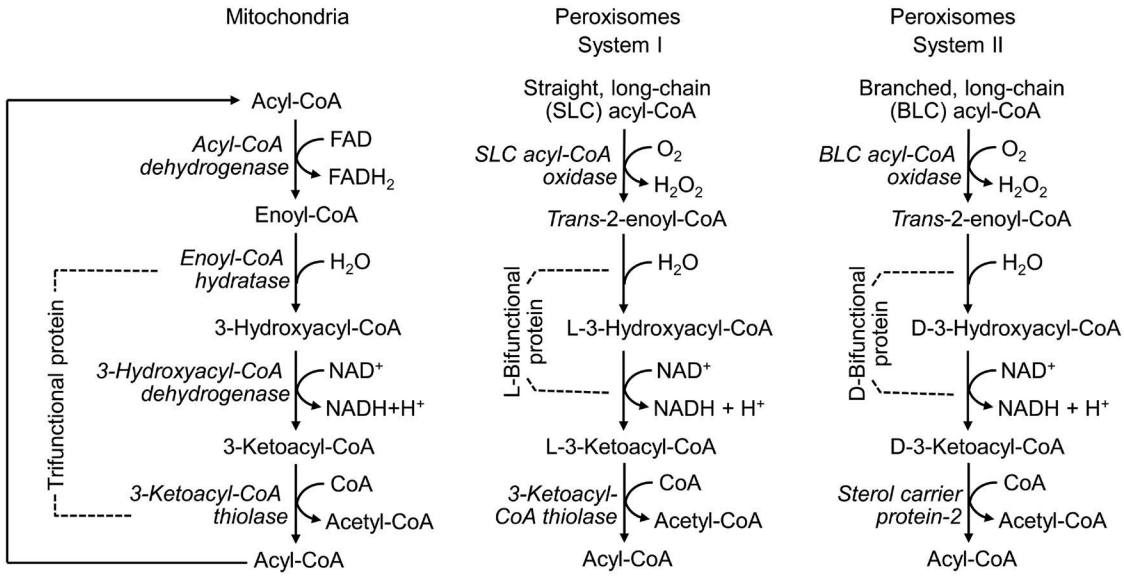


FIGURE 6.27 Peroxisomal ß-oxidation of long and very long-chain fatty acids in comparison with mitochondrial ß-oxidation. In animals, long- and very long-chain fatty acids ($\geq C_{20}$), as well as dicarboxylic acids, branched long-chain fatty acids, branched very long-chain fatty acids, and bile acid derivatives, are β-oxidized by two peroxisomal pathways. The resulting shorter-chain fatty acyl CoA products enter the mitochondria for final oxidation to CO_2 and water.

TABLE 6.9
Expression and Ligands of Peroxisome Proliferator Activated Receptors in Animal Tissues

PPAR	Expression in Tissues	Ligands
PPAR-α:	Mainly in hepatocytes and skeletal muscle; also in the heart, proximal tubular epithelium of kidney, brown adipose tissue, adrenal gland, and small intestine. Up-regulation of fatty acid oxidation and ketogenesis during fasting, and of fatty acid oxidation in the fed state.	Fenofibrate (Tricor), glitazones, and Gemfibrozil (synthetic); ω3 PUFAs, other long-chain fatty acids, high-fat diet; adrenal steroid dehydroepiandrosterone; and eicosanoids derived from arachidonic acid (natural)
PPAR-β (PPAR-δ)	Most abundant in skeletal muscle; widely expressed in animal tissues, such as heart, liver, kidneys, and Adipose tissue. Increasing cell differentiation; decreasing cell proliferation; promoting glucose and fatty acid oxidation.	GW1516 (synthetic) (now abandoned due to its carcinogenic effect); ω3 PUFAs and their metabolites (natural ligands)
PPAR-γ1	Virtually all tissues. Increasing epithelial cell Differentiation.	Thiazolidinediones (glitazones, synthetic), ω3 PUFAs
PPAR-γ2	Mainly in white adipose tissue. Increasing adipogenesis; improving insulin sensitivity.	Thiazolidinediones (glitazones, synthetic), ω3 PUFAs
PPAR-γ3	Mainly in M2-macrophages and white adipose tissue (anti-inflammatory)	Thiazolidinediones (glitazones, synthetic), ω3 PUFAs

Sources: Puri (2011), Reddy and Hashimoto (2001), and Van Veldhoven (2010).
Note: PPAR, peroxisome proliferator-activated receptors; PUFAs, polyunsaturated fatty acids.

and 3; Table 6.9). PPAR-α, which promotes fatty acid oxidation, is expressed mainly in the liver and skeletal muscle. PPAR-γ1 is present in all tissues, PPAR-γ2 (up-regulating fat storage) is predominant in the WAT, and PPAR-γ3 (anti-inflammatory) is highly expressed in M2-macrophages and the WAT. Among animal tissues, PPAR-δ/β (mimicking the effect of exercise) is most abundant in skeletal muscle. PPAR ligands, which are designated as peroxisome proliferators, include a broad spectrum of synthetic and naturally occurring compounds [e.g., thiazolidinediones (glitazones), DHA, and EPA], as well as eicosanoids and their metabolites. These substances enter the nucleus of the cell to exert their physiological or pharmacological effects.

Peroxisomal system I is transcriptionally induced by PPAR-α ligands. As a member of the nuclear hormone receptor superfamily, PPAR-α is highly expressed in hepatocytes, BAT, enterocytes, proximal tubular epithelium of the kidneys, adrenal gland, and heart muscle, and, to a lesser extent, in skeletal muscle. Synthetic PPAR-α ligands include hypolipidemic drugs (e.g., fenofibrate or Tricor, and gemfibrozil), certain food flavors, and leukotriene D_4 receptor antagonists. Natural/biological PPAR-α ligands include LCFAs and VLCFAs (particularly ω3 PUFAs), a high-fat diet, the adrenal steroid dehydroepiandrosterone, and eicosanoids derived from arachidonic acid via the lipoxygenase and cyclooxygenase pathways (Puri 2011). In general, PPAR-α ligands exhibit little obvious structural similarity, and the only shared chemical feature is that they have the potential to be transformed into a carboxylic acid derivative. Activated PPAR-α interacts with 9-*cis*-retinoic acid receptor (RXR) to form PPAR-α/RXR heterodimers, which then bind with the peroxisome proliferator response elements localized in numerous gene promoters to increase the expression of all enzymes of peroxisomal system I. PPAR-α is activated under conditions of energy deprivation to β-oxidize fatty acids and produce ketone bodies by promoting the uptake, utilization, and catabolism of fatty acids. As a result, synthetic PPAR-α ligands, such as fenofibrate and gemfibrozil, can reduce plasma concentrations of triglycerides and total cholesterol.

In addition to PPAR-α, PPAR-γ and PPAR-δ (all are nuclear receptors) have been identified in animals. There are three PPAR-γ proteins (PPAR-γ1 PPAR-γ2 and PPAR-γ3) which differ at their 5-prime ends, with each under the control of its own promoter. PPAR-γ is expressed predominantly in the WAT, liver, mammary glands, urinary bladder, colonic mucosa, and M2-macrophages, and may play an important role in adipogenesis, epithelial differentiation, and anti-inflammatory response. Thus, PPAR-γ can improve insulin sensitivity in obese subjects. PPAR-δ is widely expressed in most tissues, and its function may be to promote epithelial cell development and cell proliferation, as well as glucose and fatty acid oxidation.

Role of Peroxisomal β-Oxidation in Ameliorating Metabolic Syndrome

The primary function of the peroxisomal β-oxidation is to shorten VLCFAs so that they can be further degraded to CO_2 and water in mitochondria. Physiologically or pharmacologically active PPAR ligands promote the deposition of plasma fatty acids as TAGs in the WAT, the oxidation of fatty acids in the liver and skeletal muscle, and anti-inflammatory reactions in macrophages and the circulatory system. For example, some antidiabetic drugs (e.g., glitazones) bind PPAR-α and PPAR-γ, causing a marked increase in peroxisomes and a decrease in the circulating levels of TAGs, VLDLs, and LDLs (Puri 2011). These drugs can also attenuate the long-chain fatty acyl-CoA-induced activation of NFκB in skeletal muscle, thereby improving the insulin signaling pathway (DeFronzo 2010). Thus, like metformin and sulfonylureas, PPAR ligands or agonists aid in

reducing the circulating levels of glucose and ameliorating dyslipidemia in obese and diabetic animals, reducing the risk for the development of cardiovascular disease.

Production and Utilization of Ketone Bodies in Animals

Production of Ketone Bodies Primarily by the Liver

Under certain metabolic conditions (e.g., fasting, a low ratio of insulin/glucagon, under-feeding, or energy deficits as occurring during lactation, pregnancy, and exercise), most animal species (e.g., cattle, sheep, goats, rats, dogs, and chicks) are capable of producing large amounts of ketone bodies from fatty acid-derived acetyl-CoA (Beitz 1993). This mitochondrial process occurs mainly in the liver (predominantly periportal hepatocytes) and is illustrated in Figure 6.28, with HMG-CoA synthase being a rate-controlling enzyme (Hegardt 1999). The conversion of acetyl-CoA to acetoacetate involves the formation of acetoacetyl-CoA and HMG-CoA by thiolase, HMG-CoA synthase, and HMG-CoA lyase. Finally, acetoacetate is reduced by D-β-hydroxybutyrate dehydrogenase to form β-hydroxybutyrate, and this reversible reaction is present in all cells with mitochondria. The oxidation of LCFAs to ketone bodies requires O_2 but does not produce CO_2. Note that β-hydroxybutyrate is chemically not a ketone according to the International Union of Pure and Applied Chemistry (IUPAC) nomenclature, although this metabolite has been so called for historical reasons.

$$\text{Acetoacetate} + \text{NADH} + \text{H}^+ \rightleftharpoons \beta\text{-Hydroxybutyrate} + \text{NAD}^+ \left(\text{Mitochondrial } \beta\text{-hydroxybutyrate dehydrogenase}\right)$$

$$\text{Palmitate}\left(C_{16}H_{32}O_2\right) + 7\ O_2 \rightarrow \text{Acetoacetate}\left(C_4H_6O_3\right) + 4\ H_2O$$

$$\text{Long-chain fatty acids} + O_2 \rightarrow \text{Ketone bodies}\left(\text{but no } CO_2\right)$$

Ketogenesis occurs primarily in the same periportal hepatocytes of the liver as the mitochondrial β-oxidation of fatty acids and, to a much lesser extent, in colonocytes. Ruminal epithelial cells are also capable of producing both acetoacetate and β-hydroxybutyrate. The synthesis of ketone bodies is associated with the production of H^+. Thus, a high rate of ketogenesis in uncontrolled diabetes, during early lactation in cows, and during late pregnancy results in severe ketosis (Beitz 1993). If the H^+ is not buffered or removed, a low pH in the blood is life-threatening as in patients with type I diabetes. In animal production, ketosis causes a decreased milk yield in cattle, as well as pregnancy toxemia in sheep and goats (particularly multi-fetal pregnancies).

Interestingly, ketogenesis in young or adult pigs is very limited due to a low or negligible activity of HMG-CoA synthase (Duée et al. 1994). The mitochondrial HMG-CoA

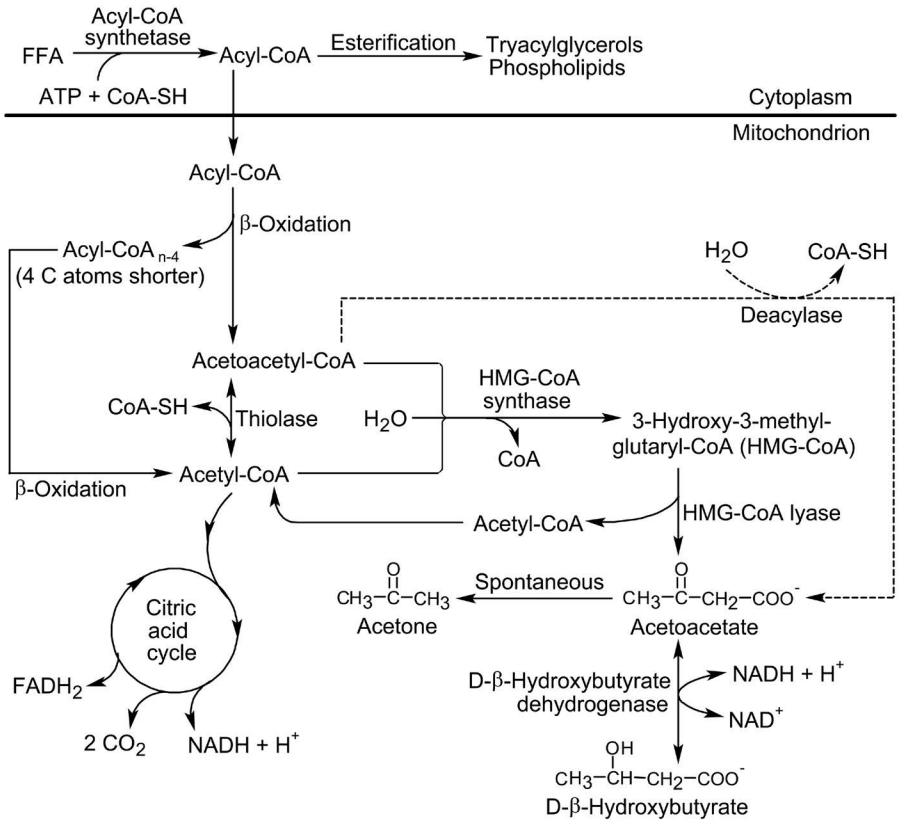


FIGURE 6.28 Mitochondrial production of ketone bodies (acetoacetate, D-β-hydroxybutyrate, and acetone) from acetyl-CoA in animals. All these reactions occur in the mitochondria of mammals, birds, and fish. In pigs, ketogenesis is limited due to a very low activity of HMG-CoA synthase.

TABLE 6.10
Concentrations of Ketone Bodies in the Plasma of Young Chickens and Piglets Fasted for 12 and 24 Hours

	Chickens[a]		Piglets[b]	
Nutritional Status	**D-β-Hydroxybutyrate**	**Acetoacetate**	**D-β-Hydroxybutyrate**	**Acetoacetate**
Fed	0.15 ± 0.002	0.11 ± 0.01	0.071 ± 0.004	0.054 ± 0.003
12-h fast	1.34 ± 0.01	0.78 ± 0.04	0.073 ± 0.005	0.056 ± 0.004
24-h fast	2.63 ± 0.02	0.89 ± 0.05	0.076 ± 0.005	0.058 ± 0.004

Values, expressed as mM, are means ± SEM, n = 10.

[a] Blood samples were obtained from 10-day-old male broiler chickens that were fed or had been fasted for 12 h or 24 h. (Wu and Thompson 1987)

[b] Blood samples were obtained from 7-day-old male piglets that were fed by sows or had been fasted for 12 h or 24 h. Ketone bodies were analyzed as described by Wu et al. (1991).

synthase protein in the liver of 48 h-old unsuckled pigs is barely detectable. Thus, the concentrations of ketone bodies are very low in the plasma of pigs (<0.2 mM) even during a 2-day or longer period of food deprivation (Gentz et al. 1970), although these metabolites are also produced from the catabolism of ketogenic amino acids. Fasting for 12 or 24 h did not affect the circulating levels of acetoacetate or D-β-hydroxybutyrate in young pigs (Table 6.10). This metabolic feature in pigs, coupled with their lack of BAT, increases the risks for hypoglycemia as well as high rates of morbidity and mortality during the neonatal period.

Regulation of Hepatic Ketogenesis

Regulation of hepatic ketogenesis involves multiple tissues: (a) the WAT, which supplies free fatty acids to the liver; (b) the liver, which either oxidizes fatty acids or synthesizes TAGs; and (c) the skeletal muscles and other tissues (e.g., heart, kidneys, brain, and small intestine) that oxidize ketone bodies. At the cellular level, ketogenesis is affected by (a) the intracellular concentrations of fatty acids, acetyl-CoA, OAA, and malonyl-CoA and (b) the activities of CPT-I and HMG-CoA synthase. All of these factors are subject to metabolic control by insulin, glucagon, and the ratio of insulin to glucagon (Figure 6.29).

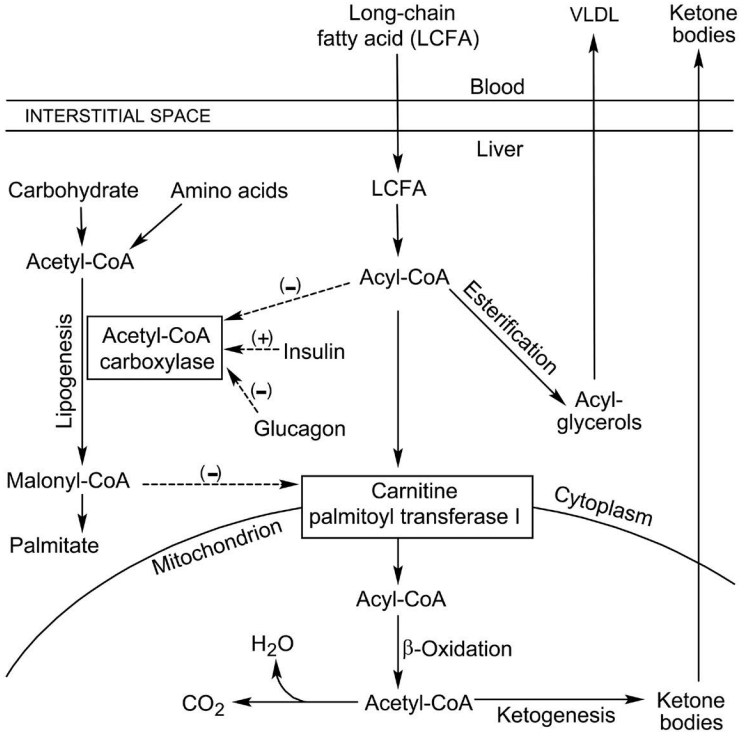


FIGURE 6.29 Regulation of mitochondrial ketogenesis in the liver of animals. The rates of hepatic ketogenesis are affected by the concentrations of free fatty acids in plasma, the hepatic oxidation of fatty acids to form acetyl-CoA, and the expression of the required proteins in hepatocytes. All of these factors are influenced by insulin, glucagon, and the ratio of insulin to glucagon. VLDL, very low-density lipoprotein.

A substantial increase in the production of ketone bodies does not occur in vivo unless blood levels of free fatty acids produced from the lipolysis of TAGs in the WAT are markedly elevated, as occurs during food deprivation (Prior and Smith 1982). As shown in Table 6.10, 12- and 24-h fasting in 10-day-old chickens increases the plasma concentration of acetoacetate by 6.1- and 7.1-fold, respectively, and of D-β-hydroxybutyrate by 7.9- and 16.5-fold, respectively. Increasing intracellular concentrations of OAA through the intravenous administration of sodium pyruvate, sodium propionate, or glucose along with an electrolyte solution, or through dietary supplementation with propylene glycol (a synthetic organic compound), corn syrup, or molasses (sources of digestible carbohydrates), can effectively treat ketosis in ruminants. The conversion of propylene glycol into L-lactate and then D-glucose for the inhibition of hepatic ketogenesis is outlined in Figure 6.30. A reduction in the availability of malonyl-CoA due to the inhibition of ACC promotes the oxidation of LCFAs and the production of ketone bodies. Finally, intravenous or intramuscular administration of insulin reduces hepatic ketogenesis and the circulating levels of ketone bodies in patients with type I diabetes. The relationship between metabolic states and hepatic ketogenesis is summarized in Table 6.11. Note that high-fat or high-protein feeding increases, but high-carbohydrate ingestion decreases, hepatic ketogenesis through altering the circulating levels of hormones (mainly insulin and glucagon) and substrates (Kellard et al. 2020; Moon and Koh 2020).

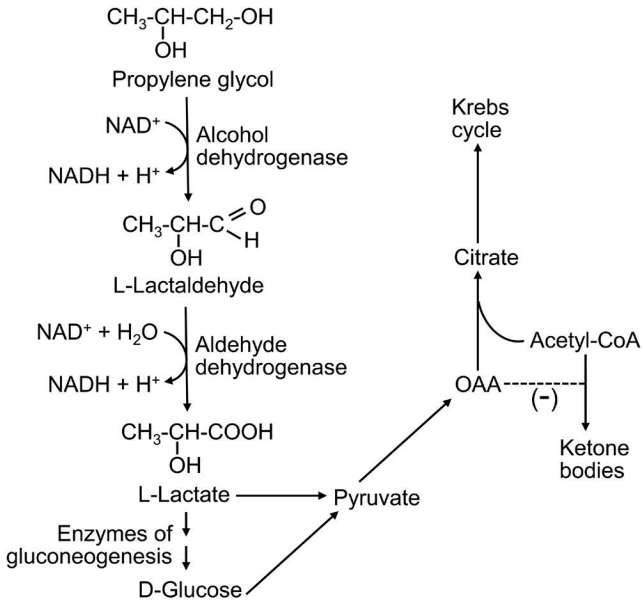


FIGURE 6.30 Metabolism of propylene glycol to pyruvate and glucose for inhibiting hepatic ketogenesis in ruminants.

Utilization of Ketone Bodies by Extrahepatic Tissues

The liver of animals cannot oxidize ketone bodies to acetyl-CoA, because of the lack of enzymes [3-ketoacid-CoA transferase (also known as succinyl-CoA:3-ketoacid-CoA transferase) and acetoacetyl-CoA synthetase] for the conversion of acetoacetate to acetoacetyl-CoA (Robinson and Williamson 1980). In contrast, many extrahepatic tissues, which include the brain, skeletal muscle, heart, kidneys, WAT, and small intestine, possess one or both of these two enzymes for the activation of acetoacetate to form acetoacetyl-CoA (Figure 6.31). The 3-ketoacid-CoA transferase and acetoacetyl-CoA synthetase are localized in the mitochondrial matrix and the cytosol of all extrahepatic tissues, respectively. Thus, in the presence of CoA-SH, acetoacetate and D-β-hydroxybutyrate are extensively oxidized in the extrahepatic tissues.

Ketone bodies are not metabolic waste. They are the major energy source for the brain during prolonged fasting when blood glucose concentrations are low. Ketone bodies inhibit the catabolism of protein, amino acids, and glucose in extrahepatic tissues to spare these nitrogen reserves

TABLE 6.11
Nutritional and Hormonal Regulation of Hepatic Ketogenesis in Animals

	Hormone Concentration					
Feeding or Diabetes	Insulin	Glucagon	Malonyl-CoA Concentration	Fatty Acid Synthesis	Fatty acid Oxidation	Ketogenesis
High carbohydrate	↑	↓	↑	↑	↓	↓
High-fat[a]	↑	↑	↓	↓	↑	↑
High-protein[b]	↑	↑	↓	↓	↑	↑
Starvation[c]	↓	↑	↓	↓	↑	↑
Uncontrolled diabetes	↓	↑	↓	↓	↑	↑

[a] Inducing insulin resistance and inhibiting acetyl-CoA carboxylase activity.
[b] Consuming an isocaloric diet with increased protein but decreased carbohydrate intake without changes in fat intake.
[c] Starvation increases the circulating level of growth hormone, thereby promoting lipolysis and fatty-acid release from the white adipose tissue to support hepatic ketogenesis.
Note: ↑, increase; ↓, decrease.

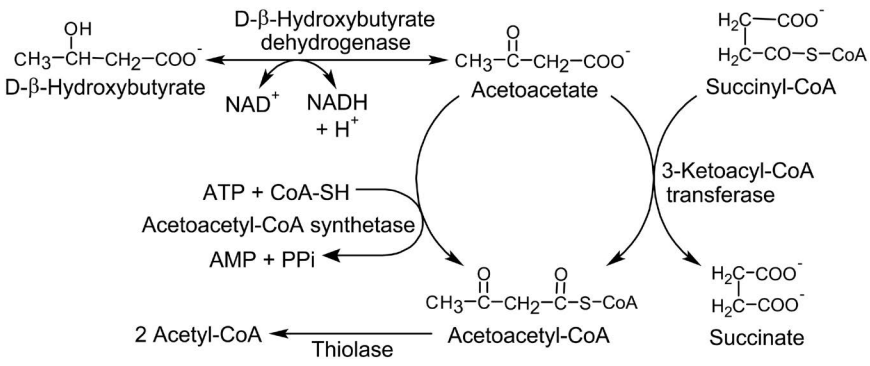


FIGURE 6.31 Utilization of ketone bodies by extrahepatic tissues. All these reactions occur in the tissues of mammals, birds, and fish, except for the livers. The liver lacks the enzyme for converting acetoacetate to acetyl-CoA, and, therefore, cannot utilize the ketone bodies.

and nutrients in the case of starvation (Thompson and Wu 1991). Ketone bodies can be regarded as important metabolic fuels and signals. For example, ß-hydroxybutyrate can protect neurons against the toxicity induced by the administration of 1-methyl-4-phenylpyridinium (heroin analog) or a fragment of amyloid protein (Kashiwaya et al. 2000). Also, high circulating levels of acetoacetate and ß-hydroxybutyrate, which are achieved through the consumption of ketogenic diets, can protect individuals with epilepsy from seizures (Clanton et al. 2017).

α-Oxidation of Fatty Acids

Although mitochondrial ß-oxidation is the major pathway for the oxidation of SCFAs, MCFAs, and LCFAs in animals, the α-oxidation of fatty acids can occur in the brain, liver, kidneys, and skin as a quantitatively minor pathway (Van Veldhoven 2010). In α-oxidation, one carbon is removed at a time from the carboxyl end of the molecule to generate CO_2. The α-oxidation of fatty acids occurs primarily in the peroxisome and, to a lesser extent, the smooth ER, and does not either require CoA intermediates after the initial step of activation or generate high-energy phosphate compounds (Jansen and Wanders 2006). Fe^{2+}, Mg^{2+}, and O_2 participate in this metabolic pathway. The discovery of high amounts of phytanic acid in the kidney, liver, and brain of patients suffering from *Refsum's Syndrome* prompted extensive research on its catabolism via the α-oxidation pathway. In addition to animals, bacteria also possess enzymes for α-oxidation in the cytosol, but this metabolic pathway is negligible in ruminal microbes as noted previously.

Phytanic acid (3,7,11,15-tetramethylhexadecanoic acid; a multi-branched C_{20} fatty acid containing 4 methyl residues on a C_{16} backbone) was known to be a component of cow's milk in the 1950s (Hansen and Shorland 1951). Phytanic acid is formed from phytol (3,7,11,15-tetramethylhexadec-*trans*-2-ene-1-ol) by bacteria. The steps of α-oxidation of phytanic acid include: (a) the activation of the fatty acid to phytanoyl-CoA by ACS_L and the transport of the acyl-CoA into peroxisomes; (b) 2-hydroxylation of phytanoyl-CoA by phytanoyl-CoA hydroxylase (Fe^{2+}) to 2-hydroxyphytanoyl-CoA; (c) the conversion of 2-hydroxyphytanoyl-CoA into pristanal and formyl-CoA by 2-hydroxyphytanoyl-CoA lyase (thiamine pyrophosphate, Mg^{2+}); (d) the dehydrogenation of pristanal by an NAD^+-linked aldehyde dehydrogenase (Figure 6.32). Formyl-CoA is hydrolyzed to formic acid and CoA-SH. It is now known that *Refsum's Syndrome* results from impaired catabolism of phytanic acid via α-oxidation in affected patients (Jansen and Wanders 2006).

Other examples of α-oxidation are possibly the conversion of palmitic acid ($C_{16:0}$), stearic acid ($C_{18:0}$), and arachidic acid ($C_{20:0}$) to pentadecanoic acid ($C_{15:0}$), heptadecanoic acid ($C_{17:0}$), and nonadecanoic acid ($C_{19:0}$), respectively, in animal species. Heptadecanoic and nonadecanoic acids may also be derived from the chain elongation of pentadecanoic acid. Interestingly, there is evidence that pentadecanoic acid, heptadecanoic acid, and nonadecanoic acid (which are all odd-numbered LCFAs), along with a group of other unidentified fatty acids, may be attractants for mosquitos (De Obaldia et al. 2022). The more of this mixture of fatty acids on the skin of people, the more attractive to mosquitoes they are. Mutant mosquitoes that lack LCFA receptors did not show the same preferences (De Obaldia et al. 2022). This may also be true for nonprimate animals.

ω-Oxidation of Fatty Acids

Another minor pathway for fatty acid oxidation is known as ω-oxidation, which is brought about by hydroxylase enzymes involving cytochrome P-450 in the ER and production of H_2O_2 (Van Veldhoven 2010). In ω-oxidation, the $-CH_3$ group of fatty acids is converted to a $-CH_2OH$ group that is subsequently oxidized to –COOH, thus producing a dicarboxylic acid. The enzymes for ω-oxidation are localized in the ER of the liver and kidneys, instead of the mitochondria for the β-oxidation (Kroetz and Xu 2005). The three steps of ω-oxidation are: (a) hydroxylation by mixed function oxidase, (b) oxidation by NAD^+-linked alcohol dehydrogenase, and (c) oxidation by NAD^+-linked

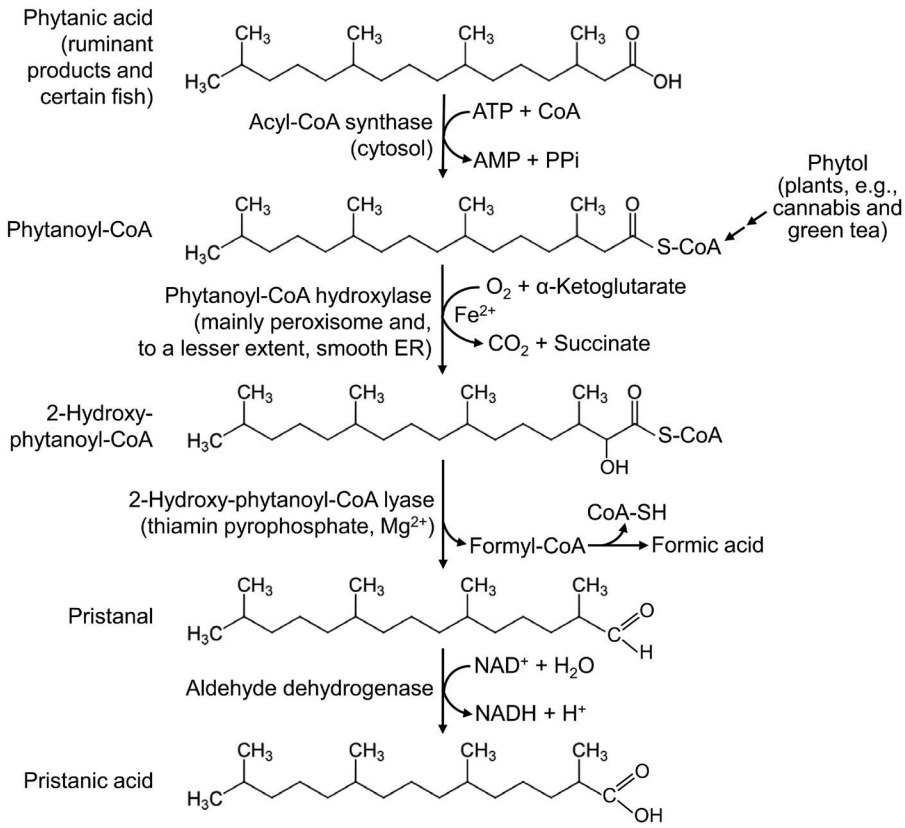


FIGURE 6.32 α-Oxidation of phytanic acid in animals. This metabolic pathway occurs primarily in the peroxisome and, to a lesser extent, the smooth endoplasmic reticulum, and requires Fe^{2+}, Mg^{2+}, and O_2. Through each process of α-oxidation, one carbon is removed from the carboxyl end of a long-chain fatty acid to generate formate.

aldehyde dehydrogenase. The long-chain dicarboxylic acid is usually ß-oxidized (primarily in peroxisomes) to form adipic (C_6) and suberic (C_8) acids, which are excreted in the urine. ω-Oxidation is normally a minor catabolic pathway for fatty acids but becomes more important when mitochondrial β-oxidation is defective.

Measurements of Fatty Acid Oxidation and Lipolysis

The oxidation of fatty acids in cells and tissues can be measured with the use of [^{14}C] or [^{3}H]-labeled fatty acid as a substrate, followed by the collection of $^{14}CO_2$ or $^{3}H_2O$. The carbon-1 and other carbons of fatty acids can be labeled with ^{14}C, but a labeled ^{3}H should not be in the carboxyl group because it can be exchanged with H^+ in water. The radioactivity of ^{14}C or ^{3}H is measured by a liquid scintillation counter. The rate of fatty acid oxidation is calculated as the amount of radioactivity in the labeled product of fatty acid oxidation ($^{14}CO_2$ or $^{3}H_2O$) divided by the specific radioactivity of the labeled precursor. When mass spectrometry is available as an analytical tool, ^{14}C and ^{3}H can be replaced by ^{13}C and ^{2}H in labeled substrates, respectively.

In adipocytes and muscle cells that lack glycerol kinase, the release of glycerol by the cells is a useful indicator of lipolysis. However, this approach is not valid for measuring lipolysis in the liver, which contains a high level of glycerol kinase activity. Whole-body lipolysis can be quantified with the use of a glycerol tracer. Regional lipolysis in the WAT and skeletal muscle of live animals can be determined by arteriovenous sampling or microdialysis techniques for glycerol measurement.

METABOLISM AND FUNCTIONS OF EICOSANOIDS

Synthesis of Bioactive Eicosanoids and Cannabinoids from PUFAs

Bioactive eicosanoids are mainly synthesized from arachidonic acid ($C_{20:4}$ ω6), but quantitatively minor eicosanoids are formed from EPA ($C_{20:5}$ ω3; a metabolite of α-linolenic acid) or dihomo-γ-linolenic acid ($C_{20:3}$ ω6, a metabolite of linoleic acid) in the smooth ER. These precursor fatty acids are normally bound to the cell membrane and nuclear membrane, and must be released from their membrane sites by phospholipase (e.g., phospholipase A_2) to be metabolized through various pathways for the synthesis of series 1, 2, or 3 of bioactive prostanoids [including prostaglandins (PGs) and thromboxanes (TXs)], series 3, 4 or 5 leukotrienes, and lipoxins (Calder 2020; Field et al. 1989; Hou et al. 2016).

Although animal cells constitutively generate many kinds of eicosanoids, their biosynthesis is enhanced when the cells are exposed to pathogens, trauma, ischemia, chemotactic factors, cytokines, or growth factors (Calder 2020; Christmas 2015). Four families of enzymes initiate the metabolism of arachidonic acid to eicosanoids: (a) cyclooxygenases (COX): COX-1 (constitutive) or COX-2 (inducible), which convert arachidonic acid into prostanoids (Figure 6.33); (b) lipoxygenases, which convert arachidonic acid into 5-hydroperoxyeicosatetraenoic acid (5-HPETE) as the common precursor of 5-hydroxyeicosatetraenoic acid (5-HETE), 5-oxo-eicosatetraenoic acid (5-oxo-ETE), leukotrienes, and lipoxins (Figure 6.34); (c) cytochrome P450 monooxygenases, which convert arachidonic acid into 20-HETE and 19-HETE; and (d) epoxygenases, which convert arachidonic acid into nonclassic eicosanoid epoxides [e.g., 5,6-epoxyeicosatrienoic acid (5,6-EET) and 14,15-EET]. COX, the lipoxygenases, and the phospholipases are tightly regulated because oxidation by COX or lipoxygenase generates highly reactive oxygen species and peroxides. Aspirin and indomethacin are potent inhibitors of COXs (Powell and Rokach 2015). The prostanoid metabolites of arachidonic acid have two double bonds (2-series prostanoids; e.g., PGE2, PGD2, and TXA2). Dihomo-γ-linolenic acid and EPA are metabolized by pathways similar to those for arachidonic acid to produce prostanoid metabolites with one double bond (1-series prostanoids; e.g., PGE_1, $PGF_{1\alpha}$, and TXA_1) and three double bonds (3-series prostanoids; e.g., PGE_3, $PGF_{3\alpha}$, and TXA_3), respectively. In addition, the metabolism of EPA and DHA generates, respectively, E and D series resolvins with 3 hydroxyl groups, whereas the metabolism of DHA also produces protectins and maresins with two hydroxyl groups (Serhan 2014). Lipoxins,

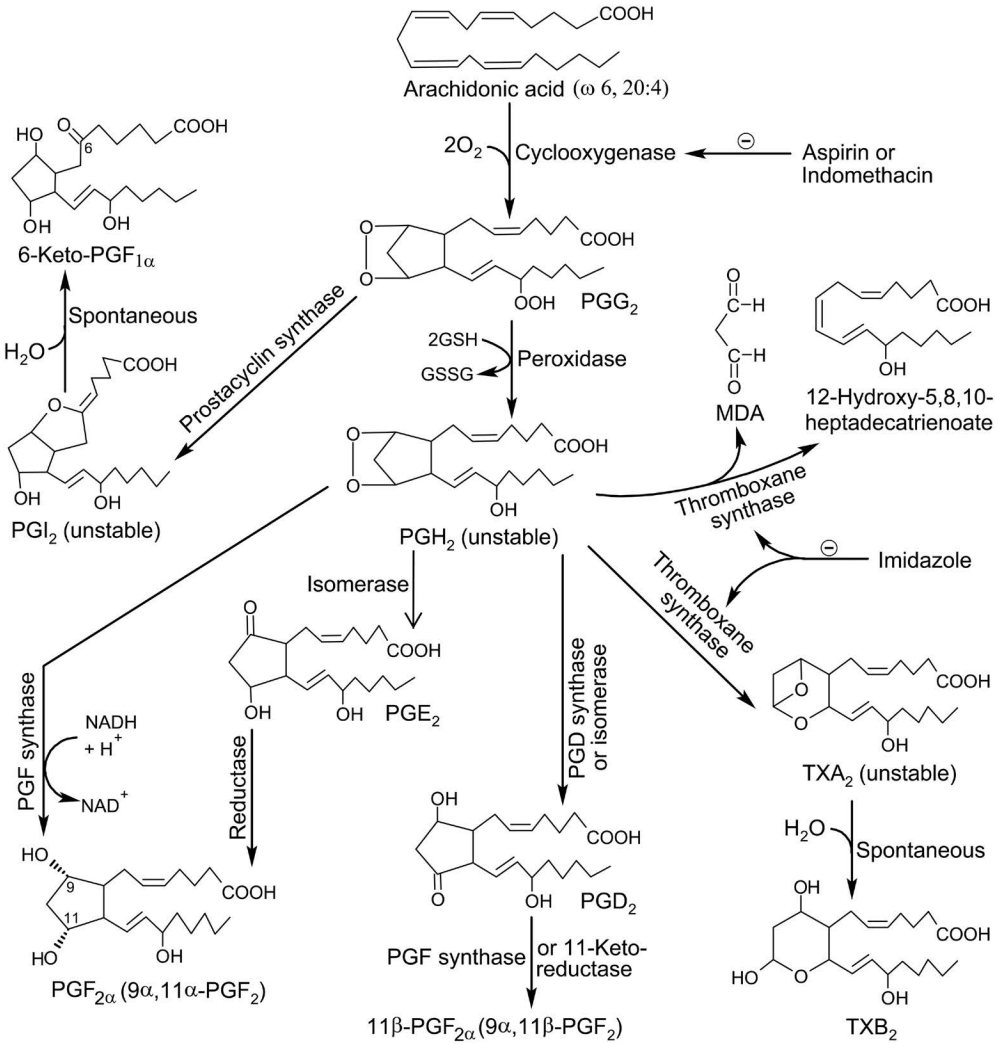


FIGURE 6.33 Metabolism of arachidonic acid to series 2 eicosanoids to prostaglandins (PGs) and thromboxanes in animals through the actions of constitutive and inducible cyclooxygenases. These pathways occur virtually in all cell types to form PGs, thromboxane A (TXA), and thromboxane B (TXB), which are all series 2 eicosanoids with two double bonds. PGI_2 is spontaneously converted into 6-keto-$PGF_{1\alpha}$. GSH, glutathione (reduced form); GSSG, glutathione disulfide (oxidized form).

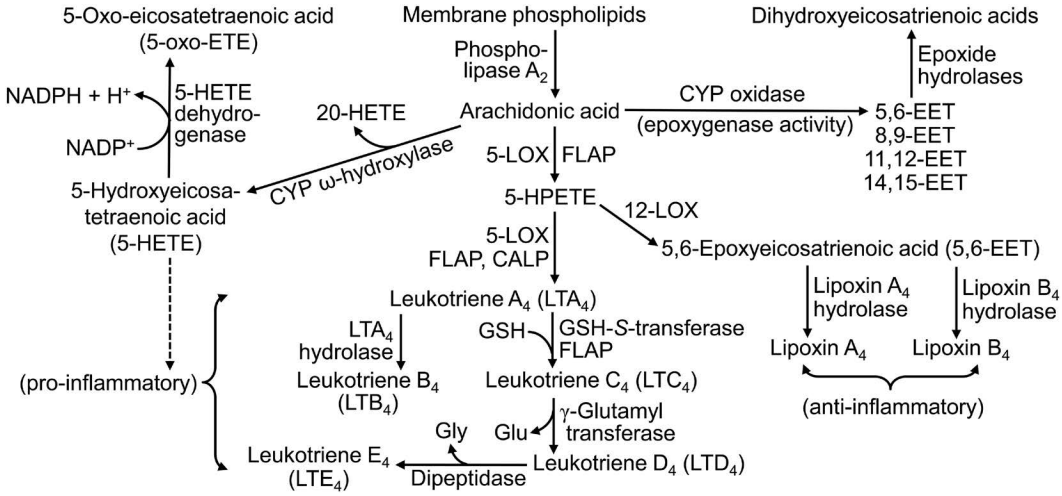


FIGURE 6.34 Metabolism of arachidonic acid to leukotrienes, lipoxins, 5-hydroxyeicosatetraenoic acids (5-HETE), and epoxyeicosatrienoic acids (EET) in animals. 5-Lipoxygenase concerts arachidonic acid into 5-hydroperoxyeicosatetraenoic acids (5-HPETE), which is the common intermediate for syntheses of leukotrienes and lipoxins. Glutathione (GSH) is required for the formation of leukotrienes. Both 5-lipoxygenase-activating protein (FLAP) and coactosin-like protein (CALP) enhance 5-lipoxygenase activity (5-LOX). FLAP also activates GSH-*S*-transferase. 12-Lipoxygenase (12-LOX) oxidizes 5-HPETE to 5,6-EET, which undergoes hydrolysis to yield lipoxin A_4 or B_4. Leukotrienes (A_4, B_4, C_4, D_4, and E_4) and 5-HETE are proinflammatory, whereas lipoxins (A_4 and B_4) are anti-inflammatory.

resolvins, protectins, and maresins are collectively coined as specialized pro-resolving mediators.

Arachidonic acid is also converted into physiologically important cannabinoids, such as anandamide and 2-arachidonoylglycerol, via different metabolic pathways in animal tissues (Figure 6.35). Phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidylserine (PS) participate in the synthesis of these eicosanoids (Wang and Ueda 2009). Note that arachidonic acid is incorporated into 1-arachidonoyl-PC and 2-arachidonoyl-phosphatidic acid, as described previously in the synthesis of TAGs (Figure 6.21).

Degradation of Bioactive Eicosanoids and Cannabinoids

Bioactive eicosanoids are rapidly degraded by animal cells via modification (e.g., the hydroxylation of the hydrocarbon chain) through the cytochrome P450 detoxification system, conjugation with glutathione (GSH) or glucuronides for excretion, and oxidation via the peroxisomal β-oxidation pathway (Turgeon et al. 2003). Among all tissues, the lungs have the highest rates of degradation of the eicosanoids. In animals, the plasma half-lives of most eicosanoids range from a few seconds to a few minutes. This is important to

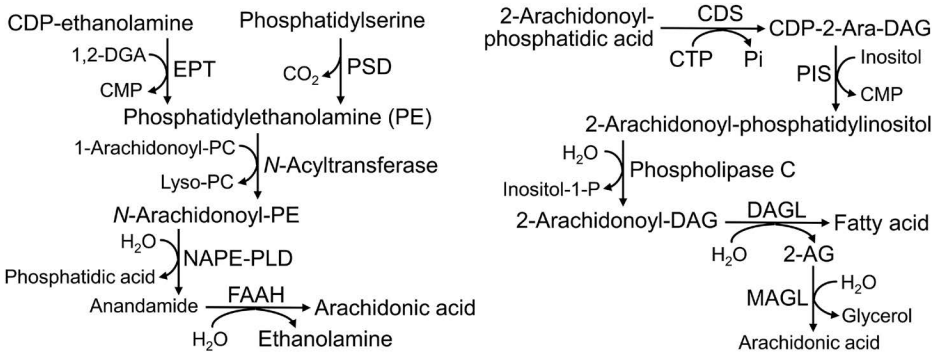


FIGURE 6.35 Synthesis and degradation of cannabinoids in animal tissues. Anandamide and 2-arachidonoylglycerol (2-AG) are two physiologically important cannabinoids synthesized from arachidonic acid (Ara) via different metabolic pathways. CDP, cytidine diphosphate; CDS, CDP-diacylglycerol synthase; CMP, cytidine monophosphate; CTP, cytidine triphosphate; DAG, diacylglycerol; DAGL, DAG lipase; EPT, ethanolamine phosphotransferase; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; NAPE-PLD, *N*-acyl phosphatidylethanolamine (NAPE)-hydrolyzing phospholipase D (PLD); PC, phosphatidylcholine; PI, phosphatidylinositol; PIS, PI synthase; and PS, phosphatidylserine; PSD, PS decarboxylase.

minimize the circulating levels of inflammatory eicosanoids and prevent any pathological effects of other eicosanoids.

Cytochrome P450 enzymes are crucial for converting PUFAs into some biologically active eicosanoids as noted previously and can also metabolize the eicosanoids into inactive compounds for excretion (Christmas 2015). Cytochromes P450 are hemoproteins containing heme as a cofactor. The term P450 is derived from the spectrophotometric peak of the cytochrome protein at the wavelength of its absorption maximum (450 nm) in the reduced state and bound with carbon monoxide. The cytochrome P450 system refers to a series of enzymes [e.g., cytochrome P450 ω-hydroxylase (a subset of monooxygenases), cytochrome P450 monooxygenase, and epoxygenase] in an NADPH-dependent electron transfer chain. The most common reaction in the cytochrome P450 system is catalyzed by monooxygenase (either cytochrome P450 ω-hydroxylase or cytochrome P450 monooxygenase), which inserts one oxygen atom from O_2 into the aliphatic position of an organic substance and the other oxygen atom into water (Powell and Rokach 2015). After eicosanoids are deactivated by the cytochrome P450 enzymes, the metabolites are excreted primarily in urine and, to a lesser extent, in feces.

$$\text{Organic substance} + O_2 + \text{NADPH} + H^+ \rightarrow \text{Organic substance-OH} + H_2O + \text{NADP}^+$$

Conjugation of eicosanoids with GSH and glucuronic acid occurs primarily in the liver and results in the formation of mercapturates (Figure 6.36) and glucuronides, respectively. The GSH conjugates are formed through the actions of GSH S-transferase, γ-glutamyl transpeptidase, cysteinyl glycine dipeptidase, and N-acetyl transferase. The glucuronide-conjugation (glucuronidation) reaction is catalyzed by uridine diphosphate (UDP)-glucuronosyltransferase, which transfers the glycosyl group from a nucleotide sugar (UDP-glucuronic acid) to a wide variety of compounds (including eicosanoids) to form water-soluble derivatives (e.g., glucuronides). The end products of GSH conjugation and glucuronidation are excreted mainly in urine and, to a lesser extent, in feces.

Eicosanoids and their metabolites can be degraded via the peroxisomal β-oxidation pathway to generate shorter-chain-CoA and acetyl-CoA (Van Veldhoven 2010). These products then enter the mitochondria for β-oxidation to CO_2 and water. Thus, through the cooperation of both peroxisomes and mitochondria, eicosanoids can be completely oxidized in animals. This assumes physiological importance when the activities of cytochrome P450, GSH conjugates, and glucuronidation pathways are reduced.

Animal tissues possess enzymes to release arachidonic acid from cannabinoids (Wang and Ueda 2009). Specifically, in response to physiological needs, fatty acid amide hydrolase converts anandamide into arachidonic acid and ethanolamine, whereas monoacylglycerol lipase hydrolyzes 2-arachidonoylglycerol to arachidonic acid and glycerol (Figure 6.35). Estrogen, progesterone, and leptin increase the expression of fatty acid amide hydrolase to affect ovulation, implantation, and uterine development (Xiao et al. 2002). The cannabinoid-degrading enzymes are potential treatment targets for a variety of pathological conditions, including pain, inflammation, and cancer.

Physiological Functions of Eicosanoids and Related Eicosanoic Acids

Eicosanoids exert specific effects on target cells close to the site of their production (Hou et al. 2016). These compounds are considered "local hormones", which participate in intercellular and intracellular signal cascades to exert effects on inflammation, fever, hemodynamics, blood clotting, immune system modulation, reproduction, cell growth,

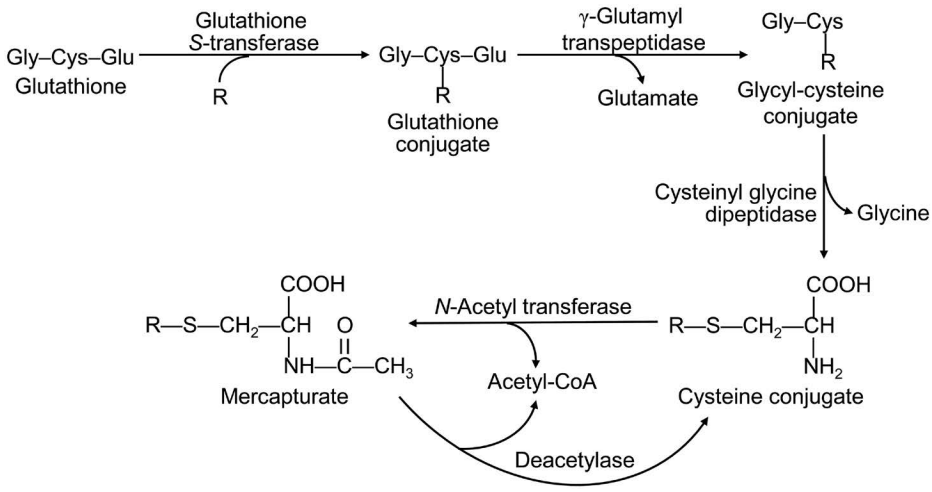


FIGURE 6.36 Conjugation of eicosanoids with glutathione (GSH) to form mercapturate in the liver. R = substances [e.g., various electrophiles, physiological compounds and metabolites (estrogen, prostaglandins, leukotrienes, and melanins), and xenobiotics (e.g., bromobenzene and acetaminophen)] that can conjugate with glutathione in hepatocytes for excretion in urine and feces.

TABLE 6.12
Physiological Functions of Eicosanoids

Eicosanoid	Physiological Functions
PG-I_2	Relaxes arterial smooth muscle; Inhibits platelet aggregation
TX-A_2	Contracts arterial smooth muscle; Activates platelet aggregation; Blood clotting; Allergic reactions
PG-E_2	Contracts smooth muscle cells; Promotes inflammation; Induces fever; Pain perception
PG-E_1	Relaxes smooth muscle cells; Improves erectile function
PG-$F_{2\alpha}$	Promotes uterine contraction; Induces labor; Enhances bronchoconstriction
PG-D_2	Relaxes smooth muscle cells; Inhibits platelet aggregation; Inhibits neurotransmitter release; Allergic reactions; Promotes hair growth
20-HETE	Promotes vasoconstriction; Inhibits platelet aggregation
LT-B4	Chemotactic factor for leukocytes; Activator of leukocytes; Promotes inflammation
LT-C4, D4	Enhances vascular permeability; Promotes vascular smooth muscle contraction; Allergic reactions
LT-E4	Enhances vascular permeability; Promotes airway mucin secretion
Lx-A4, B4	Inhibits the function of pro-inflammatory cells
14,15-EET	Promotes vasodilation; Inhibits platelet aggregation; Inhibits the function of pro-inflammatory cells
SPMs	Evokes anti-inflammatory responses and contributes to inflammation resolution

Note: EET, Epoxyeicosatrienoic acids; HETE, Hydroxyeicosatetraenoic acid; LT, leukotriene; Lx, lipoxin; PG, prostaglandin; SPMs, lipoxins, resolvins, protectins and maresins; TX, thromboxane

neurotransmission, and biological clock (Table 6.12). PGs bind to (a) plasma membrane G-protein-coupled receptors, which stimulate the formation of cAMP (e.g., PGE_1, PGI_2, and PGD_2), the phosphatidylinositol-4,5-bisphosphate signal pathway (TXA_2, PGE_2, and $PGF_{2\alpha}$), and intracellular Ca^{2+} release; or (b) PPAR-γ, which influences gene transcription, depending on cell type and eicosanoid. Some leukotrienes also act via specific G-protein-coupled receptors on the plasma membrane. Examples of the effects of eicosanoids are highlighted herein. First, the excessive production of PGE_2 by immunocytes and other cell types in response to inflammation and infection causes fever through its binding to E-class prostanoid (EP) receptors in the hypothalamus of the brain, which subsequently raises the set point of the hypothalamic thermostat for body temperature. Thus, aspirin, which inhibits PG synthesis, effectively alleviates fever. Second, $PGF_{2\alpha}$ plays a primary role during parturition by binding to its G protein–coupled receptor in the myometrium of the uterus, thereby activating phospholipase C and stimulating contractions in myometrial smooth muscle. Third, like nitric oxide, PGI_2 is a vasodilator and a potent inhibitor of platelet aggregation in blood vessels, therefore facilitating blood flow, reducing blood pressure, and preventing thrombosis. PGI_2 acts on its G-protein-coupled receptor on the cell membrane, whereas nitric oxide stimulates the soluble guanylyl cyclase in the cytosol to elicit physiological responses.

In addition to the receptor-mediated actions of eicosanoids, their metabolism in animals is affected by arachidonic acid (an ω6 PUFA) and EPA (an ω3 PUFA), therefore eliciting diverse biological responses to inflammation. For example, ω3 PUFA can inhibit the synthesis of pro-inflammatory eicosanoids from arachidonic acid and vice versa (Clandinin et al. 1993). Thus, excess ω6 PUFAs promote inflammation, but ω3 PUFAs suppress inflammation in animals. The interlinking mechanisms responsible for the anti-inflammatory actions of ω3 PUFAs include: (a) alterations in the cell membrane composition of phospholipid fatty acids, (b) the disruption of lipid rafts, (c) the inactivation of the pro-inflammatory transcription factor NFκB to reduce the expression of inflammatory genes, (d) the activation of the anti-inflammatory transcription factor PPAR-γ, and (e) the binding to the G-protein-coupled receptor GPR120 to decrease the production of PGE2 and other pro-inflammatory cytokines (Calder 2015).

PHOSPHOLIPID AND SPHINGOLIPID METABOLISM

Phospholipid Metabolism

As noted in Chapter 3, phospholipids consist of a glycerol molecule, two fatty acid molecules, and a phosphate group. They are amphipathic molecules forming the lipid bilayer of the plasma membrane and contribute to the physicochemical properties of the membrane (e.g., the membrane fluidity). Therefore, phospholipids influence the conformation and function of membrane-bound proteins (e.g., hormone receptors, ion channels, and nutrient transporters). In addition, as precursors for the synthesis of signaling molecules, phospholipids regulate gene expression and nutrient metabolism. Dietary intakes of nutrients, such as protein, fatty acids (both saturated and polyunsaturated), vitamins (e.g., A, E, and folate), and microminerals (e.g., zinc and magnesium), affect the composition and metabolism of phospholipids in animals (Gimenez et al. 2011).

Synthesis of Phospholipids

In bacteria (as those in the digestive tract of animals) and yeast, the de novo synthesis of phospholipids involves the formation of phosphatidate (DAG-3-P) from dihydroxyacetone-P (an intermediate of glycolysis) and fatty acyl-CoA, leading to the generation of cytidine diphosphate (CDP)-DAG (Figure 6.37). Because bacteria and yeast contain the Mn^{2+}- and CDP-DAG-dependent PS synthase, they are capable of synthesizing PS from CDP-DAG and serine. This enzyme, however, is absent from mammals, birds, and fish. Thus, despite the presence of enzymes for converting glucose, fatty acyl-CoA, and serine into CDP-DAG in their smooth ER, animal cells do not synthesize PS, PE, or PC from CDP-DAG. However, in animals, CDP-DAG is the

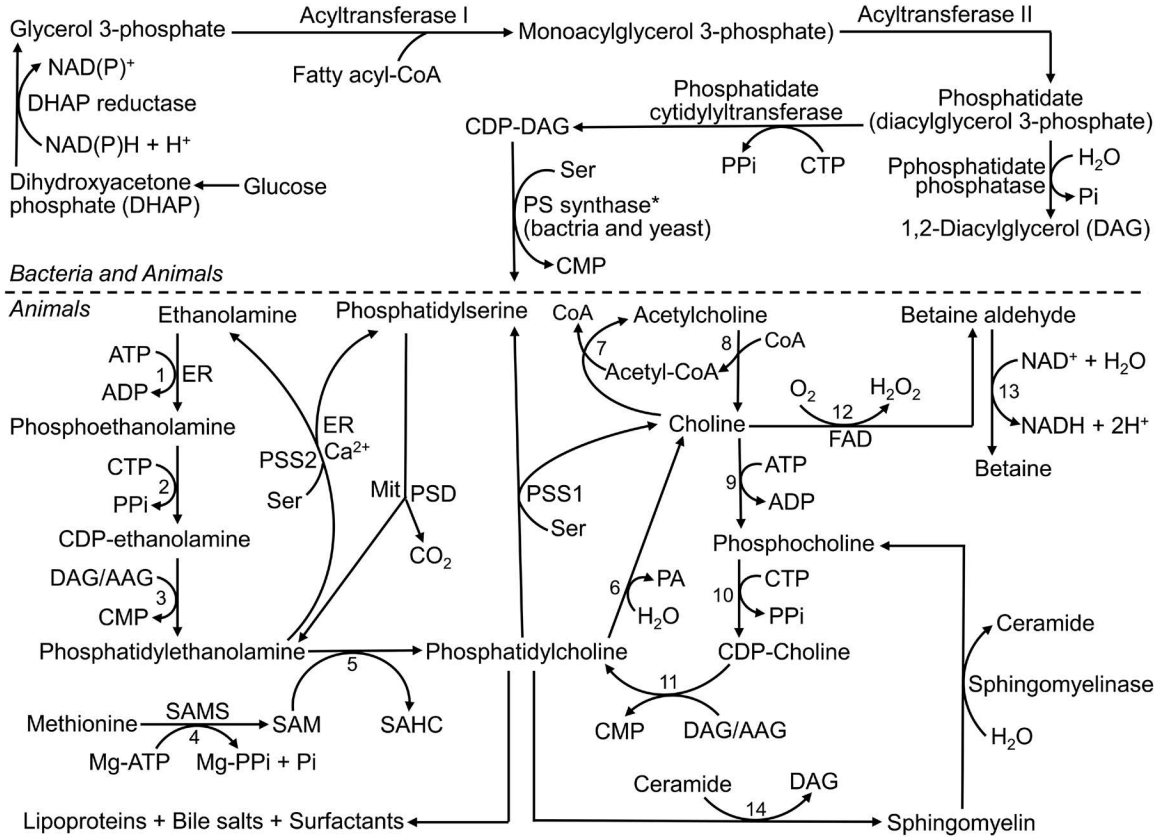


FIGURE 6.37 Phospholipid metabolism in animals and yeast. The reactions below the broken line occur in animal cells, whereas those above the broken line take place in bacteria and animal cells except for the Mn^{2+}- and CDP-DAG-dependent PS synthase. This enzyme is absent from animal cells, as indicated by the asterisk (*). The enzymes catalyzing the indicated reactions are (1) ethanolamine kinase, (2) CTP:phosphoethanolamine cytidylyltransferase, (3) CDP-ethanolamine:1,2-diacylglycerol ethanolamine phosphotransferase, (4) *S*-adenosylmethionine synthase, (5) phosphatidylethanolamine *N*-methyltransferase, (6) various phospholipases and lysophospholipases, (7) choline acetyltransferase, (8) choline acetyltransferase, (9) choline kinase, (10) CTP:phosphocholine cytidylyltransferase, (11) CDP-choline:1,2-diacylglycerol cholinephosphotransferase, (12) choline oxidase, (13) betaine aldehyde dehydrogenase, and (14) phosphatidylcholine:ceramide cholinephosphotransferase. With the exception of PSD (which is located in the inner mitochondrial membrane), the enzymes for phospholipid synthesis are present in the smooth endoplasmic reticulum. AAG, 1-*O*-alkyl-2-acetyl-*sn*-glycerol; CTP, cytidine triphosphate; CDP, cytidine diphosphate; CMP, cytidine monophosphate; DAG, diacylglycerol; PA, phosphatidate; PSD, phosphatidylserine decarboxylase; PSS1, phosphatidylserine synthase 1; PSS2, phosphatidylserine synthase 2; SAHC, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine; SAMS, *S*-adenosylmethionine synthase.

precursor for the synthesis of cardiolipin (an abundant phospholipid in mitochondrial membranes) in the mitochondrial inner membrane and of PI in the ER (Figure 6.38).

In animals, there are two pathways for PE production: (a) de novo synthesis from ethanolamine and DAGs in the smooth ER and (b) the decarboxylation of PS to form PE in the inner membrane of mitochondria (Tatsuta et al. 2014). PE serves as the common precursor for the synthesis of PS, PC, and sphingomyelin (Figure 6.37). Specifically, PE is converted into PC by PE *N*-methyltransferase, which requires *S*-adenosylmethionine (a metabolite of methionine) as the cofactor. For PS synthesis, PS synthase 1 exchanges serine in PC for choline, whereas PS synthase 2 exchanges serine in PE for ethanolamine. These two distinct enzymes are not related to the Mn^{2+}- and CDP-DAG-dependent PS synthase in bacteria and yeast (Vance and Tasseva 2013). PC is hydrolyzed by various phospholipases and lysophospholipases to form choline and phosphatidate. The latter is recycled to CDP-DAG in the smooth ER, as noted previously. This, along with the conversion of PC-derived sphingomyelin into phosphocholine, helps to conserve phospholipids in the body.

Most lipids (including phospholipids) are synthesized in the ER and transported to the mitochondria. However, cardiolipin is formed from CDP-DAG, and PS is converted into PE in the inner membrane of mitochondria. Thus, the intracellular transport of phospholipids from the sites of their synthesis to their destinations is essential for the formation and function of all cell membranes. It appears that, under physiological conditions, de novo synthesis of phospholipids cannot meet the needs of animals, including calves, cats, dogs, fish, fur animals, horses, mice, pigs,

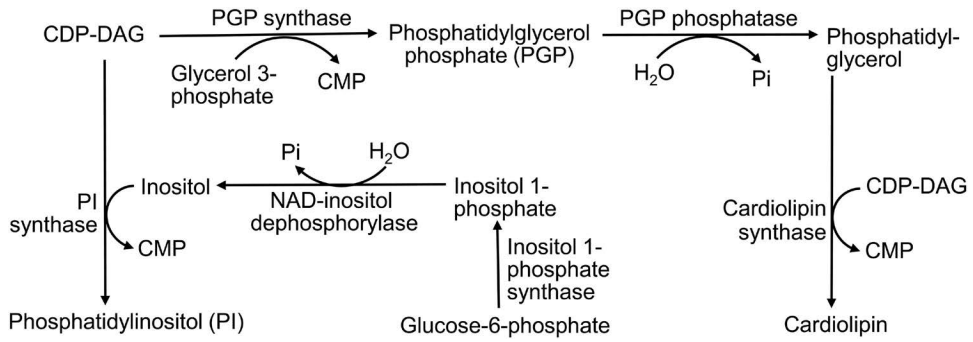


FIGURE 6.38 Synthesis of cardiolipin and phosphatidylinositol from CDP-DAG in animal cells. The reactions for the conversion of CDP-DAG into cardiolipin occur in the inner membrane of mitochondria, whereas phosphatidylinositol is generated from glucose-6-phosphate in the smooth endoplasmic reticulum. CDP, cytidine diphosphate; CMP, cytidine monophosphate; DAG, diacylglycerol.

poultry, and rats (Gimenez et al. 2011). Therefore, diets must supply these nutrients to the animals to ensure their optimal growth, development, and health.

Sources of Ethanolamine and Choline in Animals

Plants and algae can produce ethanolamine from serine via a soluble serine decarboxylase. In contrast, animal cells and bacteria lack serine decarboxylase and, therefore, cannot directly decarboxylate serine to form ethanolamine. However, in both animals and bacteria, serine and palmitoyl-CoA are converted into ceramide through a series of enzyme-catalyzed reactions in the smooth ER, where ceramide is metabolized to form fatty aldehyde and phosphoethanolamine (Figure 6.39). PS synthase 2 converts PE and serine into PS and ethanolamine. Bacteria can also synthesize PS from glucose, serine and acyl-CoA, and the PS is metabolized to PE and then ethanolamine (Figure 6.33). Note that although a small amount of ethanolamine can be generated via the breakdown of sphingolipids by sphingosine-1-P lyase, this reaction does not result in a net de novo synthesis of ethanolamine from endogenous sphingolipids. Furthermore, dietary phospholipids and ethanolamine can be additional sources of ethanolamine in animals. The concentration of ethanolamine in the plasma of nonruminants is usually low (2–10 μM; Wu 2022). In ruminants, because ruminal bacteria can de novo synthesize ethanolamine from glucose, fatty acids, and serine, the plasma concentrations of ethanolamine range from 30 to 100 μM, depending on diet, age, and physiological status (Kwon et al. 2003).

PC, which is formed from PE and PS, is the endogenous source of choline (Figure 6.33). Ultimately, serine and methionine are required for the synthesis of choline in animals. This pathway is most active in the liver among animal tissues. Under physiological conditions, the de novo synthesis of choline cannot meet the needs of animals and, therefore, their diets must provide choline (Zeisel 1981). This is particularly important for fetuses and neonates (including preruminants) because their small-intestinal microbial activity is either absent or too low to synthesize a significant amount of choline. Because choline is a substrate for the syntheses of PC and acetylcholine, a deficiency of choline impairs neurological development, cognitive function and nerve impulse transmission, as well as causing muscle damage and the abnormal deposition of TAGs in the liver (called

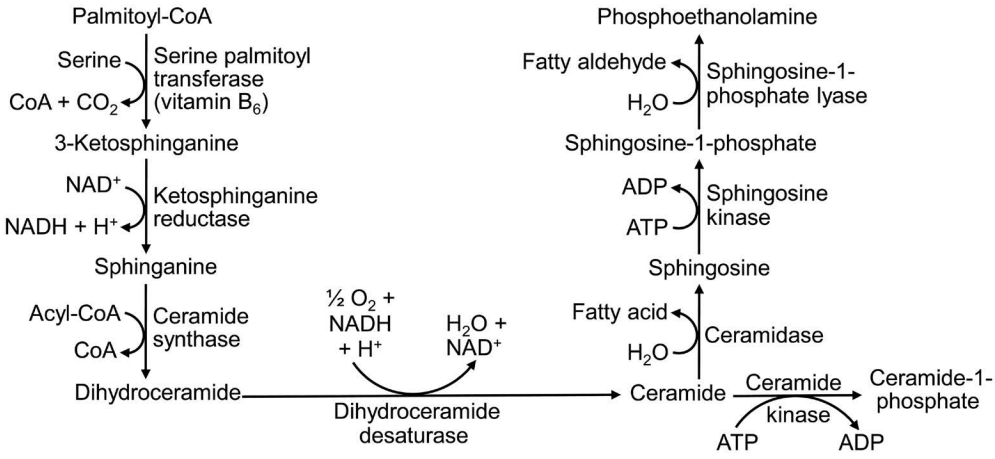


FIGURE 6.39 De novo synthesis of ceramide from palmitoyl-CoA and L-serine, as well as ceramide degradation in animal cells. These reactions occur in the smooth endoplasmic reticulum.

nonalcoholic fatty liver disease). Good dietary sources of choline are eggs, meat, poultry, fish, cruciferous vegetables, peanuts, and dairy products.

Sphingolipid Metabolism

Sphingolipids have an important role in nutrition and physiology because their metabolism produces diverse molecules that serve as structural components (sphingomyelin and glycosphingolipids in cell membranes) and signaling agents (e.g., sphingosine, ceramide, and sphingosine-1-P) in cells. In animals, the brain is particularly enriched in sphingolipids (Denisova and Booth 2005). These lipids are formed from phospholipids and such raw materials as serine and fatty acids. Specifically, PC:ceramide cholinephosphotransferase converts PC and ceramide into sphingomyelin. Additionally, animal cells can de novo synthesize sphingolipids (e.g., ceramide) from serine and palmitoyl-CoA in the ER (Figure 6.39). The synthesized sphingolipids are then imported into mitochondrial and other membranes. These lipids help to provide a waterproof skin layer, and also participate in a variety of cellular signaling to regulate differentiation, proliferation, and apoptosis of cells.

Hydrolysis of sphingolipids is initiated in lysosomes. For example, sphingomyelin is hydrolyzed by the lysosomal sphingomyelinase to form phosphocholine and ceramide (Figure 6.33). A complex sphingolipid is ultimately broken down into sphingosine, which is then reused by reacylation to form ceramide (the salvage pathway). Of note, a critical step in sphingolipid metabolism is catalyzed by sphingosine-1-P lyase, which is widely present in the ER of tissues. This enzyme cleaves the phosphorylated sphingoid bases to yield PE and a fatty aldehyde. The latter is either hexadecenal or hexadecanal in the case of sphingosine-1-P or dihydrosphingosine-1-P as the substrate, respectively. The intracellular concentrations of sphingolipids depend on the balance between the rates of their synthesis and catabolism, as well as their dietary intake and intestinal digestion and absorption.

There is no known dietary requirement of animals for sphingolipids. The ingested sphingolipids are hydrolyzed in the intestine to ceramides and sphingoid bases for absorption into the enterocytes (Vesper et al. 1999). Results of animal studies indicate that dietary supplementation with sphingolipids can suppress colon carcinogenesis, reduce serum LDLs, and elevate serum HDLs. Thus, sphingolipids may be a "functional" constituent of food for the improvement of animal health. In the absence of sphingolipid intake from diets, animals cannot maintain the homeostasis of these lipids.

Ganglioside Metabolism

Gangliosides are acidic glycosphingolipids (consisting of ceramide and oligosaccharide) highly abundant in the central nervous system and play an important role in brain development (Breiden and Sandhoff. 2018). These molecules contain one or more sialic acids linked on the hexose sugar chain and are anchored in cell membranes by their ceramide component. The biosynthesis of gangliosides is initiated with the formation of the ceramide in the smooth ER, followed by sequential glycosylation reactions catalyzed by membrane-bound glycosyltransferases in the Golgi and the trans-Golgi network (TGN). The newly formed gangliosides are transported to the plasma membrane for structural and signaling functions. To maintain their intracellular homeostasis, gangliosides undergo degradation within lysosomes by specific enzymes for the hydrolysis of hydrolyze ganglioside monosialic acids (GM). For example, β-hexosaminidase A (a hydrolyzing enzyme) removes the terminal *N*-acetylgalactosaminyl residue from ganglioside GM2 (β-D-GalNAc-(1→4)-[α-Neu5Ac-(2→3)]-β-D-Gal-(1→4)-β-D-Glc-(1→1)-N-octadecanoylsphingosine). The latter is an intermediate in the synthesis and degradation of a higher ganglioside, such as GM1 ganglioside (monosialotetrahexosylganglioside).

Abnormal metabolism of gangliosides results in two diseases: gangliosidoses and Gaucher's disease. Gangliosidoses are a group of inherited lipid storage disorders caused by the excessive accumulation of gangliosides in the lysosomes of the brain, spinal cord, liver, spleen, kidney, heart, and skeletal muscle (d'Azzo et al. 2001). The diseases result from the lack of or low activity of enzymes to hydrolyze GM, which then accumulate in the lysosome to destroy the nerve. GM1 and GM2 gangliosidoses result from the deficiency of β-galactosidase and β-hexosaminidase to break down GM1 gangliosides and GM2 gangliosides, respectively. Reducing dietary intake of gangliosides and related lipids may help ameliorate this metabolic disorder.

Glucosylceramides are precursors of gangliosides in animals. Gaucher's disease (glucocerebrosidosis or glucosylceramide lipidosis) results from the accumulation of glucosylceramides. This disorder occurs because of a deficiency of β-glucocerebrosidase for the degradation of glucocerebroside that is derived from gangliosides (Jolly and Walkley 1997). As a result, the lipid accumulates in the lysosomes of certain organs (particularly the spleen and liver), resulting in their enlargement and dysfunction. Affected animals include dogs, pigs, and sheep. Reducing dietary intake of glucosylceramides and related lipids may help ameliorate this metabolic disorder.

METABOLISM OF STEROID HORMONES

Synthesis of Progesterone and Glucocorticoids

The cytochrome P450 (CYP) enzymes, as well as hydroxysteroid dehydrogenases (HSDs) and steroid reductases are responsible for the synthesis of steroid hormones from cholesterol in a cell-specific manner (e.g., adrenal gland, testes, and ovaries; Figure 6.40). These enzymes are highly substrate-selective and include NADPH-dependent steroid dehydrogenases and reductases. The initial step of the synthetic pathways, i.e., the conversion of cholesterol into pregnenolone, is catalyzed by CYP11A1 (cholesterol monooxygenase; cholesterol side-chain cleavage). This enzyme

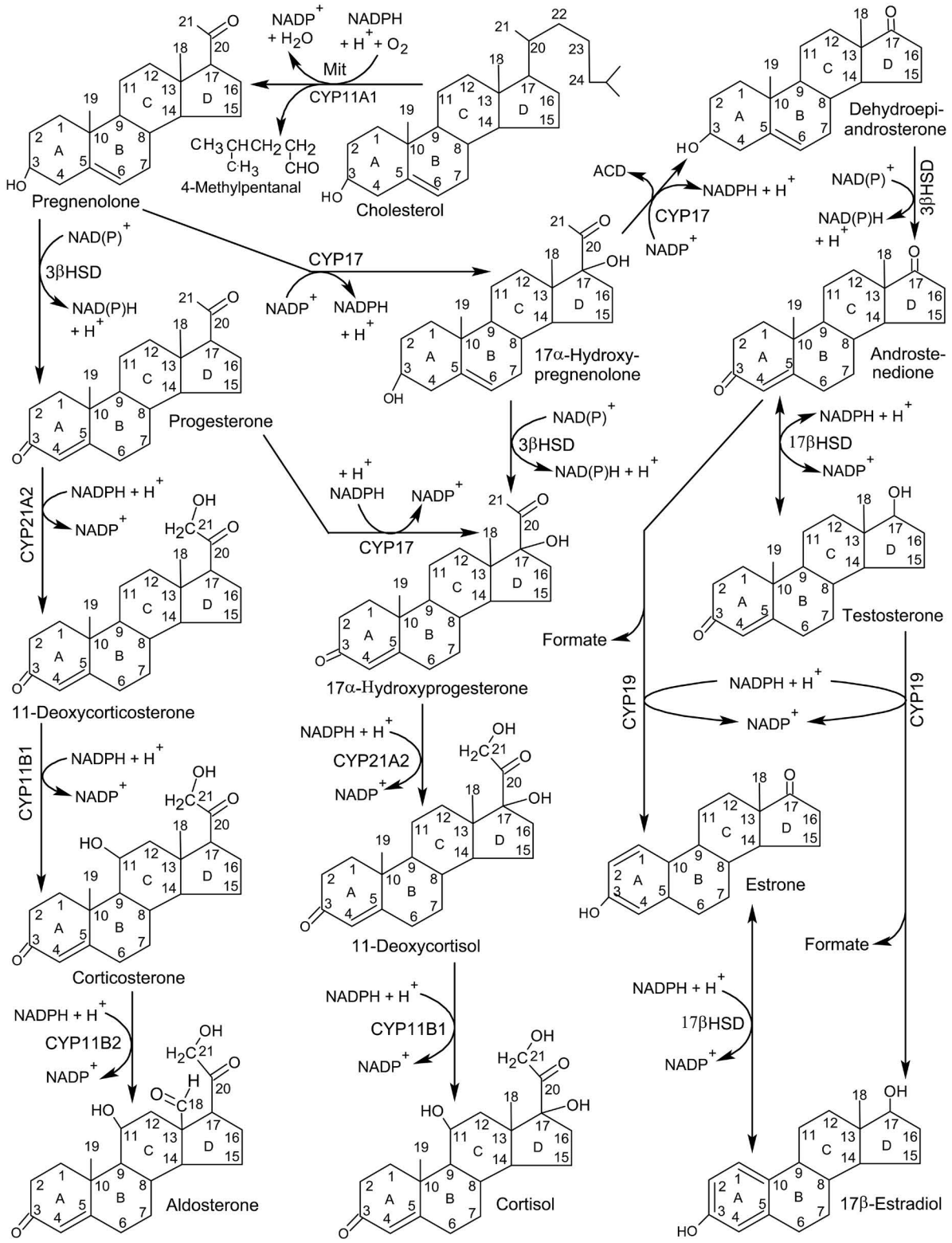


FIGURE 6.40 Biosynthesis of steroid hormones from cholesterol in a cell-specific manner. The adrenal cortex produces glucocorticoids (corticosterone, aldosterone, and cortisol) and progesterone. The production of the adrenal hormones in the context of the adrenal gland is stimulated by adrenocorticotropic hormone (ACTH) or under stress conditions (e.g., heat stress). High levels of glucocorticoids induce a catabolic response in animals, which can be ameliorated by dietary supplementation with their structural analogs, such as Yucca extracts.

is bound to the mitochondrial inner membrane of all steroidogenic tissues. Pregnenolone is converted to (a) progesterone by 3β-hydroxysteroid dehydrogenase (3β-HSD), a non-CYP450 enzyme present in both mitochondria and the ER of the placenta and steroidogenic tissues; and (b) 17α-hydroxy-pregnenolone by CYP17, which is a single protein with both 17α-hydroxylase and 17,20-lyase activities, and is expressed in the ovaries, testes, and adrenal cortex (Sanderson 2006). In the adrenal cortex, progesterone is metabolized to corticosterone, aldosterone, and cortisol, whereas 17α-hydroxy-pregnenolone is converted into cortisol. The circulating levels of adrenal hormones vary with species. For example, cortisol and corticosterone are the predominant glucocorticoids in the plasma of pigs and chickens, respectively. The production of glucocorticoids in the context of the adrenal gland is stimulated by adrenocorticotropic hormone (ACTH) or under stress conditions (e.g., heat stress). High levels of glucocorticoids induce a catabolic response in animals, which can be ameliorated by dietary supplementation with the structural analogs of the hormones, such as the *Yucca schidigera* extracts.

Synthesis of Testosterone and Estrogen

Cholesterol-derived 17α-hydroxy-pregnenolone is the common intermediate for the synthesis of testosterone by Leydig cells in the testes and of estrogen by granulosa cells in the ovaries (Figure 6.38). These are anabolic hormones that promote protein synthesis and cell growth. Luteinizing hormone (LH) stimulates testosterone synthesis by binding to its receptor on the Leydig cell membrane, which causes the activation of cAMP-dependent protein kinase (Sanderson 2006). Testosterone and follicle-stimulating hormone (FSH), which act through the activation of cAMP-dependent protein kinases, are required to produce mature sperm and achieve full reproductive potential in males. Dietary intake of nutrients (particularly protein, arginine, vitamin A, vitamin E, zinc, and PUFAs) is critical for spermatogenesis. Estrogen synthesis is increased by LH and FSH through the upregulation of expression of aromatase and 17β-HSD, which promote the conversion of estrone into estradiol. Maternal nutrition can affect the production of female reproductive hormones. Interferences with the biosynthesis of sex hormones by some dietary and environmental nonnutrient factors (e.g., toxins) will impair reproduction in both males and females.

FAT DEPOSITION AND HEALTH IN ANIMALS

Regulation of Fat Accretion in Tissues

The deposition of fats in animals depends on the balance between the rates of TAG synthesis and catabolism (Figure 6.41). When dietary lipids are not completely oxidized to CO_2 and water, fatty acids are deposited in tissues (mainly adipose tissue) as TAGs (Jobgen et al. 2006). Likewise, when there is excess acetyl-CoA relative to its oxidation via the Krebs cycle, the acetyl-CoA is converted to fatty acids and cholesterol. Fatty acids are then used to form TAGs for deposition in the WAT and other tissues. In lean and obese animals (e.g., adult rats) that are capable of de novo hepatic synthesis of LCFAs, this pathway contributes to 9% and 44% of TAG secretion by their liver, respectively (Azain et al. 1985). A high ratio of insulin/glucagon stimulates anabolic pathways, including those for TAG synthesis in the liver or WAT and TAG deposition in the WAT, whereas a lower ratio of insulin/glucagon (e.g., due to a decreased supply of dietary energy) favors catabolic responses (Table 6.7). This may explain the findings that given the same intake of energy and nutrients, growing rats fed two spaced meals per day (meal eating) for 2 weeks, which resulted in pulse spikes of plasma insulin concentrations (and also increased pentose-cycle activity in the liver and WAT), stored 91% more body fat than those fed more frequently smaller meals throughout the day (nibbling; Cohn and Joseph 1960). In addition, insulin,

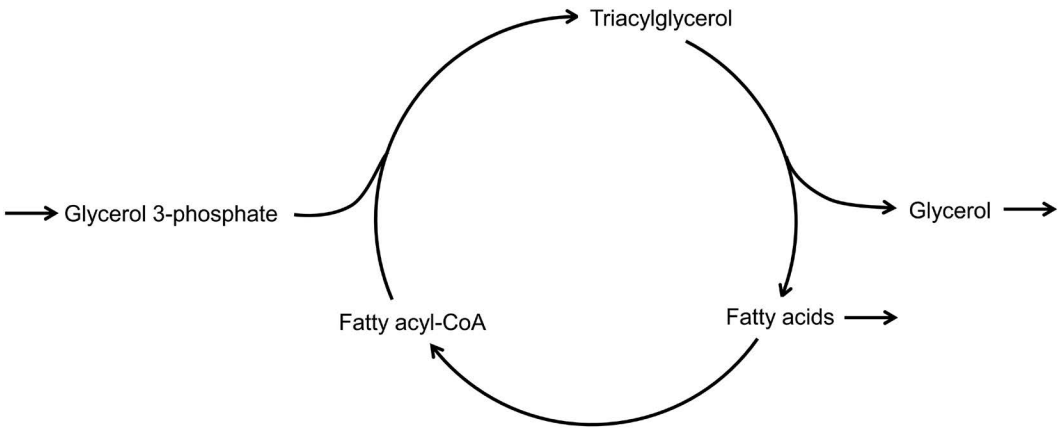


FIGURE 6.41 Intracellular turnover of triacylglycerols (TAG) in animals. The balance between the rates of fat synthesis and catabolism determines the deposition of TAG in cells and the whole body. White adipose tissue lacks glycerol kinase and releases glycerol. The latter is taken up by the liver, kidneys, and other tissues for utilization.

glucocorticoids, and PPAR-γ promote the differentiation of adipocytes so that they become mature cells for TAG synthesis and storage. Because of hyperglycemia and insulin resistance, obese animals or animals with type I or type II diabetes mellitus usually exhibit dyslipidemia, which results in impaired LDL metabolism, oxidative stress, and increased risk for cardiovascular disease (Figure 6.42). High concentrations of fatty acids and TAGs in the plasma can contribute to pancreatic disorders (e.g., inflammation and tumor development). Like overfeeding, excessive mobilization of LCFAs from the WAT during a prolonged period of severe inadequate intake of dietary energy can also result in dyslipidemia, such as the fatty liver (i.e., hepatic lipidosis) that commonly occurs in dairy cows during early lactation (Bode et al. 2004). This metabolic disorder develops when the hepatic uptake of lipids from the blood exceeds the oxidation and secretion of lipids by the liver and is usually preceded by high concentrations of LCFAs in plasma. Thus, either over-nutrition or under-nutrition negatively affects the health and production performance of animals.

A major issue in the production of livestock, poultry, and fish is that excessive amounts of subcutaneous WAT (e.g., backfat and abdominal fat) are deposited in their market-weight bodies (Etherton and Walton 1986; Li et al. 2021). Let us use the pig as an example. Pigs start to accumulate large amounts of body fat beginning at 45 kg BW, and that fat content increases disproportionately 10-fold between 45 and 115 kg (market weight) BW. Strikingly, in market-weight pigs, the subcutaneous adipose tissue represents 70% of the carcass lipid. Because consumers are demanding low-fat meats, excessive subcutaneous adipose tissue is trimmed at slaughter to meet government and/or consumers' requirements. The more backfat a market-weight pig has, the lower the price or grade. Deposition of subcutaneous adipose tissue not only decreases the proportion of lean tissue in the carcass and economic returns, but also potentially reduces the use of dietary energy for muscle protein accretion. Therefore, minimizing excess subcutaneous and abdominal WAT is of enormous importance for improving the quality and economic returns of the swine industry worldwide. This also applies, in many countries, to the production of ruminants, poultry, and fish.

As the impact of global warming on animal health and productivity is a growing concern worldwide, studies have identified the effects of heat stress on fat metabolism in humans, livestock, poultry, fish, and crustaceans. Elevated temperatures cause mitochondrial dysfunction and electron leakage, resulting in the release of superoxide anion ($O_2^{\bullet}$; an oxygen radical) from mitochondria into the cytosol. $O_2^{\bullet}$ is converted by superoxide dismutase (both mitochondrial and cytosolic enzymes) into H_2O_2 plus O_2. H_2O_2 reacts spontaneously with either ferrous iron (Fe^{2+}) to yield hydroxyl radicals ($^{\bullet}OH$ and $HO_2^{\bullet}$) or NO to generate peroxynitrite ($ONOO^-$). The excess production of these potent oxidants in the face of a reduced antioxidative capacity leads to oxidative stress, damage

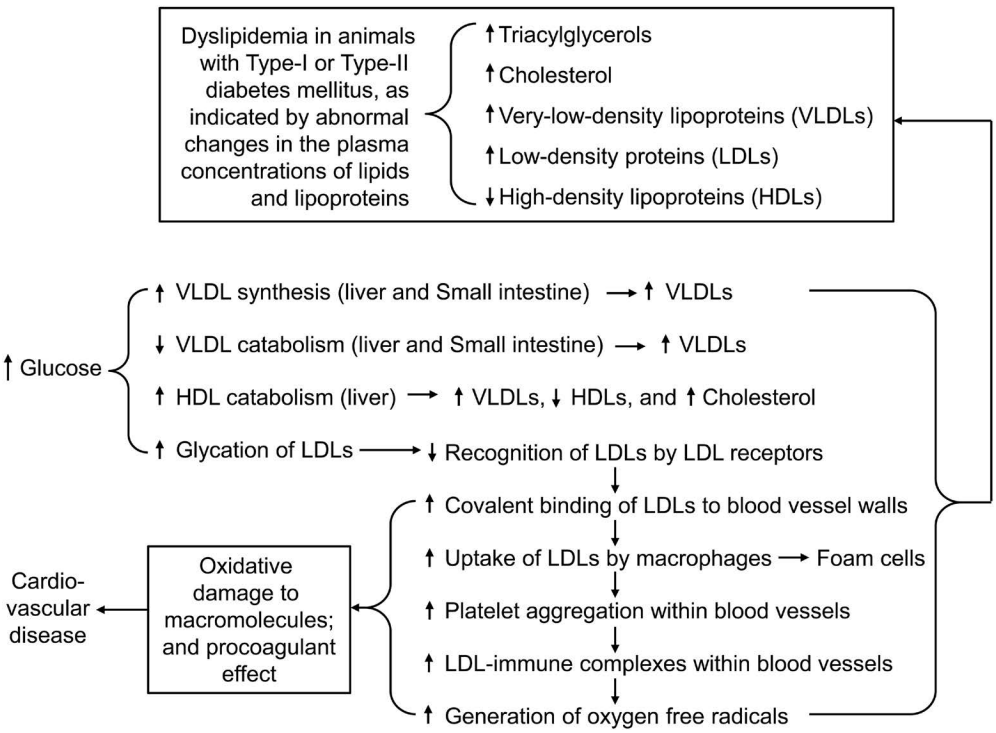


FIGURE 6.42 Dyslipidemia in animals with type I or type II diabetes mellitus. Glycosylated LDL proteins are not recognized by their receptors, resulting in high concentrations of LDLs in the blood, as well as oxidative stress and increased risks for cardiovascular disease.

to proteins and lipids, the injury and dysfunction of cell membranes and organelles, the impairment of cell signaling, and insulin resistance. Thus, there are reports that the expression and abundance of key lipogenic genes (e.g., PEPCK and FAS) are enhanced in the WAT of pigs with heat stress (Qu and Ajuwon 2018), while the expression of ATGL and lipolysis in this tissue decreased (Pardo et al. 2022; Sanz Fernandez et al. 2015), in favor of excessive TAG storage.

Hormonal and nutritional means have been explored to regulate fat content in farm animals. Specifically, increasing the circulating level of growth hormone or dietary intake of protein and amino acids can potentially reduce fat deposition in animals (Bergen and Mersmann. 2005; McKnight et al. 2010; Wray-Cahen et al. 2012). For example, intramuscular administration of recombinant porcine somatotropin to 74-kg barrows (50 μg/kg BW/day) for 24 days increases the percentage of protein and water in the body and skeletal muscle, while reducing the percentage of fats in the whole body and the WAT (Kramer et al. 1993). Similarly, intramuscular administration of recombinant porcine somatotropin (3 mg/day) to 47-kg barrows and gilts for 5 weeks improved gain by 11% and feed efficiency by 22%, while decreasing feed intake by 22%, adipocyte diameter by 7.2%, and backfat accretion by 93% (Lee et al. 1994). Compared with pigs fed a 14% CP diet, feeding a high-protein diet (20% CP) enhances protein gain and reduces TAG accretion in growing pigs. Furthermore, diets containing unsaturated fatty acids can enhance the β-oxidation of LCFAs while reducing abdominal fat deposition and fatty acid synthesis in animals (e.g., pigs and chickens), as compared to diets containing saturated fats (Sanz et al. 2000). Interestingly, dietary supplementation with 1% CLA (60% CLA isomers) to growing barrows (with a mean initial BW of 53 kg) for 6 weeks increases the size of intramuscular adipocytes and marbling, a desirable outcome in meat quality (Barnes et al. 2012).

Finally, supplementing 1% L-arginine to the diet of 110-day-old barrows for 60 days decreases serum TAG concentration by 20% and whole-body fat content by 11%, while increasing whole-body skeletal muscle mass by 5.5% (Tan et al. 2009). The effects of L-arginine on reducing lipids and improving the efficiency of protein deposition in finishing pigs have also been detected by the metabolomic analysis of serum samples (He et al. 2009). Unexpectedly, intramuscular lipid content is 70% greater in arginine-supplemented pigs than in controls to beneficially improve meat quality (Tan et al. 2009), indicating that lipid metabolism and its regulation vary with the anatomical location of WAT. Because muscle lipid represents only ~3% of carcass fats, it has little impact on whole-body lipid content in arginine-supplemented pigs (Tan et al. 2009). Similar beneficial effects of dietary arginine on lipid metabolism have been reported in growing fish such as Nile tilapia (Li et al. 2020). The underlying mechanisms are likely complex but may involve (a) activation of the AMPK and mTOR cell signaling pathways; (b) increases in lipolysis in the WAT, mitochondrial biogenesis, the oxidation of fatty acids to CO_2 and water in tissues (primarily the liver and skeletal muscle), and partitioning of dietary energy towards protein synthesis in skeletal muscle; and (c) decreases in fatty acid synthesis and lipogenesis in the liver and/or WAT (Jobgen et al. 2022a,b, 2023; McKnight et al. 2010; Tan et al. 2012). Likewise, results of recent studies indicate that supplementing 1% glycine to corn- and soybean meal-based diets between weaning and market weight enhances the mTOR cell signaling and antioxidative responses to reduce WAT deposition in pigs (particularly those with IUGR), leading to improved meat quality (He et al. 2023; 2024b,c).

Metabolic Disorders (Diseases) Related to Fatty Acid Metabolism

Lipid metabolism depends on enzymes, substrates, coenzymes, and cofactors. All of these factors are adversely affected by dietary deficiencies or excesses of nutrients. Inherited or acquired disorders in their metabolism cause multi-organ dysfunctions and/or characteristic diseases in animals (Table 6.13). Metabolic disorders (diseases) related to abnormal fatty acid metabolism are highlighted in the following paragraphs. Knowledge of metabolic disorders is necessary to prevent or treat them and to enhance the efficiency of animal production.

Obesity

A chronic imbalance between energy intake and expenditure in an animal causes excessive fat deposition in the body, leading to obesity, dyslipidemia, insulin resistance, type II diabetes mellitus, and reproductive dysfunction (Jobgen et al. 2006; Walzem and Chen 2014). Companion animals (e.g., dogs, cats, and untrained horses) have a higher risk for obesity than livestock and poultry that are processed for meat production at a relatively young age. Pigs have no BAT and naturally start to accumulate large amounts of WAT beginning at 45 kg BW. When fed conventional corn- and soybean meal-based diets, gilts (offspring of Landrace × Yorkshire) are obese at the time of breeding (~8 months of age) when they have 36% body fat (Rozeboom et al. 1995). In addition, significant body fat accumulation in beef cattle occurs only when they are fed grain-based (finishing) diets (typically in feedlots). Hormonal, nutritional (e.g., dietary arginine supplementation), and exercising strategies have been explored to regulate fat content and reduce obesity in farm and laboratory animals and in humans (Jobgen et al. 2023; Pojednic et al. 2022). The underlying mechanisms include (a) increases in the activation of AMPK, cAMP-dependent, and cGMP-dependent cell signaling pathways; the expression of genes for mitochondrial biogenesis and substrate (e.g., glucose, fatty acids, and glycerol) oxidation; and lipolysis, glycogenolysis, and antioxidative responses; and (b) decreases in the formation of malonyl-CoA, the synthesis of TAGs, and oxidative stress.

TABLE 6.13
Lipids-Related Metabolic Disorders (Including Diseases) in Ruminants and Nonruminants

Metabolic Disorder	Cause	Major Symptoms
Bovine fatty liver syndrome	Excess LCFAs in liver	Hepatic dysfunction, ketosis, and reduced lactation
Cancers (high risks for pancreatic and colon cancers)	Oxidative stress and obesity	Weight loss, muscle loss, bone loss, the dysfunction of multi-organs, metabolic disorders, and death
Cardiovascular disorders	Excess lipids in blood and NO deficiency	Hypercholesterolemia, hypertension, stroke, heart attack, impaired blood flow, and death
Essential fatty acid deficiency Syndrome	Deficiency of ω3/ω6 PUFAs	Skin lesions, growth restriction, reproductive failure
Fatty liver hemorrhagic syndrome (hens)	Stress (e.g., heat stress)	Excess fats in liver and hemorrhage in laying hens
Fatty liver and kidneys in chickens[a]	Deficiency of biotin	Abnormal liver and kidneys, and high lipid deposits
Gangliosidoses	Impaired hydrolysis of GM due to enzyme deficiencies	Excessive lysosomal accumulation of gangliosides; destroys neurons in the brain and spinal cord
Gaucher's disease	Deficiency of GCBase	Excess glucocerebroside in lysosomes; defects in brain
Ketosis in lactating dairy cows	Excess KB production	Metabolic acidosis, low appetite, and reduced lactation
Low-fat milk syndrome (cows)[b]	Low fiber/high-starch intake	Low ruminal pH, low Ac/Prop, and reduced lactation
Obesity (chronic excessive energy)	Energy intake > expenditure	Excess fat, dyslipidemia, and insulin resistance
Pregnancy toxemia[c]	Excess KB production	Metabolic acidosis, as well as maternal and fetal deaths
Yellow fat disease (steatitis)[d]	Excess intake of OUSFAs and low intake of Vit E	Deposition of yellowish pigment in white adipose tissue, oxidative stress, and possibly myopathy
Systemic metabolic acidosis	Blood pH < 7.35	Increased concentrations of ketone bodies, decreased concentrations of bicarbonate, and reduced pH in blood

[a] A deficiency of biotin impairs carboxylation reactions catalyzed by pyruvate carboxylase, acetyl-CoA carboxylase, propionyl-CoA carboxylase, and ß-methylcrotonyl-CoA carboxylase.
[b] This nutritional condition results in high concentrations of *trans*-10, *cis*-12 conjugated linoleic acid (CLA) in the rumen.
[c] Pregnancy toxemia also occurs in other mammals, such as beef cows, dairy cows, dogs, and mares during pregnancy.
[d] The disease occurs most commonly in nonruminants (e.g., cats, dogs, ferrets, fish, foals, horses, mink, pigs, poultry, rabbits, rats, and reptiles) and rarely in ruminants.
Note: Ac, acetate; GCBase, β-glucocerebrosidase that hydrolyzes glucocerebroside; GM, ganglioside monosialic acid; GPUT, galactose-1-phosphate uridylyltransferase, KB, ketone bodies (acetoacetate, β-hydroxybutyrate, and acetone); LCFAs, long-chain fatty acids; OUSFAs, oxidized unsaturated fatty acids; Prop, propionate; PUFAs, polyunsaturated fatty acids [e.g., α-linolenic acid (ω3) or linoleic acid (ω6) in all animals; and also arachidonic acid (ω6) in cats]; Vit, vitamin.

Essential Fatty Acid Deficiency Syndrome

Linoleic acid and α-linolenic acid are nutritionally essential fatty acids for all animals. Linoleic acid is the precursor for the synthesis of ω6 PUFAs (e.g., arachidonic acid), whereas α-linolenic acid is the precursor for the formation of ω3 PUFAs (e.g., EPA and DHA). In cats, which lack Δ^6-desaturase to convert linoleic acid into arachidonic acid, the latter is also a nutritionally essential fatty acids. Arachidonic acid, EPA, and DHA are essential components of cell membranes and are important bioactive lipids, as noted previously. In addition, linoleic acid is an important component of skin ceramides and, therefore, essential for skin barrier function (Djuricic and Calder 2021). When a deficiency of linoleic acid or α-linolenic acid occurs, animals exhibit numerous symptoms, including skin lesions, growth restriction, reproductive failure, increased susceptibility to infection, thrombocytopenia, impairment of neurological development, alopecia (hair loss), and poor wound healing (Spector and Kim 2015). These health problems also occur in cats with an inadequate intake of arachidonic acid. Dietary supplementation with the deficient essential fatty acids (e.g., a rumen-protected form in the case of ruminants) can effectively treat those syndromes.

Bovine Fatty Liver Syndrome

In dairy cows, there is an excessive mobilization of adipose tissue during early lactation (a period of inadequate energy intake) to release a large amount of LCFAs (Bobe et al. 2004). When the rate of hepatic uptake of lipids from the blood exceeds the rate of oxidation of fatty acids plus secretion of lipids by the liver, fat is accumulated in the liver, resulting in fatty liver syndrome (hepatic lipidosis). Affected cows are more likely to suffer from peri-parturient metabolic, health, and production problems, such as milk fever, ketosis, impaired hepatic function, low feed intake, mastitis, metritis, impaired lactation, and reduced reproductive performance. Fatty liver syndrome commonly occurs in obese high-producing cows, with mortality being as high as 25%. However, despite the name of this metabolic disorder, affected cows may not always be visibly overweight at calving time. Prevention of obesity or an overweight condition

is key to reducing the incidence of bovine fatty liver syndrome. Dietary supplementation with citrulline or rumen-protected arginine may stimulate the hepatic oxidation of fatty acids to minimize risks of dyslipidemia in lactating cows, as reported for nonruminants (Wu 2020). Other treatments may include (a) intravenous administration of glucose (60 g/h) and KCl; (b) intramuscular administration of Protamine zinc insulin (150–200 units); and (c) oral administration of propylene glycol (as a precursor of glucose; 0.5 to 1 L per day) along with intramuscular administration of ACTH (600 units on day 1; 400 units on days 2 and 3; none on day 4; and 200 units on day 5) to promote the release of amino acids from skeletal muscle that can be used for gluconeogenesis (Haass 1984).

Low-fat Milk Syndrome (Milk Fat Depression) in Dairy Cows

This disorder refers to a reduction in concentrations of milk fats (up to ≥50%) with little or no change in concentrations of milk lactose or protein (Bauman and Griinari 2003). Affected lactating cows often consume diets with low fiber content but high amounts of concentrates and unsaturated fatty acids. In grazing cows, milk fat depression may occur at some critical times of the year (e.g., early spring and autumn) and in regions where pasture grasses contain a low amount of fiber. Affected cows have a severely low ruminal pH and their rumen produce a relatively high amount of *trans*-10, *cis*-12 CLA. The latter inhibits fatty acid synthesis by mammary epithelial cells. At a concentration of ≤0.2 g/100 g of total fatty acids in the abomasum of cows, this CLA can induce milk fat depression (Bauman and Griinari 2003). Prevention of low-fat milk syndrome includes either an adequate intake of dietary fiber or dietary supplementation with rumen-protected palmitic acid (412 g/day; Granados-Rivera et al. 2017).

Ketosis in Lactating Dairy Cows

Ketosis is defined as elevated concentrations of ketone bodies (acetoacetate, β-hydroxybutyrate, and acetone) in the blood. This disorder results from the excessive oxidation of fatty acids to acetyl-CoA relative to the oxidation of acetyl-CoA to CO_2, leading to the conversion of acetyl-CoA into acetoacetate and β-hydroxybutyrate in the liver. In cows, ketosis usually occurs during early lactation when their appetite is depressed after calving and dietary energy intake cannot meet the increasing metabolic demands for milk production (Gordon et al. 2013). Because of a negative energy balance, cows mobilize their fat reserves to meet the needs of the brain, lactating mammary glands, muscles, and other tissues for energy. Factors that reduce feed intake and impair gluconeogenesis will increase risks of ketosis. Treatment should be based on the primary cause of the negative energy balance and ketosis. Increasing concentrations of glucose in the plasma through oral administration of propylene glycol (e.g., 300–900 ml once daily) or intravenous administration of 250 g glucose (i.e., 500 ml of 50% Dextrose) can help to restore physiological concentrations of glucose in the blood and to inhibit hepatic ketogenesis (Gordon et al. 2013).

Pregnancy Toxemia

This disorder refers to ketosis in gestating dams. Pregnancy toxemia is most prevalent in ewes and does carrying multiple fetuses during late gestation, but also occurs in other mammals, such as beef cows, dairy cows, dogs, mares, and women during pregnancy. In multi-fetal pregnancies, risks for ketosis increase when concentrations of glucose in maternal plasma are low, e.g., <30 mg/100 ml, which is lower than the normal values of 40–60 mg glucose/100 ml in gestating ewes and does (LeValley 2010). In addition, overly fat ewes and does are also susceptible to pregnancy toxemia under stressful conditions such as cold weather, heavy rain, and transportation because excessive fat mobilization in the WAT releases a large amount of free fatty acids. Pregnancy toxemia may be prevented by adding molasses or grain concentrates to diets for promoting hepatic gluconeogenesis. Treatment may include the oral administration of 200 ml of propylene glycol or corn syrup (rapid sources of energy and glucose) along with 3–4 L of an electrolyte solution, 4 times daily (LeValley 2010).

Fatty Liver Hemorrhagic Syndrome in Laying Hens

This disorder is common in prolific laying hens that are housed in cages, fed high-energy diets, and challenged with heat stress. Affected birds exhibit fatty liver syndrome, which is characterized by excessive fats in the liver and abdomen, as well as possibly pale combs (Hansen and Walzem 1993). The enlarged liver is prone to oxidative damage and hemorrhage. Hepatic bleeding often occurs in hens when they lay eggs, resulting in a high rate of mortality. The fatty liver hemorrhagic syndrome may be prevented or alleviated through dietary supplementation with either arginine (to reduce fat deposition) or Yucca extracts (to antagonize glucocorticoids), as well as improved housing and management.

Fatty Liver and Kidney Syndrome in Chickens

This disorder is caused by a deficiency of biotin in chickens (particularly 2- to 5-week-old chickens). The liver and kidneys of affected birds are pale and swollen, while having abnormally high lipid deposits (Balnave et al. 1977). In addition, concentrations of pyruvate and lactate are increased due to reduced pyruvate carboxylase activity, but concentrations of HCO_3^- and glucose are reduced in these tissues. The resulting lactic acidosis is a major factor contributing to high rates of morbidity and mortality in affected birds. The nutritional fatty liver and kidney syndrome in chickens can be prevented by dietary supplementation with biotin.

Yellow Fat Disease

This disorder (also called steatitis or pansteatitis) results from an excessive intake of oxidized unsaturated fatty

acids and deficiencies of vitamin E or other antioxidants in spoiled diets. Affected animals exhibit severe inflammation, lipid peroxidation, the deposition of the "ceroid" pigment, a rubbery texture, and yellowish appearance in the WAT (Harari 2018). These symptoms occur commonly in nonruminants (e.g., cats, dogs, ferrets, fish, foals, horses, mink, pigs, poultry, rabbits, rats, and reptiles) but rarely in ruminants. In some animals, myopathy may be observed. Yellow fat disease can be treated with oral administration of vitamin E (e.g., 15 and 30 mg/day for mink and cats, respectively) along with removal of the offending unsaturated fatty acids in feedstuffs.

High Risks for Cardiovascular Disease and Cancers

Excessive accumulation of lipids (primarily cholesterol, LCFAs, and TAGs) in the blood can partially or completely block blood vessels and induce oxidative stress in tissues, while reducing the oxidation of fatty acids and glucose, the synthesis and availability of NO in the vasculature, blood flow, and the delivery of O_2 and nutrients to tissues such as the brain, heart, and skeletal muscle (Bird et al. 2021; Jackson et al. 2024; Jobgen et al. 2006; McGlory et al. 2019). The adverse outcomes are obesity, insulin resistance, the development of cardiovascular diseases (e.g., hypertension, stroke, coronary and peripheral arterial disease, ischemia/reperfusion injury, heart failure, erectile dysfunction, and preeclampsia), and increased risks for cancers, particularly pancreatic and colon cancers. Thus, mitigating dyslipidemia is essential for optimal health in all animals.

SUMMARY

Fat-derived LCFAs and SCFAs are the major energy sources for nonruminants and ruminants, respectively. In nonruminants and preruminants, the digestion of lipids starts in the mouth and the stomach and is completed primarily in the upper part of the small intestine with the aid of taurine or glycine-conjugated bile salts and pancreatic secretions (including lipases and bicarbonate). Micelles consisting of lipid digestion products (MAGs, LCFAs, phospholipids, and cholesterol) pass the unstirred water layer, and the lipid components are absorbed through the apical membrane of enterocytes via simple diffusion and protein carriers. In ruminants, the digestion of TAGs in the rumen is limited (a maximum of 7% fats in the diet) but generates similar products but no MAGs, and the resulting LCFAs (containing biohydrogenated and conjugated LCFAs) are absorbed in the small intestine as in nonruminants. In all animals, the absorption of glycerol as well as SCFAs and MCFAs into enterocytes is mediated by protein carriers.

Once inside enterocytes, TAGs are resynthesized for the assembly of chylomicrons, nascent VLDLs, and premature HDLs. These lipoproteins are secreted into the lacteals (most nonruminant mammals and all ruminants) or the portal vein (poultry), and then enter the blood circulation at the junction of the jugular and subclavian veins. During their transport in the blood, mature HDLs released from the liver transfer Apo C-II and E to chylomicrons and VLDLs, and these fat-rich lipoproteins are then hydrolyzed extracellularly by LPL localized on the luminal surface of the microvascular endothelium within extrahepatic tissues, such as skeletal muscle, heart, WAT, kidneys, and macrophages. The resulting chylomicron remnants are taken up mainly by the liver and, to a lesser extent, by macrophages, whereas the resulting VLDL remnants (LDLs primarily plus IDLs) are mainly taken up by the liver and, to a lesser extent, by peripheral tissues (e.g., skeletal muscle, heart, WAT and kidneys) for catabolism in a tissue-dependent manner. Because the adult liver lacks LPL, hepatic lipase plays an important role in the clearance of TAGs from the blood. HDLs, released from the small intestine and liver, collect cholesterol from the peripheral tissues and transport it to the liver. In the liver, cholesterol is synthesized from acetyl-CoA and converted into bile acids or bile alcohols for excretion in the feces. Cholesterol is also used for the synthesis of all steroid hormones. Phospholipids, particularly phosphatidylcholine, play an important role in TAG digestion, as well as interorgan cholesterol and lipoprotein metabolism. In all animal cells, PUFAs can be metabolized to produce eicosanoids with diverse physiological functions, such as vasodilation, smooth muscle contraction, and mediation of pro- or anti-inflammatory responses.

In the fed and fasting states of animals, fatty acids are oxidized to CO_2 and water primarily via the mitochondrial β-oxidation pathway. Minor pathways for the catabolism of LCFAs and VLCFAs are the β-oxidation and ω-oxidation pathways in the peroxisome and the smooth ER, respectively. In most animals, except for pigs, under the conditions of fasting, energy deficits (e.g., lactation, late pregnancy, and intensive exercise), and type I diabetes mellitus, fatty acids are oxidized to acetoacetate and D-β-hydroxybutyrate. These ketone bodies serve as major energy substrates for the brain and extrahepatic tissues, as well as cell signaling molecules. In the fed state, saturated and monounsaturated LCFAs are synthesized primarily from glucose and amino acids in nonruminants, but from SCFAs and lactate in ruminants, in a tissue-dependent manner. When the dietary intake of energy exceeds energy expenditure, TAGs accumulate in the WAT and liver. The deposition of TAGs in the body depends on the balance between their synthesis and catabolism, which are under regulation by hormones (insulin, growth hormone and glucocorticoids), amino acids (e.g., arginine) and metabolites (e.g., NO and CO) via cell signaling involving protein kinases A and G (Figure 6.43). Excess deposition of TAGs in tissues results in obesity and can lead to insulin resistance and dyslipidemia, as well as increased risk for cardiovascular disease, fatty liver, and pancreatic disorders (Table 6.13). Interorgan metabolism of lipids plays an important role in the maintenance of their whole-body homeostasis in the health of animals, including mammals, birds, fish, and crustaceans.

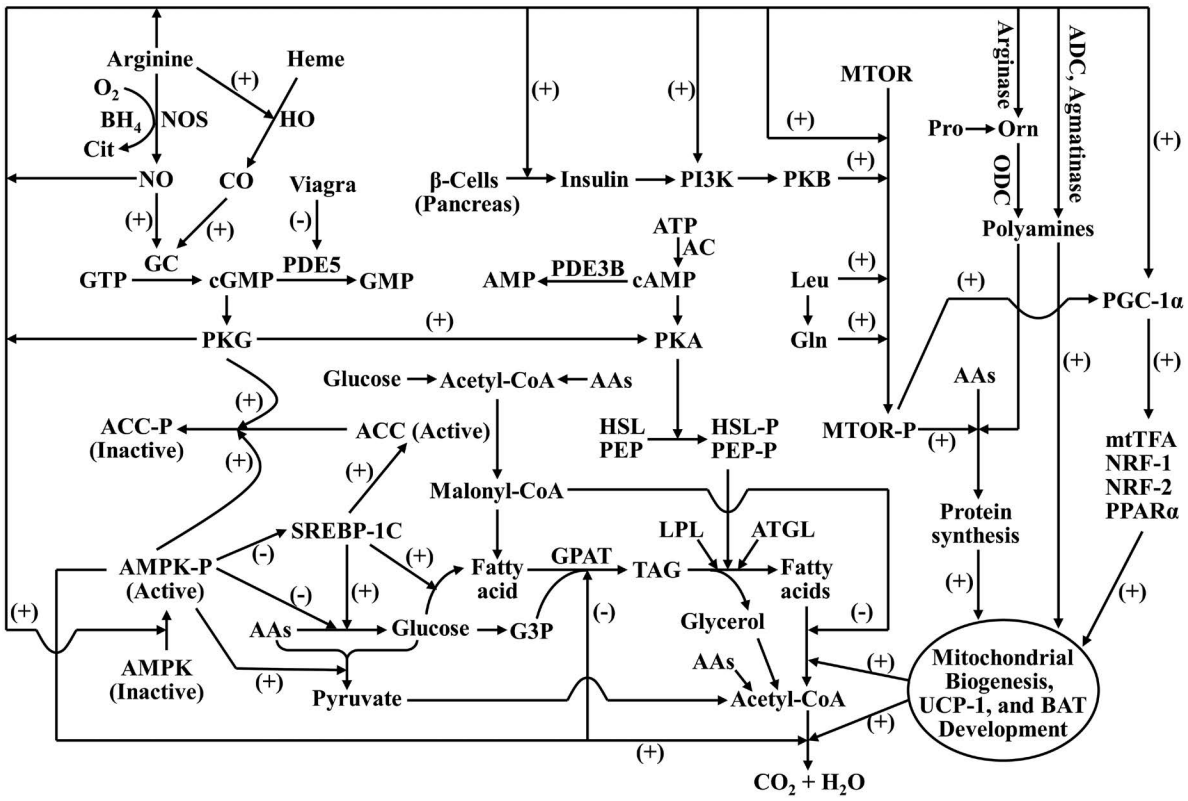


FIGURE 6.43 Regulation of fat deposition in animals through cell signaling involving cAMP- and cGMP-dependent protein kinases. AC, adenylyl cyclase; ACC, acetyl-CoA carboxylase; ADC, arginine decarboxylase; AMPK, AMP-activated protein kinase; ATGL, adipose triglyceride lipase; BAT, brown adipose tissue; BH4, tetrahydrobiopterin; Cit, citrulline; GC, guanylyl cyclase; G3P, glycerol-3-phosphate; GPAT, glycerol-3-phosphate acyltransferase; HO, heme oxygenase; HSL, hormone-sensitive lipase; LPL, lipoprotein lipase; MTOR, mechanistic target of rapamycin; mtTFA, mitochondrial transcription factor A; NO, nitric oxide; NOS, nitric oxide synthase; NRF, nuclear respiration factor; ODC, ornithine (Orn) decarboxylase; PDE5, phosphodiesterase 5; PDE3B, phosphodiesterase 3B; PEP, perilipins; PGC-1a, peroxisome proliferator-activated receptor-γ (PPAR-γ) coactivator 1α; PKA, cAMP-dependent protein kinase A; PKG, cGMP-dependent protein kinase G; PPAR-α, peroxisome proliferator activated receptor-α; Pro, proline; SREBP-1C, sterol regulatory element binding protein-1c; TAG, triacylglycerols; UCP-1, uncoupling protein-1. (Adapted from Wu 2022.)

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7 Nutrition and Metabolism of Protein and Amino Acids

Protein, which consists of amino acids linked by peptide bonds, is a major component of animals. Their growth depends on the deposition of protein in their tissues such as skeletal muscle, bone, and the small intestine (Bergen 2008; Buttery 1983; Reeds et al. 1993). Dietary protein is hydrolyzed by proteases and peptidases (oligo-, tri-, and di-peptidases) to generate tripeptides, dipeptides, and free amino acids in the lumen of the gastrointestinal tract (GIT). These digestion products are either utilized by bacteria in the small intestine or absorbed into enterocytes (Dai et al. 2011). The absorbed amino acids that are not degraded by the small intestine (including its mucosa and luminal microbes) enter the portal vein for utilization (including protein synthesis) by extraintestinal tissues. Except for the absorption of intact immunoglobulins by the small intestine of mammalian neonates, dietary protein has no nutritive value to animals unless it is digested (Chiba et al. 1991; Wu et al. 2014a). Thus, animals have dietary requirements for amino acids but not protein or nitrogen (N). The content, digestibility, and proportions of amino acids in dietary protein are the determinants of its nutritive quality.

Amino acids contain N and sulfur (S), as well as hydrocarbon skeletons, and cannot be replaced by any other nutrients (e.g., carbohydrates and lipids). Whether the hydrocarbon skeletons are synthesized de novo by animal cells depends on the amino acid and animal species (Baker 2009; Reeds et al. 2000). Adequate intake of dietary amino acids is essential for the optimal growth, development, and health of all animals. Thus, protein undernutrition causes stunting, anemia, physical weakness, edema, vascular dysfunction, and impaired immunity (Hou and Wu 2018a,b; Waterlow 1995). Note that Kwashiorkor (a disease in humans) results primarily from a severe deficiency of dietary protein, whereas marasmus (another disease in humans) results from severe deficiencies of both protein and energy (Wu 2022). On the other hand, excess protein intake leads to nutrient wasting and environmental pollution, as well as digestive, hepatic, renal, and vascular abnormalities. Thus, optimizing the recommendations of dietary amino acid requirements for animals is important for maximizing their growth, production performance, and feed efficiency, while improving their health and resistance to infectious disease. This is also essential for the precision feeding of animals (Eugenio et al. 2022; Pomar and Remus 2019).

Amino acids were traditionally classified as nutritionally essential or nonessential for animals solely based on N balance or growth. Nutritionally essential amino acids (EAAs) are defined as those amino acids whose carbon skeletons are not synthesized at all or adequately in animal cells. The so-called nutritionally nonessential amino acids (NEAAs) have not been considered by the U.S. National Research Council (NRC) for any animal species because they were assumed to be sufficiently synthesized de novo. However, a growing body of literature shows that this assumption is invalid because the supply of the synthesizable amino acids in diets cannot maximally support the survival, growth, reproduction, or lactation performance of animals (He et al. 2024a,b; Hou et al. 2015a; Li et al. 2022; McKnight et al. 2010). The different functions of individual amino acids beyond serving as the building blocks for proteins must be considered when formulating diets for livestock, avian, and aquatic species, as well as companion animals, to improve the efficiency of their nutrient utilization and well-being (Beaumont and Blachier 2020; Hu et al. 2022; Posey and Davis 2023; Wang et al. 2017; Wu 2022). It is now recognized that animals do have dietary requirements of all proteinogenic amino acids and that the long-standing term "NEAA" is a misnomer in the nutritional sciences (Hou and Wu 2017; Wu and Li 2022). These key aspects of amino acid nutrition and metabolism in animals are highlighted in this chapter. The standard abbreviations of all amino acids are used throughout the text to facilitate reading.

DIGESTION OF PROTEIN AND ABSORPTION OF SMALL PEPTIDES AND FREE AMINO ACIDS IN NONRUMINANT MAMMALS AND BIRDS

Protein intake by animals is often expressed per kilogram of body weight (BW). On an as-fed basis, swine diets usually contain 12–20% crude protein (CP): for example, 20% at weaning (21 days of age), 14% during the finishing period, 12% during gestation, and 18% during lactation. The CP content (on an as-fed basis) in chicken diets is usually 22%, 20%, and 18%, respectively, for starters (1–21 days of age), growers (22–35 days of age), and finishers (36–49 days of age). Equine diets usually contain 10–14% CP, but horses can digest 20% protein in diets [on a dry matter (DM) basis]. Fish diets usually contain 26–55% CP, depending on species and age (NRC 2011). In nonruminants, the digestion of dietary protein starts in the stomach, and most digestion occurs in the small intestine (Eugenio et al. 2022). Events of this process include (a) the denaturing of dietary protein and activation of zymogens by gastric acid in the stomach, followed by local digestion with active gastric proteases to form large peptides, and (b) the hydrolysis of the large peptides and the undigested proteins in the small intestine by the pancreas- and enterocyte-derived proteases. Proteases

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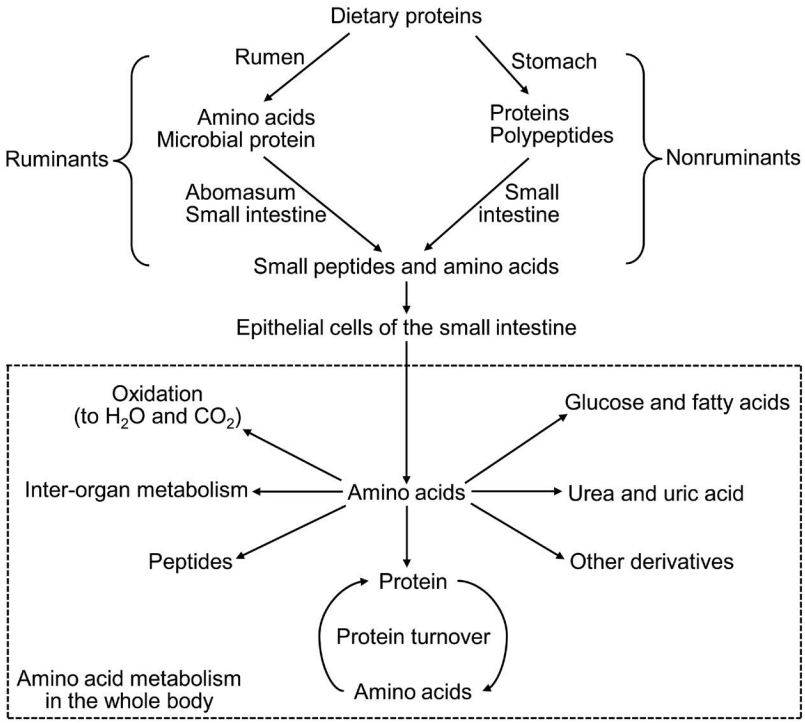


FIGURE 7.1 Overview of protein metabolism in animals. Ruminants digest dietary protein in the rumen, as well as rumen-escaped and microbial proteins in the abomasum and the small intestine. In nonruminants, the digestion of dietary protein is initiated in the stomach and completed in the small intestine. In all animals, the products of protein digestion (small peptides and free amino acids) are absorbed into the epithelial cells (enterocytes) of the small intestine and utilized by the whole body.

are enzymes that degrade proteins by hydrolysis of peptide bonds, whereas oligo-, tri-, and di-peptidases hydrolyze oligo-, tri-, and di-peptides, respectively. The products of protein digestion are absorbed into the enterocytes via amino acid and peptide transporters (Matthews 2000). The form of amino acids (e.g., intact protein, protein hydrolysates, peptides, or free amino acids) in diets can affect the postprandial appearance of amino acids in plasma, concentrations of insulin and other hormones in plasma, and the dynamics of amino acid metabolism for protein deposition (Eugenio et al. 2022). An overview of protein digestion and absorption is outlined in Figure 7.1. Among the different segments of the small intestine, the jejunum is the major site for terminal protein digestion and the absorption of the digestion products, but these processes also take place in the duodenum and ileum (Sauer et al. 2000). The digestibility of dietary protein is affected by biological (e.g., species and strains of animals, physiological status, and individual variations), environmental (e.g., ambient temperatures, pollution, and noise), and dietary factors (e.g., the form, odor, texture, and particle size of feed).

There are many bacteria (both species and number) throughout the nonruminant digestive tract, and a rather high population in the lower small intestine. These intestinal bacteria play a role in the digestion of food (including protein) by breaking down the food matrix (especially plant fiber), releasing proteins, and secreting proteases/peptidases. Following the release of amino acids due to the bacterial action, the amino acids can be taken up by the bacteria for protein synthesis (growth), catabolism, or conversion into new amino acids. There may be some absorption of bacteria-derived amino acids in the small and large intestines, although the amounts are not considered to be nutritionally significant. Overall, intestinal bacteria degrade more amino acids than those synthesized by the microbes plus those absorbed by the gut. Measures of protein digestibility are based on the disappearance of amino acids in the digestive tract, with disappearance being assumed to equate with absorption. This is not an accurate method because some of the dietary amino acids may have been catabolized by intestinal bacteria. The complex role that microbes play in food (including protein) digestion is not fully understood.

Digestion of Protein in the Stomach of Nonruminant Mammals and Birds

Secretion of Gastric Hydrochloric Acid (HCl)

In nonruminants, the digestion of dietary protein in the stomach requires not only active gastric proteases (pepsins and rennin) but also HCl. This acid is produced from NaCl and carbonic acid (H_2CO_3) by parietal cells in the gastric glands to create an acidic environment (e.g., pH = 2–2.5 in adults; equivalent to 10^{-2} to $10^{-2.5}$ M HCl). The stimulatory or inhibitory factors for the control of gastric acid secretion act primarily through affecting the production of gastrin

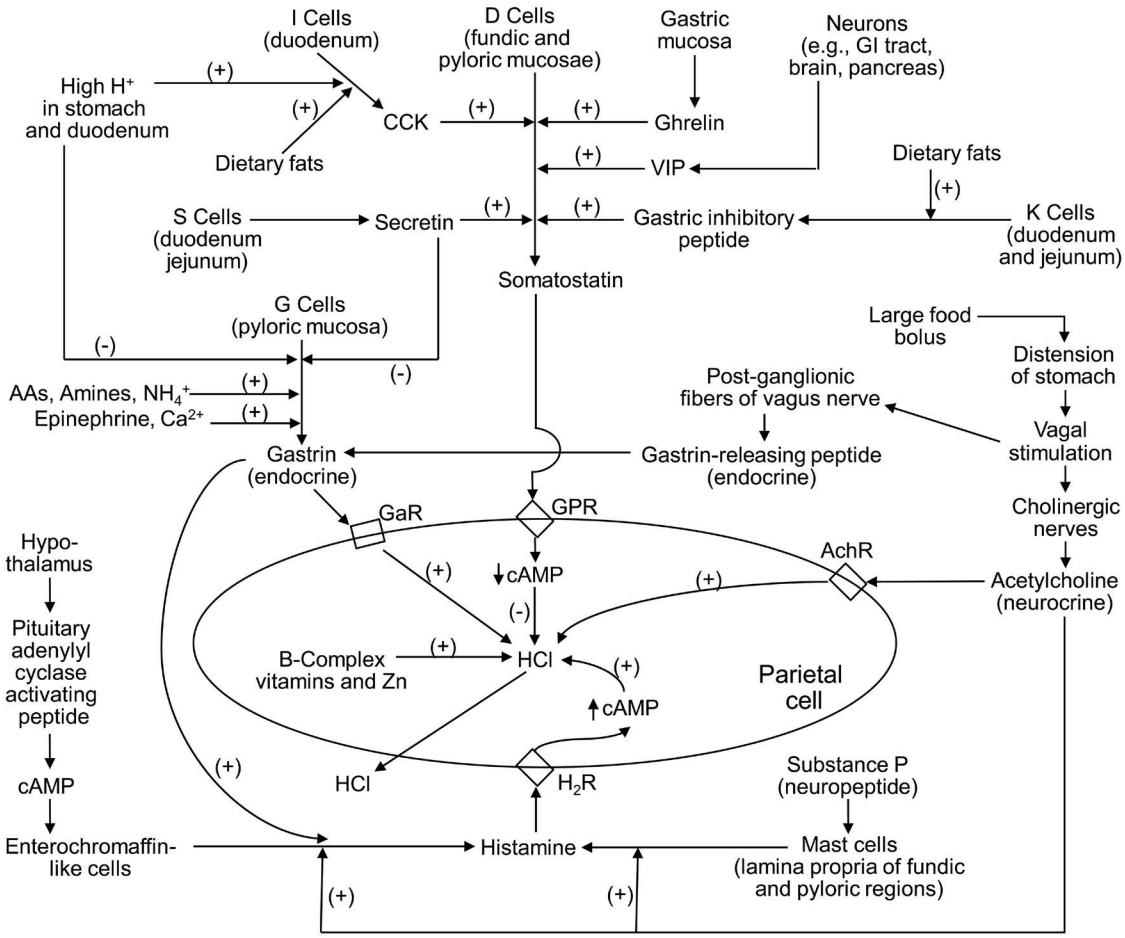


FIGURE 7.2 The regulation of gastric HCl secretion by stimulatory or inhibitory factors. These factors act primarily by affecting the production of gastrin (stimulatory) or somatostatin (inhibitory) by the mucosae of the stomach and the duodenum. AAs, amino acids; AchR, acetylcholine receptor; CCK, cholecystokinin; GI, gastrointestinal; GaP, gastrin receptor; GPR, G-protein-coupled receptor; VIP, vasoactive intestinal peptide. (+), stimulatory; (–), inhibitory.

(stimulatory) or somatostatin (inhibitory) in the mucosa of the stomach and duodenum (Figure 7.2).

1. *Stimuli of gastric acid secretion*. Neural, hormonal, and nutritional stimuli of gastric secretion enhance the generation of one or more of the following molecules: (a) gastrin (endocrine; secreted by gastrin G-cells; primary stimulus), (b) acetylcholine (neurocrine; released by the vagus nerve and enteric system), and (c) histamine (nutritional and paracrine; released from gastric enterochromaffin-like cells; Cranwell 1995; Yen 2001). These signaling molecules bind to their respective receptors on the plasma membrane of the parietal cell to increase histamine release and cyclic adenosine monophosphate (cAMP) production (Figure 7.2).
2. *Inhibitors of gastric acid secretion*. The factors inhibiting gastric HCl secretion include (a) slow gastric emptying, (b) high H^+ concentrations in gastric and duodenal contents (stimulating secretin release), (c) some nutritional factors (e.g., dietary protein and fat intakes, and feed particle size), and (d) many gastrointestinal peptides of different sources (Figure 7.2). Examples of these peptides are secretin (released by D-cells in the duodenum and jejunum), gastric inhibitory peptide (GIP, also known as glucose-dependent insulinotropic peptide; released by K-cells in the duodenum and jejunum), vasoactive intestinal peptide (released by the GIT, brain, pancreas, heart, and many other tissues), peptide YY (released by L-cells of the ileal mucosa), and somatostatin (secreted by endocrine cells of the gastric epithelium; primary inhibitor). Secretin, GIP, and vasoactive intestinal peptide all stimulate the release of somatostatin, which binds to the G-protein-coupled receptor on the plasma membrane of parietal cells on the stomach mucosa and inhibits adenylyl cyclase activity (i.e., cAMP production).

Different dietary factors inhibit gastric HCl secretion through different mechanisms. Examples include (a) food-induced distension through mechanoreceptors to stimulate secretin release; (b) high concentrations of fat digestion products through the activation of cholecystokinin (CCK) release from intestinal enteroendocrine I cells in the duodenum; (c) dietary deficiencies of protein,

zinc, and B-complex vitamins by impairing various metabolic pathways; (d) large feed particles due to their rapid passage out of the stomach and possible damage to its epithelium; and (e) small feed particles because of their prolonged retention in the stomach (particularly the esophageal region) and damage to its epithelium (gastric ulceration) (Rojas and Stein 2017).

Digestive Function of Gastric HCl and Gastric Proteases

Gastric HCl has two main digestive functions: (a) to convert inactive gastric proteases [pepsinogens A, B, C, and D, and pro-rennin (pro-chymosin); collectively called zymogens] to active proteases [pepsins A, B, C, and D, and rennin (chymosin)] (Figure 7.3); and (b) to denature dietary proteins so that they lose their natural folded structures to expose their peptide bonds to the active proteases for hydrolysis. All these zymogens are synthesized and released by the chief cells of the gastric glands in the fundic and pyloric regions (Cranwell 1995). Rennin is a mammalian enzyme and is not synthesized by birds.

The gastric protease precursors are activated by gastric HCl through limited auto-catalysis. Pepsin A (optimum pH = 2) is the major type of pepsin. As endopeptidases, pepsins break down peptide bonds between a hydrophobic amino acid and a preferably aromatic amino acid (e.g., phenylalanine, tryptophan, or tyrosine) from the inside of protein to generate large peptides (Figure 7.4). Pepsins are inhibited by high pH and by pepstatin. The latter is a hexa-peptide that contains an unusual amino acid, statine [(3S,4S)-4-amino-3-hydroxy-6-methylheptanoic acid)] and was originally isolated from *Actinomyce* cultures.

Developmental Changes of Gastric Proteases in Nonruminant Mammals and Birds

Not all aspects of developmental changes in gastric proteases are known for each species. Available data from most species studied to date indicate that the abundances of pepsinogens A, B, and C in the stomach are very low at birth but increase markedly after birth. Here, let us use pigs as an example. Pepsinogens A and C in the stomach are barely detectable at birth, but their concentrations (mg/g tissue) increase progressively in the entire mucosa of the gastric fundic region between birth and day 160 of age (Cranwell 1995). Likewise, pepsinogen B in the entire mucosa of the fundic region of the stomach is negligible at birth and increases markedly between birth and day 30 of age. Of note, the concentration of the mucosal pepsinogen B (mg/g tissue) peaks at day 30 of age, then declines progressively to a medium level at day 60 of age, and thereafter remains at the medium level until day 160 of age. Starting from day 30 of age, pepsinogen A is the most abundant gastric protease in the gastric mucosa among all the zymogens.

Enteral feeding and glucocorticoids play an important role in the induction of gastric pepsinogens A, B, and C in postnatal animals. In contrast, the expression of gastric pro-rennin increases progressively in fetal pigs during the last trimester of gestation and peaks at birth (Yen 2001). As a result, in pigs, the concentration (mg/g tissue) of pro-rennin in the entire wall of the fundic region of the stomach is greatest at birth but declines progressively to a very low level at day 60 of age and then remains at the minimal level until day 160 of age. These patterns in the development of gastric zymogens play an important role in the adaptation of mammals to the shift of dietary protein sources from milk to primarily the ingredients of plant origin.

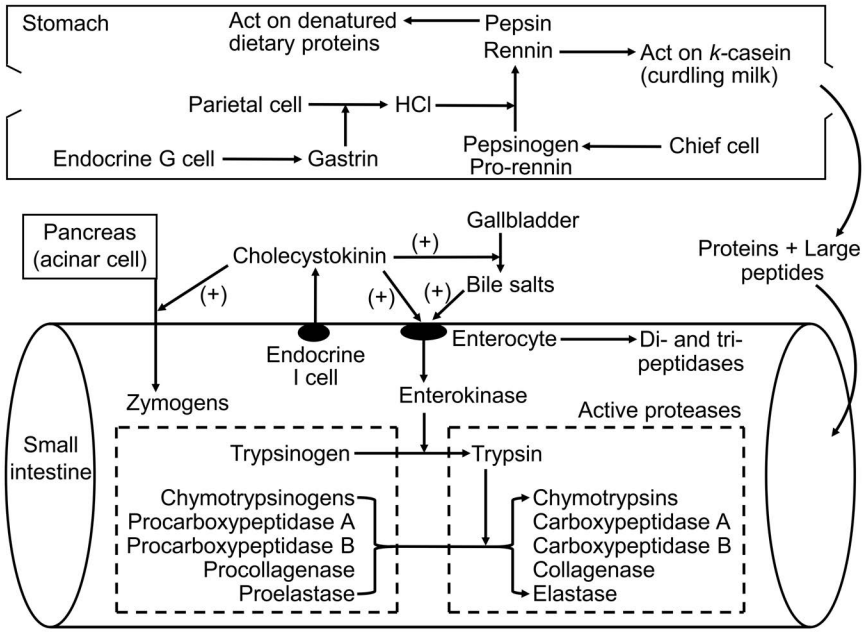


FIGURE 7.3 Conversion of zymogens to active proteases by HCl within the stomach through limited auto-catalysis.

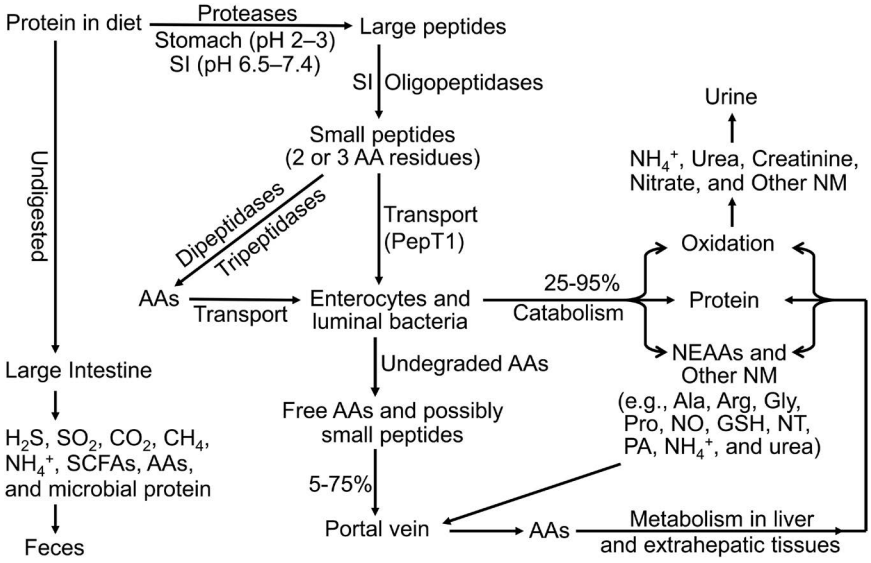


FIGURE 7.4 Digestion of dietary protein in the gastrointestinal tract of the small intestine in monogastric animals, including humans. All diet-derived AAs undergo various degrees of catabolism by luminal bacteria, and some of them are oxidized by enterocytes. For example, 95% of dietary glutamate is utilized by the small intestine, and only 5% of dietary glutamate enters the portal circulation. AA metabolites are excreted in feces and urine. AAs, amino acids; GSH, glutathione; NEAAs, nutritionally nonessential amino acids; NM, nitrogenous metabolites; NT, nucleotides; PepT1, H^+ gradient-driven peptide transporter 1; SCFAs, short-chain fatty acids; SI, the small intestine.

As noted previously, the amount of rennin within the stomach of adult mammals is low or negligible. However, rennin (optimum pH = 3–4) plays a crucial role in curdling or coagulating milk proteins in the stomach of mammalian neonates. Rennin has a weak proteolytic activity. There are three isoforms of rennin, i.e., rennins A, B, and C (Rampilli et al. 2004). In the milk of most mammals, the major protein is casein, which exists in four major types: α-s1, α-s2, β, and κ. In milk, κ casein interacts with α and β caseins to form water-soluble micelles. As an endopeptidase, rennin partially degrades the κ casein, converting it into para-κ casein and a smaller protein (Brinkhuis and Payens 1985). Consequently, para-κ casein cannot stabilize the micellar structure of caseins, resulting in the precipitation of the hydrophobic α and β caseins by Ca^{2+} to form a curd. The casein curd is then degraded by pepsins to yield large peptides. If milk proteins are not coagulated in the stomach, they will rapidly flow out of the stomach and will not be subject to initial digestion by pepsins and may also pass through the small intestine quickly. In animals that lack rennin, milk is partially coagulated by the action of pepsins.

Developmental Changes of Gastric Proteases in Avian Species

Avian species exhibit a different pattern of changes in the development of gastric proteases than mammals. Studies with chickens and quails indicate that the specific activities of pepsinogens A, B, and C in the proventriculus (glandular stomach) increase progressively during embryonic development (Yasugi and Mizuno 1981) and reach a temporary peak several days before hatching. Interestingly, in both chickens and quails, the specific activities of the gastric acid proteases increase 30-fold within 24 h after hatching, in comparison with the values at birth, regardless of enteral feeding. The rapid shift in the expression of these gastric enzymes is likely mediated by hormones such as glucocorticoids. This, along with the grinding function of the gizzard, prepares the birds for post-hatching consumption and utilization of solid foods.

Regulation of the Secretion of Gastric Proteases in Nonruminat Mammals and Birds

The amounts and activities of proteases and peptidases in the stomach of nonruminants are affected by (a) dietary protein intake, (b) enteral feeding and glucocorticoids, (c) release of acetylcholine from the vagus nerve, (d) concentrations of HCl in the stomach, and (e) secretion of CCK, gastrin, and other gastrointestinal peptides (Raufman 1992). Because gastric proteases are proteins, dietary provision of amino acids has a profound effect on stimulating the synthesis of these enzymes by the chief cells of the gastric mucosa (San Gabriel and Uneyama 2013). For example, in most teleost fish, pancreatic digestive proteases are produced and secreted from the pancreas in response to the first feeding (Rønnestad et al. 2013). In addition, dietary protein intake increases gastric acid secretion to activate gastric proteases and facilitate protein hydrolysis. Factors that stimulate the secretion of gastric proteases include amino acids, histamine, acetylcholine, gastrin, gastrin-releasing peptide, vagal stimulation, CCK, secretin, and vasoactive intestinal peptide. Most of these factors also enhance gastric acid production; some exceptions are CCK, secretin, and vasoactive

intestinal peptide, which have opposite effects (Figure 7.2). In contrast, somatostatin and peptide YY inhibit the secretion of gastric proteases and acid.

Digestion of Proteins in the Small Intestine of Nonruminant Mammals and Birds

Flow of Digesta from the Stomach into the Small Intestine for Proteolysis

Dietary proteins that are resistant to pepsins in the stomach, together with the large polypeptides resulting from enzymatic hydrolysis by pepsins in the stomach, enter the duodenum. In the lumen of the duodenum, the proteins and the large polypeptides are further broken down by pancreas- and small-intestinal mucosa-derived proteolytic enzymes in the alkaline medium (owing to bile salts, pancreatic juice, and duodenal secretions). The extracellular proteolysis occurring in the duodenum is limited due to the short length of this intestinal segment in animals (Barrett 2014). The chyme moves into the jejunum, where most proteolysis takes place due to its long length and high protease activities. Continuous digestion of protein and polypeptides can occur in the ileum if their degradation is not completed in the jejunum (including its luminal microbes).

Release of Pancreatic Pro-Proteases into the Lumen of the Duodenum

The acinar cells of the pancreas secrete inactive proteases (pro-proteases) into the lumen of the duodenum. They are the zymogens of endopeptidases (trypsin, chymotrypsins A, B, and C, and elastase) and exopeptidases (carboxypeptidases A and B; Table 7.1). These inactive enzymes become active in the duodenal lumen by a cascade of limited proteolysis [the removal of an N-terminal oligopeptide (2–6 amino acid residues) from each zymogen]. First, enterokinase, which is synthesized and released by enterocytes of the duodenum, converts trypsinogen into trypsin (the active enzyme) through the removal of an N-terminal hexapeptide. Trypsin then converts other pancreatic zymogens into active forms such as chymotrypsins A, B, and C, elastase, and carboxypeptidases A and B. This series of protease activations is controlled by bile acids and CCK, which

TABLE 7.1
Digestive Proteases and Peptidases in the Stomach and the Small Intestine

Enzyme	Site of Production	Recognized Amino Acid Residues in Peptide Bonds	Optimal pH
	Proteases in the Lumen of the Stomach		
Pepsins A, B, C and D[a]	Mucosa of stomach	Aromatic and hydrophobic amino aids (most efficient)	1.8–2
Renins A, B and C[a]	Mucosa of stomach	Weak proteolytic activity; clot milk protein	1.8–2
	Proteases in the Lumen of the Small Intestine		
Trypsin[a]	Pancreas	Arginine and lysine	8–9
Chymotrypsins A, B and C[a]	Pancreas	Aromatic amino acids and methionine	8–9
Collagenase[a]	Pancreas	Peptide bonds formed from a neutral amino acid (NAA) and Gly in a Pro-NAA-Gly-Pro sequence	7.5
Elastase[a]	Pancreas	Aliphatic amino acids	8.5
Carboxypeptidase A[b]	Pancreas	Aromatic amino acids	7.2
Carboxypeptidase B[b]	Pancreas	Arginine and lysine	8.0
Aminopeptidases[b]	Enterocytes of small intestine	Amino acids with free NH_2 groups	7.0–7.4
	Oligopeptidases, Tripeptidases, and Dipeptidases in the Lumen of the Small Intestine		
Oligopeptidase A	Enterocytes of small intestine	A broad spectrum of oligopeptides	6.5–7.0
Oligopeptidase B	Enterocytes of small intestine	Basic amino acids in oligopeptides	6.5–7.0
Oligopeptidase P	Enterocytes of small intestine	Pro or OH-Pro in oligopeptides	6.5–7.0
Dipeptidases	Enterocytes of small intestine	Dipeptides (not containing imino acids)	6.5–7.5
Tripeptidases	Enterocytes of small intestine	Dipeptides (not containing imino acids)	6.5–7.5
Prolidase I (dipeptidase)[c]	Enterocytes of small intestine	Proline or hydroxyproline	7.2
Prolidase II (dipeptidase)[d]	Enterocytes of small intestine	X-Hydroxyproline	8.0

[a] Endopeptidase (also called proteinase in the literature). Collagenase is a metalloprotease.

[b] Exopeptidase (a metalloprotease). Examples are aminopeptidases A, B, N, L, and P, which cleave an acidic amino acid (e.g., Asp or Glu), basic amino acid (e.g., Arg or Lys), neutral AA (e.g., Ala or Met), Leu, and Pro, respectively, from the N-terminus of a polypeptide. Aminopeptidase M removes any unsubstituted amino acid (including Ala and Pro) from the N-terminal position of a polypeptide; it is both an amino- and an iminopeptidase.

[c] Reacts with all imino dipeptides at high activity.

[d] Exhibits very low activity toward Gly-Pro, and reacts with X (a nonglycine amino acid; e.g., methionine or phenylalanine)-Hydroxyproline at high activity.

stimulate the production of enterokinase by the duodenal enterocytes and of proteases by the pancreas (Figure 7.1). The active pancreatic proteases are present in the small-intestinal lumen, and some of them may be associated with the brush-border surface of the enterocytes.

Release of Proteases and Oligopeptidases from the Small-Intestinal Mucosa into The Intestinal Lumen

The mucosae of the duodenum, jejunum, and ileum release the active forms of proteases and oligopeptidases into the brush-border surface, some of which are present in the intestinal lumen. Therefore, no activation of these enzymes is required for their proteolytic actions. Aminopeptidases are exopeptidases that hydrolyze a protein or large peptide from the N-terminus. Although some of the intestinal aminopeptidases (e.g., aminopeptidases A, B, N, and P) have broad substrate specificity, others (e.g., alanine aminopeptidase, leucine aminopeptidase, Met aminopeptidase, and *N*-formylmethionine aminopeptidase) are highly specific toward the N-terminus containing a specific amino acid (e.g., alanine, leucine, Met, or formylmethionine; Giuffrida et al. 2014; Sherriff et al. 1992). *N*-Formylmethionyl oligopeptides arise from N-terminal peptide sequences of bacterial precursor proteins. As their names imply, aminopeptidases A, B, and N cleave peptide bonds at the N-terminus adjacent to an anionic, basic, or neutral amino acid, respectively. Aminopeptidase P cleaves a peptide bond at the N-terminus adjacent to proline or hydroxyproline. Many, but not all, of aminopeptidases are zinc metalloenzymes.

Oligopeptidases hydrolyze peptide bonds in oligopeptides consisting of 2–20 amino acid residues (Giuffrida et al. 2014). Oligopeptidase A acts on a broad spectrum of oligopeptide substrates. However, oligopeptidase B hydrolyzes a peptide bond on the carboxyl side of a basic amino acid in an oligopeptide, whereas prolyl oligopeptidase (prolyl endopeptidase) cleaves proline or hydroxyproline from the inside of an oligopeptide. As alluded to previously, oligopeptidases are bound to the brush-border surface of the enterocytes.

Extracellular Hydrolysis of Proteins and Polypeptides in the Small Intestine

All the proteases and peptidases present in the small intestine have an optimum pH ranging from 6.5 to 9.0, which is similar to the luminal fluid pH of the duodenum (pH 6.5), jejunum (pH 7.0), and the terminal ileum (pH 7.4) in birds and nonruminant mammals, and of the intestine of many fish species (pH 6.5–7.4). Trypsin, chymotrypsins, and elastase (all endopeptidases) specifically cleave amino acid residues in peptide bonds residing inside a protein or polypeptide; namely, basic amino acids, aromatic amino acids plus Met, and aliphatic amino acids, respectively (Figure 7.5). Carboxypeptidases A and B (exopeptidases) catalyze, respectively, the hydrolysis of an aromatic (or aliphatic) amino acid (e.g., phenylalanine or alanine) and a basic amino acid (lysine, arginine, or histidine) from the C-terminal position of a protein or polypeptide (Figure 7.5). Aminopeptidases (exopeptidases) cleave an amino acid from the N-terminal position of a polypeptide. For example, aminopeptidases A, B, N, L, and P remove an acidic amino acid (e.g., aspartate or glutamate), basic amino acid (e.g., arginine or lysine), neutral amino acid (e.g., alanine or methionine), leucine, or proline, respectively, from the N-terminus of a polypeptide. Interestingly, aminopeptidase M (originally found in the so-called microsomes) cleaves any unsubstituted amino acid (including alanine and proline) from the N-terminal position of a polypeptide and therefore acts as both amino- and iminopeptidases.

Through their collective actions, the proteases present in the small intestine ultimately generate oligopeptides, which are hydrolyzed by oligopeptidases into small peptides (consisting of 2–6 amino acid residues) and free amino acids in the intestinal lumen. The small peptides containing 4–6 amino acid residues are further hydrolyzed by peptidases

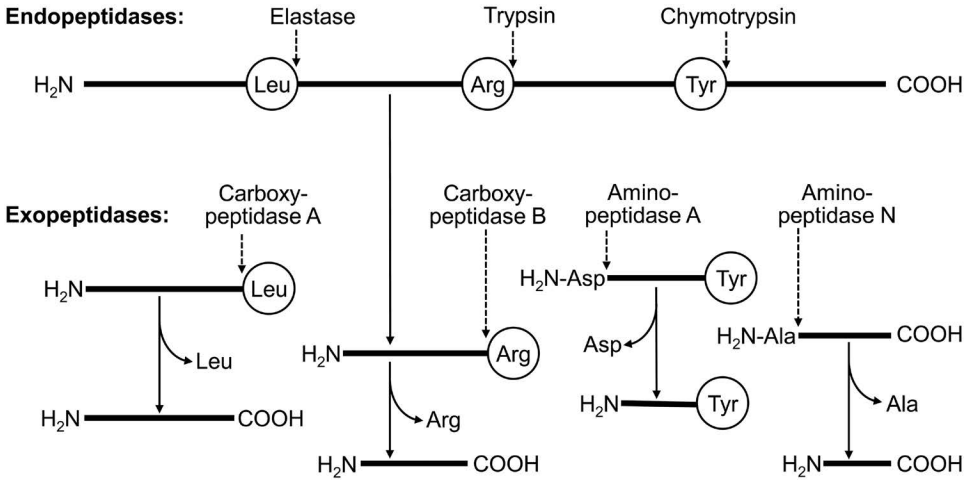


FIGURE 7.5 Specific cleavage of peptide bonds by trypsin, chymotrypsins, and elastase (all endopeptidases), as well as carboxypeptidases (Carboxy) A and B (all exopeptidases).

(primarily bound to the brush-border of enterocytes and, to a lesser extent, in the intestinal lumen) to form free amino acids, dipeptides, and tripeptides (Giuffrida et al. 2014). Dipeptides (not containing imino acids, i.e., proline or hydroxyproline) and tripeptides are hydrolyzed by mucosa-derived dipeptidases and tripeptidases, respectively. Dipeptides containing an imino acid are cleaved by mucosa-derived prolidases. Prolidase I hydrolyzes all dipeptides containing an imino acid, whereas prolidase II has a stricter substrate requirement [i.e., acting only on dipeptides containing a nonglycine amino acid (e.g., methionine or phenylalanine) and an imino acid]. All peptidases are highly active on the apical membrane of the enterocyte at birth and remain so throughout life (Austic 1985). The products of protein digestion in the lumen of the small intestine consist of approximately 20% free amino acids and 80% di- and tripeptides.

Developmental Changes in Extracellular Proteases in the Small Intestine of Nonruminant Mammals

Nonruminant mammals (e.g., pigs) are born with relatively low activities of chymotrypsins A, B, and C, elastase, and carboxypeptidases A and B in the small intestine, but a well-developed intestinal peptidase capacity (Austic 1985; Cranwell 1995). After birth, the total activities of all pancreatic proteases measured in the whole small-intestinal contents increase with age before weaning, decrease markedly during the first 3–7 days postweaning, and rise again thereafter during the growing period, as shown for pigs, guinea pigs, and rabbits (Debray et al. 2003; Owsley et al. 1986; Tarvid 1992; Yen 2001). The small-intestinal mucosa-derived proteases exhibit a pattern of changes similar to the pancreas-derived proteases (Austic 1985). The development of intestinal proteases has important implications for protein digestion. For example, young mammals effectively digest nonimmunoglobulin proteins in colostrum (100%; Lin et al. 2009) and milk (92%; Mavromichalis et al. 2001). However, these animals have a lower ability to hydrolyze plant-sourced proteins than adults (Wilson and Leibholz 1981). This is because young animals have underdevelopments in gastrointestinal mucosal and microbial proteases before weaning, and they do not possess strong teeth to grind solid materials (e.g., cereal grains). Young mammals cannot use any proteins that are entrapped inside cereal grains due to the lack of exposure of the proteins to digestive proteases. This is because the plant cell walls are barely broken down and the dietary nonstarch polysaccharides (NSPs) that are bound to plant proteins are barely degraded (Yen 2001). However, as alluded to previously, young mammals can utilize processed plant-sourced proteins in the matrix of the ingredients containing little or none of the following hyperallergic or antinutritional factors: trypsin inhibitors, glycinin, β-conglycinin, phytate, oligosaccharides (raffinose and stachyose), and saponins (Sauer and Ozimek 1986). After weaning, pigs adapt to solid diets and develop a relatively high ability to digest proteins in corn grains, soybean meal (SBM), sorghum grains, and meat and bone meal (MBM), with their true ileal digestibilities ranging from 85% to 90% (Table 7.2).

After weaning, horses gradually develop a high capacity for digesting dietary protein in the large intestine and are able to digest plant-sourced feed (e.g., forages, grasses, corn, SBM, wheat, and barley). In horses, total tract protein digestibility is 85–90% for SBM and cereal grains (e.g., corn, oats, and sorghum), with prececal digestibility (occurring in the small intestine) being greater than 50%; 78% for alfalfa, with prececal digestibility being 28%; and 60% for coastal bermudagrass, with prececal digestibility being 17% (Spooner 2012). Thus, in these animals, dietary protein is hydrolyzed mainly in the small intestine when they consume cereal grains but in the large intestine when they consume forages. The process of protein digestion in the large intestine of horses and rabbits is similar to that in the rumen.

Developmental Changes in Extracellular Proteases in the Small Intestine of Avian Species

Total protease activities in the avian small intestine increase markedly between birth and 21 days of age (Noy and Sklan 1995). For example, total trypsin activity is 5- to 6-fold greater at day 10 than at day 1 post-hatching, and total chymotrypsin activity rises gradually from shortly after hatching to day 14 post-hatching and then remains constant until day 23 post-hatching (Nitzan et al. 1991; Nir et al. 1993). In chickens, total carboxypeptidase A activity peaks on the first day after hatching, declines during the first week post-hatching, and then remains at a constant level until 56 days post-hatching (Tarvid 1991). As in mammals, intestinal peptidase activity is high in poultry at birth (Tarvid 1992). This developmental pattern of intestinal proteases, coupled with a functional gizzard to grind the ingested cereal grains, allows newborn birds to utilize a solid corn- and SBM-based diet, albeit at a lower rate (e.g., compared with older animals). Accordingly, the true digestibility of plant-sourced CP in chickens increases from 78% at 4 days of age to 92% at 21 days of age (Noy and Sklan 1995). Similarly, 3-week-old broiler chickens can effectively utilize plant-sourced proteins, as indicated by a digestibility coefficient of 85–90% for individual amino acids (Table 7.2). In birds, as reported for mammals, luminal bacteria in the small intestine can be a significant source of proteases and peptidases for the hydrolysis of dietary protein and peptides (Dai et al. 2015), and the feedstuff matrix can also affect the digestibility of dietary protein (Moughan et al. 2014; Sauer et al. 2000).

Regulation of Activities of Small-Intestinal Proteases

The amounts and activities of proteases and peptidases in the small-intestinal tract are affected by dietary factors through (a) the direct inhibition of proteases, (b) the regulation of gene expression, and (c) the regulation of CCK secretion. For example, increasing dietary protein levels stimulates the synthesis and secretion of proteolytic enzymes by the pancreas and small-intestinal

TABLE 7.2
True Ideal Digestibilities of Amino Acids in Ingredients for Chicken and Swine Diets[a]

	Broilers (21 Days of Age)				Pigs (50–65 Days of Age)			
Amino Acid	Corn Grain	Soybean Meal	Sorghum Grain	Meat and Bone Meal	Corn Grain	Soybean Meal	Sorghum Grain	Meat and Bone Meal
	% of ingested amino acid							
Alanine	87.6	88.9	85.4	90.2	88.5	89.0	87.2	90.5
Arginine	88.4	90.6	87.2	91.4	89.3	90.2	88.4	91.3
Asparagine	86.5	88.3	85.9	90.6	86.8	88.5	86.0	90.2
Aspartate	87.2	89.5	86.1	90.2	86.3	88.2	85.8	89.7
Cysteine	85.1	86.4	84.8	89.4	86.0	87.1	85.1	89.0
Glutamine	88.6	89.5	87.6	90.8	87.7	89.2	86.8	90.8
Glutamate	89.2	90.2	88.4	91.2	88.1	89.6	87.4	91.0
Glycine	86.4	88.3	85.7	90.5	86.6	88.0	85.7	89.5
Histidine	85.5	87.4	84.9	89.6	87.0	88.5	86.2	90.2
Isoleucine	88.7	89.3	88.0	90.8	88.2	88.9	87.6	90.5
Leucine	88.2	89.0	87.6	90.3	87.8	89.6	86.4	90.6
Lysine	85.0	88.4	84.3	90.0	84.5	89.8	83.7	90.4
Methionine	87.5	90.1	86.8	90.6	88.6	89.1	87.4	90.5
Phenylalanine	89.1	90.3	88.5	90.9	89.5	90.0	88.9	91.0
Proline	86.8	88.0	85.9	89.4	86.4	87.2	86.0	89.2
Hydroxyproline	---	---	---	88.7	---	---	---	88.4
Serine	88.4	90.2	87.5	91.1	88.6	89.0	87.9	90.6
Threonine	85.2	86.5	84.8	89.3	84.9	86.8	84.3	88.5
Tryptophan	86.0	87.2	85.3	89.0	85.2	88.1	84.6	89.7
Tyrosine	88.5	89.6	88.0	91.4	89.0	90.2	88.2	91.0
Valine	88.2	89.8	87.6	90.7	87.1	88.7	86.1	90.3

Source: Wu et al. (2014).

[a] Except for glycine, all amino acids are L-isomers.

mucosa (Yen 2001). In contrast, raw soybeans and other leguminous seeds contain protease (e.g., trypsin and chymotrypsin) inhibitors, leading to the depression of protein digestion and animal growth. Because protease inhibitors are heat-sensitive, soybeans must be heated at an appropriate temperature before they are used to feed animals. Fatty acids with $\geq C_{12}$ (including oleic acid and linoleic acid) stimulate CCK production by intestinal enteroendocrine I cells (McLaughlin et al. 1998) and the small intestine in animals (Chelikani et al. 2004). An underlying mechanism responsible for the effect of fatty acids (e.g., linoleic acid) is the activation of the transient receptor potential channel type M5 (TRPM5; Shah et al. 2012). Through the CCK-induced production of pancreatic proteases, dietary fatty acids, particularly ω6 and ω9 unsaturated fatty acids, can enhance the digestibility of dietary protein in nonruminant animals.

Protein Digestibility vs. Dietary Amino Acid Bioavailability

The small intestine is the terminal site for the digestion of dietary protein in nonruminants. As noted in Chapter 1, digestion is defined as the chemical disintegration of foodstuffs in the digestive tract into smaller molecules that are suitable for assimilation by the animal. In the small intestine, the digestion of dietary protein is a chemical process for the hydrolysis of di-, tri-, oligo-, and polypeptides. The term "amino acid digestibility", which has long been used in describing the hydrolysis of dietary protein, is a misnomer in animal nutrition. This is because amino acid digestibility actually does not refer to the process of amino acid breakdown into smaller molecules in the GIT. The term amino acid digestibility should be replaced by "protein digestibility" or "coefficient of amino acid release from protein". The concept of protein digestibility differs from that of dietary amino acid bioavailability (Rutherfurd and Moughan 2012). The latter can be defined as the proportion of an ingested amino acid that is absorbed in the GIT and utilized by the tissues of animals. Thus, the bioavailabilities of dietary amino acids represent the combined results of digestion, absorption, and metabolism of dietary amino acids (e.g., lysine in its natural and modified forms) in animals.

Catabolism of Free Amino Acids and Small Peptides by Luminal Bacteria in the Small Intestine

The lumen of the small intestine harbors numerous species of bacteria (Chapter 1), which can degrade

free amino acids and small peptides. Dai et al. (2011) reported high rates of the utilization of glutamine, lysine, arginine, threonine, leucine, isoleucine, valine, and histidine by luminal bacteria from the small intestine of pigs. Except for the formation of glutamate and its metabolites (alanine and aspartate) from glutamine, and of L-ornithine from arginine, there was no net production of any amino acids from glutamine, lysine, arginine, threonine, leucine, isoleucine, valine, and histidine by pig intestinal microbes. These authors also found that small-intestinal bacteria, particularly *Streptococcus sp.*, *Megasphaera elsdenii, Escherichia coli*, and *Klebsiella sp.* degraded amino acids at different rates in a species-dependent manner and that protein synthesis was a major pathway for amino acid metabolism in all the bacterial species studied. Of note, Yang et al. (2014) demonstrated that bacteria from the small-intestinal lumen of pigs degraded amino acids, but bacteria that tightly adhered to the small-intestinal epithelial wall of pigs might be able to synthesize amino acids, suggesting variations in amino acid metabolism by bacteria from different niches. Dai et al. (2012a) reported that the oxidation of lysine, threonine, and arginine accounted for 10% of their utilization in the pure cultures of *Streptococcus sp.*, *E. coli*, and *Klebsiella sp.* from the jejunum and ileum of growing pigs. The values were either higher or lower in mixed bacterial cultures from the jejunum or ileum, depending on amino acids, bacterial species, and the gut segment; for example, 15% for lysine in the mixed jejunal or ileal bacteria; 5.5% and 0.3% for threonine in the mixed jejunal and ileal bacteria, respectively; and 20% for arginine in the mixed ileal bacteria. Among all metabolic pathways in the intestinal bacteria, protein synthesis accounted for various percentages of amino acid utilization: 50–70% for leucine, 25% for threonine, proline, and methionine, 15% for lysine and arginine, and 10% for glutamine (Dai et al. 2012a). Some in vivo studies indicate that 10–30% of dietary amino acids (in the form of free amino acids and small peptides), particularly those that are not synthesized by eukaryotes, are degraded by the small-intestinal microbes in postweaning pigs (Stoll and Burrin 2006). This may also be true for other species, but experimental data are currently not available. Those free amino acids and small peptides that are derived from protein digestion are utilized by the small-intestinal bacteria mainly for protein synthesis (Dai et al. 2010). The microbial protein enters the large intestine primarily for excretion in feces.

Absorption of Small Peptides and Amino Acids by the Small Intestine of Nonruminant Mammals and Birds

Absorption refers to the movement of the products of digestion across the intestinal mucosa into the vascular system for utilization by animals. The cells responsible for nutrient absorption are enterocytes, which contain the apical membrane and the basolateral membrane. There is a lateral space between two enterocytes. The apical membranes of the enterocytes form microvilli, which increase the area for absorption. Absorption of amino acids can occur through two routes: (a) nutrients may pass completely through the enterocytes, entering at the apical membrane and leaving through the basolateral membrane into the lateral space (transcellular absorption); and (b) alternatively, nutrients may move through the tight junctions directly into the lateral space (paracellular absorption). The latter is represented by the receptor-mediated absorption of intact proteins (e.g., immunoglobulins) by enterocytes in mammalian neonates before the *"gut closure"* (Chapter 1). Amino acids and possibly a small amount of small peptides absorbed into the lamina propria enter the venule and then the portal vein (Figure 7.6).

Transport of Di- and Tri-Peptides by Enterocytes

Absorption of tripeptides and dipeptides occurs through the apical-membrane Na^+-independent, H^+-driven peptide transporter 1 (PepT1), which is expressed primarily in the small intestine. However, peptide absorption is indirectly coupled to Na^+ transport into enterocytes because the needed protons are provided by Na^+/H^+ exchange. Importantly, tripeptides and dipeptides are absorbed intact from the lumen of the small intestine into its enterocytes, where they are hydrolyzed by cytosolic peptidases to form free amino acids (Zhanghi and Matthews 2010). PepT1 does not transport a peptide consisting of 4 or more amino acids (Gilbert et al. 2008). The formation of small peptides in the small intestine can reduce its luminal osmolality and maintain the homeostasis of its enterocytes. Due to the high activity of intracellular peptidases in the small intestine, it is unlikely that a nutritionally significant quantity of peptides in the lumen of the healthy gut can transcellularly enter the portal vein or the intestinal lymphatics. This is consistent with the absence of PepT1 from the basolateral membrane of the enterocytes (Matthews 2000). However, it is possible that a limited, but physiologically significant, amount of special small peptides may be absorbed intact from the luminal content to the bloodstream through M cells, exosomes, and enterocytes via transepithelial cell transport. Examples of these unique small peptides may include those containing an imino acid (e.g., Gly-Pro-Hydroxyproline, a degradation product of collagen) or a formyl amino acid (e.g., *N*-formyl-Met-Leu-Phe, a bacterial peptide serving as a chemotactic for leukocytes; Dorward et al. 2015). Diet-derived peptides can exert their bioactive (e.g., physiological and regulatory) actions at the level of the small intestine, and the intestine-generated signals can be transmitted to the brain, the endocrine system, and the immune system to beneficially impact the whole body.

Di- and tripeptides are absorbed into the enterocytes of the small intestine faster than their constituent free amino acids (Matthews 2000). The high rate of transport of these small peptides minimizes their exposure to luminal bacteria for catabolism and improves the balance of free amino acids in the portal vein. This indicates the

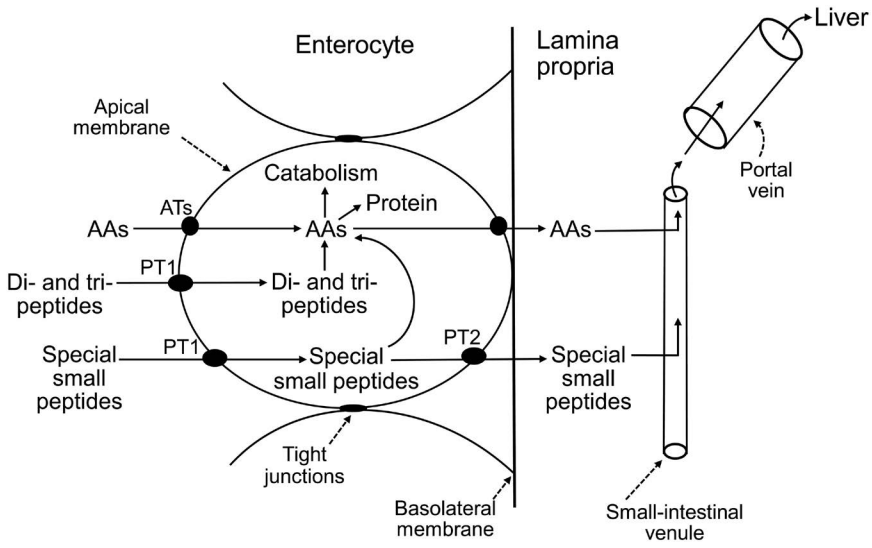


FIGURE 7.6 Absorption of small peptides and free AAs by enterocytes of the small intestine. After entering enterocytes, free AAs are either used for protein synthesis or degraded, and the remaining AAs exit into the lamina propria of the intestinal mucosa. Enterocytes rapidly hydrolyze the absorbed di- and tri-peptides into free AAs. Significant amounts of some special small peptides may escape catabolism inside the enterocytes and exit into the lamina propria. ATs, AA transporters; PT1, peptide transporter 1; PT2, peptide transporter 2.

importance of small peptides in animal nutrition. The concept of peptide transport was generally accepted by the scientific community only in the late 1970s after many years of considerable debate since the first observation of possible intestinal peptide absorption in the late 1950s. Animals will gain more protein and grow faster if they are fed a diet consisting of intact proteins or peptides, in comparison with animals fed the same diet except that the amino acids are provided in the free form. For example, the weight gain was 62% higher and urinary N excretion was 25% lower in young rats fed a 15% casein diet, in comparison with an isonitrogenous diet containing similar amounts of free amino acids (Daenzer et al. 2001). Also, 5- to 20-kg pigs fed a diet containing 11.9% casein, 9.1% amino acids, and 1% $NaHCO_3$ exhibited an 18% increase in daily weight gain and a 20% increase in gain:feed ratio, compared with pigs fed diets containing 20% amino acids (Officer et al. 1997).

Like other epithelial cells (Parks et al. 2000), the absorption of luminal glutathione (GSH; a tripeptide consisting of a γ-glutamyl isopeptide bond) across the apical membrane of the enterocyte involves the membrane-bound γ-glutamyltransferase and cysteinylglycinase (cysteinylglycine dipeptidase). These two enzymes hydrolyze the extracellular GSH to its constituent amino acids (glutamate, cysteine, and glycine), which are then absorbed rapidly into the enterocyte via the actions of their various transporters. However, GSH can be rapidly transported intact as a tripeptide across the apical membrane of the enterocyte into the cell via a transport system that does not recognize normal dipeptides and tripeptides or oxidized-glutathione (GS-SG; Ganapathy et al. 2006). In addition, the apical GSH transport system is not energized by a Na^+, K^+, or H^+ gradient but can be activated by Na^+ and K^+ as substrates (Ganapathy et al. 2006). The basolateral membrane of the enterocyte contains a GSH transport system that is active, Na^+ dependent, and electrogenic. This basolateral transport system takes up GSH from the blood into the enterocyte and releases GSH from the enterocyte into the blood. Thus, an inhibition of γ-glutamyltransferase by acivicin does not prevent the transport of GSH intact by the epithelial cells (Parks et al. 2000; Vincenzini et al. 1992).

Transport of Free Amino Acids by Enterocytes

The absorption of amino acids is rapid in the duodenum and jejunum but slower in the ileum. Different amino acids are absorbed by enterocytes at different rates (Bröer and Palacín 2011). There are four mechanisms for the transport of amino acids into the enterocytes through their apical membrane: (a) Na^+-independent system (facilitated system), (b) Na^+-dependent system (active transport), (c) simple diffusion (a passive, nonsaturable transport system without a need for carrier proteins), and (d) the γ-glutamyl cycle. Some amino acid transports are either uniporters (namely the flux of amino acids only in one direction) or antiporters [namely the flux of two substances (e.g., arginine and glutamine, glutamate/cystine, and glutamine/H^+) in the opposite direction]. Simple diffusion and the γ-glutamyl cycle are insignificant for amino acid transport by enterocytes and other cell types under physiological conditions. In contrast, apical-membrane Na^+-dependent and Na^+-independent amino acid transporters (Table 7.3) are responsible for the uptake of ~60% and 40%, respectively, of free amino acids from the lumen of the small intestine into enterocytes. Examples of these apical-membrane amino

TABLE 7.3
Amino Acid Transporters in the Apical Membrane of Enterocytes and in Other Cell Types of Animals

Transport System	Gene Name	Major Amino Acids	Comments
		Na^+-independent System	
asc	SLC7A10	Small AAs (Ala, Ser, Cys, Gly, Thr)	Transports small neutral AAs and D-Ser
$b^{0,+}$	SLC7A9	Neutral (0) and basic (+) AAs	Also transports cystine
L	SLC7A5	Large neutral AAs (e.g., Leu, Phe, Met)	Also transports ABHDC and His
L	SLC7A8	All neutral AAs except for Pro	Has a greater affinity for large neutral AAs
L	SLC43A1	Large neutral AAs	Has a greater affinity for BCAAs
X_c^-	SLC7A11	Acidic AAs (Glu and Asp)	Also transports cystine
y^+ (CAT-1)	SLC7A1	Cationic AAs (e.g., Arg, His, Lys, Orn)	Also transports neutral AAs
y^+ (CAT-2)	SLC7A2	Cationic AAs (e.g., Arg, His, Lys, Orn)	Also transports neutral AAs
y^+ (CAT-3)	SLC7A3	Cationic AAs (e.g., Arg, His, Lys, Orn)	Also transports neutral AAs
y^+L	SLC7A6	Cationic AAs and large neutral AAs	Also transports Gln
y^+L	SLC7A7	Cationic AAs and large neutral AAs	Also transports Ala, Gln, and Cys
		Na^+-dependent System	
A	SLC38A1	Small and neutral AAs (e.g., Ala, Met)	Also transports His, Asn and Cys
A	SLC38A2	Small and neutral AAs (e.g., Ala, Met)	Also transports His, Asn, Cys, and Pro
ASC	SLC1A4	Small AAs (Ala, Ser, and Cys)	Also transports Thr
ASC	SLC1A5	Small AAs (Ala, Ser, and Cys)	Also transports Cys, Gln and Thr
BETA	SLC6A1	γ-Aminobutyrate (GABA)	Na^+- and Cl^--dependent transporter
BETA	SLC6A6	Taurine transporter; also β-Ala, GABA	Na^+- and Cl^--dependent transporter
BETA	SLC6A11	GABA, betaine, taurine	Na^+- and Cl^--dependent transporter
B^0	SLC6A15	Neutral AAs (e.g., BCAAs, Met, Pro)	Na^+- and Cl^--dependent transporter
$B^{0,+}$	SLC6A14	All neutral and basic AAs, β-Ala	Na^+- and Cl^--dependent transporter
Gly	SLC6A9	Gly and sarcosine	Inhibited by lithium
IMINO	SLC6A20	Pro, OH-Pro	Also transports neutral AAs (e.g., Gly)
N (SN1)	SLC38A3	Gln, Asn, Cit, His	Also transports Ala
N (SN2)	SLC38A5	Gln, Asn, Cit, His	Also transports Ala, Ser, Gly
NBB	SLC6A19	All neutral AAs (brush border transporter)	Bound to apical membrane of enterocytes
Phe	Unknown	Phe and Met (brush border transporter)	Bound to apical membrane of enterocytes
X_{AG}^-	SLC1A1	Asp and Glu	Also transports Cys, cysteine, and D-Asp
X_{AG}^-	SLC1A2	Asp and Glu	Also transports Cys, cysteine, and D-Asp
X_{AG}^-	SLC1A3	Asp and Glu	Also transports D-Asp

Note: ABHDC, 2-aminobicyclo[2,2,1]heptane-dicarboxylic acid; BCAAs, branched-chain AAs; SLC, solute carrier

acid transporters (transmembrane proteins) are given in Figure 7.7. The conventional names of amino acid transporters are based on their major substrates, whereas the Gene Nomenclature Committee groups amino acid transporters into solute carrier (SLC) families according to their gene sequence similarity. For example, SLC7A1, SLC7A2, and SLC7A3 encode the cationic amino acid transporter (CAT)-1, CAT-2, and CAT-3 proteins, respectively, in the conventional system y^+ for the transport of basic amino acids by cells.

Several characteristics of the apical-membrane amino acid transport are noteworthy. First, one amino acid can be transported by multiple systems. For example, glutamine is taken up by the apical and basolateral membranes of the enterocyte through Na^+-dependent systems N (SN1 and SN2), B^0, $B^{0,+}$, and A, as well as Na^+-independent systems $b^{0,+}$, L, and y^+L (Wu 2022). In addition, branched-chain amino acids (BCAAs) are transported by systems L, y^+L, B^0, and $B^{0,+}$. Amino acid transporters have preferences for their substrates. For example, B^0AT1 transports all neutral amino acids, albeit to a varying extent. V_{max} values appear to be fairly similar, but affinities are different for each amino acid. The order of preference is Met = Leu = Ile = Val > Gln = Asn = Phe = Cys = Ala > Ser = Gly = Tyr = Thr = His = Pro > Trp > Lys (Bröer 2008). The redundancy of transporters for any amino acid reduces the risk of cell uptake failure, which could occur if there were only one transporter for an amino acid.

Second, amino acid transporters exhibit cell- and tissue-specific differences in their expression, which play an important role in interorgan metabolism of amino acids. The nutritional and physiological significance of this

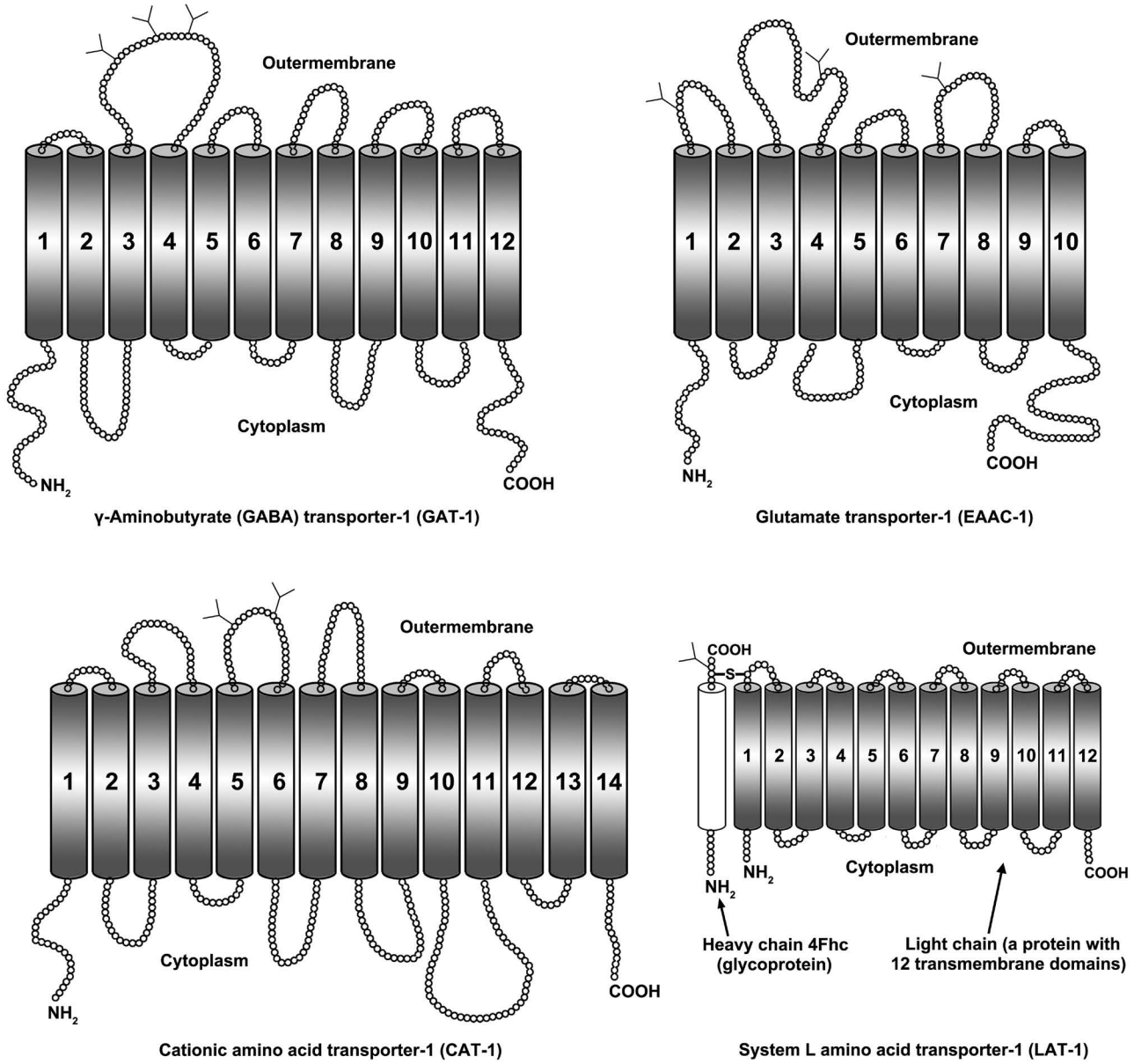


FIGURE 7.7 Examples of transporters (transmembrane proteins) of acidic, basic, and neutral amino acids in animal cells. (Adapted from Bröer and Palacín 2011; Hyde et al. 2003.)

phenomenon can be illustrated by the findings that mammalian hepatocytes have a low abundance of cationic amino acid transporters (CAT-1, 2, and 3 proteins) and therefore a low ability to take up extracellular basic amino acids. Because the mammalian hepatocytes have a very high ability to degrade the basic amino acids, their limited uptake by these cells minimizes their irreversible loss in the liver, thereby maximizing their availability from the diet, the small intestine, and the blood to extrahepatic cells and tissues.

Third, the same amino acid may not be transported by all cells in an animal. For instance, macrophages and endothelial cells readily take up extracellular L-citrulline, but there is little uptake of this amino acid by the mammalian liver. This ensures that the small intestine-derived citrulline is fully available for arginine synthesis in extrahepatic tissues and cells. Fourth, the Na^+-dependent amino acid transport systems co-transport both amino acids and Na^+ (all soluble in water) into the cell in an ATP-dependent manner, which affects electric potential across the plasma membrane and the influx of water (Hyde et al. 2003). The Na^+ that has entered the cell along with an amino acid will exit the cell through the basolateral membrane Na^+/K^+-ATP pump (also called Na^+/K^+-ATPase), which transports 3 Na^+ ions out of the enterocyte in exchange for the influx of 2 K^+ ions. The energy that powers this transport process is provided by the hydrolysis of ATP into ADP plus inorganic phosphate (Pi). The Na^+-dependent uptake of 1 mol of amino acid from the

intestinal lumen into the enterocytes requires 1 mol of ATP. Thus, when glutamine or glutamate is added to the mucosal side of the small intestine, the electric current of the plasma membrane and water absorption are increased as the amino acid enters the cell.

In a Na^+-dependent transport system, an amino acid transporter binds Na^+ first and then an amino acid. The binding of the transporter to Na^+ increases its affinity for the amino acid substrate. The binding of the amino acid transporter to both the Na^+ and the amino acid results in a further conformational change of the transporter protein, leading to the entry of both substrates from the intestinal lumen into the cytoplasm of the enterocytes. Thus, the rapid and complete absorption of most amino acids is dependent on the electrochemical gradient of Na^+ across the enterocytes and also contributes to the generation of the osmotic gradient that drives water absorption by the cells.

Some of the apical membrane amino acid transporters may also be localized in the basolateral membrane of the enterocytes for the efflux of amino acids from the cells into the interstitial space of the mucosal lamina propria. The basolateral membrane of the enterocyte contains additional sets of amino acid transporters that export amino acids from the cells into the lamina propria, and those that have been characterized are shown in Table 7.4. The basolateral membrane amino acid transporters transport amino acids out of the enterocytes in a Na^+-dependent or independent manner. To maintain the balance of electrolytes within the enterocytes, 3 Na^+ ions that have entered the cells from the intestinal lumen via Na^+-dependent amino acid transporters are transported out of the cells by the basolateral membrane Na^+/K^+-ATPase in exchange for the influx of 2 K^+ ions. The basolateral membrane of the enterocytes is more permeable to amino acids than their apical membrane or nonepithelial membranes. Thus, simple diffusion of amino acids from the enterocytes into the lamina propria can occur but is quantitatively minor for the intestinal absorption of amino acids across the enterocytes, compared to that by the basolateral-membrane amino acid transporters.

Polarity of Enterocytes in Amino Acid and Peptide Transport

The apical membrane (facing the intestinal lumen) of the enterocytes can rapidly absorb luminal amino acids (Yen et al. 2004). During the digestion period after enteral feeding, dietary amino acids enter the portal vein down their concentration gradients. Therefore, there is a positive influx of amino acids from the intestinal lumen to the liver. In the postabsorptive state, there is limited intestinal absorption of amino acids in nonruminants. In contrast, the basolateral membrane (facing the blood) of enterocytes takes up a large amount of glutamine, but no nutritionally significant quantity of other amino acids, from the arterial blood during the postabsorptive period in growing pigs (Wu et al. 1994b). This is due to a high abundance of glutamine transporters, but the limited expression of nonglutamine transporters, in the basolateral membrane of the enterocyte. It is also possible that, in the postabsorptive state, the concentrations of nonglutamine amino acids in the interstitial fluid of the mucosal lamina propria are much lower than those in the cytosol of the enterocytes. This would hinder the transport of amino acids via Na^+-dependent or independent transporters against the concentration gradients of the amino acids. Thus, an enteral diet must be provided adequately to

TABLE 7.4
Amino Acid (AA) Transporters in the Basolateral Membrane of Enterocytes

Transport System	Isoform	Major AAs	Na^+ Dependence	Notes
A	---	Neutral AAs (including Gln)	Yes	Efflux from enterocytes
ASC	---	Ala, Ser and Cys	Yes	Efflux from enterocytes
asc-Like	---	Ala, Ser and Cys	No	Efflux from enterocytes
Gly	GLY1	Glycine	Na^+, Cl^-	Efflux from enterocytes
L	LAT2-4F2hc	Large neutral AAs	No	Efflux from enterocytes
N	---	Gln, Asn, Cit, and His	Yes	Efflux from enterocytes
TAT1[a]	---	Aromatic AAs (Trp, Tyr, and Phe)	No	Efflux from enterocytes
SNAT2[b]	---	Neutral AAs	Yes	Efflux from enterocytes
y^+L	Y^+LAT1	Cationic AAs	No	Efflux from enterocytes
		Large neutral AAs	Yes	Influx from blood to enterocytes
	Y^+LAT2[c]	Cationic AAs	No	Efflux from enterocytes
		Large neutral AAs	Yes	Influx from blood to enterocytes
y^+	CAT-1	Cationic AAs	No	Efflux from enterocytes

[a] T-type AA transporter-1.
[b] SNAT, sodium-coupled neutral AA transporter. SNAT1 and 4 are expressed in extraintestinal tissues.
[c] Expressed in both epithelial and nonepithelial tissues.

postnatal animals as the source of exogenous amino acids to support the maintenance, growth, function, and health of the small-intestinal mucosa. Like adult rats, the small intestine of young pigs takes up 30% of glutamine from the arterial blood in the postabsorptive state (Wu et al. 1994b), indicating an important role of the gut in whole-body glutamine utilization.

The small-intestinal mucosa does not take up small peptides from the arterial blood. This is because the basolateral membrane of the enterocytes lacks PepT1. As noted previously, the basolateral membrane of the enterocytes may express other peptide transporters. For example, Shepherd et al. (2002) reported the presence of a peptide transporter in the basolateral membrane of the enterocytes in the rat small intestine. These authors observed the transepithelial transport of a nonhydrolysable dipeptide, D-phenylalanine–L-glutamine (1 mM), from the lumen of the small intestine into the vascular perfusate. There is also evidence that the basolateral membrane of the colonocyte (the human intestinal cell line Caco-2) may contain a Na^+-independent peptide transporter to transport small peptides out of the cells into the lamina propria of the large intestine (Irie et al. 2004). A dipeptide [glycine-sarcosine (*N*-methylglycine)] enters Caco-2 cells through their apical membrane and exits the cells through their basolateral membrane in a pH-dependent manner. Interestingly, the K_M for the efflux of the dipeptide is lower than that of its influx. The molecular characteristics of the peptide transporter in the rat small intestine or the Caco-2 cells have not been identified.

Metabolism of Amino Acids in Enterocytes

As summarized in Table 7.5, available evidence shows (a) no degradation of asparagine, cysteine, histidine, lysine, phenylalanine, threonine, tryptophan, and tyrosine; (b) a very low rate of degradation of alanine, glycine, methionine, and serine; (c) high rates of the degradation of arginine (after weaning only), proline, and BCAAs; and (d) very high rates of the degradation of aspartate, glutamate, and glutamine as the major metabolic fuels in enterocytes from pigs, rats, sheep, and calves (Chen et al. 2007, 2009; He and Wu 2022; Wu et al. 2005a). In the intestinal mucosa, methionine is metabolized to provide *S*-adenosylmethionine (SAM), which is decarboxylated in the cytosol to yield CO_2 and the decarboxylated SAM. The latter is used for the synthesis of polyamines (putrescine, spermidine, and spermine), as well as DNA and protein methylation. In enterocytes, the rate of SAM (and thus methionine) decarboxylation is quantitatively very low, and there is no oxidation of other methionine carbons to CO_2 (Chen et al. 2009). In these cells, proline is extensively oxidized by mitochondrial proline oxidase to form pyrroline-5-carboxylate (P5C), which is the precursor of ornithine and citrulline. However, the oxidation of proline to CO_2 by these cells is limited due to the very low activity of P5C dehydrogenase (Wu 1997). In porcine, rat, ovine, and bovine enterocytes, citrulline is synthesized from glutamine, glutamate, and proline via the formation of ornithine, whereas alanine is generated from glutamine and glutamate (Dillon and Wu 2021; Wu 1997; Wu et al. 2021). Although mitochondria-generated ornithine is effectively channeled for citrulline production in these cells, extracellular ornithine is not utilized by them for citrulline synthesis and, instead, is metabolized to form proline because of the complex compartmentalization of ornithine degradation (Wu et al. 1994a). The rates of glutamate utilization by the small intestine of sow-reared pigs are summarized in Table 7.6. In contrast, chicken enterocytes do not synthesize ornithine, citrulline, or arginine from glutamine, glutamate, and proline; have a very low rate of glutamine degradation; have a very limited ability to catabolize arginine via NO synthases; and do not degrade arginine via arginase, asparagine, cysteine, histidine, lysine, phenylalanine, proline, threonine, tryptophan, and tyrosine (He et al. 2021a,b,c; Furukawa et al. 2021; Wu et al. 1995). Likewise, the enterocytes of newborn and adult horses do not form citrulline or arginine from glutamine/glutamate and proline (Martinez et al. 2022). Thus, there are marked differences in intestinal mucosal amino acid metabolism between mammals and birds. Results from studies of pigs, rats, and humans reveal that dietary amino acids undergo first-pass metabolism in the portal-drained viscera (PDV; including enterocytes and other

TABLE 7.5
Metabolic Fates of Amino Acids in Enterocytes of the Small Intestine of Ruminants and Nonruminants

Metabolic Fates	Amino Acids
Neither synthesized nor degraded	Asparagine, Cysteine, Histidine, Tryptophan, Tyrosine
Synthesized but not degraded	None
Degraded but not synthesized	Branched-Chain Amino Acids (Isoleucine, Leucine, Valine), Lysine, Methionine[a], Phenylalanine, Threonine
Both degraded and synthesized (species-specific)	Alanine, Arginine[b], Aspartate, Citrulline, Glutamate, Glutamine[c], Glycine[d], Ornithine[e], Proline[f], Serine[d]

Source: Chen et al. (2007, 2009), He et al. (2021c), Wu et al. (2005a, 2024).

[a] Limited degradation. Little or no CO_2 is produced from methionine in the enterocytes of ruminants and nonruminants.

[b] Synthesis from citrulline. Limited catabolism via nitric oxide synthases and no degradation via arginase in the enterocytes of birds.

[c] A low rate of degradation in the enterocytes of birds.

[d] Limited degradation in the enterocytes of ruminants and nonruminants.

[e] Degraded but not synthesized in the enterocytes of birds.

[f] No degradation via proline oxidase in the enterocytes of birds.

TABLE 7.6
Utilization of L-Glutamate (Glu) by the Small Intestine of Sow-Reared 21- to 35-Day-Old Pigs

Variable	Amount (g/day)
Glu provision from milk to the small intestine (SI)[a]	6.44
Amount of milk Glu entering the portal vein[b]	0.32
Utilization of total milk Glu by the SI[b]	6.12
Glu needed for accretion of intracellular constituent proteins[c]	0.13
Glu needed for production of mucin proteins exported by SI into the intestinal lumen[d]	0.046
Glu needed for production of membrane-bound mucin proteins in the SI mucosa[e]	0.31
Glu metabolized to CO_2, amino acids, lactate, and glutathione[f]	5.95

Source: Hou and Wu (2018).

[a] Calculated for the sow-reared 21- to 35-day-old pig with the average body weight of 7.92 kg on the basis of the following: Glu content in whole milk = 4.80 g/L; average milk intake = 1.345 L/day (170 ml/kg BW/day, i.e., 190 ml/kg BW/day on day 21 and 150 ml/kg BW/day on day 35); Glu intake from whole milk = 816 mg/kg BW/day (6.46 g/day; 4.80 × 1.345 L = 6.46); true ileal Glu digestibility = 99.7%; availability of dietary Glu to the SI = 6.44 g/day (i.e., 6.46 × 99.7% = 6.44 g/d).

[b] The rate of utilization of dietary Glu by the SI = 95% of enteral intake; utilization of dietary Glu by the SI = 6.12 g/day (i.e., 6.46 × 99.7% × 95% = 6.12); the flux of plasma Glu into the SI = 0.00. A negligible amount of Glu is formed from intestinal metabolism of AAs in pigs.

[c] The SI gain = 11.1 g/day; protein content in the SI = 13.9%; Glu content in proteins of SI tissues = 8.57 g/100 g protein; Glu needed for accretion of intracellular constituent proteins = 0.13 g/day (i.e., 11.1 × 13.9% × 8.57% = 0.13).

[d] The rate of mucin proteins exported into the SI lumen = 0.14 g/kg BW/day; production of mucin protein exported into the SI lumen = 1.11 g/day (i.e., 0.14 × 7.92 = 1.11); Glu content in mucins = 4.1% (i.e., 4.1 g/100 g mucin protein); Glu needed for production of exported mucin protein = 0.046 g/day (i.e., 1.11 × 4.1% = 0.046).

[e] The mucosal weight of the SI = 76 g (25% of the SI weight; 25% × 302 g = 76 g); protein content in the SI mucosa = 14%; mucin protein content in the SI mucosa = 76 mg/g mucosa protein (7.6%); membrane-bound mucin protein in the SI mucosa = 0.81 g (i.e., 76 × 14% × 7.6% = 0.81); fractional rate of synthesis of membrane-bound mucin protein in the SI = 414%/day; membrane-bound mucin protein synthesized by the SI = 3.35 g/day (i.e., 0.81 × 414% = 3.35); Glu needed for production of membrane-bound mucin protein = 0.14 g/day (i.e., 3.35 × 4.1% = 0.14); Glu needed for production of membrane-bound mucin = 0.18 g/day (i.e., 0.04 + 0.14 = 0.18); Glu needed for production of intestinal protein = 0.31 g/day (i.e., 0.13 + 0.18 = 0.31).

[f] The rate of intestinal oxidation of Glu to CO_2 = 3.09 g/day (5.95 × 52% = 3.09); synthesis of Orn, Cit, and Arg = 0.32 g/day (i.e., 5.95 × 5.4% = 0.32); proline synthesis = 0.31 g/day (i.e., 5.95 × 5.2% = 0.31); Ala plus lactate synthesis = 0.62 g/day (i.e., 5.95 × 10.4% = 0.62); synthesis of glutathione (GSH) = 0.60 mmol/kg BW/day, with 1 mol of GSH containing 1 mol of Glu; Glu required for GSH synthesis = 0.70 g/day (i.e., 0.60 × 147 × 7.92 = 698 mg); synthesis of other nitrogenous substances (e.g., Asp and Asn) = 0.45 g/day (i.e., 5.95 × 7.5% = 0.45); other organic acids (α-KG, malate, pyruvate, and succinate) = 0.46 g/day (i.e., 5.95 × 7.7% = 0.46).

cells of the small-intestinal mucosa) at various rates (Figure 7.8).

Studies with pigs graphically illustrate developmental changes in amino acid metabolism in the small-intestinal mucosa. For example, in porcine enterocytes, the rates of citrulline and arginine synthesis from glutamine are greatest at birth, decline progressively thereafter, reach the lowest level at 14 to 21 days of age, and are induced after weaning (Wu and Morris 1998). Between 0 and 21 days of age, proline is a major substrate for citrulline and arginine syntheses in these cells (Wu 1997). Enterocytes in neonatal pigs convert almost all citrulline into arginine due to high argininosuccinate synthase (ASS) and argininosuccinate lyase (ASL) activities, and release almost all the synthesized arginine due to the near absence of arginase activity. This helps to maximize the availability of the diet- and small intestine-derived arginine to extraintestinal tissues. Enterocytes in postweaning pigs convert only a small percentage of the synthesized citrulline (10%) into arginine because of reduced ASS and ASL activities, and instead release 90% of the synthesized citrulline from the cells. After weaning, the kidneys express high activities of ASS and ASL to readily convert citrulline into arginine in their proximal tubules with limited arginase activity, and release nearly all the synthesized arginine into the blood circulation. Such metabolic adaptation to weaning is of nutritional significance to spare the intestine-synthesized citrulline because arginase is induced in the enterocytes of postweaning mammals, including pigs (Wu 1995). Arginase hydrolyzes arginine into ornithine and urea, which would contribute to a loss of the citrulline synthesized from glutamate, glutamine, and proline in the enterocytes of postweaning pigs. Although proline and arginine are actively degraded by enterocytes of postweaning mammals, these two amino acids support the growth and remodeling of the small intestine partly through polyamine synthesis. In postweaning pigs, <20% of the extracted amino acids are utilized for intestinal mucosal protein synthesis. Thus, not all amino acids that enter enterocytes from the small-intestinal lumen exit into the portal circulation.

Limited Use of Dietary Ammonia and Urea as Sources of Amino Acids in Nonruminant Mammals and Birds

Growing nonruminant mammals and birds can utilize a small amount of dietary ammonia-N for the synthesis of NEAAs when diets are severely deficient in amino acids. For example, the addition of N in the form of diammonium citrate (providing 1.45% and 2.9% CP) to SBM- and cornstarch-based diets containing a low CP (only 10.19%) level can be used for the synthesis of NEAAs in a dose-dependent manner by growing pigs (15- to 27-kg BW; Mansilla et al. 2017). Similarly, supplementing 2.15% diammonium citrate to a purified diet containing 8% EAAs (a low amino acid level) enhanced the growth of young rats during weeks 0 to 4 postweaning (Lardy and Feldott 1950). Likewise, young chicks can use dietary diammonium citrate (1.51% and 3.02%) for enhancing weight gain in a dose-dependent

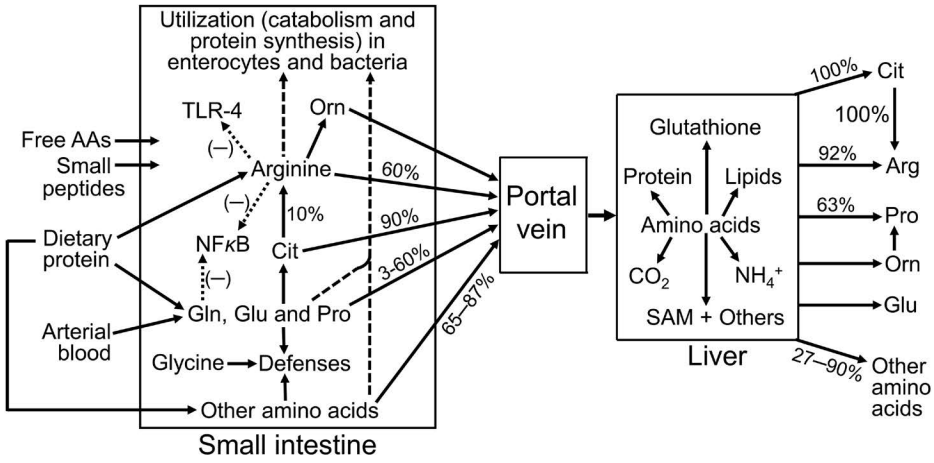


FIGURE 7.8 The first-pass metabolism of amino acids (AAs) in the portal-drained viscera of animals. In the small intestine, the hydrolysis of dietary protein by extracellular proteases and peptidases releases AAs and small peptides into the lumen. Enterocytes take up these digestion products, use some AAs via catabolism and protein synthesis at various rates, and release the remaining AAs to the portal vein. Arginine, glutamine, and glycine play important roles in antioxidative defense in these cells through inhibiting Toll-like receptor-4 (TLR-4), nuclear factor kappa B (NF*k*B), and oxidant-induced cell injury, respectively. In addition, other cell types (e.g., paneth cells) in the intestinal mucosa use dietary AAs for the production of proteins (e.g., defensins). Degradation of diet-derived AAs also occurs in the luminal bacteria of the gut. In post-weaning mammals (e.g., pigs, rats, and humans), about 3% (e.g., glutamate) to 87% (e.g., tryptophan) of dietary AAs enter the portal vein. All citrulline, 92% arginine, 63% proline, and 27–90% of other AAs bypass the liver to enter the systemic blood circulation for metabolic utilization.

manner when fed purified diets containing only 10% amino acids (Featherston et al. 1962). Interestingly, under this feeding condition, adding 0.8% urea to the low-amino acid diets also improved the growth of chicks, likely due to the synthesis of amino acids by intestinal microbes, but dietary supplementation with 1.5% urea had no further effect (Featherston et al. 1962). Note that (a) urea is rapidly absorbed by the small intestine into the blood and excreted primarily in the urine; (b) animal cells lack urease, but the intestinal microbes contain urease to hydrolyze urea into ammonia plus CO_2; and (c) when fed low-CP or low-amino acid diets supplemented with nonamino acid N (e.g., urea or ammonia), young nonruminant animals had suboptimum growth rates possibly due to moderate ammonia toxicity (Grimson and Bowland 1971; El Boushy 1980; Rose et al. 1949). In support of this view, 7 out of 8 ponies (up to 136-kg BW) fed a single dose of 450 g of urea died due to severe ammonia toxicity (Hintz et al. 1970). Thus, unlike ruminants, nonruminant mammals (e.g., pigs and horses) and birds (e.g., chickens and ducks) have a limited ability to use dietary ammonia or urea.

DIGESTION OF PROTEIN AND ABSORPTION OF SMALL PEPTIDES AND FREE AMINO ACIDS IN PRERUMINANTS

Digestion of Proteins in the Abomasum and Small Intestine in Preruminants

In preruminants, milk or a liquid diet largely bypasses the reticulorumen and flows from the mouth directly into the abomasum (Chapter 1). The processes for the digestion of dietary protein by proteases in the abomasum and the small intestine in preruminants are the same as those in preweaning nonruminant mammals. The concentrations of proteolytic enzymes in the gastric and small-intestinal contents of preruminants are similar between birth and weaning (Porter 1969). Young calves and lambs have sufficient activities of the digestive proteases to hydrolyze the ingested milk protein, and an addition of exogenous proteases to diets does not enhance its digestibility. In preruminants that consume solid feed, dietary amino acids undergo only limited catabolism in the rumen (Wu et al. 2024).

The pH of the GIT plays an important role in protein digestion in preruminants as in preweaning nonruminant mammals. In preruminants, the abomasal contents have pH ~2.0 before feeding (which aids in the activation of gastric proteases) and a pH ~6.0 immediately after milk consumption due to dilution by a large volume of the neutral milk. The gastric solution has a gradual reduction in pH to the prefeeding value 5 h after milk consumption because of gastric emptying and HCl secretion (Porter 1969). Because saliva and gastric mucosal secretions are continuously added to the abomasal fluid, the total volume of the chyme leaving the abomasum is about twice the volume of the consumed milk. In young calves, the rate of the flow of abomasal contents into the duodenum peaks in the first hour after feeding, and the flow of the chyme into the jejunum is greatest during the first 4–5 h after feeding. Because there is continuous secretion of the pancreatic juice and bile into the lumen of the duodenum, feeding has little effect on pH values (7.0–7.4) in the distal small intestine.

Like young nonruminant mammals, preruminants are well adapted to the digestion of milk protein. The true digestibility of natural milk protein in young calves is 100% (Gerrits et al. 1997). However, the proteins of overheated milk are less well digested than natural milk. Pasteurization of milk does not affect its protein digestibility in preruminants. In contrast to milk, preruminants cannot break down the cell walls of cereal grains and, therefore, cannot use the proteins in unprocessed cereal grains or in raw soybeans. These animals also have a limited ability to use even soy flour (finely ground, defatted SBM) if it contains trypsin inhibitors and other factors that cause depression in food intake and poor growth. Liquid soy flour is an ineffective source of protein for preruminants because such a diet is unpalatable to the animals and often causes severe diarrhea. Of interest, Colvin and Ramsey (1968) reported that the nutritive value of trypsin inhibitor-free soya flour could be improved by treatment with acid at pH 4 for 5 h at 37°C. This is likely due to the denaturation of plant-sourced proteins and the partial inhibition of nontrypsin antinutritional factors.

Absorption of Protein Digestion Products by the Small Intestine in Preruminants

Healthy young preruminants have a high capacity for absorbing the products of protein digestion in the small intestine, particularly the jejunum (Porter 1969). As in preweaning monogastric mammals, di- and tripeptides as well as free amino acids are produced from the terminal digestion of dietary protein in the small intestine. These digestion products are absorbed into the enterocytes through the H^+-driven PepT1 for the small peptides and various amino acid transporters for free amino acids (Table 7.3). There is evidence that large amounts of di- and tri-peptides in the lumen of the small intestine are absorbed rapidly into its enterocytes in young calves (Koeln et al. 1993). In these animals, as reported for nonruminants, the apical membrane of the enterocytes has at least four classes of Na^+-dependent amino acid transporters, one each for acidic, basic, neutral, and β-amino acids (Matthews 2000). Dietary sodium intake is necessary for the rapid and complete absorption of dietary amino acids from the lumen of the small intestine into the lamina propria. Thus, because amino acid transport by the enterocytes drives the absorption of water across the cells into the blood, some amino acids (e.g., glycine and glutamine) are added, along with D-glucose and NaCl, to rehydration solutions to treat diarrhea in young calves (Naylor et al. 1997).

DIGESTION OF PROTEIN AND ABSORPTION OF SMALL PEPTIDES AND FREE AMINO ACIDS IN RUMINANTS

CP is an important concept in ruminant nutrition (Chapter 4). Utilization of dietary protein and nonprotein N (NPN) in ruminants involves the rumen, abomasum, and the small intestine (Figure 7.9). Adequate intake of rumen-degradable protein (RDP) is critical for maximizing

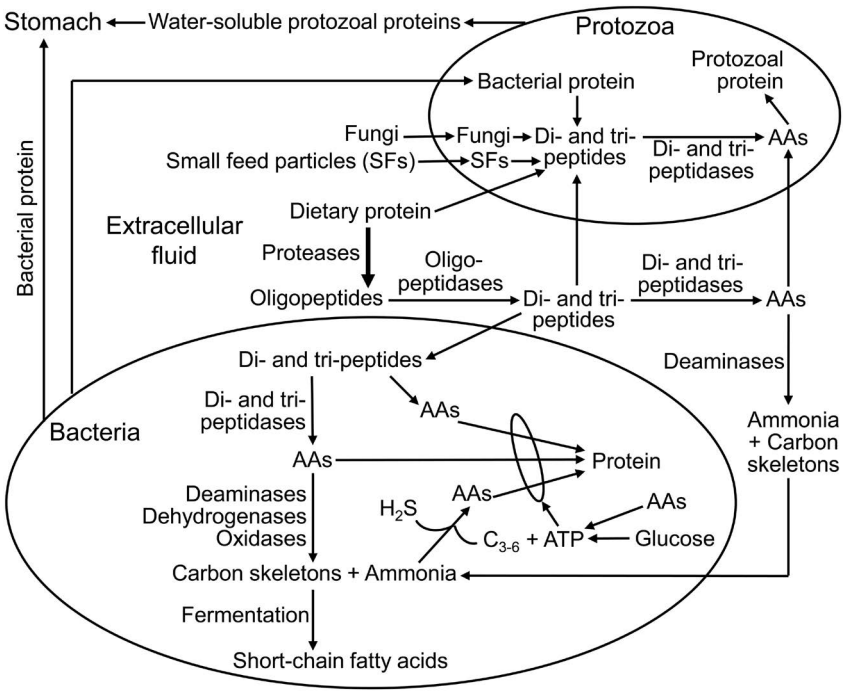


FIGURE 7.9 Fermentative digestion of protein in the rumen. Dietary proteins are hydrolyzed by extracellular proteases and oligopeptidases of primarily bacterial origin to form di- and tripeptides and some amino acids (AAs). Some AAs can be degraded to form ammonia and the corresponding α-ketoacids. Bacteria use small peptides, Aas, and ammonia to synthesize new proteins. Protozoa engulf bacteria, fungi, small feed particles, and insoluble proteins and use these substrates for synthesizing protozoal proteins.

ruminal microbial growth, feed intake, and the digestibility of nutrients (particularly protein and fiber; Firkins and Yu 2015). Depending on its type, protein may undergo extensive extracellular degradation by proteases and peptidases of bacterial origin. The resultant products serve as precursors for intracellular amino acid and protein synthesis, primarily in bacteria. Bacteria are either engulfed by protozoa in the rumen or flow along with the protozoa into the abomasum to provide the ruminant with microbial (bacterial and protozoal) proteins. The latter are hydrolyzed by proteases and peptidases in the stomach and the small intestine. Microbial proteins typically supply most of the amino acids needed by ruminants fed roughages, but rumen-undegraded protein (RUP) may provide >50% of the absorbed amino acids in high-producing dairy cows fed a high-concentrate diet (Schwab 2003). In healthy ruminants, the true digestibility of plant-source protein and rumen-protected high-quality protein in diets at the end of the ileum (after passing through the forestomachs, abosamum, duodenum, and jejunum) is 62–66% and 85–90%, respectively (Chalupa and Sniffen 1994). Much evidence shows that cost-effective ruminant production with high economic returns should be based on (a) meeting the N needs of ruminal fermentation from low-CP diets and (b) providing adequate and balanced amounts of amino acids (the amino acid profile of microbes being complemented with RUP) for absorption from the small intestine into the blood circulation (Bergen 2021; Cao et al. 2021; NRC 2001; Titgemeyer 2003).

Degradation of Dietary Protein in the Rumen

Extracellular Proteolysis by Bacterial Proteases and Peptidases

The rumen harbors various species and strains of anaerobic bacteria, methanogenic archaea, protozoa, and fungi (Firkins and Yu 2015). Approximately 40% or more of the bacteria isolated from ruminal fluid have proteolytic activity toward extracellular protein because they release high-activity endoproteases and peptidases (including oligo-, tri-, and dipeptidases). Entodiniomorphid protozoa also secrete endo proteases and peptidases into the ruminal fluid. Numerous endoproteases are bound extracellularly to the cell membranes of bacteria and protozoa (Firkins and Mackie 2020). The major proteolytic microbes are saccharolytic bacteria (*Ruminobacter amylophilus, Butyrivibrio fibrisolvens,* and *Prevotella ruminicola*), with *P. ruminicola* being the most numerous proteolytic bacteria (Firkins and Mackie 2020; Nagaraja 2016). *R. amylophilus* is the most active proteolytic bacteria in cultures, but its number in the rumen is low. Cellulolytic bacteria and archaea have low protease activity. Extracellular fungal proteases are also active in the rumen. Feed proteins, large peptides, or oligopeptides (consisting of 5 or more amino acid residues) do not enter bacteria because both Gram-negative and positive bacteria have a size exclusion of ~5 amino acids (Higgins and Gibson 1986).

Bacteria play a major role in the ruminal degradation of dietary protein, as well as other potential sources of proteins (Broderick et al. 1991; Russell et al. 1992; Wallace 1994). The latter include the endogenous proteins of saliva (e.g., 5.4 g/day in cattle fed forage diets), epithelial cells sloughed from the ruminal epithelium (e.g., 5 g/day in a cow and steer), and the remains of the lysed ruminal microbes. The extracellular degradation of the proteins in the rumen is initiated by the proteases of bacterial origin. Approximately 90% and 10% of the total proteolytic activity are associated with the cell surface of bacteria and the cell-free fraction, respectively (NRC 2001). Water-soluble proteins are adsorbed to bacteria, which are attached to water-insoluble proteins. The hydrolysis of proteins by extracellular proteases (including carboxypeptidases and aminopeptidases) yields oligopeptides, which are further degraded by oligopeptidases to form small peptides (consisting of 2–3 amino acid residues) and some free amino acids (Figure 7.9). Some di- and tripeptides are hydrolyzed extracellularly by di- and tripeptidases, respectively, into free amino acids. The major sources of bacterial proteases and peptidases are summarized in Table 7.7.

TABLE 7.7
Major Sources of Microbial Proteases and Peptidases in the Rumen

Proteases (cell-bound and secreted): Protein → Oligopeptides

Saccharolytic bacteria (*R. amylophilus*[b], *S. bovis*, *E. budayi*, *Clostridium spp*, *B. fibrisolvens*[a], *L. multipara*, *D. ruminantium*, *and P. ruminicola*); other bacteria (*S. ruminantium*, *E. ruminantium*, and *Fusobacterium spp.*); ciliate protozoa (*E. caudatum*, *O. caudatus*, *E. maggii*, *E. medium*, *P. multivesiculatum*, and *E. simplex*); and fungi (*P. communis*, *N. frontalis*, and *O. joyonii*)

Oligopeptidases: Oligopeptides → Di- and tri-peptides → Amino acids

Saccharolytic bacteria (*R. amylophilus*, *S. bovis*, and *P. ruminicola*) and small entodiniomorphid protozoa

Di- and tri-peptidases: Di- and tri-peptides → Amino acids

Saccharolytic bacteria (*L. multipara*, *D. ruminantium*, *P. ruminicola*[a], and *F. succinogenes*); other bacteria (*M. elsdenii* and *S. ruminantium*); ciliate protozoa (*E. caudatum* and *Isotricha spp.*)

Deaminases: Amino acids → Ammonia

Saccharolytic bacteria (*B. fibrisolvens* and *P. ruminicola*); other bacteria (*P. anerobius, C. aminophilum*[b], *M. elsdenii, S. ruminantium*, and *C. sticklandii*); ciliate protozoa (*E. caudatum*)

Source: Broderick et al. (1991), Russell et al. (1992), and Wallace (1994).

[a] High numbers but low activity: *Butyrivibrio fibrisolvens, Megasphaera elsdenii, Prevotella ruminicola* (>60% of ruminal bacteria), *Selenomonas ruminantium,* and *Streptococcus bovis* (>10^9 cells/ml ruminal fluid; 10 to 20 nmol of ammonia/min per mg of protein). Quantitatively, they are the major fermenters of amino acids in the rumen.

[b] Low numbers but high activity: *Clostridium aminophilum, Clostridium sticklandii,* and *Peptostreptococcus anaerobius* (10^7 cells/ml ruminal fluid; 300 nmol of ammonia/min per mg of protein).

Extracellular and Intracellular Degradation of Amino Acids and NPN into Ammonia in the Rumen

Free amino acids may undergo extensive extracellular degradation to ammonia and α-ketoacids [e.g., pyruvate, oxaloacetate (OAA), α-ketoglutarate (α-KG), and branched-chain α-ketoacids (BCKAs)] by enzymes (including amino acid deaminases, dehydrogenases, and oxidases) derived from saccharolytic bacteria and hyperammonia-producing bacteria (Firkins and Mackie 2020). Some amino acids may enter these microbes for active intracellular metabolism, yielding ammonia plus their carbon skeletons (Wu 2022). The carbon skeletons are converted into CO_2, CH_4, and short-chain fatty acids (SCFAs). Assuming that 10% of dietary CP is converted into SCFAs in the rumen, it can be estimated that dietary protein and amino acids provide only a small amount (2–3%) of the SCFAs produced in the rumen under normal feeding conditions (e.g., dietary CP = 10–18% of DM, depending on animal species and production stage). Monensin and essential oils are known to reduce bacterial population in the rumen and inhibit amino acid deamination independent of, or concomitant with, their effects on protozoa (Bergen and Bates 1984; Firkins et al. 2007).

NPN substances [e.g., ammonia, nitrate, nitrite, small peptides, nucleic acids (up to 20% NPN), glucosamines, uric acid, and allantoin] are present in the rumen, where they can be metabolized extracellularly to ammonia by enzymes released primarily from bacteria (Vogels and Van der Driet 1976). The ruminal bacteria can also take up the NPN substances and convert them into ammonia. Bacteria, and to a lesser extent, archaea and fungi convert extracellular and intracellular ammonia into amino acids. These reactions are illustrated in Figure 7.10.

Utilization of Urea, Nitrate, and Nitrite

Urea is hydrolyzed into ammonia and CO_2 by urease. This enzyme is absent from animal cells (Wu 2022). Nitrate is reduced to nitrite by nitrate reductase, and nitrite is then converted into ammonia by nitrite reductase. These reactions consume $2H^+$ and, therefore, H_2, because $2H^+$ and H_2 are interconverted by bacterial hydrogenase and ferredoxin hydrogenase (Averill 1996). In the rumen, nitrate-utilizing bacteria include *Wolinella succinogenes*, *Veillonella parvula*, and *Selenomonas ruminantium* (Yang et al. 2016). Thus, although H_2 is a major product of fermentation by isolated protozoa, fungi, and some bacteria, it does not accumulate in the rumen because of its immediate use by the mixed populations of microbes (Moss et al. 2000). Likewise, in the rumen, some nitrate and nitrite are reduced to nitric oxide (NO), NH_3, and N_2O (Yang et al. 2016), and there is little production or fixation of N_2 (Nagaraja 2016; Pun and Satter 1975). In the presence of ammonia, S, and carbohydrates, ruminal microbes can synthesize all proteinogenic amino acids, as well as most physiologically important nonproteinogenic amino acids except for taurine (Bergen 2021; Liu and Masters 2000).

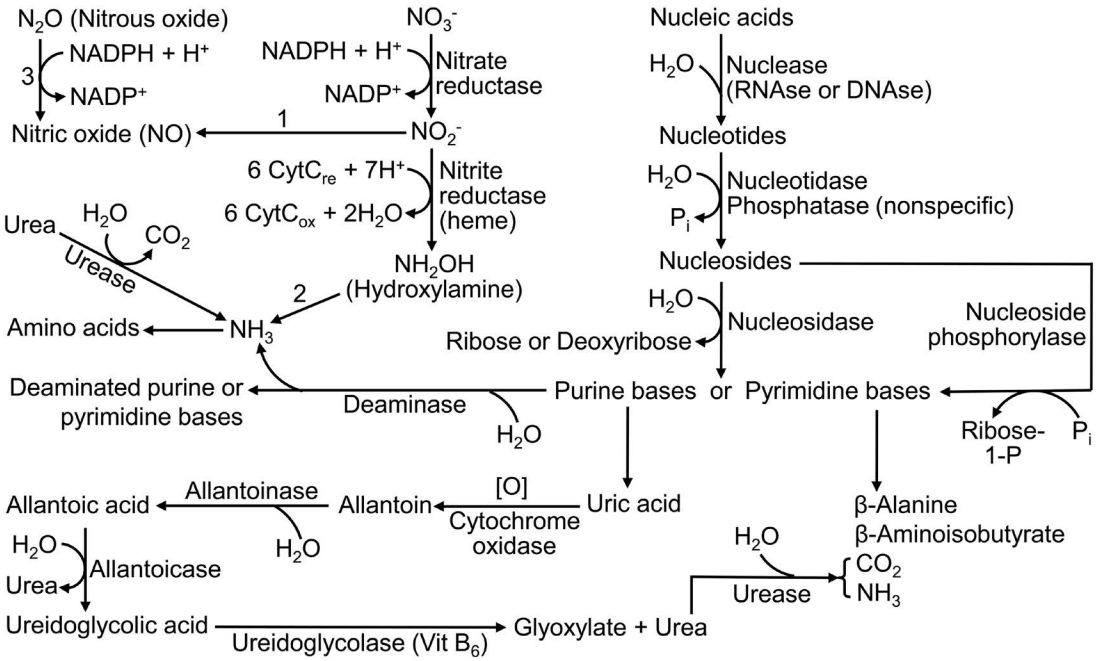


FIGURE 7.10 Extracellular degradation of NPN substances into ammonia in the rumen. These reactions are catalyzed extracellularly by enzymes of primarily bacterial origin. (1) mammalian and bacterial nitrite reductase (Cu^{2+} or heme); (2) hydroxylamine reductase (cytochdrome C); and (3) nitric oxide (NO) reductase (cytochrome C). $CytC_{ox}$, oxidized cytochrome C; $CytC_{re}$, reduced cytochrome C.

$NO_3^- + 2e^- + 2H^+$ (from NADPH + H^+)
$\rightleftharpoons NO_2^- + H_2O + NADP^+$
[Bacteria, nitrate reductase (Mo)]

$NO_2^- + 2e^- + 2H^+ \rightleftharpoons NO + H_2O$
[Bacteria, nitrite reductase (heme)]

$NO_2^- + 2e^- + 2H^+ \rightleftharpoons NO + H_2O$
[Bacteria, nitrite reductase (copper)]

$2NO_2^- + 4e^- + 3H_2O \rightleftharpoons N_2O + 6OH^-$
[Bacteria, nitrite reductase (copper)]

$NO_2^- + 6e^- + 7H^+ \rightarrow NH_3 + 2H_2O$
[Bacteria, nitrite reductase (cytochrome C)]

$2NO + 2e^- + 2H^+ \rightleftharpoons N_2O + H_2O$
[Bacteria, nitric oxide reductase (heme)]

$2NO + 2e^- + 2H^+$ (from NADPH + H^+)
$\rightleftharpoons N_2O + H_2O + NADP^+$
[Fungi, nitric oxide reductase]

H_2 + Oxidized ferredoxin (Fd_{ox})
$\rightleftharpoons 2H^+$ + Reduced ferredoxin (Fd_{red})
[Bacteria, ferredoxin hydrogenase (Fe-S center)]

$2H_2 + NAD^+ + 2\ (Fd_{ox}) \rightleftharpoons 2H^+ + NADH + H^+ + 2Fd_{red}$
[Bacteria, hydrogenase (Fe-S center)]

$H_2 \rightleftharpoons 2H^+$ (Bacteria, [Fe-Fe] hydrogenase (Fe-S center) or [Ni-Fe] hydrogenase (Fe-S center))

$H_2 \rightleftharpoons 2H^+$ (Bacteria, [Fe] hydrogenase (containing no Fe-S center)

The reduction potentials (E^o) for the microbial production of CH_4, HS^-, nitrate, and ammonia at pH 7.0 (Lam and Kuypers 2011) are shown as follows. The greater the value of the reduction potential, the easier the reductive reaction.

$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$	(E^o = –0.30 V)
$SO_4^{2-} \rightarrow HS^-$	(E^o = –0.23 V)
$NO_2^- \rightarrow NH_4^+$	(E^o = –0.34 V)
$NO_3^- \rightarrow NH_4^+$	(E^o = –0.36 V)
$NO_3^- \rightarrow NO_2^-$	(E^o = –0.42 V)

Thus, the reduction of nitrate or nitrite to NH_3 is energetically more favorable than the reduction of CO_2 plus H_2 to CH_4. As a result, CH_4 production is substantially reduced when nitrate is added to the rumen (Moss et al. 2000). Because nitrate can act as an alternative H_2 sink in the rumen, dietary supplementation with appropriate doses of nitrate (<1.5% of DM) to ruminants can reduce CH_4 emissions and improve production performance (Lee and Beauchemin 2014). A disadvantage of this method is nitrate or nitrite poisoning because of a slow reduction process for nitrate or nitrite by ruminal bacteria (Nagaraja 2016). Specifically, when the rate of nitrate reduction to nitrite in the rumen is greater than the rate of nitrite reduction to NH_3, nitrite is accumulated and then absorbed across the ruminal wall into the blood (Yang et al. 2016). In the blood, nitrite binds to red blood cells and converts the ferrous (Fe^{2+}) form of hemoglobin to its ferric (Fe^{3+}) form (methemoglobin), whereas nitrite itself is oxidized to nitrate. Because methemoglobin cannot carry oxygen, high levels of methemoglobin will result in toxicity syndromes, including reduced food intake, breathing difficulties, brain damage, or even death. To reduce nitrate or nitrite toxicity in ruminants, a strategy of acclimation over two weeks or longer has been implemented in some studies (Lee and Beauchemin 2014). Note that methylene blue (1 mg/kg BW, intravenous administration) can be used to treat nitrate or nitrite poisoning by rapidly converting methemoglobin into hemoglobin (Van Dijk et al. 1983).

Utilization of Nucleic Acids

In the rumen, nucleic acids (5–10% of dietary N) are degraded by nucleases (e.g., ribonucleases and deoxyribonucleases) into nucleotides, which are further hydrolyzed by nucleotidases and nonspecific phosphatases into nucleosides and Pi (Nagaraja 2016). The nucleosides are degraded into (a) purine or pyrimidine bases plus ribose or deoxyribose by nucleosidases or (b) purine or pyrimidine bases plus ribose-1-phosphate by nucleoside phosphorylase. Purines are converted sequentially into uric acid, allantoin, allantoic acid, ureidoglycolic acid, and urea plus glycoxylate. Pyrimidine bases are metabolized to β-alanine, β-aminoisobutyrate, ammonia, and CO_2. All these products have been found in the rumen of ruminants (Vogels and Van der Driet 1976). Indeed, 42% of the ruminal coliform isolates are capable of growing on a medium that contains uric acid as the primary source of carbon and N. The predominant uricolytic organism is *Paracolobactrum aerogenoides*, but *S. ruminantium* can also use adenine and uric acid, but not allantoin, xanthine, or uracil, as N sources. The collective actions of ruminal bacteria benefit their ruminant hosts through the conversion of NPN into ammonia, amino acids, and protein.

Lack or Limited Degradation of Extracellular Citrulline and Glutamate in the Rumen

Recent studies reveal that the ruminal microbes of cattle and sheep extensively hydrolyze arginine and glutamine but do not degrade extracellular citrulline and only have a limited ability to metabolize extracellular glutamate due to limited or no uptake (Gilbreath et al. 2019, 2020a,b). This seminal finding refutes the traditional view that all dietary amino acids are extensively catabolized by ruminal microbes. Therefore, unencapsulated citrulline or glutamate can be directly supplemented to the diets of ruminants for improving their health and productivity. It is possible that

extracellular aspartate also undergoes little or no degradation by microbes in the rumen; however, direct evidence is lacking.

Intracellular Protein Synthesis from Small Peptides, Amino Acids, and Ammonia in Microbes

Extracellular di- and tripeptides, free amino acids, and ammonia in the rumen are taken up primarily by the bacteria, where the peptides are hydrolyzed to free amino acids and ammonia is used for the formation of free amino acids, followed by the conversion of the free amino acids into protein (McDonal et al. 2021). Bacteria-sourced protein can be identified using diaminopimelic acid (DAPA; synthesized from L-aspartate, pyruvate, succinyl-CoA, and L-glutamate) as a biomarker (Wu 2022). DAPA is present as both L- and D-isomers in the peptidoglycans of all Gram-negative and some Gram-positive bacteria, but not archaea. The peptidoglycan accounts for 10% and 90% of the cell wall of Gram-negative and positive bacteria, respectively. Thus, DAPA can be used to estimate the outflow of bacterial biomass from the rumen. In addition to bacteria, isotrichid protozoa take up a small amount of extracellular di- and tripeptides and free amino acids but no extracellular ammonia, whereas entodiniomorphid protozoa engulf particulate matter (e.g., bacteria and feed). Furthermore, ruminal fungi use a small amount of extracellular di- and tripeptides, free amino acids, and ammonia for protein synthesis. Ammonia is the preferred N source for the growth of many ruminal bacteria and archaea, with 60–80% of their protein N being formed from ammonia (Firkins and Mackie 2020).

Before their use for protein synthesis in all microbes, the intracellular small peptides must be hydrolyzed into free amino acids, and ammonia converted into free amino acids. Within these cells, free amino acids are used via multiple metabolic pathways for the production of (a) proteins and peptides; (b) new amino acids (including all L-amino acids and, to a much lesser extent, some D-amino acids); (c) ammonia, NO, and H_2S; (d) nucleic acids; (e) amines; (6) α-ketoacids such as pyruvate, OAA, α-KG, and BCKAs; and (f) SCFAs. The energy required for amino acid and protein syntheses, as well as amino acid transport into the cells, is provided primarily from the fermentation of dietary carbohydrates and, to a lesser extent, amino acid catabolism. In addition, many minerals (e.g., magnesium, manganese, zinc, S, and cobalt) participate in these synthetic processes. Thus, adequate intakes of fermentable carbohydrates and digestible minerals are essential for maximum microbial protein synthesis in the rumen. In all microbes, protein synthesis is maximized when the release of amino acids from dietary protein matches ATP production from the degradation of carbohydrates.

About 90% of the total N in the ruminal content exists in an insoluble form (e.g., microbial proteins, smaller particles of undigested dietary protein, and sloughed ruminal epithelial cells). N in the soluble pool (~10% of the total ruminal N) consists of ammonia N (~70%) and a mixture of free amino acids and peptides. Ammonia N is present in the rumen in concentrations varying between 20 and 500 mg per liter, depending on diet and time after feeding. A minimum concentration of ruminal ammonia (50 mg/L fluid) is necessary for bacteria to grow well in the rumen. Maximum concentrations of ammonia are usually reached about 2 h after ingestion of a protein-containing diet. Amino acid- and peptide-N are present at much lower concentrations (usually <20 mg/L) than those of ammonia in the rumen (McDonal et al. 2021). The proportion of microbial protein from ammonia in the rumen depends on N sources: for example, ranging from 26% when high concentrations of peptides and free amino acids are present (as in steers fed diets supplemented with high-protein concentrates) to 100% when urea is the sole source (Salter et al. 1979). In lactating cows fed an 18%-CP alfalfa hay diet, 33% of microbial N is derived from ammonia (Hristov et al. 2005). At typical concentrations of peptides and amino acids in the rumen, ~80% of the microbial N is derived from ammonia (Firkins et al. 2007). In the rumen, 1 mol of urea is hydrolyzed by urease to 2 mol of NH_3 plus 1 mol of CO_2, with the NH_3 being either utilized for bacterial amino acid and protein syntheses or absorbed into the blood (Figure 7.9). Intact microbial protein accounts for >50% of total N in the rumen (Bergen and Bates 1984).

The most important initial reaction for microbial assimilation of ammonia is catalyzed by glutamate dehydrogenase (GDH) to produce glutamate, which is then utilized to synthesize glutamine, alanine, aspartate, and asparagine by glutamine synthetase (GS), glutamate-pyruvate transaminase, glutamate-OAA transaminase, and asparagine synthetase, respectively (Figure 7.11). Glutamate is also formed from glutamine and α-KG by glutamate synthase. This glutamate family of amino acids (glutamate, glutamine, alanine, aspartate, and asparagine) serves as substrates for the synthesis of all other amino acids by microbes in the presence of fermentable carbohydrates and S in the rumen. There is evidence that $NADP^+$-dependent GDH plays an anabolic role as its enzymatic activity increases with excess ammonia concentrations, but NAD^+-dependent GDH contributes to glutamate catabolism (Firkins and Mackie 2020). Sulfur is needed for the synthesis of methionine and cysteine and can be supplied as sulfate or protein. Sulfate (SO_4^{2-}) is converted to sulfite (SO_3^{2-}) in ruminal microbes via three sequential reactions involving ATP-dependent sulfurylase, ATP-dependent adenosine-5'-phosphosulfate kinase, and NADPH-dependent 3'-phosphoadenosine-5'-phosphosulfate reductase (Wu 2022). Sulfite is reduced to sulfide (S^{2-}) by NADPH-dependent sulfite reductase in the microbes, and hydrogen sulfide (H_2S) is required for the conversion of serine into cysteine (Figure 7.10). Elemental S, two forms of calcium sulfate (hydrated, $CaSO_4.2H_2O$; and anhydrous, $CaSO_4$), and ammonium sulfate [$(NH_4)_2SO_4$] are effective sources of sulfide for ruminal bacteria. The diets for ruminants should contain N and S at a ratio (g/g) ranging from 12:1 to 16:1, depending on the desired levels of their growth and lactation performance.

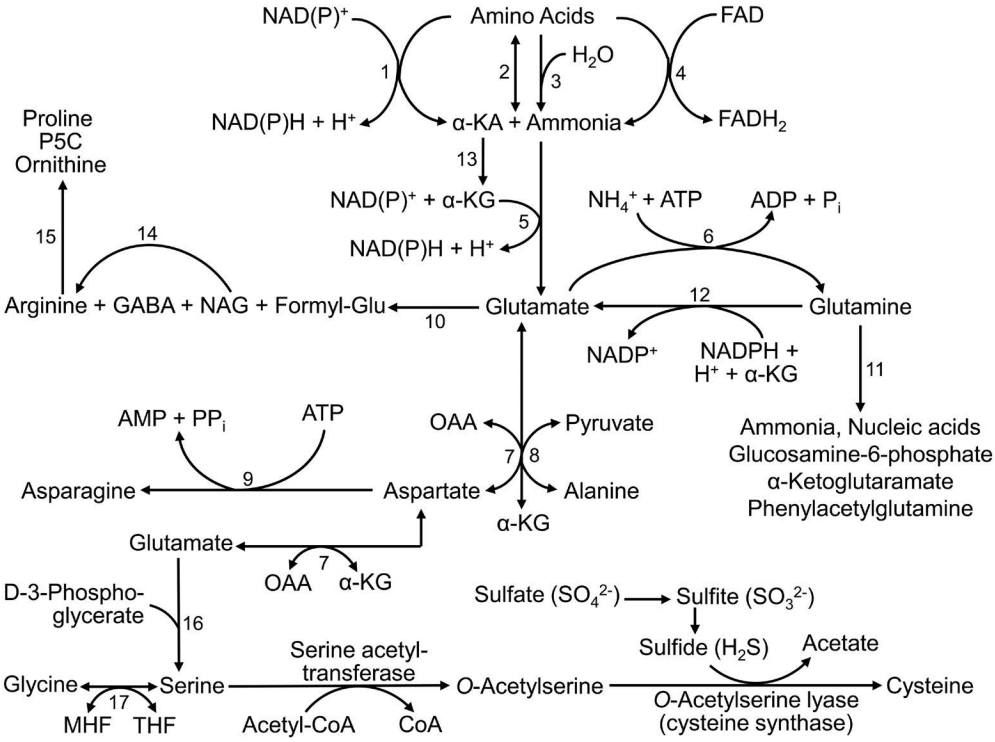


FIGURE 7.11 Production and utilization of ammonia by microorganisms in the rumen of ruminants. GABA, γ-aminobutyrate; Formyl-Glu, formylglutamate; α-KA, α-ketoacids; α-KG, α-ketoglutarate; NAG, N-acetylglutamate; P5C, pyrroline-5-caroxylate. The enzymes that catalyze the indicated reactions are: (1) amino acid dehydrogenases; (2) amino acid transaminases; (3) amino acid deaminases; (4) amino acid oxidases; (5) glutamate dehydrogenase; (6) glutamine synthetase; (7) glutamate-oxaloacetate transaminase (aspartate transaminase); (8) glutamate-pyruvate transaminase (alanine transaminase); (9) asparagine synthetase; (10) the syntheses of NAG, GABA, P5C, and formyl-Glu from glutamate are catalyzed by NAG synthase, glutamate decarboxylase, γ-glutamyl kinase plus glutamyl semialdehyde dehydrogenase, and complex enzymes, respectively; (11) a series of enzymes required in multiple pathways; (12) glutamate synthase (also known as NADPH-dependent glutamine:α-ketoglutarate amidotransferase; glutamine + 2 α-ketoglutarate + NADPH + $H^+ \rightarrow$ 2 glutamate + $NADP^+$); and (13) the conversion of α-ketoacids to α-ketoglutarate via various reactions; (14) the enzymes for converting NAG into arginine; (15) arginase, ornithine aminotransferase, and P5C reductase; and (16) the enzymes for converting D-3-phosphoglycerate and glutamate into serine; (17) serine hydroxymethyltransferase. MTF, N^5-N^{10}-methylene tetrahydrofolate; NAG, *N*-acetyl-glutamate; OAA, oxaloacetate; P5C, pyrroline-5-carboxylate; and THF, tetrahydrofolate.

Role of Ruminal Protozoa in Intracellular Protein Degradation

In the rumen, protozoa are active in ruminal protein degradation, and because of their large size, can account for about 25% of the total microbial mass in the rumen, depending on the type of rations (Firkins et al. 2007). They engulf and digest [through the actions of their hydrolytic enzymes (including lysozymes to break down bacterial cell walls)] bacteria, fungi, small feed particles, and water-insoluble proteins (Jouany 1996). Protozoa are active in degrading extracellular water-insoluble proteins, but poorly degrade extracellular water-soluble proteins due to their limited engulfment (Firkins and Yu 2015). Like bacteria, protozoa actively deaminate amino acids to form ammonia plus their carbon skeletons. However, in contrast to bacteria, protozoa cannot synthesize amino acids from ammonia (Jouany 1996) and instead release large amounts of peptides, amino acids, and ammonia (representing ~50% of total ingested protein) into ruminal fluid. Because of their secretory activity, substantial autolysis, and apoptosis, protozoa are a source of high-activity peptidases in ruminal fluid for the extracellular hydrolysis of peptides. Thus, through a series of actions (e.g., the engulfment of bacteria, extracellular protein, intracellular proteolysis, the release of proteolysis products, and their reuptake), protozoa are key players in the intra-ruminal recycling of N. There is evidence that ≥65% of protozoal protein recycles within the rumen under normal nutritional and physiological conditions (Punia et al. 1992). Such an extensive N cycle, along with the selective retention of isotrichid protozoa in the rumen, functions to (a) convert insoluble proteins in the bacterial and fungal membranes and in ruminal fluid into soluble cytosolic proteins; (b) supply a utilizable source of N to generate small peptides, amino acids, and ammonia for sustaining bacterial growth; and (c) conserve dietary and endogenous N. This helps to explain, in part, why defaunation (the removal of protozoa) decreases protein degradation, as well as the concentrations of peptides, amino acids, and ammonia in the rumen, while enhancing N balance in ruminants (Firkins et al. 2007; Males and Purser 1970).

Role of Ruminal Fungi in Intracellular Protein Degradation

Fungi can account for ~5% of the ruminal microbial mass in the ruminants fed lignified fiber diets, but their numbers are substantially reduced when diets rich in concentrates are fed. Fungi are capable of intracellular proteolysis, peptidolysis, and deamination (Broderick et al. 1991), but their role in degrading ruminal feed protein (extracellular protein) is limited because the extracellular protease activity of fungal origin is low in the rumen (Brock et al. 1982). In evaluating the seven most common strains of ruminal fungi in culture, Michel et al. (1993) found that only one strain (i.e., *Piromyces sp.*) exhibited proteolytic activity toward extracellular casein. All seven strains of fungi had intracellular aminopeptidase activity but no carboxypeptidase activity. It is likely that ruminal fungi do not play a significant role in directly degrading feed proteins in the rumen. However, these cells can make an indirect contribution to the extracellular proteolysis by taking up di- and tripeptides, free amino acids, and ammonia from ruminal fluid and reducing the concentrations of these products in ruminal fluid. Ruminal fungi themselves contribute to the protein supply of the host by serving as a source of high-quality microbial protein for digestion in the abomasum and small intestine (Gruninger et al. 2014).

Major Factors Affecting Protein Degradation in the Rumen

The most important factors affecting microbial protein degradation are the types of dietary protein and carbohydrates, their metabolism to ammonia or lactate, ruminal pH, and microbial number and activity (including the predominant microbial population) in the rumen (Firkins and Yu 2015). Other influential factors include the particle size of the ration, ruminal passage rate, and environmental temperatures. Overall, the microbial ecosystem, including the populations of not only bacteria but also protozoa and fungi, is crucial for the fermentative digestion of protein in the rumen (Nagaraja 2016).

Effects of Type of Dietary Protein on Its Degradation in the Rumen

Different dietary proteins have different rates of degradation in the rumen (Wallace 1994). The rates of ruminal degradation are 200–300%/h for soluble proteins (e.g., globulins and some albumins); 5–15%/h for most albumins and glutelins; 0.1–1.5%/h for prolamines, extensins, prolamines, plant cell-wall protein, and denatured proteins; and 0.0%/h for Maillard products, ligin-bound proteins, and tannin-bound proteins (Chalupa and Sniffen 1994; Ghorbani 2012). Consistent with the current literature, several definitions on ruminal protein digestion are based on CP content in diets and digesta: (a) RDP, which replaces the earlier term degraded intake protein (DIP); (b) RUP, which replaces the earlier term rumen-undegraded intake protein (UIP); (c) rumen-synthesized microbial protein (MCP); and (d) metabolizable protein (MP; the true protein that is postruminally digestible), which is not measurable in feed but can be estimated or calculated as 0.64 × MCP (NRC 2001). The rationale for the latter calculation is that microbial CP (as provided by ruminal bacteria and protozoa) contains 80% true protein, and the true protein of microbial CP is 80% digestible in the small intestine (NRC 2001). For ruminants fed diets containing proteins (e.g., rumen-protected protein and some animal-sourced proteins such as blood meal and feather meal) that escape degradation in the rumen, MP includes both MCP and UIP. The latter refers to dietary protein that is not degraded by ruminal microbes and is available for postruminal digestion.

$$\begin{aligned}&\text{Metabolizable protein (MP)}\\&\quad=\text{Microbial protein (MCP)}\\&\qquad+\text{Rumen-undegraded intake protein (UIP)}\end{aligned}$$

The proportion of total dietary protein that is degraded in the rumen varies from 70–85% for most diets to 30–40% for less soluble proteins (Table 7.8). Rates of protein

TABLE 7.8
Degradation of Dietary Protein and NPN Substances in the Rumen of Cattle

Ingredient	CP content (%)	RDP (%)	RUP (%)
Plant-Sourced Protein Ingredient			
Alfalfa hay	22	84	16
Brewer's grains	29.2	34	66
Canola meal	40.9	68	32
Corn distiller's grains	30.4	26	74
Corn gluten meal	66.3	41	59
Soybean meal	52.9	80	20
Soypass®	52.6	34	66
Silage			
Alfalfa	19.5	92	8
Barley	11.9	86	14
Corn	8.6	77	23
Other Plant-Sourced Ingredient			
Barley grain	13.2	67	33
Barley straw	4.4	30	70
Timothy hay	10.8	73	27
Animal-Sourced Protein Ingredient			
Blood meal	93.8	25	75
Hydrolyzed feather meal	85.8	30	70
Fish meal	67.9	40	60
Meat & Bone meal	50	47	53
Nonprotein Nitrogen (NPN)			
Urea	291	100	0

Source: Chalupa and Sniffen (1994) and Ghorbani (2012).

Note: CP, crude protein; RDP, rumen-degraded protein; RUP, rumen-undegraded protein.

degradation in the rumen depend on residence time in the rumen, proteolytic activity of ruminal microbes, the type of protein, and the level of feeding. For example, some proteins of animal origin (e.g., feather meal, MBM, and blood meal) are much more resistant to ruminal degradation than plant proteins. Much evidence shows that dietary RDP feeds the ruminal bacteria and ensures an adequate supply of microbial protein to the small intestine, whereas dietary RUP (digestible in the abomasum and small intestine) complements the microbial protein for the provision of protein (the quality of which varies with RUP in diets) to the host ruminant (NRC 2001).

Effects of Type of Carbohydrate on Microbial Protein Synthesis in the Rumen

As the major source of energy for ruminal microbes and an effector of ruminal pH, dietary carbohydrate supply profoundly influences the utilization of ruminal NH_3 for microbial protein synthesis (Hristov et al. 2005). Compared with neutral detergent fiber (NDF), intraruminal provision of glucose to lactating cows reduced (a) ruminal ammonia-N flux (including the transfer of blood urea into the rumen), (b) irreversible disappearance of ruminal ammonia-N from the rumen (including the ammonia absorbed into the blood through the ruminal epithelium plus the ammonia converted into microbial protein), (c) ruminal acetate and ammonia concentrations, and (d) ruminal pH. However, intraruminal administration of glucose increased: (a) ruminal butyrate concentration, (b) microbial N concentration in the rumen, (c) microbial N flow out of the rumen, and (d) the percentage of bacterial N derived from ammonia in lactating cows. Starch had similar effects on the measured variables to those of glucose, except that ruminal ammonia flux or ruminal acetate concentration did not differ between starch and NDF feeding (Table 7.9). Glucose or starch feeding, which decreases ruminal pH, may alter the number and activity of butyrate-, acetate-, and/or ammonia-producing bacteria in the rumen (Firkins et al. 2007). Thus, appropriate amounts of readily fermentable carbohydrates in the diets of ruminants likely inhibit ammonia production from amino acids and enhance microbial protein synthesis in the rumen.

Effects of Dietary Concentrate and Forage Intake on Proteolytic Bacteria in the Rumen

Adequate intake of dietary protein (e.g., 14% CP in the diet of growing beef and 18% CP in the diet of high-producing lactating cows; expressed on the basis of DM content) is essential for the optimal growth and proteolytic activity of ruminal bacteria. Feeding an appropriate amount of concentrates to ruminants increases the total microbial population in the rumen, including some active proteolytic bacteria that are also amylolytic bacteria (e.g., *P. ruminicola, R. amylophilus,* and *Streptococcus bovis*; Ghorbani 2012). Under these conditions (e.g., in high-producing dairy cows), the ruminal fermentation of dietary protein and the engulfment of bacteria by protozoa are favored, as reported for hay-fed sheep supplemented with or without concentrates (Table 7.10). Likewise, consumption of fresh forage enhances the proportion of proteolytic bacteria relative to the total microbial population in the rumen, which is also beneficial for the ruminal fermentation of low-quality protein (Chalupa and Sniffen 1994). Very high rates of intake of dietary starch rapidly reduce the pH and the number of proteolytic bacteria in the rumen, which, in turn, adversely affects ruminal protein degradation.

Nutritional Importance of Protein Digestion in the Rumen

Ruminants are unique in digestive physiology in that they can convert NPN (e.g., urea, ammonia, and nitrate), roughages, and forages into bacterial and protozoal proteins, which are digested terminally in the small intestine into constituent small peptides and amino acids. The peptides and amino acids are used for the synthesis of high-quality animal proteins in skeletal muscle, lactating mammary glands, and other tissues. Thus, ruminants can convert low-quality protein and nonprotein materials (including nitrogenous wastes) into high-quality products (e.g., meat and milk) for human consumption without a need to compete with monogastric animals or humans for foods or natural resources. This underscores the nutritional and economic significance of the digestion of plant proteins by ruminants. An understanding of these complex processes will aid in (a) increasing the supply of all amino acids to the small intestine for absorption and utilization for tissue protein synthesis, (b) sustaining regional and global animal production, and (c) providing high-quality animal protein to improve human growth, development, and health.

A major loss of dietary protein in ruminants occurs due to inefficiencies of ruminal digestion and, as in all other animals, extensive catabolism of amino acids in the GIT and suboptimal rates of protein synthesis in tissues (particularly skeletal muscle; Wu et al. 2014b). For example, only 30–35% of feed protein is captured in milk, and the remaining 65–70% is lost as nitrogenous compounds in the feces and urine. In ruminant diets, soluble protein should account for 30–32% of total CP or about half of the RDP level, whereas RUP should be 32–39% of total CP in the diet (Fellner 2002). Such diets should meet dietary requirements for not only NDF and starches but also total CP, RDP, and RUP. This is because (a) total CP provides N sources for microbial protein synthesis in the rumen, which is tightly coupled with ATP production from the glycolysis of glucose and its fermentation to SCFAs in the microbes; (b) RDP is essential for sustaining microbial growth and mass in the rumen; (c) RUP supplies high-quality protein for digestion in the abomasum and small intestine; and (d) dietary NDF helps to maintain a rumen environment that is favorable for fermentation. Consistent with this notion is the finding that, in lactating cows that are fed diets with CP content between 9% and 17% and without supplemental fats, milk protein yield increases by 0.02% unit for a 1% unit increase in dietary CP (Emery 1978).

TABLE 7.9
Effects of Dietary Carbohydrates on the Use of Ruminal Ammonia N in Lactating Dairy Cows

Variable	Glucose	Starch	NDF	Mix	SEM
Dry matter (DM) intake, kg/day	21.8	21.4	22.6	22.5	0.48
Organic matter (OM) intake, kg/day	19.9	19.8	20.4	20.3	0.43
NDF intake, kg/day	8.3^{m}	8.3^{m}	12.4^{a}	9.6^{b}	0.23
Nitrogen (N) intake, kg/day	0.634	0.630	0.662	0.651	0.02
Ruminal true digestibility of DM, %	64.1	61.9	60.9	63.2	1.56
Ruminal true digestibility of OM, %	66.5	64.0	64.7	67.0	1.85
Ruminal true digestibility of NDF, %	57.0	59.6	56.5	64.8	3.39
Ruminal true digestibility of N, %	57.5	59.1	66.6	58.8	2.97
Total-tract apparent digestibility of DM, %	71.5^{B}	73.6^{A}	77.9^{A}	75.6^{A}	1.92
Total-tract apparent digestibility of OM, %	74.2	75.9	78.9	77.6	1.97
Total-tract apparent digestibility of NDF, %	57.1^{b}	61.2^{b}	73.5^{a}	68.0^{a}	3.19
Total-tract apparent digestibility of N, %	74.8^{b}	75.6^{b}	86.6^{a}	$79.1^{a,b}$	1.60
Ammonia-N in ruminal fluid, mM	8.5^{b}	9.6^{b}	16.4^{a}	12.4^{a}	1.05
Ruminal ammonia-N flux, g/day	350^{b}	487^{a}	533^{a}	525^{a}	39.1
Ruminal ammonia-N flux as % of dietary N intake	55^{b}	77^{a}	80^{a}	80^{a}	5.9
Irreversible disappearance of ruminal ammonia-N, g/day	230^{B}	343^{A}	320^{A}	$294^{A,B}$	26.7
Bacterial N from ruminal ammonia-N, %	38.3^{b}	60.1^{a}	$48.3^{a,b}$	$47.3^{a,b}$	1.91
Microbial N in ruminal fluid, g	180	143	140	198	21.4
Microbial N in ruminal fluid as % of total ruminal non-ammonia N	59.3	49.7	49.7	61.0	6.34
Microbial N in ruminal fluid, g/kg of truly digested OM	15^{a}	15^{a}	12^{c}	14^{b}	0.8
Microbial N derived from ruminal ammonia-N, g/day	75^{b}	111^{a}	74^{b}	$89^{a,b}$	10.4
Microbial protein N from ruminal bacterial N, %	44.8	46.3	46.3	43.3	1.91
Microbial protein N from ruminal N, %	17.1^{c}	27.7^{a}	22.4^{b}	$20.8^{b,c}$	1.82
Microbial protein synthesis from ruminal ammonia-N, %	33^{a}	33^{a}	23^{b}	30^{a}	2.4
Microbial N flow out of the rumen, g/day	197^{a}	185^{a}	153^{b}	188^{a}	9.7
Total protozoa in ruminal fluid, $\log_{10}$/ml	5.26	5.21	5.42	5.49	0.13
Total SCFAs in ruminal fluid, mM	122^{b}	$139^{a,b}$	$136^{a,b}$	145^{a}	3.08
Acetate in ruminal fluid, mM	74.0^{b}	95.4^{a}	94.5^{a}	97.0^{b}	2.45
Propionate in ruminal fluid, mM	22.1	23.0	23.2	24.0	1.28
Butyrate in ruminal fluid, mM	22.2^{a}	15.0^{c}	12.4^{d}	18.9^{b}	0.81
Isobutyrate, mM	0.63^{c}	1.29^{b}	1.57^{a}	1.22^{b}	0.08
Valerate, mM	2.84	2.67	2.61	2.74	0.11
Isovalerate, mM	0.61^{b}	1.73^{a}	1.68^{a}	1.37^{a}	0.13
Acetate/Propionate, mol/mol	3.36^{B}	4.26^{A}	4.08^{A}	4.06^{A}	0.21
pH in ruminal fluid	6.00^{c}	6.19^{b}	6.41^{a}	6.05^{c}	0.049
Milk N yield (i.e., milk true protein yield/6.38, g/day)	97	89	90	83	9.7
Urinary allantoin, g/day	57.1	70.4	58.6	66.6	4.96
Total N loss in urine plus feces, g/day	435	441	427	453	18.6
Total N loss in urine plus feces as % of dietary N intake	69.2	70.6	64.7	69.6	3.85

Source: Hristov et al. (2005). Late-lactation Holstein cows were fed a 22.1%-CP diet (73.2% chopped alfalfa hay, 20.6% tested carbohydrate, 3.6% ring-dried blood meal, 0.4% monosodium phosphate, 0.9% sodium bicarbonate, and 1.3% mineral-vitamin premix; DM basis) (chopped) at 12-h intervals (DM intake = 22.2 kg/day). Glucose, cornstarch, NDF, or a mix of apple pectin + glucose + cornstarch + NDF (25% each) was introduced intraruminally at 20.6% of DM intake twice daily before each feeding.

[a-d:] Within a row, means not sharing the same superscript letter differ ($P \leq 0.05$).

[A-B:] Within a row, means not sharing the same superscript letter differ ($P < 0.10$).

Note: NDF, neutral-detergent fiber (provided from white-oat fiber); SCFAs, short-chain fatty acids; SEM, pooled standard error of the mean.

TABLE 7.10
Effects of Dietary Concentrates on Microbial Protein Synthesis in the Rumen of Hay-Fed Mature Sheep

Variable	Hay (100%)	60% Hay + 40% Concentrates[a]
Organic matter intake, g/day	804	750
Nitrogen intake, g/day	21.7	21.2
Ruminal retention time of particles, h	19.9	31.6
Ruminal retention time of solutes, h	11.8	10.6
Ruminal pH	6.48	6.08
Ruminal ammonia-N, mg/L fluid	192	225
Ruminal microbial N, g		
Total microbial N	18.3	24.0
Bacterial N	10.9	8.54
Protozoal N	6.93	14.9
Fungal N	0.41	0.56
% of total microbial N in ruminal digesta		
Fungal N	2	2
Protozoal N	38	62
Bacterial N	60	36
Flow of ammonia-N out of the rumen, g/day	2.80	2.2
Duodenal digesta ammonia-N, mg/L fluid	96.5	105.5
Duodenal digesta microbial N, g/day		
Total microbial N	14.8	15.1
Bacterial N	13.9	13.0
Protozoal N	0.82	1.73
Fungal N	0.16	0.35
% of total microbial N flow (duodenal digesta)		
Fungi	1	2
Protozoa	5	12
Bacteria	94	86
Flow of ammonia-N to duodenum, g/day	0.98	0.99

Source: Faichney et al. (1997).

[a] Dietary concentrates contained (g/kg; on an as-fed basis): barley, 825; soybean meal, 110; dried molasses, 47; and mineral-vitamin mix, 18.

Protecting High-Quality Protein and Amino Acid Supplements from Ruminal Degradation

High-quality dietary protein is required to support maximal growth, reproduction, and lactation performance of ruminants (e.g., beef, dairy cows, sheep, and goats). Degradation of such protein by ruminal microbes results in a substantial amount of waste, as not all dietary protein is utilized for microbial protein synthesis. Additionally, microbial protein synthesis requires large amounts of energy, and the efficiency of energetic transformations for protein synthesis from amino acids is usually less than 75%. Furthermore, supplements of crystalline amino acids in the diets of ruminants are not efficacious because of their rapid degradation in the rumen (Tedeschi and Fox 2016). Thus, high-quality protein or supplemental amino acids (e.g., arginine, histidine, lysine, and methionine) in diets should be protected from rumen degradation. Several approaches have been developed to reduce the degradation of protein in the rumen. These methods include the treatment of protein by mild heating, chemical treatment, addition of polyphenolic phytochemicals, and physical encapsulation (e.g., rumen-protected protein). In the small intestine, the coating lipids are readily broken down by lipase to release proteins or amino acids. Similarly, nearly all free amino acids in diets do not escape the rumen into the abomasum, and supplemental amino acids for ruminants should be protected from ruminal degradation and generally recognized as safe (GRAS) to ensure both high efficacy and safety. Rumen-protected amino acids should be stable, particularly when they are incorporated into silage- or forage-based total mixed rations.

Heating

Heating is one of the earliest methods used to increase the escape of high-quality protein (e.g., casein) from the rumen. The underlying principle is the Maillard reaction, which involves a reaction between certain amino acids [particularly the ε-amino groups ($-NH_2$) of lysine residues in protein] and carbonyl compounds (–HC=O), usually reducing sugars (e.g., glucose; Chapter 4). The initial reaction is the formation of a Schiff base, followed by Amadori rearrangement of the Schiff base to generate an Amadori compound. The formation of the Schiff base is reversible. However, further heating results in the production of melanoidin polymers that are nutritionally unavailable to animals. Therefore, an appropriate heating procedure should be developed to balance a reduction in RUP digestibility (attributed to the Maillard reaction) and an increase in RUP supply. In addition, heating helps to denature protease inhibitors in feedstuffs.

Chemical Treatments

Chemical treatments of dietary protein aid in reducing its solubility and its susceptibility to proteases in the rumen. An example of chemical treatment is the use of formaldehyde (HCHO). An advantage of this method is that most of the formaldehyde-protein reactions are unstable at low pH such as the pH in the abomasum and the upper part of the small intestine. Therefore, formaldehyde-treated proteins are susceptible to degradation by proteases in the abomasum and the small intestine. However, formaldehyde is a potential carcinogen and is a hazard to animals and humans. Other chemicals and methods used for treating dietary protein (e.g., SBM) include NaOH, ethanol, propionic acid, and calcium lignosulfonate, as well as extrusion and expeller processing (Waltz and Stern 1989). To date, a common method to chemically treat a protein ingredient (e.g., oilseed meal) for manufacturing a rumen-protected product is to mix the feedstuff with calcium lignosulfonate (a byproduct of the wool pulp industry containing a variety of sugars, mainly D-xylose), followed by appropriate heating.

Polyphenolic Phytochemicals

Tannins (natural phytochemicals) are often used to protect protein degradation in the rumen. They spontaneously react with proteins primarily through hydrogen bonding to generate a water-insoluble complex. Such a tannin-protein complex is resistant to ruminal proteases at pH 5.5–6.5. However, in the lumen of the abomasum (pH 2–3), protein is dissociated from the tannins and is available for degradation by proteases. In addition, tannins, like saponins, reduces the population of protozoa in the rumen, thereby reducing the catabolism of dietary protein in ruminants (Firkins and Mackie 2020). Thus, high-quality proteins can be effectively protected from degradation in the rumen and become available for digestion in the abomasum and then in the small intestine. In practice, tannins may be added directly to feedstuffs for ruminants.

Physical Encapsulation of Protein or Amino Acids

Early work in the 1970s indicated that certain protein ingredients of animal origin are resistant to ruminal degradation (Tamminga 1979). Animal-sourced protein is more resistant to bacterial proteases than plant-sourced protein because of its water insolubility that results from heating during the rendering process to remove any potential pathogens and inactivate any latent proteases from the animal's tissues. To date, the methods for physical encapsulation of proteins or amino acids include blood spraying and the use of a hydrogenated lipid layer. Protein supplements are sprayed with blood, followed by heating the mixture to dryness to effectively coat the surface of proteins. Proteins or amino acids can also be coated with hydrogenated lipids (e.g., lecithin or soy oils) to form microcapsules. All these encapsulated proteins and amino acids are not colonized to the surface of ruminal bacteria and are less accessible to proteases or amino acid deaminases in the ruminal fluid.

Inhibition of Amino Acid Degradation

The conversion of amino acids to their carbon skeletons and ammonia is catalyzed by amino acid deaminases. Thus, inhibition of these enzymes in the rumen can increase the availability of dietary amino acids for absorption in the small intestine. In support of this view, dietary supplementation with an inhibitor of amino acid deaminases (e.g., diphenyliodonium chloride) can enhance the flow of high-quality protein from the rumen into the abomasum and the small intestine, thereby improving growth performance in beef cattle fed diets containing high-quality protein. However, an inhibition of amino acid deaminases can reduce the availability of ammonia for amino acid synthesis by ruminal bacteria and, therefore, is not beneficial for ruminants fed diets containing low-quality protein.

Flow of Microbial Protein from the Rumen into the Abomasum and Duodenum

Ruminal bacteria and protozoa undergo turnover in the rumen to grow new cells and replace the old ones. In addition, microbial lysis occurs within the rumen due to the presence of autolytic enzymes and bacteriocins as well as bacteriophages and mycoplasmas. Furthermore, ruminal microbes flow out of the rumen into the abomasum and then the duodenum, with the rates for bacteria being greater than those for protozoa. The mean retention times of bacteria and protozoa in the rumen of roughage-fed ruminants are ~20 and 35 h, respectively (Ffoulkes and Leng 1988). Thus, as shown for mature sheep (Table 7.10), protozoal N may account for ~25–40% of total microbial N in the rumen, but only 1% of total microbial N in the duodenal digesta. Of note, Sok et al. (2017) estimated that (a) 40% of the bacteria in the duodenum are present in the fluid phase, which means that they pass with the gastric liquid phase; and (b) 60% of bacteria and most entodiniomorphid protozoa (>90% of protozoa) are found in the particulate phase of the duodenum. Note that the duodenal digesta also consists of saliva, bile salt, pancreatic and small-intestinal secretions, and epithelial cells sloughed from the stomachs and duodenum.

The composition of 9 proteinogenic amino acids is similar, but that of 11 proteinogenic amino acids differs between ruminal bacterial and protozoal proteins (Table 7.11). Specifically, the content of alanine, glycine, methionine, threonine, tryptophan, and valine is 60%, 27%, 13%, 9%, 27%, and 20% greater in ruminal bacterial protein than that in ruminal protozoal protein, respectively. In contrast, the content of glutamate + glutamine, isoleucine, lysine, and phenylalanine is 12%, 20%, 43%, and 11% greater in ruminal protozoal protein than that in ruminal bacterial protein, respectively. Thus, compared with bacterial protein, protozoal protein may have a better nutritive value for the small intestine and the host, lending additional support for the nutritional importance of the engulfment of bacteria by protozoa in the rumen. Much evidence shows that feeding different dietary rations to ruminants can affect the amount of microbial proteins flowing from the rumen into the duodenum but do not generally influence the amino acid composition or quality of the microbial proteins (Bergen et al. 1968a; Bergen 2015; Fenderson and Bergen 1972; Flores et al. 1986). Ruminal microbial protein is not ideal for the maximal growth performance of ruminants (Titgemeyer 2003).

In contrast to common belief, the content of only ten amino acids is similar between ruminal bacteria protein and casein: histidine, leucine, lysine, methionine, cysteine, phenylalanine, serine, tryptophan, tyrosine, and valine (Bergen 2015). Notably, the content of alanine, arginine, aspartate, asparagine, glycine, and threonine is much higher, but the content of glutamate, glutamine, and proline is much lower in ruminal microbial protein than that in casein (Bergen 2015). Because S-containing amino acids in casein are inadequate for the maximum growth of young animals (Wu 2022), this protein is not a good representative of amino acids in the whole body of growing livestock, including ruminants and swine.

Because of the addition of endogenous proteins into the duodenum, the composition of most amino acids is similar between the duodenal digesta and the animal body, except

TABLE 7.11
Amino Acid (AA) Composition (g/100 g of the Total AAs) of Proteins in Feeds, Digestive Tract Bacteria, Casein, Whole Body, and Skeletal Muscle of Ruminants

	Feed[a]		Ruminal Mixed Bacteria[a]		Ruminal Protozoa		Duodenal Digesta		Casein	Whole Body[e]		Skeletal Muscle[e]	
AA	Cattle	Sheep	Cattle	Sheep	Cattle[b]	Sheep[c]	Cattle[b]	Sheep[d]	Cattle[d]	Cattle	Sheep	Cattle	Sheep
Ala	8.02	6.53	6.72	6.74	4.29	4.1	5.4	5.53	2.79	7.12	6.65	5.55	5.52
Arg	5.18	5.91	5.01	5.03	4.54	4.9	5.0	4.97	3.42	7.02	6.80	6.57	6.58
Asn	4.71	5.13	5.36	5.34	---	---	---	3.96	2.57	3.67	3.58	4.18	4.16
Asp	6.58	5.83	6.75	6.74	---	---	---	4.85	3.90	4.48	4.37	5.16	5.15
Asx	11.29	10.96	12.11	12.08	13.18	11.6	7.7	8.81	6.47	8.15	7.95	9.34	9.31
Cys	1.61	1.87	1.49	1.48	1.49	---	---	1.39	0.43	1.34	1.46	1.35	1.38
Gln	5.95	9.02	5.13	5.11	---	---	---	6.16	11.3	4.91	5.09	5.64	5.66
Glu	10.8	7.85	7.99	8.02	---	---	---	7.93	9.43	8.02	8.32	9.32	9.35
Glx	16.75	16.87	13.12	13.13	14.36	15.1	14.4	14.09	20.7	12.93	13.41	14.96	15.01
Gly	4.93	4.90	5.07	5.06	4.38	4.1	7.4	7.02	1.87	11.33	11.30	4.18	4.17
His	2.28	2.18	2.07	2.05	1.83	2.1	2.0	2.16	2.80	2.53	2.12	3.95	3.94
Ile	4.47	4.20	5.51	5.53	6.49	6.8	5.7	5.73	4.94	2.81	3.60	5.15	5.13
Leu	8.67	8.32	7.66	7.67	7.80	8.5	9.1	8.74	8.87	6.93	6.94	8.33	8.34
Lys	4.66	4.98	7.70	7.70	10.78	11.3	6.2	6.57	7.53	6.46	6.10	9.03	9.02
Met	1.79	1.63	2.40	2.42	2.16	2.1	1.8	1.81	2.65	1.88	1.90	3.17	3.18
Phe	5.30	4.90	5.16	5.13	5.63	5.8	5.4	5.34	4.90	3.65	3.46	4.18	4.19
Pro	4.90	7.93	3.66	3.67	3.55	3.7	6.9	4.78	10.9	8.15	8.55	4.08	4.06
Ser	4.56	5.05	4.62	4.65	4.25	4.5	5.2	4.85	5.11	4.40	4.52	4.41	4.38
Thr	4.78	3.81	5.57	5.52	4.97	5.2	5.8	5.38	4.12	4.03	3.68	4.59	4.61
Trp	1.53	1.24	1.38	1.39	---	1.05[g]	---	1.36	1.25	1.14	1.14	1.25	1.26
Tyr	3.42	3.73	4.63	4.65	4.75	5.4	5.1	5.03	5.09	2.53	2.70	3.75	3.76
Val	5.91	4.98	6.11	6.08	5.16	5.0	6.3	6.32	6.06	3.93	4.26	5.94	5.96
Hyp	ND	ND	ND	ND	---	---	---	0.12	0.14	3.67	3.48	0.21	0.20

[a] Gilbreath et al. (2021); Adult Angus × Hereford steers (mean body weight of 538 kg) consumed daily 14.02 kg (dry matter) of Bermudagrass hay and 0.506 kg (dry matter) of dried distillers grains with solubles. Adult Suffolk female sheep (60 to 65 kg) consuming daily 23 g feed (as-fed basis)/kg body weight of a soybean hull-, wheat middling-, and corn-based diet.

[b] Sok et al. (2017).

[c] Chamberlain et al. (1986).

[d] Gilbreath et al. (2020). Adult Suffolk female sheep (60 to 65 kg) consumed the same diet as indicated above. Duodenal digesta samples were obtained at 4 h after feeding for AA analysis. Values were calculated on the basis of the molecular weights of intact AAs.

[e] Wu et al. (2024).

[g] Fenderson and Bergen (1972). Under the same experimental conditions, the content of Trp in ovine ruminal bacterial protein (1.33 g/100 g protein) is 27% greater than that in ovine ruminal protozoal protein (1.05 g/100 g protein).

Note: Asx, Asn + Asp; Glx, Gln + Glu; Hyp, 4-hydrocyproline; ND, not detected; ---, data are not available.

for the following. First, the content of arginine, glycine, and proline is lower, but that of three BCAAs, phenylalanine, and threonine is higher in the duodenal digesta compared to the animal body. Thus, like nonruminants (Wu 2022), ruminants must synthesize arginine, glycine, and proline, with endogenous N donors probably being BCAAs, and inadequate synthesis of arginine, glycine, and proline may limit the maximum growth and production performance of ruminants.

Digestion of Microbial and Feed Proteins in the Abomasum and Small Intestine

Digestion of protein in the abomasum and small intestine of ruminants is largely similar to that in the stomach and small intestine of nonruminants, with two exceptions for extracellular hydrolysis of bacterial and protozoal proteins. Namely, in contrast to the stomach of nonruminants, the abomasum of ruminants (e.g., cattle, sheep, and deer) secretes large amounts of gastric lysozymes, which efficiently break down bacterial cell walls by hydrolyzing the β-1,4 glycosidic linkages of membrane polysaccharides (Irwin 1995; Irwin and Wilson 1990). The gastric lysozymes are most active at pH 2–3, in contrast to other lysozymes that exhibit highest activities at pH 7.4 and have broader pH optima. Interestingly, these enzymes are unusually resistant to cleavage by pepsin, thereby prolonging their presence in the lumen of the stomach. Of note, colobine monkeys, which are also foregut fermenters, produce high levels of gastric

lysozymes (Irwin 1995). Thus, the diverse foregut fermenters share a digestive strategy to digest bacterial protein. Upon exposure to gastric proteases, proteins of rumen bacterial origin and feed proteins are hydrolyzed by proteases to large peptides.

The digestion of ruminal protozoa in the abomasum is different from that of ruminal bacteria, due to marked differences in the structures of their cell membranes. The cell membrane of ruminal protozoa and the pellicle (a thin layer outer membrane supporting the cell membrane) of various ruminal protozoa (primarily ciliates) do not consist of β-1,4-linked polysaccharides or lignin. Therefore, no lysozymes are required to break down the protozoal cell membrane. Through the transit of digesta, ruminal protozoa flow to the omasum, where they become inactive and start to disintegrate (Hungate 1966). In the abomasum, protozoal proteins are denatured by HCl, followed by enzymatic attack by pepsin to form large peptides (Hook et al. 2012). The protozoal protein that escapes degradation in the abomasum is further degraded by proteases (e.g., trypsin and chymotrypsin) in the small intestine to generate large peptides.

In the small intestine of ruminants, the bacteria- and protozoa-derived large peptides are degraded by oligopeptidases to yield di- and tripeptides and free amino acids, as in nonruminants. These digestion products are absorbed into enterocytes, where the small peptides are hydrolyzed to free amino acids. The amino acids that are not utilized by the enterocytes are transported through the lamina propria to the intestinal venule, which leads to the portal vein. As in nonruminants, the enterocytes of ruminants [e.g., mature sheep (Wolff et al. 1972) and growing cattle (Lescoat et al. 1996)] take up a large amount of glutamine but no nutritionally significant quantity of other amino acids from the arterial blood during the postabsorptive period.

The true digestibility of microbial protein, which is determined at the terminal ileum after the correction for the flow of endogenous protein, is 85% in mature sheep (Storm and Orskov 1983) and 80% in cows (NRC 2001). Similarly, Larsen et al. (2001) reported that the true digestibility of microbial protein was ~80% in lactating cows fed barley plus concentrate, as measured for most amino acids (e.g., arginine, 79.5%; aspartate plus asparagine, 80.4%; glycine, 79.3%; isoleucine, 80.4%; leucine, 80.4%; lysine, 81.6%; methionine, 78.3%; and valine, 78.9%). The true digestibility of protozoal protein in ruminants is approximately 90% (McDonald et al. 2021), which is about 10–15% higher than that of bacterial protein (Bergen and Purser 1968; Bergen et al. 1968b).

Digestion and Absorption of Nucleic Acids in the Small Intestine

The dietary nucleic acids that escape the rumen, as well as the bacterial and protozoal nucleic acids that leave the rumen, are hydrolyzed extracellularly to nucleotides in the small intestine by pancreatic nucleases (e.g., ribonuclease and deoxyribonuclease that are released from the pancreas) and by intestinal phosphodiesterases (released from the small intestine). Thereafter, specific pancreatic nucleotidases and nonspecific phosphatases (released from the pancreas) further degrade nucleotides to nucleosides and Pi. The digestibility of nucleic acids in the small intestine of ruminants is 80–90% (McDonald et al. 2021; Stentoft et al. 2015).

The nucleosides and Pi are transported, respectively, into the enterocytes by apical membrane Na^+-dependent nucleoside transporters [concentrative nucleoside transporter 1 (CNT1; selective for purine nucleoside) and CNT2 (selective for pyrimidine nucleoside)] and three Na^+-dependent phosphate transporters (NaPi2b, PiT1, and PiT2). The nucleosides that are not absorbed directly are degraded extracellularly by both pancreatic nucleosidases and small intestinal mucosa-derived nucleoside phosphorylases to form purine or pyrimidine bases. The purine or pyrimidine bases are then transported by Na^+-dependent nucleobase transporters into the enterocytes. Like preruminants, the small intestine of ruminants has a high capacity to absorb nucleosides and their bases. In dairy cows, the hepatic removal of purines and pyrimidines is almost the same as their net PDV release (Stentoft et al. 2015).

$$\text{Nucleic acid} + H_2O \rightarrow \text{Nucleotide}$$
$$(\text{Nuclease; RNAse or DNAse})$$
$$\text{Nucleotide} + H_2O \rightarrow \text{Nucleoside} + \text{Pi}$$
$$(\text{Nucleotidase})$$
$$\text{Nucleoside} + H_2O \rightarrow \text{Purine or pyrimidine base} + \text{Ribose or deoxyribose}$$
$$(\text{Nucleosidase})$$
$$\text{Nucleoside} + P_i \rightarrow \text{Purine or pyrimidine base} + \text{Ribose-1-phosphate}$$
$$(\text{Nucleoside phosphorylases})$$

Nitrogen Recycling in Ruminants and Its Nutritional Implications

Ammonia plays an important role in interorgan amino acid metabolism in ruminants. The ammonia that is produced in the rumen but is not utilized for synthesis of amino acids or polypeptides in microbes is absorbed into the blood circulation and converted into urea (Figure 7.12). Ammonia in the ruminal fluid may also be utilized by ruminal epithelial cells for biosynthetic processes (including the production of urea, glutamate, and glutamine). Ammonia in the plasma is taken up by the liver for the synthesis of urea via the urea cycle. Some urea is excreted into the urine, and up to 70% of urea undergoes recycling through the blood circulation, depending on dietary CP intake (Wu 2022). About 20% of the circulating urea is taken up by the intestine, where it is hydrolyzed by microbial urease to form ammonia plus CO_2. Ammonia, which takes up H^+ to form NH_4^+, can increase the pH of the ruminal fluid. Some urea in the blood enters the rumen through saliva, and ~15% of the urea is recycled

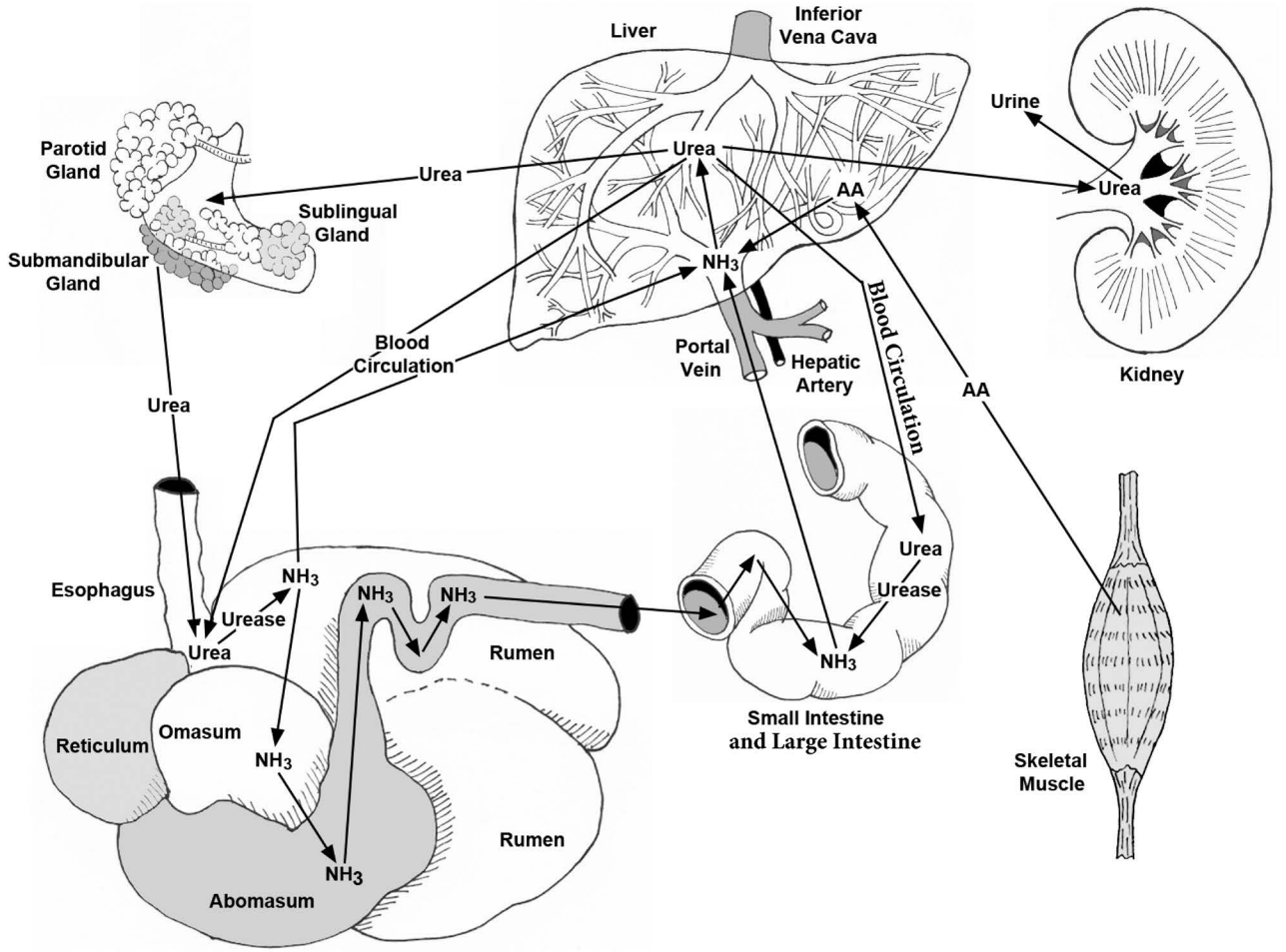


FIGURE 7.12 Urea recycling in ruminants. Some of the urea formed in the liver from ammonia via the urea cycle enters the saliva, rumen, small intestine, and large intestine through blood circulation. In the rumen and intestinal lumen, urea is hydrolyzed by urease to ammonia, with some of the ammonia being used for synthesis of amino acids and proteins by microbes. The remaining ammonia re-enters the liver for the resynthesis of urea, and some of the urea returns to the rumen and intestine, as noted previously. This process, known as urea recycling, helps maximize the efficiency of nitrogen utilization in ruminants.

to the rumen via saliva. In the rumen, urea is hydrolyzed by microbial urease to form ammonia plus CO_2. The recycled N helps conserve ammonia for biosynthetic processes in the rumen. Because microbial urease hydrolyzes urea into ammonia, urea can be used as a source of N for the diets of ruminants. Urea is well utilized by ruminal microbes when diets contain <13% CP. Thus, urea has been fed to ruminants as a NPN supplement for more than 100 years. Results of extensive research indicate that urea may represent 15–25% of CP in diets for beef cattle, cows, and sheep (Kertz 2010).

Urea is a natural substance in forages and is a major form of NPN in conventional ruminant diets. Corn silage and alfalfa may contain up to 50% and 20% of NPN, respectively. Efficacy and safety of dietary supplementation with urea to diets for cattle and sheep depend on many factors, including (a) the dosage (usually ≤1% in a concentrate-containing diet; ≤2% in low-CP hay) and frequency, (b) percentages of dietary carbohydrates and CP as well as their digestibilities in the rumen, and (c) adequate supply of phosphorus, S, and trace minerals. It should be borne in mind that the toxicity of ammonia can occur when its concentrations in the rumen are very high. The underlying mechanisms involve (a) a substantial increase in the ruminal pH (urea → 2 NH_3 + CO_2; NH_3 + H^+ → NH_4^+), leading to reductions in bacterial growth and synthetic activity in the rumen; (b) removal of α-KG from the Krebs cycle, thereby interfering with ATP production by cells, particularly those in the central nervous system; (c) disturbance of acid-base balance in the circulation; and (d) enhanced synthesis of glutamine, which inhibits the synthesis of NO from arginine in endothelial cells, thereby reducing blood flow and oxygen supply to vital organs, particularly the brain. It is advantageous to supply urea as a liquid feed supplement with molasses (2.5–10% of total dietary DM intake) and anhydrous phosphoric acid (orthophosphoric acid, a nontoxic, white solid at a temperature <42°C; 0.1–0.3% of total dietary DM intake; Broderick and Radloff 2004; Dixon 2013;

Kertz 2010). This is because (a) molasses serves as an energy source for microbial utilization of ammonia and protein synthesis, and (b) phosphoric acid lowers ruminal fluid pH. Due to concerns over ammonia toxicity, NPN should contribute to <30% of CP in ruminant diets.

FERMENTATION OF PROTEIN AND AMINO ACIDS IN THE LARGE INTESTINE OF MAMMALS AND BIRDS

Nonruminant Mammals and Birds

The large intestine of nonruminants contains relatively large amounts of microbes that can ferment the dietary protein that escapes the small intestine. The biochemical processes of N metabolism in the large intestine are similar to those in the rumen, but the hindgut fermentation of dietary protein in nonruminants (including pigs, horses, rabbits, cats, dogs, and chickens) is much less effective than ruminal fermentation (Hendriks et al. 2012; Mok and Urschel 2020). The resulting products include mainly microbial protein but also low-molecular-weight metabolites such as ammonia, amino acids, urea, nitrite, nitrate, H_2S, CO_2, methane, skatole (3-methyl-indole) and other indoles, and SCFAs (Wu 2022). The amounts of free amino acids represent <1% of the hindgut fermentation products of protein. Note that skatole and related indoles (lipid-soluble metabolites of tryptophan) serve as signaling molecules in bacterial communications and are responsible, in part, for the odor of feces. After absorption by the large intestine into the blood, skatole and other indoles are degraded in the liver. However, both androstenone and testosterone (testicular hormones) inhibit the hepatic degradation of skatole and other indoles, therefore allowing them to be stored in white adipose tissue (WAT) and resulting in an unpleasant odor called "boar taint" in pork from intact male pigs. This explains why barrows are raised for meat in the swine industry. Nutritional methods to alleviate or prevent the boar taint include (a) the optimization of dietary intakes of tryptophan and other amino acids and (b) dietary supplementation with either 10% alfalfa (a source of fiber) or 0.2–0.3% oregano essential oil for boars may reduce bacterial tryptophan metabolism in the large intestine and help delay their pubertal development (i.e., the production of sex hormones) so as to alleviate or prevent the boar taint (Marro et al. 2024).

Compared to enterocytes, the epithelial cells of the cecum and colon express much lower levels of amino acid and peptide transporters and, under normal feeding conditions, are exposed to only a limited amount of luminal substrates. The large intestine cannot absorb intact protein and take up nutritionally insignificant quantities of free amino acids as well as di- and tripeptides in animals (including pigs and humans) under normal feeding conditions (Bergen and Wu 2009; van der Wielen et al. 2017). Thus, the hindgut fermentation of dietary protein has a limited role in providing amino acids to the host animals. This disadvantage is partially overcome in some animals (e.g., rabbits) that practice coprophagia (also known as coprophagy, meaning the eating of feces). The coprophages excrete both hard pellet feces and soft feces and consume the soft feces as a source of food protein, which is digested in the stomach and the small intestine.

Ruminants

In ruminants, dietary nitrogenous compounds that are not absorbed by the small intestine will enter the large intestine, where they are fermented by microbes to form primarily protein and low-molecular-weight substances (e.g., ammonia, H_2S, and SCFAs; Bergen and Wu 2009). Other sources of N in the large intestine are derived from the recycling of blood urea and ammonia, as well as endogenous protein (e.g., sloughed epithelial cells, enzyme secretions, and mucoproteins). Neither the nitrogenous compounds that are bound to lignin nor the terminal products of the Maillard reaction will be degraded by microbes in the large bowel. The microbial protein synthesis (which helps to remove excessive ammonia from the hindgut) and fecal N excretion increase as the amount of dietary fermentable carbohydrates reaching the cecum and colon increases. As in nonruminants, the microbial protein synthesized in the large intestine is not absorbed into colonocytes and, therefore, has little nutritive value to the host.

DIGESTION OF PROTEIN AND ABSORPTION OF SMALL PEPTIDES AND FREE AMINO ACIDS IN FISH AND CRUSTACEANS

Mechanisms responsible for the digestion and absorption of dietary proteins in fish and crustaceans are similar to those in nonruminant mammals except for the following (Gatlin et al. 2007; Li et al. 2021b,c; Mai et al. 2022). First, carnivorous aquatic species, whose diets are predominantly composed of protein on a DM basis, may produce higher amounts of proteolytic enzymes (e.g., trypsin and chymotrypsin) than omnivorous aquatic species. Second, in fish without a stomach, the hydrolysis of all dietary protein is initiated and completed in the intestine, and this process takes a much longer time (up to 8 h post feeding) as compared with nonruminant animals such as pigs and chickens (up to 4 h post feeding). Third, in crustaceans, the digestion of dietary protein occurs in the pyloric region of the stomach, where zymogens (released primarily by the F-cells of the hepatopancreas) are activated via limited proteolysis in the presence of HCl (formed by the gastric epithelial cells) and stored as active proteases. Additionally, in crustaceans, peptidases (released as active enzymes primarily by the F-cells of the hepatopancreas) are fully active in the pyloric region of the teleost stomach. Fourth, in crustaceans, the products of gastric protein digestion move to the hepatopancreas for absorption into its R-cells and then into the

hemolymph. Fifth, in crustaceans, the midgut tube and hindgut play a minor role in the digestion of dietary protein or the absorption of its products.

Developmental Changes of Gastric Proteases in Fish and Crustaceans

As noted in Chapter 1, not all fish have a stomach, and agastric fish will not develop a stomach or synthesize gastric proteases even at the adult stage. Precocious fish have a functional stomach at the onset of first exogenous feeding, whereas altricial larvae develop a functional stomach during metamorphosis. In precocious and altricial fish, there are no pepsins at the larval stage due to the absence of a functional stomach. For example, pepsins are not found in the stomach of larval striped bass or hybrid striped bass (HSB) until day 16 after hatching, which corresponds to the development of their stomach (Gabaudan 1984). This organ slowly produces pepsin-like enzymes and develops acidification capacity during metamorphosis (Rønnestad et al. 2013). Active pepsins are present in the functional stomach with an acidic environment (e.g., pH 2–3). Although the stomach of most teleosts exhibits similarities in their anatomical and functional ontogeny, there are inter-species variations in the sequence and time of appearance of pepsins.

Developmental Changes in Extracellular Proteases in the Intestine of Fish and Crustaceans

Fish larvae obtain nutrients from the yolk sac for the first several days (e.g., 4–5 days post hatching in striped bass and HSB) as they undergo metamorphosis and digestive tract development (García-Gasca et al. 2006; Pedersen et al. 1987). Thereafter (just before the initiation of exogenous feeding), the pancreas of fish synthesizes significant amounts of pancreatic proteases. The abundances of these enzymes increase progressively as the larvae develop. For example, in striped bass and HSB, the specific activities of pancreatic trypsin, chymotrypsin, aminopeptidases, elastase, and carboxypeptidases A and B at day 32 post hatching (as larval development nears completion) are 65–300% greater than those at day 4 (Baragi and Lovell 1986). After the intestine is developed, the pancreas secretes these enzymes into the intestinal lumen, which helps fish to hydrolyze the ingested dietary protein. Depending on fish species and physiological conditions, the quality and quantity of dietary protein, as well as other dietary and environmental factors, the true digestibility of dietary protein in fish [e.g., HSB and largemouth bass (LMB)] generally ranges from 80% to 90% (Gatlin and Wu 2025; NRC 2011; Ribeiro et al. 2011). These values are similar to those for terrestrial animals (Wu 2022). However, the rates of protein digestion plus amino acid absorption (expressed as mol/kg BW/h) are much lower in fish than in mammals and birds (Li et al. 2121b).

BIOAVAILABILITY OF DIETARY AMINO ACIDS TO EXTRA-DIGESTIVE ORGANS IN ANIMALS

Net Entry of Dietary Amino Acids from the Small Intestine into the Portal Vein

Enterocytes release the absorbed, undegraded amino acids into the interstitial space of the lamina propria, where they are taken up by the residing capillaries into the intestinal venule. Through the blood circulation, amino acids are transported to the small vein and then the portal vein. Because enterocytes, luminal bacteria, and the lamina propria in the mammalian small intestine extensively or partially degrade certain amino acids (Burrin and Davis 2004; He and Wu 2022; Wu 1998), not all the amino acids released from the hydrolysis of dietary protein enter the portal circulation (Table 7.12). For example, in 30-day-old growing pigs, 96% glutamate, 95% aspartate, and 67% glutamine but only 13% alanine, 13% asparagine, 17% cysteine, and 18% glycine in the digestible diet are metabolized by the gut during their first pass into the portal circulation (Hou et al. 2016a). In 60-kg growing pigs fed every 8 h (3 times daily) a 16%-CP diet (daily feed intake = 3.6% of BW), hardly any dietary aspartate, glutamine and glutamate (free or peptide-bound) in the small-intestinal lumen is absorbed to the portal blood (Figure 7.13). On average, ~50% of dietary amino acids do not enter the portal vein in postweaning pigs, adult pigs, and adult rats. Thus, in swine that are fed diets adequate in protein, all proteinogenic amino acids except for arginine and alanine exhibit a negative balance across the portal

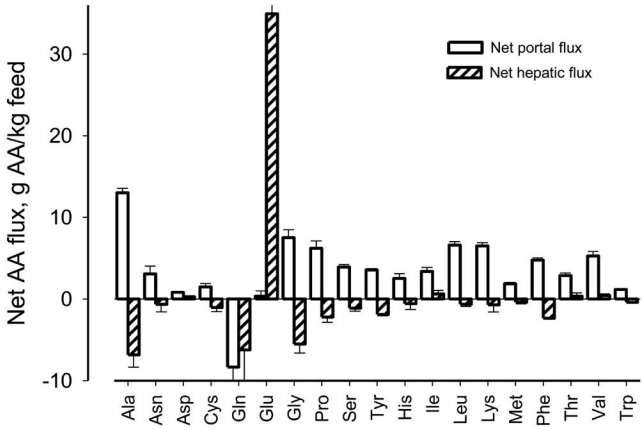


FIGURE 7.13 Net portal flux (open bar) and net hepatic flux (filled bar) of glucose and amino acids (AA) in 60-kg growing pigs fed a 16%-CP diet at the rate of 3.6% of body weight per day. Feed was divided into 3 equally sized meals per day at 8-h intervals. A positive flux denotes the net release, and a negative flux the net uptake across the tissues. Hardly any of the dietary Asp, Gln, and Glu (free or peptide-bound) in the small-intestinal lumen is absorbed into the portal blood. The liver takes up Gln from the portal vein, but releases both Asp and Glu. The absolute amount of amino acid fluxes varies markedly among different amino acids. Each data point represents mean ± SEM, n = 8. [Reproduced from Kristensen and Wu (2012), which is freely available in the public domain.]

TABLE 7.12
Metabolism of Amino Acids in the Small Intestine and Liver of Growing Pigs and in the Portal-Drained Viscera (PDV) and Liver of Lactating Cows

	Growing Pigs			Lactating Cows[e]		
Amino Acid	Catabolism in the Small Intestine (SI)[a]	Oral Bioavailability[b]	Net Uptake (+) or Release (–) by Liver[c]	PDV Recovery of AAs Absorbed from the SI (%)	Net PDV Flux of AAs (g/h)[f]	Net Uptake or Release by Liver[c]
Ala	13	87	+52	61	7.54	+11
Arg	40	60	+8	49	---	+4.9
Asn	13	87	+56	---	---	+17
Asp	95	5	–0.30 g/kg feed[d]	20	---	+2.4
Citrulline	10	90	0.0	85	---	+0.1
Cys	17	83	+70	---	0.47	---
Gln	67	33	+6.4 g/kg feed[d]	---	0.61	+5.7
Glu	96	4	–35 g/kg feed[d]	12	1.38	–24
Gly	18	82	+73	37	3.24	+15
His	20	80	+9	42	1.51	+4.8
Ile	35	65	+9	38	3.73	+1.9
Leu	34	66	+9	34	5.77	+2.1
Lys	25	75	+10	45	5.23	+2.2
Met	21	79	+17	56	2.09	+9.9
Phe	22	78	+51	45	4.20	+12
Pro	40	60	+40	24	---	+6.5
Ser	17	83	+28	58	4.28	+19
Thr	28	72	+10	38	3.16	+5.3
Trp	19	81	+35	---	1.51	+4.2
Tyr	20	80	+54	58	3.88	+11
Val	35	65	+10	29	3.82	+1.3

Source: Doepel et al. (2007), Hou et al. (2016), Kristensen and Wu (2012), Lescoat et al. (1996), and Reynolds (2006).

[a] % of the AA present in the small-intestinal lumen. AA catabolism occurs in the small-intestinal bacteria plus the small-intestinal mucosa.

[b] % of the AA orally administered to the pig.

[c] % of the portal vein flux, unless specified otherwise. (+) denotes net uptake, and (–) net release.

[d] Net uptake or net release per kg feed consumed by growing pigs fed a 16%-CP diet.

[e] Cows consumed 19.4 kg dry matter/day and produced 16.5 kg milk/day.

[f] Net PDV flux in the lactating cow which consumed 18 kg dry matter/day (30.6 Mcal/day net energy and 2,067 g/day metabolizable protein) and produced 40 kg milk/day.

vein (i.e., output < input; indicating utilization by the small intestine); both arginine and alanine show a positive balance across the portal vein (i.e., output > input) because of their net synthesis by enterocytes (Wu 2022). In ruminants, diet- or microbial protein-derived glutamate and glutamine are extensively utilized (primarily via catabolism) by the mucosa of the small intestine (Wu et al. 2024) and therefore nearly all of the two amino acids do not enter the portal vein under normal feeding conditions (Burrin et al. 1991; Doepel et al. 2007; Reynolds 2006; Taylor-Edwards et al. 2012). In poultry, whose enterocytes have a limited ability to degrade many amino acids (including arginine and glutamine), it is likely that >50% of dietary amino acids can enter the portal vein (He et al. 2021b, 2022; Wu et al. 1995).

The bioavailabilities of dietary amino acids in the blood circulation can be increased by both feed antibiotics and functional amino acids through an inhibition of intestinal catabolism in mucosal cells and luminal bacteria (Wu 1998). For example, the net utilization of lysine, leucine, valine, ornithine, and serine by jejunal or ileal mixed bacteria was decreased by 0.5 to 5 mM glutamine in a concentration-dependent manner (Dai et al. 2013a). These effects of glutamine may be mediated, in part, by the production of glutamate. In addition, exposure to 0.5 to 5 mM arginine decreased the rates of net catabolism of (a) threonine, glycine, phenylalanine, and BCAAs by *E. coli* from the pig jejunum and ileum, and (b) lysine, threonine, isoleucine, leucine, glycine, and alanine by jejunal or ileal mixed bacteria (Dai et al. 2012b). Dietary supplementation with 1–2% monosodium glutamate increased the concentrations of alanine, arginine, asparagine, citrulline, glutamine, histidine, lysine, methionine, proline, tryptophan, and tyrosine in the

plasma of lactating sows (Rezaei et al. 2022a). Likewise, dietary supplementation with 1.54–3.07% BCAAs [leucine, isoleucine, and valine; respective ratios (g/g) = 1.00:2.56:1.23] increased the concentrations of alanine, arginine, asparagine, citrulline, glutamine, isoleucine, leucine, proline, tryptophan, and valine in the plasma of lactating sows (Rezaei et al. 2022b).

Extraction of Amino Acids from the Portal Vein by the Liver

Amino acids from the portal vein are first exposed to hepatocytes. In growing pigs, the liver has net uptakes of basic and neutral proteinogenic amino acids from the portal vein at different proportions of their net portal-vein fluxes, the net release of acidic amino acids, and no uptake of citrulline (Wu 2022). Generally, the rates of hepatic uptake of amino acids in decreasing order are: small neutral amino acids > large neutral amino acids > BCAAs = basic amino acids. Based on the portal and hepatic fluxes of amino acids, Kristensen and Wu (2012) classified them into three groups. The first group of amino acids includes those that are absorbed from the small intestine into the portal vein and taken up by the liver at ≥17% of their net portal fluxes: alanine, asparagine, cysteine, glycine, methionine, phenylalanine, proline, serine, tryptophan, and tyrosine. The second group of amino acids is those that are absorbed from the small intestine into the portal vein and taken up by the liver at ≤10% of their net portal fluxes: arginine, histidine, isoleucine, leucine, lysine, threonine, and valine. The third group of amino acids is those that exhibit little entry from the small-intestinal lumen into the portal vein: aspartate, glutamine, and glutamate. The 60-kg pig liver receives little dietary aspartate, glutamine, and glutamate from the portal vein as noted previously but takes up a relatively large amount of glutamine (6.4 g/kg feed intake) from the portal vein (ultimately the arterial blood) and releases 35 g of glutamate and 0.3 g of aspartate per kilogram of feed intake as a result of hepatic amino acid metabolism. Among all amino acids, the percentages of hepatic uptakes of glycine (73%) and cysteine (70%) are greatest in the growing pigs.

In growing animals, only a small amount of amino acids is degraded in their livers. For example, in 60-kg pigs fed a 16%-CP diet, all the ammonia absorbed into the portal blood is removed during its single passage through the liver, and this organ takes up a small amount of ammonia released from peripheral tissues (Kristensen and Wu 2012). These authors have also reported that 95% of the urea-N released from the liver is accounted for by the hepatic uptake of ammonia from the portal vein and the hepatic artery in 60-kg pigs. In other words, only 5% of the urea-N released by the liver is provided from hepatic amino acid degradation when the pigs are fed a balanced diet (Figure 7.14). This is consistent with a low rate of hepatic gluconeogenesis in fed pigs (Chapter 5).

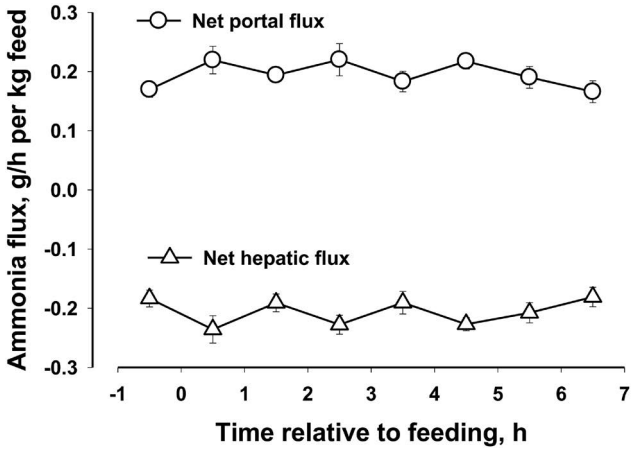


FIGURE 7.14 Net portal flux (open bar) and net hepatic flux (filled bar) of ammonia in 60-kg growing pigs fed a 16%-CP diet at the rate of 3.6% of body weight per day. Feed was divided into 3 equally sized meals per day at 8-h intervals. A positive flux denotes the net release, and a negative flux the net uptake across the tissues. Each data point represents mean ± SEM, n = 8. [Reproduced from Kristensen and Wu (2012), which is freely available in the public domain.]

ENDOGENOUS SYNTHESIS OF AMINO ACIDS IN ANIMALS

Need for Endogenous Synthesis of Amino Acids in Animals

Based on the needs of protein synthesis for EAAs, farm animals (e.g., livestock, poultry, fish, and crustaceans) are normally fed diets containing minimal levels of CP. This feeding method aims at reducing feed costs and the concentrations of ammonia in the blood circulation. However, recent studies with growing, gestating, and lactating pigs, as well as poultry and fish, indicate that the supply of dietary protein, as currently recommended by the NRC, does not meet their requirements for NEAAs (amino acids whose carbon skeletons can be synthesized de novo in the body) to achieve maximal growth and production performance (Hou et al. 2016a). First, in milk-fed piglets, the intake of dietary EAAs greatly exceeds protein accretion in the body, whereas among NEAAs, only asparagine and serine in milk exceed the needs for their accretion in the body, and other dietary NEAAs cannot meet the needs for protein accretion in the neonate (Table 7.13). These deficits are particularly severe for aspartate, glutamate, glycine, and arginine. Accordingly, de novo synthesis of these amino acids must be active in milk-fed neonates to support weight gains, with the net rates of synthesis of arginine, glutamine, and glycine being at least 0.58, 1.15, and 1.20 g/kg BW/day, respectively. For example, arginine is formed de novo from glutamate, glutamine, and proline via the intestinal-renal axis (Wu and Morris 1998), whereas 4-hydroxyproline is converted into glycine in many tissues of animals (Hu et al. 2023a,b). Second, in weanling pigs fed typical corn- and

TABLE 7.13
Metabolism of Amino Acids (AAs) in the 14-Day-Old Pig (3.9 kg Body Weight) Reared by Sows[a]

AA	AA in Sow's Milk	Dietary AA Entering the Portal Vein	AA Accretion in Extra-intestinal Tissues	Dietary AA Entering the Portal Vein/AA Accretion in Extraintestinal Tissues	Extraintestinal Metabolism of AA via Nonprotein Synthesis Pathways		
					Total Amount	Total Nitrogen (N)	Total Carbon (C)
	g/L	g/day	g/day	g/g	g/day	mmol N/day	mmol C/day
Catabolism of AAs Whose Carbon Skeletons Are Not Synthesized by Animal Cells							
Cys	0.72	0.50	0.40	1.25	0.10	0.83	2.48
His	0.92	0.76	0.63	1.21	0.13	2.51	5.03
Ile	2.28	1.41	1.07	1.32	0.34	2.59	15.6
Leu	4.46	2.78	2.06	1.35	0.72	5.49	32.9
Lys	4.08	3.09	1.82	1.70	1.27	17.4	52.1
Met	1.04	0.85	0.57	1.49	0.28	1.88	9.38
Phe	2.03	1.50	1.05	1.43	0.45	2.72	24.5
Thr	2.29	1.32	1.03	1.28	0.29	2.43	9.74
Trp	0.66	0.52	0.33	1.58	0.19	1.86	10.2
Tyr	1.94	1.43	0.80	1.79	0.63	3.48	31.3
Val	2.54	1.51	1.28	1.18	0.23	1.96	9.82
Sub-total	23.0	15.7	11.0	---	4.63	43.1	203
Net Synthesis of AAs Whose Carbon Skeletons Can Be Formed by Animal Cells[b]							
Ala	1.97	1.38	1.98	0.70	–0.80	–8.98	–26.9
Arg	1.43	1.06	2.05	0.52	2.27	13.0	0.0
Asn	2.53	2.00	1.07	1.87	–0.93	–14.1	–28.2
Asp	2.59	0.11	1.29	0.085	1.18	8.87	35.5
Glu	4.57	0.21	2.36	0.089	2.15	14.6	73.1
Gln	4.87	1.42	1.52	0.93	0.10	1.37	3.40
Gly	1.12	0.82	3.41	0.24	2.59	34.5	69.0
Pro	5.59	3.12	3.66[c]	0.85	0.37	3.21	16.1
Hyp	1.04	0.86	0.0	---	–0.86	–6.56	–32.8
Ser	2.35	1.72	1.34	1.28	–0.38	–3.62	–10.8
Sub-total	27.0	12.7	18.7	---	5.69	42.3	98.3

Source: Hou et al. (2016).

[a] The milk consumption and body-weight gain of the piglet are 913 ml/day and 235 g/day, respectively.

[b] Rates of AA formation from intestinal catabolism of dietary AAs are estimated to be 1.40, 0.17, and 2.27 g/day for Ala, Pro, and Arg, respectively, and to be negligible for other AAs. The sign "–" denotes the contribution of dietary AA for extraintestinal AA synthesis in a cell- and tissue-specific manner.

[c] Including Pro and hydroxyproline for the calculation of extraintestinal carbon and nitrogen balance.

SBM-based diets, dietary EAAs, as well as asparagine, serine, and alanine, exceed the needs for protein synthesis, but dietary aspartate, glutamate, glycine, proline, arginine, and glutamine cannot meet the needs of the growing piglets and must be synthesized in the body. Third, in gestating gilts typically fed 2 kg/day of a corn- and SBM-based diet containing 12.2% CP, all EAAs except lysine, tryptophan, and methionine exceed their uterine uptake, whereas alanine, asparagine, and serine are the only synthesizable amino acids whose supplies in the diet exceed uterine uptake. Other NEAAs (including arginine, glutamate, glutamine, and glycine) must be synthesized by the gestating dam to support fetal growth and development. For example, about 30% of dietary CP is used for glutamate and glutamine synthesis in adult pregnant or nonpregnant ewes (Table 7.14). Fourth, in lactating sows fed a corn- and SBM-based diet containing 18% CP, all dietary EAAs (except for lysine) entering the portal vein exceed their output in sow's milk, whereas dietary aspartate, glutamate, glutamine, and proline (which are the most abundant amino acids in milk protein) cannot meet their output in milk and must be synthesized de novo from dietary EAAs. Finally, in growing broiler chickens fed normal protein diets (i.e., an 18%-CP diet between 5 and 21 days of age; a 17%-CP diet between 21 and 35 days of age), glycine, glutamate, and glutamine must be synthesized from EAAs for muscle growth. In

TABLE 7.14
Estimated Flow of L-Glutamate and L-Glutamine along the Forestomach and the Small Intestine in Adult Sheep[a]

Variable	L-Glutamate	L-Glutamine
Feed intake (on an as-fed basis), kg/day	1.20	1.20
Crude protein (CP) intake, g/day	140	140
Microbial and feed proteins entering the duodenum, g/day	95.2	95.2
Nitrogen leaving the rumen as ammonia and reentering the rumen via N recycling for microbial protein synthesis, g/day	18.2	18.2
Total microbial and feed proteins in the small intestine, g/day	113.4	113.4
Digestible microbial and feed proteins in the small intestine, g/day	96.4	96.4
Glu or Gln released from digestible microbial and feed protein, g/day	7.71	5.30
Glu or Gln entering the portal vein (100% extraction by the small intestine), g/day	0	0
Glu or Gln catabolism in the whole body, g/day	51.5	46.7
Glu or Gln required for protein deposition (maintenance), g/day	0	0
Glu or Gln oxidation in the portal-drained viscera, g/day	7.71	5.30
Endogenous synthesis of Glu and Gln, g/day	43.8	41.4
Percent (%) of dietary CP used for Glu and Gln synthesis[b]	31.3	29.6

Source: Wu et al. (2024).

[a] Adult nonpregnant and nonlactating female sheep (60-kg body weight). The dietary content of CP, glutamate, and glutamine (on an as-fed basis) is 11.7%, 0.94%, and 1.10%, respectively. Microbial and feed proteins entering the duodenum represent 68% of the dietary CP intake. The N leaving the rumen as ammonia and reentering the rumen via N recycling accounts for 13% of the dietary nitrogen intake. The true digestibility of proteins in the small intestine is 85%. It is assumed that microbial protein and feed protein account for 90% and 10% of the total proteins, respectively.

[b] Calculated as (the endogenous synthesis of Glu or Gln)/CP intake × 100%.

1- to 42-day-old growing broiler chickens fed a low-protein (16.2% CP) diet, glycine, proline, alanine, glutamate, and aspartate must be synthesized from EAAs to sustain protein gains (He et al. 2021a). Thus, because dietary intake cannot meet the needs of animals for NEAAs, their endogenous synthesis and dietary supplementation are necessary for the maximum growth performance of livestock, poultry, and fish (Hou et al. 2016a).

Emerging evidence from studies with humans shows that de novo synthesis of serine is insufficient for the health of eyes, and an adequate amount of serine must be provided to both children and adults to protect the retina from damage (Gantner et al. 2019). For example, when serine is sufficient, serine palmitoyltransferase condenses serine and palmitoyl-CoA into 3-ketodihydrosphingosine, which is a precursor of sphinganine. However, when serine is deficient or a variant of serine palmitoyltransferase is expressed, this enzyme uses alanine as a substrate in addition to palmitoyl-CoA to produce 1-deoxysphinganine. The latter is highly toxic to photoreceptor cells and causes their death, resulting in retinal and peripheral neuropathies (Gantner et al. 2019).

$$\text{Serine} + \text{Palmitoyl-CoA}\ (\text{serine palmitoyltransferase}) \rightarrow 3\text{-Ketodihydrosphingosine} \rightarrow \text{Sphinganine}$$

$$\text{Alanine} + \text{Palmitoyl-CoA (either low serine or variant enzyme)} \rightarrow 1\text{-Deoxysphinganine} \rightarrow \text{Deoxysphinganin}$$

EAAs as Precursors for Synthesis of NEAAs

Four aspects of amino acid synthesis in animals are noteworthy. Namely, all animals (a) cannot synthesize de novo the carbon skeletons of the following 9 proteinogenic amino acids: histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine; (b) synthesize de novo the following 8 proteinogenic amino acids: alanine, aspartate, asparagine, glutamate, glutamine, glycine, proline, and serine (Figure 7.15); (c) hydroxylate phenylalanine into tyrosine and convert methionine into cysteine but not vice versa; and (d) synthesize arginine in a highly species-specific manner (Wu 2022). Many of the synthesized amino acids are interconvertible in animals. In nonruminants, EAAs are ultimately the sources of the amino groups in NEAAs and provide carbon skeletons for the synthesis of most NEAAs. In ruminants, although there exists a symbiotic relationship between the ruminant and its ruminal microbes (Hungate 1966), dietary amino acids are the major source of N for ruminal amino acid synthesis. Thus, if a purified diet containing only EAAs is fed to animals, there will be a great shortage of NEAAs for supporting their maximum growth and production performance. In addition to developmental changes in the expression of amino acid-synthetic enzymes, dietary intake of EAAs is by far the single most important factor affecting the de novo synthesis of NEAAs in animals. The conversion of dietary EAAs (expensive nutrients) into NEAAs requires energy and produces oxidants and therefore is not desirable for biological efficiency, economy, or animal health. Adequate provision of NEAAs in diets is nutritionally and physiologically advantageous.

Cell- and Tissue-Specific Syntheses of Amino Acids

It is important to recognize that, within the same animal, the synthesis of amino acids occurs in a cell- and

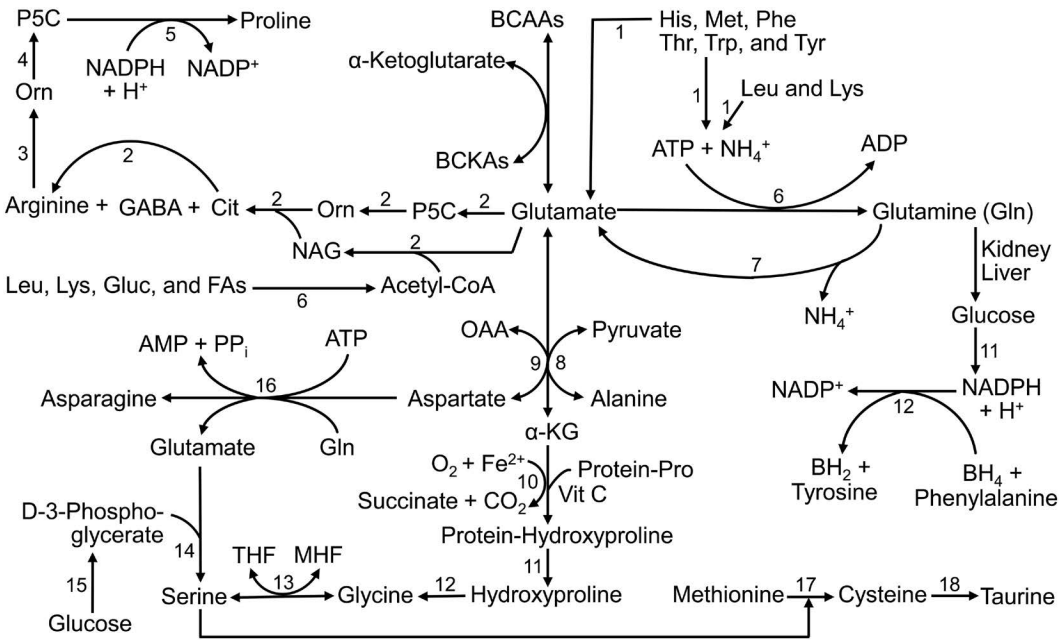


FIGURE 7.15 Synthesis of amino acids in animal cells. The enzymes that catalyze the indicated reactions are (1) a series of enzymes for amino acid degradation, (2) a series of enzymes for arginine synthesis, (3) arginase, (4) ornithine aminotransferase, (5) pyrroline-5-carboxylate (P5C) reductase, (6) glutamine synthetase, (7) phosphate-activated glutaminase, (8) glutamate-pyruvate transaminase, (9) glutamate-oxaloacetate, (OAA) transaminase, (10) prolyl hydroxylase, (11) pathways of protein degradation, (12) phenylalanine hydroxylase, (13) serine hydroxymethyltransferase, (14) a series of enzymes for serine synthesis, (15) a series of enzymes in glycolysis, (16) asparagine synthetase, (17) a series of enzymes for methionine degradation via the transsulfuration pathway, and (18) cysteine dioxygenase and cysteinesulfinate decarboxylase. These reactions are cell- and tissue-specific in animals. BCAAs, branched-chain AAs; BCKAs, branched-chain α-ketoacids.

tissue-specific manner and requires interorgan metabolism of amino acids (Gilbreath et al. 2021; He et al. 2021a, 2024; Li et al. 2021a,b; Wu et al. 2022; Zhang et al. 2021). Some NEAAs can be synthesized in certain tissues but not in others due to the lack of one or more of the required enzymes. Examples are given as follows. First, the livers of mammals can synthesize many NEAAs (e.g., alanine, glutamate, glutamine, glycine, and serine) from EAAs but are incapable of having net synthesis of citrulline or arginine under physiological conditions. This is because (a) citrulline is immediately converted into arginine via ASS and ASL, and (b) the arginine is then rapidly hydrolyzed by hepatic arginase to urea plus ornithine. The enterocytes of most mammals (including cattle, pigs, rats, and sheep) can synthesize citrulline, arginine, and proline from glutamine and glutamate, but these pathways are absent from the livers of the same animals due to the lack of P5C synthase.

Second, skeletal muscle, heart, brain, WAT, mammary tissue, and placenta can synthesize alanine, aspartate, glutamate, and glutamine from BCAAs and α-KG (Harper et al. 1984). Specifically, BCAA transaminase (located in both the cytosol and mitochondria) catalyzes the transamination of BCAAs with α-KG to form BCKAs and glutamate. This enzyme is widely expressed in many tissues, but its activity is very low in the liver under physiological conditions. Glutamate-OAA transaminase converts glutamate and pyruvate into α-KG and alanine, whereas GS synthesizes glutamine from glutamate and NH_4^+ in an ATP-dependent manner. Both alanine and glutamine are major vehicles for interorgan transport of N and are key substrates for gluconeogenesis. Furthermore, glutamine is used for both intestinal citrulline synthesis (the regulation of NO production and hemodynamics) and renal ammonia production (the regulation of acid-base balance).

Third, the small intestine of most mammals can synthesize (a) alanine, arginine, aspartate, asparagine, citrulline, ornithine, and proline from glutamate and glutamine; (b) glutamate from BCAAs plus glucose, glutamine, and proline; (c) tyrosine from phenylalanine; and (d) glycine from 4-hydroxyproline (Wu 2022). In many mammalian species, the small intestine releases alanine, arginine, citrulline, ornithine, and proline in the postabsorptive state, indicating the net synthesis of these amino acids by the gut. The pathways for arginine synthesis from glutamine, glutamate, and proline in the enterocytes of most mammals are outlined in Figure 7.16. Note that although arginine is formed as an intermediate in mammalian livers via the urea cycle, there is no net synthesis of this amino acid via this metabolic cycle under physiological conditions. Although citrulline is converted into arginine via ASS and ASL, arginine is rapidly hydrolyzed by hepatic arginase to urea plus ornithine. Thus, most mammals (e.g., mice, pigs, and rats) have

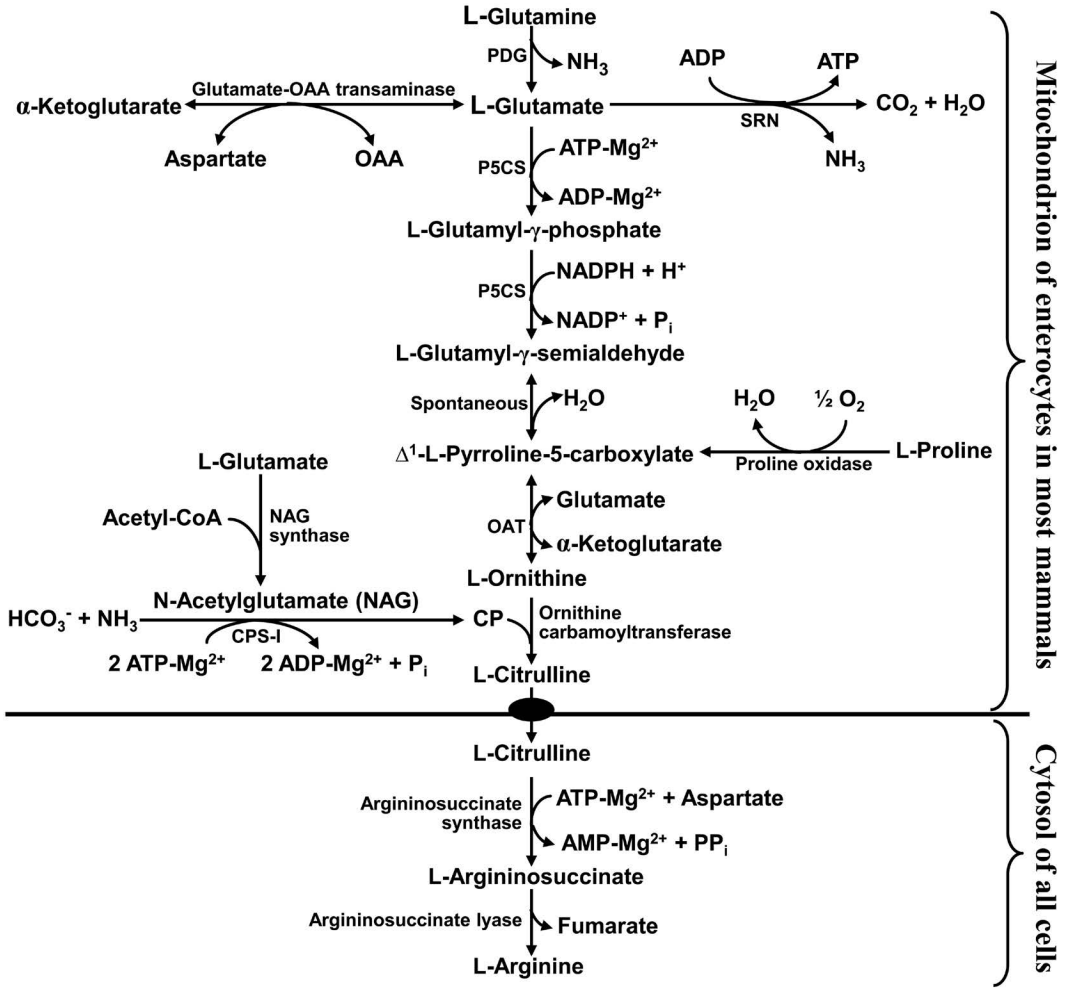


FIGURE 7.16 Synthesis of citrulline and arginine from glutamine, glutamate, and proline in most mammals (including pigs, ruminants, rats, and humans). Citrulline is synthesized from glutamine, glutamate, and proline almost exclusively in the mitochondria of enterocytes, while all cell types contain cytosolic enzymes for converting citrulline into arginine. The enzymes catalyzing the indicated reactions are (1) glutaminase (two isoforms: phosphate-independent, and phosphate-activated); (2) γ-glutamyl kinase; (3) glutamyl-γ-phosphate dehydrogenase; (4) nonenzymatic reaction; (5) ornithine aminotransferase; (6) ornithine carbamoyltransferase; (7) argininosuccinate synthase; (8) argininosuccinate lyase; (9) pyrroline-5-carboxylate reductase; (10) carbamoylphosphate (CP) synthase II; (11) a series of enzyme-catalyzed reactions involving glutamate dehydrogenase, α-ketoglutarate dehydrogenase, and succinyl-CoA dehydrogenase; (12) *N*-acetylglutamate synthase; (13) acetylglutamate kinase; (14) *N*-acetyl-γ-glutamyl phosphate dehydrogenase; (15) *N*-acetylornithine-δ-aminotransferase; and (16) acetylornithine deacetylase.

lower requirements for dietary arginine than poultry (e.g., chickens, ducks, and geese) because net arginine synthesis is present in those mammals but absent from birds.

Fourth, the kidneys can (a) synthesize alanine, aspartate, glutamate, glycine, and serine; (b) convert phenylalanine into tyrosine; and (c) form glycine and serine from 4-hydroxyproline. The kidneys also express ASS and ASL for converting the intestine-derived citrulline into arginine. These two enzymes are localized within the proximal convoluted tubules with little arginase activity to maximize the release of arginine into the blood circulation. This interorgan metabolic pathway is known as the intestinal-renal axis for arginine synthesis. Because of the high rates of blood flow through the kidney, it plays an important role in amino acid synthesis by animals.

Fifth, endothelial cells, smooth muscle cells, cardiac myocytes, macrophages, and lymphocytes can synthesize alanine, aspartate, and glutamate from glutamine via glutaminolysis. These cells also convert citrulline into arginine to conserve arginine at the expense of aspartate, thereby sustaining NO production by NO synthase. Skeletal muscle has been included as part of the immune system because (a) this organ is the major source of glutamine, and (b) lymphocytes and macrophages derive 35% and 50% of ATP from this pathway, respectively, indicating its major role in immune responses. Thus, glutamine is a common amino acid that integrates the functions of the circulatory, immune, and muscular systems in animals.

Cell- and tissue-specific synthesis of amino acids is of nutritional and physiological importance. For example, in

food-deprived subjects, alanine and glutamine account for approximately 50% of total amino acids released by the skeletal muscle. The synthesis of glutamine from NH_4^+ and glutamate plays a key role in removing ammonia from skeletal muscle, heart, and brain, with glucose being the primary source of the glutamate carbons. Of note, because of its large mass (40% and 45% of BW in neonates and adults, respectively), skeletal muscle is the major source of both alanine and glutamine in postabsorptive animals to maintain their homeostasis. In the brain, cell-specific synthesis of glutamate and glutamine is important for the synthesis of γ-aminobutyric acid (GABA; a neurotransmitter) and, therefore, for the regulation of neurological function. In gestating mammals, the release of glutamine from the placenta contributes a large amount of glutamine to the fetal circulation to support rapid fetal growth. In the fetus, glutamine is utilized for the synthesis of glutamate, aspartate, and asparagine to compensate for the inadequate uptake of these three amino acids by the gravid uterus. Finally, in lactating mammals, the active synthesis of glutamate, glutamine, and proline by mammary tissue results in their high abundances in milk to ensure the optimal growth and development of the small intestine and other tissues in neonates. Within the same animal, rates of amino acid synthesis can differ markedly among various cell types (e.g., liver, skeletal muscle, small intestine, and kidneys).

Species Differences in Syntheses of Amino Acids

There are species differences in the synthesis of some amino acids (Baker 2005; Wu 2022). Examples are given as follows. First, most mammals (e.g., cattle, dogs, humans, mice, pigs, rats, and sheep) can synthesize arginine from glutamate, glutamine, and proline via the intestinal-renal axis, albeit at insufficient rates for maximal growth or optimal health (including cardiovascular and reproductive health; Wu et al. 2018, 2021, 2022). However, some mammals (e.g., cats, ferrets, and minks) have a very limited or no ability to synthesize citrulline or arginine from glutamate, glutamine, or proline in their enterocytes and other cell types (Li and Wu 2023; Wu and Morris 1998), and this may be true in most fish (Li et al. 2021b) and crustaceans (Li et al. 2021c). Compared with cats, dogs have a greater ability to form arginine from glutamate, glutamine, and proline, but the rates of synthesis are inadequate for metabolic utilization including growth and ammonia detoxification. Furthermore, there is no synthesis of citrulline or arginine from glutamine, glutamate, or proline in the enterocytes of chickens due to the lack of P5C synthase, proline oxidase, and ornithine carbamoyltransferase (Furukawa et al. 2021; Wu et al. 1995). Likewise, in birds, the production of proline from arginine is limited because of a low arginase activity in their tissues, and there is no formation of proline from glutamate and glutamine in the whole body due to the absence of intestinal P5C synthase (He et al. 2021a).

Second, most breeds of dogs can synthesize sufficient taurine when fed methionine- or cysteine-adequate diets (Li and Wu 2023). However, a few breeds of dogs (e.g., the Newfoundland breed, golden retrievers, and American Cocker Spaniels) have been reported to have a limited ability to synthesize taurine even when fed methionine- and cysteine-adequate diets (Backus et al. 2003; Kittleson et al. 1997) possibly because of lower hepatic activities of cysteine dioxygenase and cysteine sulfinate decarboxylase due to gene mutations. In contrast, all breeds of cats have a very limited ability to convert cysteine into taurine due to low activities of hepatic cysteine dioxygenase (oxidizing cysteine to cysteinesulfinate) and cysteinesulfinate decarboxylase (converting cysteinesulfinate into taurine; Li and Wu 2023). Thus, dietary cysteinesulfinate is a poor substrate for taurine production in cats. Likewise, human infants have relatively low activities of both cysteine dioxygenase and cysteinesulfinate decarboxylase compared to adults, and therefore require the dietary intake of taurine for maintaining normal retinal, cardiac, and skeletal functions. As for cats, supplementing taurine to all-plant protein and taurine-free diets enhances growth and feed efficiency in carnivore fish (e.g., the rainbow trout and the Japanese flounder), but not the common carp, suggesting a suboptimal de novo synthesis of taurine by certain aquatic species (Li et al. 2009). Interestingly, supplementing 4.5% taurine to soy protein-based diets improves growth performance and prevents "green liver syndrome" in yellowtail (a marine carnivorous fish; Takagi et al. 2008). Of note, poultry, pigs, and ruminants, which typically consume plant-based diets with little or no taurine, can synthesize sufficient taurine when the provision of methionine or cysteine is adequate under normal production conditions.

Third, the rate of glycine synthesis is much lower than the rate of glycine utilization in poultry, neonatal pigs, growing-finishing pigs [particularly those with intrauterine growth restriction (IUGR)], human infants, HSB, and LMB (Graber and Baker 1973; He et al. 2023a; Li et al. 2023a; Rossi et al. 2021; Wang et al. 2014a; Wu et al. 2014a), but whether this is also true in other animal species is unknown. Based on the intakes of digestible amino acids and the accretion of amino acids in the bodies of 14- and 42-day-old broiler chickens fed conventional corn- and SBM-based diets, all dietary amino acids but glycine meet requirements for growth (He et al. 2021a), with data shown in Table 7.15 for 2-week-old birds. Of note, such diets provide only ~30% of the glycine required for weight gain plus uric acid production, or ~25% of the glycine needed for all metabolic pathways in 7- to 42-day-old broiler chickens (Table 7.16). Thus, the rapidly growing birds must synthesize daily at least 74–76% of the needed glycine. Similar analyses apply to growing pigs, gestating gilts, and fish fed SBM-based diets (Table 7.17). For example, glycine from sow's milk only meets 20% of the requirement of suckling piglets (Hu et al. 2023a,b; Wang et al. 2013). In addition, a conventional corn- and SBM-based diet provides only 27% of glycine required by gestating gilts, with the remaining portion (73%) being synthesized de novo. Furthermore, juvenile HSB (even those fed conventional 60% fishmeal-based diets containing 2.95% glycine) must synthesize daily 41% of the total glycine requirement (Suehs et al. 2024). When fed plant-based diets (e.g., 15% fishmeal and 58% SBM) containing 2.2% glycine, the rate of its endogenous synthesis would amount to 55% of the total glycine requirement to achieve

TABLE 7.15
Utilization of Dietary Amino Acids (AAs) by 14-Day-Old Broiler Chickens[a]

	AA in Diet		Intake of	AA in the Body		AA Accretion	Digestible AA	Percentage (%) of Digestible AA	
AA	% of Diet (As-Fed Basis)	g/100 g AA	Digestible AA in Diet (g/day)[b]	g/100 g Wet Weight	g/100 g AAs	in the Body (g/day)	Catabolized to CO_2 (g/day)	Oxidized to CO_2	Deposited in the Body
Ala	1.31	6.16	0.491	0.951	6.61	0.345	0.145	29.6	70.4
Arg	1.44	6.77	0.546	0.975	6.78	0.354	0.192	35.2	64.8
Asn	0.93	4.37	0.345	0.521	3.62	0.189	0.156	45.2	54.8
Asp	1.31	6.16	0.491	0.618	4.30	0.224	0.266	54.3	45.7
Cys[c]	0.51	2.40	0.186	0.219	1.52	0.079	0.106	57.2	42.8
Gln	1.81	8.51	0.684	0.747	5.19	0.271	0.413	60.4	39.6
Glu	1.70	7.99	0.647	1.190	8.28	0.432	0.216	33.3	66.7
Gly	0.88	4.14	0.326	1.670	11.6	0.606	–0.280	–86.0	186.3
His	0.55	2.58	0.202	0.306	2.13	0.112	0.091	44.9	55.1
Ile	0.88	4.14	0.333	0.513	3.57	0.186	0.146	44.0	56.0
Leu	1.79	8.41	0.673	0.988	6.87	0.359	0.315	46.7	53.3
Lys	1.40	6.58	0.514	0.881	6.13	0.320	0.194	37.8	62.2
Met	0.52	2.44	0.196	0.273	1.90	0.099	0.097	49.3	50.7
Phe	1.01	4.75	0.385	0.496	3.45	0.180	0.204	53.2	46.8
Pro	1.52	7.14	0.564	1.224	8.51	0.445	0.120	21.2	78.8
Ser	0.81	3.81	0.307	0.641	4.46	0.233	0.074	24.2	75.8
Thr	0.87	4.09	0.317	0.521	3.62	0.189	0.128	40.4	59.6
Trp	0.24	1.13	0.088	0.167	1.16	0.061	0.027	31.2	68.8
Tyr	0.78	3.67	0.295	0.379	2.64	0.138	0.157	53.3	46.7
Val	1.02	4.79	0.385	0.598	4.16	0.217	0.168	43.7	56.3
Hyp	ND	ND	---	0.499	3.47	0.181	–0.181	---	---

Source: He et al. (2021).

[a] Male broiler chickens (Cobb) were fed a corn- and soybean meal-based diet containing 21.5% crude protein. A negative value indicates net formation.

[b] Feed intake was 170 g/kg body weight per day. The mean body weight of 14-day-old broiler chickens was 297 g.

[c] Total cysteine (cysteine plus ½ cystine).

Note: AA, amino acid; Hyp, 4-hydroxyproline; ND, not detected.

TABLE 7.16
Use of Dietary Glycine for Growth and Uric Acid Production in Broiler Chickens Fed Corn- and Soybean Meal-Based Diets[a]

Age of Chickens (days)	Feed Intake	Digestible Glycine Intake	Glycine Accretion in the Body	Uric acid Excretion in Urine	Glycine Needed for Uric Acid Production	Glycine Needed for Weight Gain and Uric Acid Production	Dietary Glycine Meeting Glycine Needs for Weight Gain and Uric Acid Production (%)
7–14	180.5 ± 2.9	1.44 ± 0.03	2.05 ± 0.05	5.63 ± 0.19	2.51 ± 0.08	4.57 ± 0.09	30.8 ± 0.19
35–42	75.6 ± 0.55	0.51 ± 0.01	0.98 ± 0.01	1.88 ± 0.04	0.84 ± 0.02	1.82 ± 0.02	28.2 ± 0.11

Source: He et al. (2021).

[a] Values, expressed as g/kg body weight (BW)/day unless indicated otherwise, are means ± SEM, n = 4 pens. Cobb male broiler chickens were fed 21.5% and 18.4% crude-protein diets (corn- and soybean meal-based) between days 7 and 14 and between days 35 and 42 of age, respectively. There were 10 birds per pen. The mean BWs of the chickens were 36, 118, 297, 1489 and 2245 g at 0, 7, 14, 35, and 42 days of age, respectively. The content of glycine in the whole bodies of 14- and 42-day-old broilers were 1.65 and 1.68 g/100 g of BW, respectively. One mol of glycine is required to synthesize 1 mol of uric acid. Considering the oxidation of glycine to CO_2 and the use of glycine via other synthetic processes, the diet provides 26% and 24% of the glycine needed for all metabolic pathways in 7- to 14-day-old and 35- to 42-day-old broilers, respectively.

TABLE 7.17
Relative Contributions of Diets vs. Endogenous Synthesis to Meet the Glycine Requirements of Young Pigs, Gestating Gilts, and Juvenile Hybrid Striped Bass (HSB)

Variable	Milk-Fed Piglets (mg/kg BW per day)[a]	Gestating Gilts (g/day)[b]	HSB (mg/fish over 4 weeks)[i]
Glycine Provision from Diet	311	7.76	491
Total glycine from diet	349	11.0	546
Undigestible glycine in diet	38	1.54	54.6
Use by the intestinal mucosa in first-pass metabolism	---	1.70	---
Glycine Requirements for Growth and Metabolic Function	≥1,515	28.65	834
Body weight gain	1,216	2.89 (maternal)	218
Glycine oxidation to CO_2	96	0.39 (maternal)	447
Glycine utilization for creatine synthesis	46	3.81 (maternal)	31.0
Glycine utilization for purine synthesis	112	0.39 (maternal)[c]	13.4
Glycine utilization for conjugation of bile acid	16	1.18 (maternal)[d]	---
Glycine utilization for glutathione synthesis	13	0.90 (maternal)	8.2
Glycine utilization for hippuric acid production	11	0.98 (maternal)[e]	---
Glycine utilization for heme synthesis	5	0.35	0.23
Glycine utilization for serine synthesis	---	---	62.5
Loss to the surrounding water via skin, gills, and urine	---	---	37.0
Uptake of glycine by the uterus for conceptus growth	---	17.76	---
Glycine Needed from Endogenous Synthesis	≥1,204	20.89	343
Dietary serine	81	0.28[f]	13.2
Choline (via sarcosine)	36	0.64[g]	5.1
Threonine (via threonine dehydrogenase)	33	0.28[h]	6.4
4-Hydroxyproline	425	9.68	318
Unknown substrates (possibly glucose and glutamate)	629	10.01	---
Contribution of Endogenous Glycine Synthesis to Needs	80%	73%	41%

Source: Suehs et al. (2024), Wang et al. (2013), Hu et al. (2022), and Wu et al. (2018).

[a] Calculated for sow-reared 7-day-old pigs based on (a) the intake (0.78 L/day) of milk containing 1.12 g glycine/L of whole milk; (b) the digestibility of glycine is 89%; (c) the body weight (BW) gain of a piglet is 200 g/day (27.2 g protein/day); and (d) the rate of 4-hydroxyproline production is 472 mg/kg BW/day, with 90% of 4-hydroxyproline being converted into glycine.

[b] Calculated for pregnant gilts fed daily a 2-kg of corn- and soybean meal–based diet containing 12% crude protein (including 0.55% glycine). The true ileal digestibility of dietary glycine is 86%, and the bioavailability of orally administered glycine entering the portal vein of gilts is 82%. The gilt has a maternal BW of 110 kg at breeding, gestates 11 fetuses, gains 175 g of maternal tissue per day (excluding conceptus weight gain) during pregnancy. The content of glycine in maternal tissue is 16.5 g/kg tissue, and glycine accretion in the whole maternal body (excluding fetuses) is 2.89 g/day (i.e., 16.5×0.175).

[c] Calculated on the basis of the use of 1 mol of glycine for the synthesis of 1 mol of purine nucleoside, as well as the urinary excretion of purine derivatives (allantoic + uric acid + xanthine + hypoxanthine; 52 μmol/kg BW/day) by gestating sows (Orue et al. 1995).

[d] Calculated on the basis of hepatic bile acid production (3.3 mmol of bile acid/kg BW/day) by pigs (Juste et al. 1983), 86.3% of bile acid being conjugated with glycine (mol/mol), and 5% of bile acid being excreted per day (i.e., $3.3/1000 \times 86.3\% \times 5\% \times 75.1 \times 110$ kg = 1.18 g/day).

[e] Calculated on the basis of urinary excretion of hippuric acid (0.1181 mmol/kg BW/day) by pigs (Kristensen et al. 2009) and the synthesis of 1 mol of hippuric acid from 1 mol of glycine (i.e., $0.1181/1000 \times 75.1 \times 110$ kg = 0.98 g/day).

[f] A portion (1.39 g/day) of dietary serine is available for maternal tissues (containing 4.92 g serine/kg BW), with 0.86 g/day and 0.14 g/day serine being used for protein accretion and oxidation to CO_2, respectively, in maternal tissues. The amount of dietary serine available for glycine synthesis is 0.39 g/day (i.e., 1.39 – 0.86 – 0.14).

[g] Calculated on the basis of the dietary intake of choline by the gestating gilt [2.212 g (or 21.27 mmol) per day] (Li et al. 2010) and the finding that 40% of the choline administered to rats can be converted into glycine (Soloway and Stetten 1953).

[h] A portion (1.25 g/day) of dietary threonine is available for maternal tissues (containing 3.94 g threonine/kg BW), with 0.69 g/day and 0.12 g/day threonine being used for protein accretion and oxidation to CO_2, respectively, in maternal tissues. The amount of dietary threonine available for glycine synthesis is 0.44 g/day (i.e., 1.25 – 0.69 – 0.12).

[i] Calculated for juvenile hybrid striped bass (5.5 to 22.1 g of body weight) fed a 60%-fishmeal diet over a period of 4 weeks. The digestibility of glycine in the diet is 90%. The hepatopancreas and intestinal microbes are estimated to use 17 mg glycine/fish over the 4-week period.

a similar growth rate. Thus, supplementing 1% glycine to either milk- or corn- and SBM-based diets between weaning and market weight improves the growth rate, feed efficiency, and meat quality of pigs, particularly those with IUGR (He et al. 2023a). Likewise, dietary supplementation with 1% and 2% glycine enhances the growth performance of HSB fed SBM-based diets in a dose-dependent manner, while preventing intestinal hemorrhage (He et al. 2023b). It is likely that carnivores synthesize less glycine than noncarnivores per kilogram of BW because the diets for the former provide more glycine than those for the latter (Herring et al. 2021).

Fourth, cats and other members of the Felidae family synthesize: felinine from acetyl-CoA and GSH; isovalthine from cysteine and GSH; and isobuteine from valine and GSH (Hendriks et al. 2008; Rutherfurd-Markwick et al. 2005). In contrast, other mammals (including dogs and pigs) have no or limited ability to form these three special amino acids. Physiological functions of felinine, isovalthine, and isobuteine remain unknown but may serve as pheromones in cats for the purpose of territorial marking and intra-species communications (e.g., chemical signals to attract females; Miyazaki et al. 2008).

Synthesis of Amino Acids from Their α-Ketoacids or Analogs in Animal Cells and Bacteria

With the exception of four amino acids (L-arginine, L-cysteine, L-lysine, and L-threonine), all proteinogenic amino acids can be formed from (a) their α-ketoacids through L-amino acid transaminases, and (b) their D-isomers via D-amino acid oxidases (peroxisomal enzymes containing FAD as a cofactor) and L-amino acid transaminases (ubiquitously present in both mitochondria and the cytosol), which widely occur in animal tissues. The final steps for the syntheses of L-arginine, L-cysteine, and glycine in animal cells or of L-lysine and L-threonine in microbes do not involve transamination and therefore do not form from their α-ketoacids in the organisms. An α-ketoacid (KA) transaminates with an amino acid (usually glutamate) to generate a new amino acid and a new α-ketoacid. Because glutamate is synthesized from ammonia and α-KG, the conversion of an α-ketoacid into its corresponding L-amino acid drives the disposal of ammonia for the production of a new L-amino acid. This is physiologically important for patients with hepatic or renal dysfunction; the formation of a new amino acid (e.g., leucine) from its α-ketoacid (e.g., α-ketoisocaproate) not only removes ammonia but also generates a new amino acid to support protein synthesis and possibly activate anabolic metabolic pathways.

Animal cells lack D-amino acid transaminases. Therefore, D-amino acids do not undergo transamination reactions in mammalian, avian, or other vertebrate cells. However, animal tissues (e.g., liver, kidney, brain, and heart) contain D-amino acid oxidases (oxidoreductases) to oxidize D-amino acids (e.g., D-methionine) to their corresponding α-ketoacids (e.g., α-keto-γ-methyl thiobutyrate, also called 2-keto-4-methylthiobutanoic acid; KMB). Subsequently, L-amino acid transaminases catalyze the conversion of the α-ketoacid and glutamate into the corresponding L-α-amino acid (e.g., L-methionine) and α-KG. Because of technical challenges to manufacturing L-methionine through microbial fermentation, KMB is an effective precursor of L-methionine in animals, and is therefore a useful additive to the feeds for poultry and swine.

$$\begin{aligned}&\text{D-Methionine} + O_2 + H_2O + \text{FAD}\\ &\quad\rightarrow \alpha\text{-Keto-}\gamma\text{-methylthiobutyrate} + FADH_2 + H_2O_2\\ &\alpha\text{-Keto-}\gamma\text{-methylthiobutyrate} + \text{L-Glutamate}\\ &\quad\rightleftharpoons \text{L-Methionine} + \alpha\text{-Ketoglutarate}\end{aligned}$$

In recent years, two hydroxy acids of methionine (L-2-hydroxy-4-methylthiobutanoic acid and D-2-hydroxy-4-methylthiobutanoic acid) have also been used as the precursors of L-methionine through the formation of KMB in farm animals (Figure 7.17). These hydroxy acids are absorbed mainly by monocarboxylate transporter 1, coupled with the action of the Na^+/H^+ exchanger (NHE3). L-2-hydroxy acid oxidase (a H_2O_2-producing flavoenzyme in peroxisomes of tissues, mainly the liver and kidneys) oxidizes L-2-hydroxy-4-methylthiobutanoic acid into KMB, whereas D-2-hydroxy acid dehydrogenase (a H_2O_2-producing flavoenzyme in the mitochondria of tissues, mainly the liver and kidneys) converts D-2-hydroxy-4-methylthiobutanoic acid into KMB (Zhang et al. 2015). The choice of an L-methionine precursor as a feed additive depends on its cost (compared with other sources of L-methionine such as MBM, intestinal mucosal protein, and blood meal) and diets.

Bacteria (e.g., those in the intestine) contain D-amino acid oxidases, D-amino acid transaminases, and L-amino acid transaminases and can therefore convert most D-amino acids to L-amino acids. However, the extent of these reactions in the gastrointestinal microbes is unknown. The presence of D-amino acid transaminases in intestinal microbes provides an additional route for L-amino acid synthesis from D-amino acids in animals, as shown for D-tryptophan. The efficiency of utilization of D-amino acids for the synthesis of L-amino acids varies greatly with animal species and D-amino acids. For example, the efficiency of synthesis of L-leucine from D-leucine is 100%, 50%, and 15% for chicks, rats, and mice, respectively, whereas the efficiency of conversion of D-tryptophan into L-tryptophan is 20%, 100%, and 30% for chicks, rats, and mice, respectively (Wu 2022).

$$\begin{aligned}&\text{D-Tryptophan} + \alpha\text{-Ketoglutarate}\\ &\quad\rightleftharpoons \text{Indole-3-pyruvate} + \text{L-Glutamate}\\ &\text{Indole-3-pyruvate} + \text{L-Glutamate}\\ &\quad\rightleftharpoons \text{L-Tryptophan} + \alpha\text{-Ketoglutarate}\end{aligned}$$

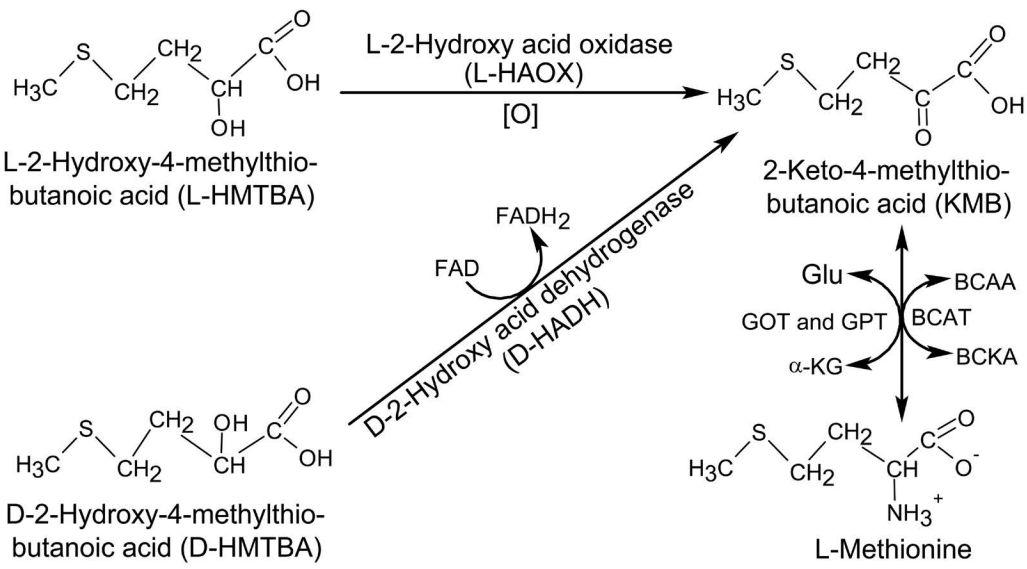


FIGURE 7.17 Synthesis of methionine from its hydroxyl ketoacids. Enzymes for these reactions occur widely in animal tissues. The conversion of L-2-hydroxy-4-methylthiobutanoic acid and D-2-hydroxy-4-methylthiobutanoic acid into L-methionine requires the cooperation of multiple organs, which include primarily the liver, kidneys, and skeletal muscle.

Syntheses of D-Amino Acids in Animal Cells and Bacteria

As noted in Chapter 4, D-amino acids are present in nature. An amino acid racemase (also known as D-amino acid racemase) converts a free L-amino acid into a free D-amino acid in animal cells and bacteria. Most amino acid racemases (including D-aspartate racemase, D-serine racemase and D-alanine racemase) depend on pyridoxal-5'-phosphate for catalytic activity. These enzymes are localized in the cytosol with their respective substrates. Only two amino acid racemases, D-aspartate racemase and D-serine racemases have been identified in mammals and birds to date. Both D-aspartate and D-aspartate racemase activity have been identified in (a) pinealocytes of the pineal gland, (b) pituicytes of the posterior pituitary gland, (c) epinephrine-producing chromaffin cells of the medulla of the adrenal glands, and (d) elongated spermatids of the testes. In the brain, endogenously synthesized D-aspartate is important for neurological function, because the entry of D-amino acids from the peripheral circulation into this organ appears to be limited by the blood-brain barrier. In addition to nervous tissues, D-aspartate racemase has been reported in the kidney and liver of mammals, which is in keeping with the presence of D-aspartate in these organs. Likewise, all animals (e.g., mammals, birds, and invertebrates) express D-serine racemase, which converts free L-serine into D-serine. This enzyme is enriched in the glial cells of the rat brain that contain high levels of D-serine.

$$\text{L-Amino acid (e.g., alanine, aspartate or serine)} \rightleftharpoons \text{D-Amino acid (e.g., alanine, aspartate, or D-serine)}$$

D-Amino acid racemase (vitamin B_6-dependen enzyme)

D-Alanine racemase converts free L-alanine into D-alanine, which is a key building block in the biosynthesis of the peptidoglycan layer in bacterial cell walls. D-Alanine racemases are ubiquitous among microbes and present in certain invertebrates, but are typically absent from mammals, birds, and fish. Bacteria (e.g., *S. faecalis, Mycobacterium tuberculosis, E. coli, Listeria monocytogenes*, and *Bacillus anthracis*) and invertebrates (e.g., *Corbicula japonica*, a brackish-water species, and crayfish) use D-alanine to grow. Thus, D-alanine racemase is an attractive target for the development of novel antimicrobials in both medicine and livestock production.

Regulation of Amino Acid Syntheses in Animals

Under normal nutritional and physiological conditions, syntheses of amino acids in animals are limited by one or more of the following factors: (a) the availabilities of their precursor amino acids (mainly those amino acids that are not synthesized by eukaryotes), (b) intracellular or intramitochondrial concentrations of co-substrates or cofactors, (c) the amounts nd specific activities of the required enzymes, (d) feed-back inhibition [e.g., inhibition of GS expression by glutamine in skeletal muscle cells (Huang et al. 2007)], and (e) allosteric inhibition. Some hormones (e.g., insulin and glucocorticoids) regulate expression of the enzymes [e.g., GS (Wang and Watford 2007)] involved in amino acid synthesis. The synthesis of citrulline and arginine by pig enterocytes and microbes is used as an example to illustrate this general principle.

First, increasing the dietary provision of glutamate, glutamine, and proline promotes intestinal citrulline and arginine synthesis in suckling, postweaning, growing, gestating, and lactating pigs (Hou et al. 2016a; Wu et al. 2018). Thus, when growing pigs (e.g., 10–20 kg) are fed a low-CP

diet (e.g., 14% CP), which supplies all EAAs at the levels of an intermediate CP-level diet (17% CP) or a normal CP diet (e.g., 20% CP) but does not meet the needs of arginine and all other NEAAs, including aspartate and glycine (Hou et al. 2016a), their growth performance, feed efficiency, and muscle protein gain are suboptimal and their fat deposition in the body is substantially increased. As dietary CP content decreases from 20% to 17% and to 14% at the same dietary levels of EAAs, but decreasing the dietary levels of NEAAs, the daily weight gain of the growing pigs decreases progressively (Li et al. 2016). Similarly, the growth performance of pigs (13–35 kg) decreases progressively as the dietary CP content decreases from 20% to 17.2%, 15.3%, and 13.9% at the same dietary levels of EAAs but decreasing the dietary levels of NEAAs (Peng et al. 2016). These results further support the notion that animals do have dietary requirements of "NEAAs" for maximal growth and development (Wu et al. 2013a, 2014a).

Second, the intestinal synthesis of citrulline and arginine requires *N*-acetylglutamate [NAG, an allosteric activator of carbamoylphosphate synthase-I (CPS-I) in mitochondria]. A substantial decrease in the mitochondrial concentration of NAG in the enterocytes of 7- to 21-day-old pigs limits citrulline and arginine synthesis, compared with newborn pigs (Wu et al. 2004), as noted previously. Because NAG is actively degraded by deacetylase in the cytosol of cells, *N*-carbamoylglutamate (NCG, a metabolically stable analog of NAG which is not a substrate of deacetylase) can be used as a dietary additive to stimulate intestinal citrulline and arginine synthesis in young pigs (Wu et al. 2004). This approach is also effective in enhancing arginine synthesis in gestating pigs and rats.

Third, either a natural surge of cortisol during weaning or intramuscular administration of cortisol increases intestinal citrulline and arginine synthesis in 14- to 21-day-old pigs through enhancing (a) expression of P5C synthase and NAG synthase and (b) mitochondrial NAG synthesis and concentration in their enterocytes. Fourth, although the purified P5C synthase is inhibited in vitro by high concentrations of ornithine (a product of P5C transamination by ornithine aminotransferase), supplementing the basal diet (containing ~1% arginine) of growing pigs with up to 2% arginine does not reduce citrulline plus arginine synthesis from glutamine or proline in their enterocytes (Wu et al. 2016). This is because the arginine-derived ornithine is extensively metabolized to proline in these cells, such that their intracellular concentrations of ornithine are not sufficiently high to inhibit P5C synthase.

Finally, in many of the metabolic pathways for the synthesis of amino acids (e.g., arginine, glutamine, and tryptophan) in intestinal bacteria, products are allosteric inhibitors of certain key enzymes (e.g., an inhibition of GS and anthranilate synthase by glutamine and tryptophan, respectively). Thus, in gastrointestinal (including ruminal) microbes, the rates of incorporation of free amino acids into protein can influence the rates of their syntheses from NPN (e.g., ammonia and urea).

DEGRADATION OF AMINO ACIDS IN ANIMALS

Partition of Amino Acids into Pathways for Catabolism and Protein Synthesis

The K_M values of aminoacyl-tRNA synthetases (enzymes initiating protein synthesis) for amino acids in cells range from 0.2 to 0.4 mM (Wolfson et al. 1998), which are similar to the intracellular concentrations of most amino acids (except for aspartate, glutamine, glutamate, glycine, proline, and alanine at 2–25 mM, and arginine (nonhepatocytes) and threonine (at 1–2 mM) but lower than the K_M values of the various enzymes initiating the degradation of amino acids (1.5–30 mM) in animal tissues (Krebs 1972). Thus, protein synthesis takes precedence over the degradation of amino acids in animals to ensure their survival and to conserve the amino acids. However, because amino acid-degrading enzymes are present in live animals, the catabolism of amino acids occurs in the body regardless of their nutritional states, with the rates being the highest immediately after feeding. Amino acids in excess of either needs for the synthesis of protein and other biologically active substances or the upper limits of intracellular concentrations in tissues must be degraded in a cell-specific manner. Although some cell types (e.g., enterocytes in mammals) depend on the oxidation of certain amino acids (glutamine, glutamate, and aspartate) as their major metabolic fuels, the use of amino acids for ATP production is energetically inefficient when compared with the oxidation of glucose and fatty acids. On average, the efficiencies of energy transfer from protein, fat, and glucose to ATP are 41.5%, 54.8%, and 55.7%, respectively (Wu 2022). In some animals such as fish and carnivorous mammals, dietary protein may provide the bulk of energy, but knowledge of their utilization of amino acids can provide a basis for the development of their alternatives (e.g., pyruvate, α-KG, or OAA) in diets. In mammals, birds, and fish, rates of degradation of amino acids generally increase with their extracellular concentrations. As indicated in Chapter 1, the carbon skeletons of amino acids must be eventually converted into acetyl-CoA for net oxidation to CO_2 and water. Thus, animals have high capacities to catabolize amino acids (e.g., arginine, glutamine, glutamate, glycine, and proline) as an adaptive response to their dietary intakes. Utilization of an amino acid via obligatory catabolism (including oxidation) and protein synthesis is a metabolic basis for amino acid requirements by an animal (Liu and Masters 2003; Wu et al. 2014a).

Cell- and Tissue-Specific Degradation of Amino Acids

The degradation of amino acids is catalyzed by specific enzymes, whose distribution varies greatly among different cells and tissues of the same species (Wu 2022). This gives rise to cell- and tissue-specific catabolism of amino acids (Table 7.18), as indicated by the following examples

TABLE 7.18
Cell- and Tissue-Specific Catabolism of Amino Acids in Mammals and Birds under Normal Physiological Conditions

	Mammals					Birds			
Amino acid	Enterocytes	Liver	Skeletal Muscle	Kidney	Mammary Gland	Enterocytes	Liver	Skeletal Muscle	Kidney
Ala	Limited	+++	Limited	++	+	Limited	+++	Limited	++
Arg	+++	+++++	No	+ [a]	+++	No	No	No	+ [a]
Asn	No	+++	No	++	No	No	+++	No	++
Asp	+++++	+++	+	++	+	?	+++	+	++
Cys	No	+++	No	++	No	No	+++	No	++
Gln	+++++	+++	++	+++	No	Limited	Limited	++	+++
Glu	+++++	+++	++	+++	+	?	+++	++	+++
Gly	Limited	+++	No	++	Limited	Limited	+++	No	++
His	No	+++	No	+	No	No	+++	No	+
Ile	+++	Limited	+++	+++	+++	+++	Limited	+++	+++
Leu	+++	Limited	+++	+++	+++	+++	Limited	+++	+++
Lys	No	+++	No	+	No	No	+++	No	+
Met	No	+++	No	+	No	No	+++	No	+
Orn	++++	+++	No	+++	+++	+	+++	No	+++
Phe	No	+++	No	++	No	No	+++	No	++
Pro	+++	++	No	++	No	?	++	No	++
Ser	Limited	+++	No	++	Limited	Limited	+++	No	++
Thr	No	+++	No	+	++	No	+++	No	+
Tyr	No	+++	No	+	No	No	+++	No	+
Val	+++	Limited	+++	+++	+++	+++	Limited	+++	+++
D-AA	Limited	+	No	++	No	Limited	+	No	++

[a] Limited activity in proximal renal tubules where synthesis of arginine from citrulline primarily occurs in kidneys, but is present at high activity in other parts of the renal tissue.

Note: D-AA, D-amino acids; "++++" to "+", enzymatic activity ranges from "very high" to "presence"; "?", unknown.

for glutamine. First, in pigs and rats, the placenta, mammary gland, and mature red blood cells do not contain the phosphate-activated glutaminase pathway for glutamine degradation, but this pathway is highly active in all other cells and tissues (including the small intestine, skeletal muscle, heart, kidneys, and immunocytes; Figure 7.18). The lack of glutamine catabolism via glutaminase in the placenta and the lactating gland ensures maximum transfer of glutamine from the mother to her fetus and newborn for their optimum growth, development, and health. Likewise, in chickens, phosphate-activated glutaminase activity is about 328% greater in breast muscle than that in leg muscles, which is consistent with much lower concentrations of glutamine in the former than those in the latter (Table 7.19). In the kidneys, glutamine plays important roles in oxidation, gluconeogenesis, and whole-body pH homeostasis. Because skeletal muscle and the heart synthesize de novo glutamine from glutamate while hydrolyzing glutamine to glutamate, the net rate of the intracellular glutamine–glutamate cycle determines the release of glutamine from these tissues. This is the major factor affecting the endogenous provision of glutamine in animals.

$$4\text{Glutamine}\left(C_5H_{10}O_3N_2\right) + 6H_2O + 12O_2 \rightarrow 6CO_2 + \text{Glucose}\left(C_6H_{12}O_6\right) + 8HCO_3^- + 8NH_4^+$$

Second, in mammals, birds, or fish, the liver exhibits only low BCAA transaminase activity as measured in the presence of saturated substrate concentrations and has a limited ability to initiate BCAA degradation under physiological conditions. In contrast, skeletal muscle, kidneys, small intestine, and many other tissues (including the lactating mammary tissue) extensively possess a high activity of BCAA transaminase to transaminate BCAAs with α-KG to form BCKAs and glutamate (Figure 7.19). However, the opposite is true for the oxidation of BCKAs by BCKA dehydrogenase, which is highly active in the liver but only has a low to medium activity in other tissues. The liver also converts the α-ketoacids of isoleucine and valine into

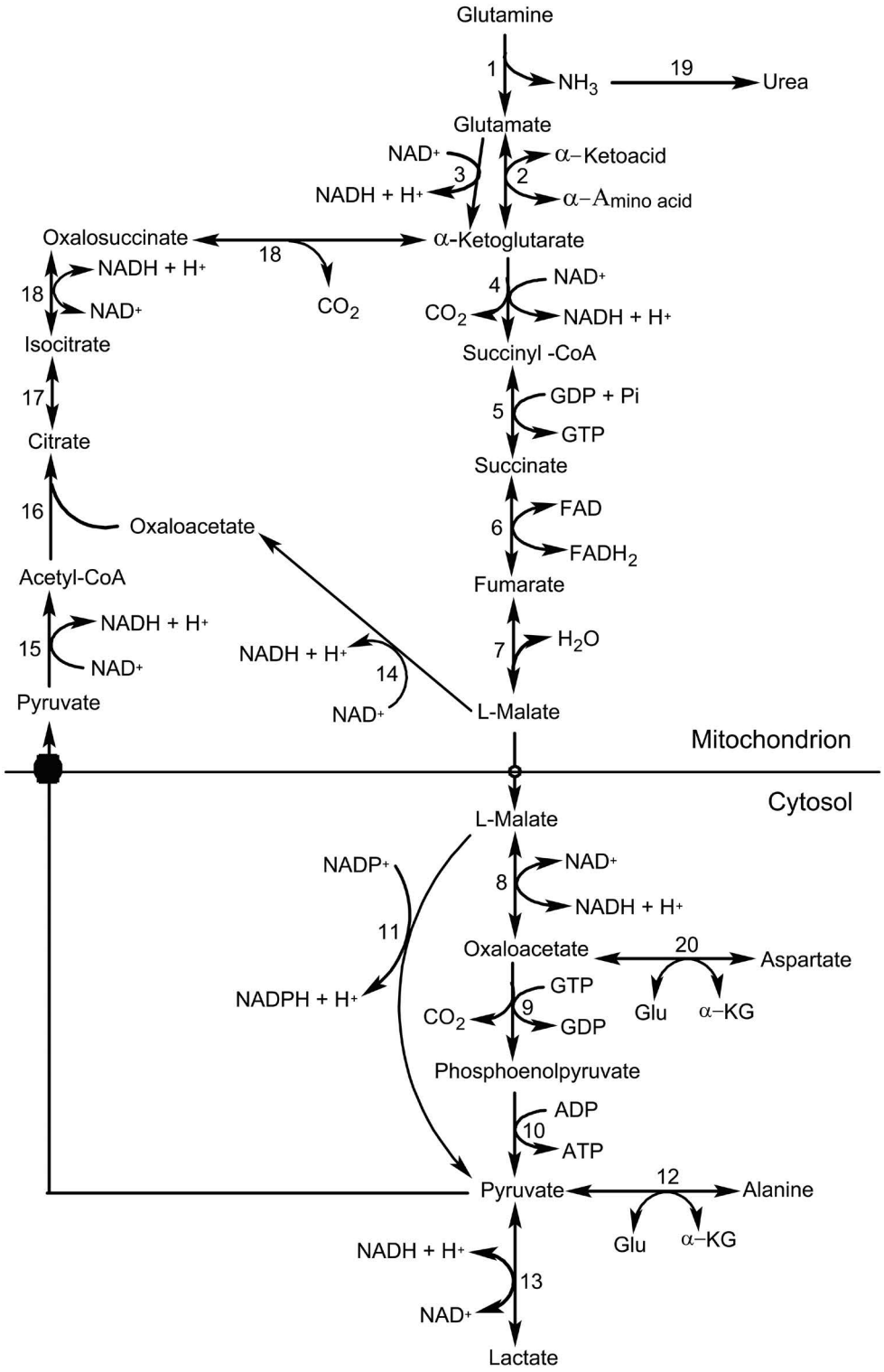


FIGURE 7.18 Glutamine degradation via the phosphate-activated glutaminase pathway in animals. The enzymes that catalyze the indicated reactions are (1) phosphate-activated glutaminase, (2) glutamate transaminase, (3) glutamate dehydrogenase, (4) α-ketoglutarate dehydrogenase, (5) succinate thiokinase, (6) succinate dehydrogenase, (7) fumarase, (8) NAD^+-linked malate dehydrogenase (cytoplasm), (9) phosphoenolpyruvate carboxykinase, (10) pyruvate kinase, (11) $NADP^+$-linked malate dehydrogenase, (12) glutamate-pyruvate transaminase, (13) lactate dehydrogenase, (14) NAD^+-linked malate dehydrogenase (mitochondria), (15) pyruvate dehydrogenase, (16) citrate synthase, (17) aconitase, (18) isocitrate dehydrogenase, and (19) conversion of ammonia into urea via the urea cycle. Glutamine degradation to form glutamate, aspartate, and alanine is known as glutaminolysis.

TABLE 7.19
Concentrations of Free Amino Acids, Activities of Enzymes, and Rates of Protein Synthesis in Skeletal Muscles of Six-Week-Old Fed or 48-h Fasted Male White Leghorn Chickens

		Concentrations of Amino Acids[a]							Activity of Enzyme[b]		
Tissue or Plasma	Nutritional State	Gln	Glu	Gly	Leu	Lys	Ser	Tau	PDG	GS	FRS[c]
GM	Fed	9.45	3.43	1.05	0.28	0.47	1.60	11.8	0.39	0.50	36.1
	48-h fasted	3.02*	1.69*	1.37	0.31	0.52	1.17*	9.20*	0.28	0.73*	13.6*
PM	Fed	1.41	0.90	0.71	0.32	0.23	0.71	0.45	1.67	0.07	10.5
	48-h fasted	1.38	0.89	0.80	0.26	0.26	0.84*	0.42	1.28	0.08	10.3
Plasma	Fed	1089	265	496	305	209	481	249	---	---	---
	48-h fasted	941	317*	709*	287	446*	503	512*	---	---	---

Source: He et al. (2021) and Watford and Wu (2005).

[a] Values are expressed as nmol/mg of wet tissue for tissues or nmol/ml for plasma.

[b] Values are expressed as unit/g of wet tissue. One unit represents 1 μmol of product formed per min at 38°C.

[c] Values are expressed as %/day.

Note: FRS, fractional rate of protein synthesis; GM, gastrocnemius muscle; GS, glutamine synthetase; ND, not detected; PAG, phosphate-activated glutaminase; PM, pectoralis muscle; * $P < 0.05$ vs. the fed group.

glucose, as well as all BCKAs into ketone bodies, depending on physiological needs.

Third, all amino acids other than BCAAs can be degraded in the liver. However, net degradation of non-BCAAs may not always occur in the liver, depending on their extracellular concentrations, as reported previously for glutamine degradation in the rat liver (Lund and Watford 1976). Specifically, in the perfused rat liver, there is no net degradation of physiological levels of glutamine (0.5–1 mM in the perfusion medium), but net degradation of glutamine to CO_2 and ammonia occurs in the liver when its extracellular concentration exceeds 1 mM. This is explained by the intercellular glutamine–glutamate cycle between periportal hepatocytes and perivenous hepatocytes in the liver (Haussinger 1990). Because the catabolism of many amino acids in the liver produces acetoacetate, which does not undergo oxidation in hepatocytes, this metabolite is oxidized to CO_2 and water by extrahepatic tissues and cells. Furthermore, among extrahepatic tissues, rates of the degradation of alanine, aspartate, glutamine, glutamate, arginine, proline, and ornithine vary considerably with different cell types. For example, in pigs, oxidation of proline to CO_2 is very limited in enterocytes due to the low expression of P5C dehydrogenase but occurs at a high rate in hepatocytes due to high P5C dehydrogenase activity.

Fourth, the liver and kidneys degrade lysine via the mitochondrial saccharopine and peroxisomal pipecolate pathways (Figure 7.20), as well as threonine via the mitochondrial threonine dehydrogenase and cytosolic dehydratase pathways (Figure 7.21), but other tissues have little or no ability to oxidize lysine or threonine to CO_2 and water

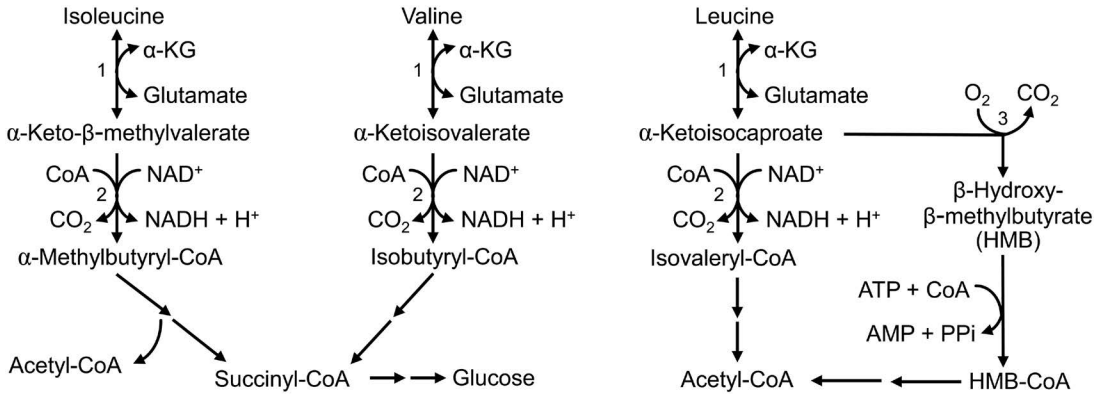


FIGURE 7.19 Degradation of branched-chain amino acids in animals. The enzymes that catalyze the indicated reactions are (1) branched-chain amino acid (BCAA) transaminase, (2) branched-chain α-ketoacid (BCKA) dehydrogenase, (3) α-ketoisocaproate (KIC) dioxygenase, and (4) β-hydroxy β-methylbutyryl-CoA (HMB-CoA) synthase.

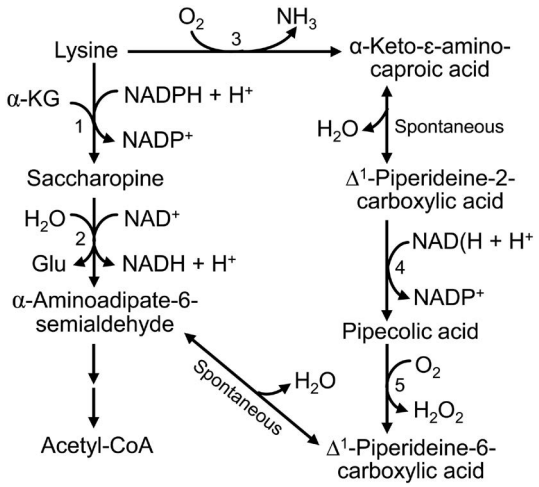


FIGURE 7.20 Lysine degradation in the liver of animals. Lysine is degraded via the mitochondrial saccharopine pathway and the peroxisomal pipecolate pathway. The enzymes that catalyze the indicated reactions are (1) lysine:α-ketoglutarate reductase, (2) saccharopine dehydrogenase (NAD^+, glutamate-forming), (3) lysine oxidase (a peroxisomal protein), (4) peperideine-2-carboxylic acid reductase, and (5) pipecolate oxidase (a peroxisomal protein). α-KG, α-ketoglutarate.

(Benevenga and Blemings 2007). Phenylalanine is converted into tyrosine in the liver, kidneys, and pancreas and, to a much lesser extent, in the small intestine, skin, and brain, but the hydroxylation of phenylalanine is absent from other tissues because of the lack of phenylalanine hydroxylase. Note that the hydroxylation of phenylalanine, as well as other aromatic amino acids and arginine, requires tetrahydrobiopterin (BH_4) as an essential factor (Figure 7.22). BH_4 is deficient under some conditions such as oxidative stress, obesity, diabetes, and endothelial dysfunction (Kelly et al. 2024).

Finally, although methionine is converted into SAM in many cell types, the liver is the only organ that can completely degrade methionine into cysteine, taurine, CO_2, and water in animals (Stipanuk 2004). The pathway for methionine catabolism via the transsulfuration pathway is outlined in Figure 7.23. Cysteine is oxidized to sulfur dioxide (SO_2), sulfide (S^{2-}; e.g., H_2S), sulfite [SO_3^{2-}; e.g., HSO_3^- (hydrogen sulfite)], and sulfate [SO_4^{2-}; e.g., H_2SO_4 (sulfuric acid)] in animals. Like methionine, tryptophan degradation can be initiated in multiple tissues (Figure 7.24), but the liver is the only organ to oxidize tryptophan to CO_2 and water. In animals, tryptophan is catabolized via the kynurenine, serotonin, and transamination pathways. The kynurenine pathway involves the deamination and decarboxylation of tryptophan to form kynurenine and occurs primarily in the liver and brain (Le Floc'h et al. 2011). The further catabolism of kynurenine produces indoleacetic acid, niacin, pyruvate, and acetyl-CoA. Quantitatively, the kynurenine pathway accounts for the degradation of over 95% of the available peripheral tryptophan. The serotonin pathway depends on the BH_4-dependent hydroxylation and vitamin B_6-dependent decarboxylation of tryptophan to generate serotonin (5-hydroxytryptamine) and takes place mainly in the neuronal cells of the GIT and brain. Serotonin is a biogenic amine that functions as a neurotransmitter and a gastrointestinal hormone. Thus, like the syntheses of amino acids, the catabolism of most amino acids takes place in a tissue- and cell-specific manner (Wu 2022).

Compartmentalization of Amino Acid Degradation in Cells

An interesting characteristic of amino acid degradation in animals is complex compartmentalization (Haüssinger 1990). This means that (a) the metabolic pathways for amino acid catabolism are specific to certain organelles, and (b) extracellularly and intracellularly derived amino acids may have very different metabolic fates. For example, in pig enterocytes, the oxidation of proline to P5C by proline

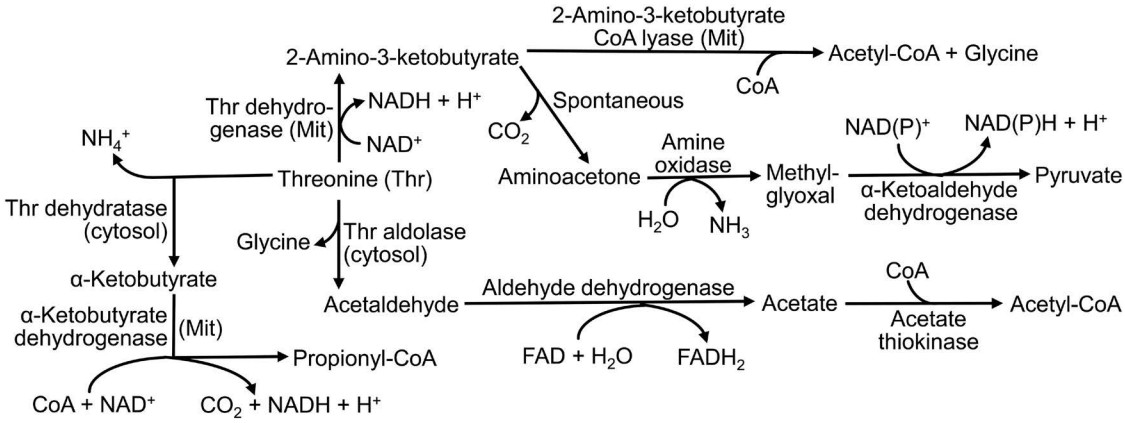


FIGURE 7.21 Threonine degradation in the liver of animals. Threonine degradation in the liver is initiated primarily by threonine dehydrogenase (a mitochondrial enzyme) and, to a much lesser extent, threonine dehydratase and threonine aldolase (cytosolic enzymes). Products of threonine catabolism include glycine, pyruvate, and propionyl-CoA.

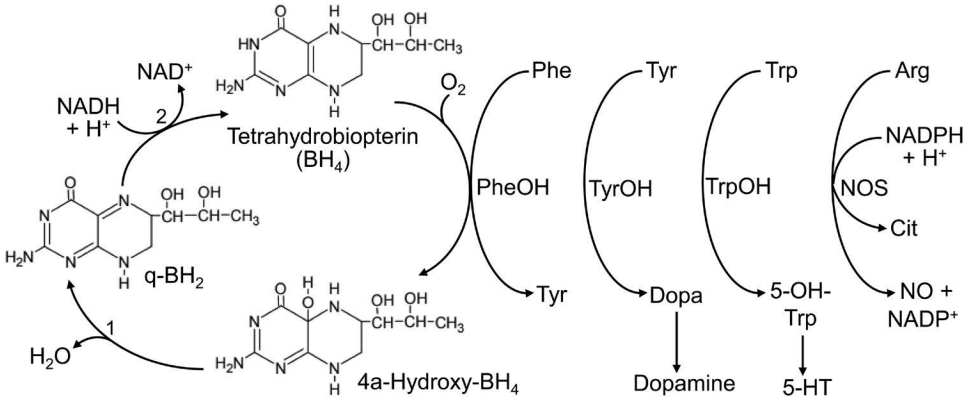


FIGURE 7.22 Hydroxylation of aromatic amino acids by hydroxylases and of arginine by NO synthase in animal tissues. These reactions require tetrahydrobiopterin as an essential cofactor. 4a-Hydroxy-tetrahydrobiopterin is an intermediate in these reactions and is subsequently converted into pterin-4a-carbinolamine. The latter undergoes dehydration to form quinonoid-7,8-dihydrobiopterin (q-BH2). The enzymes catalyzing the indicated reactions are (1) pterin-4a-carbinolamine dehydrogenase and (2) dihydrobiopterin reductase. NOS, NO synthase; PheOH, phenylalanine hydroxylase; TrpOH, tryptophan hydroxylase; TyrOH, tyrosine hydroxylase.

oxidase occurs only in mitochondria, whereas ornithine decarboxylase is expressed in the cytosol but not in mitochondria. Also, the ornithine generated from glutamine in the mitochondria of pig enterocytes, but not extracellular ornithine, is converted into citrulline in the mitochondria of these cells (Wu and Morris 1998). Thus, dietary supplementation with ornithine cannot increase the concentration of citrulline or arginine in the plasma of pigs, rats, or humans. This illustrates a challenging issue in the studies of amino acid metabolism in cells and the whole body.

Interorgan Metabolism of Dietary Amino Acids

The Intestinal-Renal Axis for Arginine Synthesis

As noted previously, dietary glutamine, glutamate, and proline, as well as arterial glutamine, are converted into citrulline and arginine in the enterocytes of most mammals, with citrulline being taken up by the kidneys and other extrahepatic tissues for arginine production. In the neonates of those citrulline-producing mammals, most of the citrulline produced by enterocytes is further

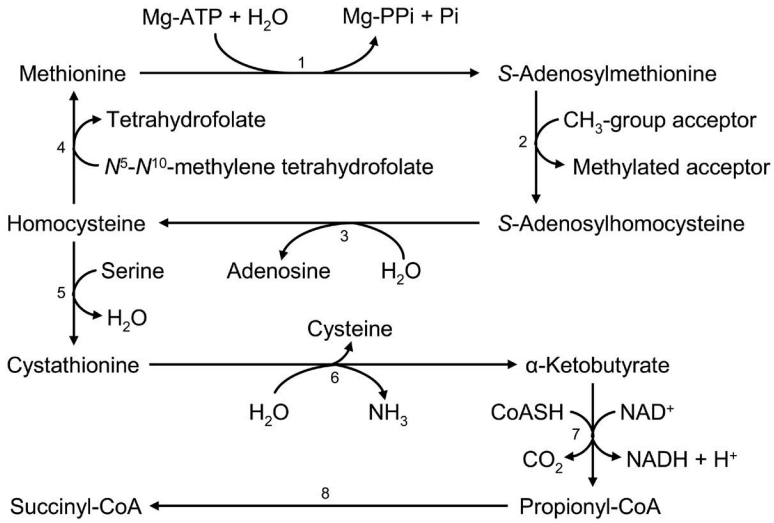


FIGURE 7.23 Methionine degradation via the transsulfuration pathway in animals. The enzymes that catalyze the indicated reactions are (1) *S*-adenosylmethionine synthase (methionine adenosyltransferase), (2) *S*-adenosylmethionine methylase, (3) *S*-adenosylhomocysteinase, (4) homocysteine methyltransferase (a vitamin B_{12}-dependent enzyme), (5) cystathionine β-synthase (a pyridoxal phosphate-dependent enzyme), (6) cystathionine γ-lyase (a pyridoxal phosphate-dependent enzyme), (7) α-ketobutyrate dehydrogenase, and (8) a series of enzymes [propionyl-CoA carboxylase, methylmalonyl-CoA racemase, and methylmalonyl-CoA mutase (a vitamin B_{12}-dependent enzyme)]. All the carbons of cysteine are derived from serine. Many tissues can convert methionine into SAM, but the liver is the only organ to oxidize methionine into cysteine, taurine, CO_2, and H_2O. Enterocytes of pigs, rats, mice, cattle, sheep, chickens, hybrid striped bass, and largemouth bass, and the intestine of shrimp and blue crabs do not convert methionine or cysteine into taurine.

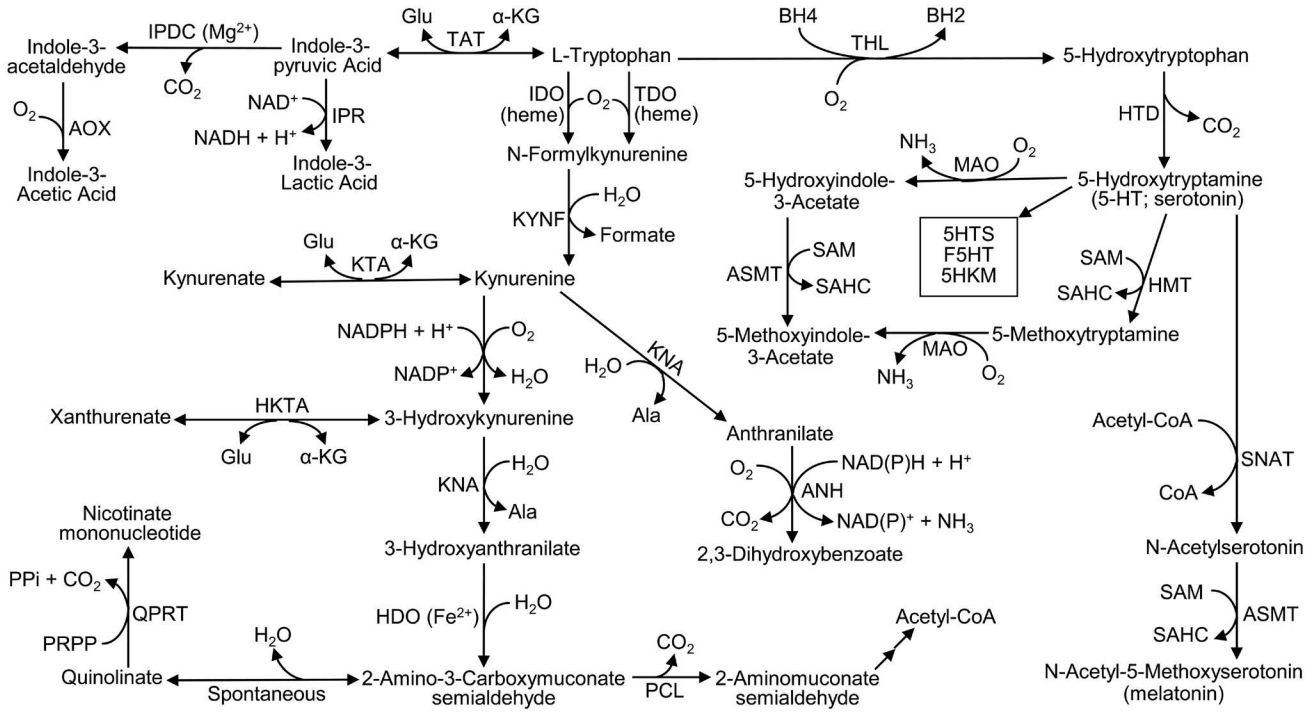


FIGURE 7.24 Tryptophan degradation in animals. Degradation of L-tryptophan is initiated by indoleamine 2,3-dioxygenase (IDO), tryptophan 2, 3-dioxygenase (TDO), and tryptophan hydroxylase (THL). These pathways are cell- and tissue-specific. Serotonin, melatonin, and their metabolites can form sulfate and glucuronide conjugates for excretion in urine and feces. Products of tryptophan catabolism include NAD, serotonin, melatonin, kynurenine, indoles, and acetyl-CoA. Many tissues can initiate Trp catabolism, but the liver is the only organ to oxidize Met into Cys, Tau, CO_2, and water. AMSR, 2-aminomuconate semialdehyde reductase; ANH, anthranilate hydroxylase [also known as Anthranilate 3-monooxygenase (deaminating)]; AOC, aldehyde oxidase; ASMT, *N*-acetylserotonin *O*-methyltransferase; F5HT, formyl 5-hydroxytryptamine; HDO, 3-hydroxyanthranilate dioxygenase; 5HKM, 5-hydroxykynuremine; HKTA, 3-hydroxykynurenine transaminase; HMT, 5-hydroxyindole-*O*-methyltransferase; 5-HT, 5-hydroxytryptamine; HTD, 5-hydroxytryptopohan decarboxylase; 5HTS, 5-hydroxytryptamine sulfate; 5-HTP, 5-hydroxy-l-tryptophan; IPDC, indole-3-pyruvate decarboxylase (a thiamine diphosphate-dependent enzyme); IPR, indole-3-pyruvate reductase; KAD, α-ketoadipate dehydrogenase; KHL, kynurenine hydroxylase; KNA, kynureninase; KTA, kynurenine transaminase; KYN, kynurenine; KYNF, kynurenine formamidase; MAO, monoamine oxidase; NADS, NAD synthase; NER, nonenzymatic reaction; NGH, NAD glycohydrolase; OCR, oxalocrotonate reductase; PCL, picolinate carboxylase; PLP, pyridoxal phosphate; PRPP, 5-phosphoribosyl-1-pyrophosphate; QPRT, quinolinate phosphoribosyl transferase; SNAT, serotonin-N-acetyltransferase; SAM, S-adenosylmethionine; SAHC, *S*-adenosylhomocyteine; SR, a series of reactions for conversion of glutaryl-CoA to acetyl-CoA; and TAT, tryptophan aminotransferase. The following enzymes require pyridoxal phosphate for catalytic activities: HTD, HKTA, KNA, KTA, and TAT. QPRT is inhibited by high concentrations of leucine.

converted into arginine locally because of high ASS and ASL activities, and only a small amount of citrulline is released from the intestine (Wu and Morris 1998). Rates of citrulline and arginine syntheses depend on developmental stages, substrate availabilities, and intestinal activities of key enzymes such as NAG synthase, P5C synthase, and proline oxidase. However, increasing dietary intake of arginine within physiological ranges does not affect intestinal synthesis of citrulline from glutamate, glutamine, or proline in pigs or rats (Wu et al. 2016) or renal conversion of citrulline into arginine (Dhanakoti et al. 1992). In the mammals that lack P5C synthase in the small-intestinal mucosa and do not synthesize citrulline from glutamate, glutamine, or proline, oral or intravenously administered citrulline is effectively used for arginine synthesis in vivo.

Renal Glutamine Utilization for Regulation of Acid-Base Balance

Through renal ammoniagenesis, glutamine plays an important role in regulating acid-base balance by producing NH_3 (ammonia), which then combines with H^+ (proton) to form NH_4^+ for excretion in the urine, as noted previously. Under acidotic conditions, which occur during intensive exercise, early lactation, and late pregnancy, and in animals with type I diabetes mellitus, renal utilization of glutamine is markedly increased to meet the need for ammonia production and therefore the removal of H^+ (Xue et al. 2010). The sources of glutamine for renal uptake are mainly the skeletal muscle, WAT and liver. In acidotic animals, suppression of glutamine degradation in the small intestine and immunocytes also increases the availability of glutamine in the blood for extraction by the kidneys.

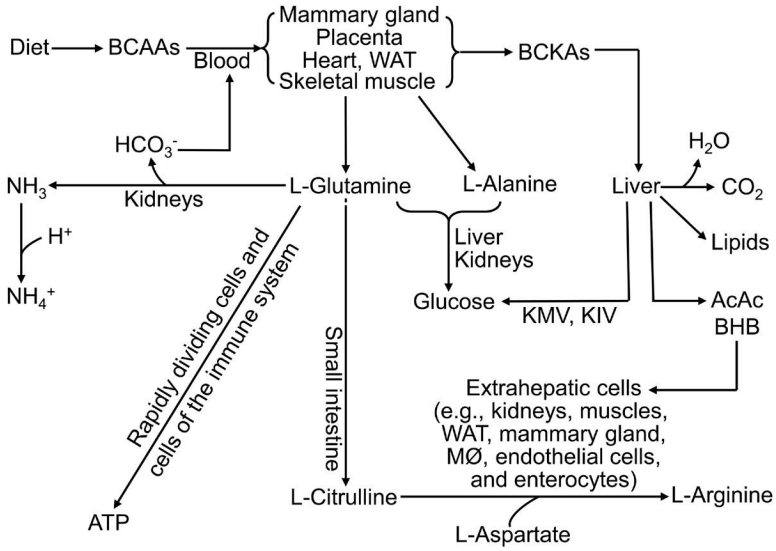


FIGURE 7.25 Interorgan metabolism of branched-chain amino acids (BCAAs) in animals. Dietary BCAAs are absorbed into the blood, largely bypass the liver, and are catabolized in skeletal muscle to synthesize glutamine and alanine. The transamination of BCAAs in the muscle also results in the release of branched-chain α-ketoacids (BCKAs). Glutamine and alanine are taken up by the liver and kidneys for glucose synthesis. Glutamine is a major metabolic fuel in enterocytes and immunocytes, a major substrate for citrulline synthesis in the small intestine of most mammals, and a major regulator of acid-base balance through renal ammoniagenesis and HCO_3^- absorption. Arginine is synthesized from citrulline in all cell types. BCKAs released from the skeletal muscle are taken up by the liver, where they are either oxidized or converted into glucose, ketone bodies, and lipids. AcAc, acetoacetate; BHB, β-hydroxybutyrate; KIV, α-ketoisovalerate; KMV, α-keto-β-methylvalerate; MØ, macrophages.

Glutamine and Alanine Synthesis from BCAAs

In mammals, most dietary glutamine does not enter the portal vein. Therefore, the circulating glutamine must be derived primarily from de novo synthesis. Dietary BCAAs absorbed from the small intestine enter the portal vein and largely bypass the liver due to the near absence of hepatic BCAA catabolism. BCAAs in the blood are taken up by extrahepatic tissues, where these amino acids are transaminated with α-KG by cytosolic and mitochondrial BCAA transaminases to form glutamate and BCKAs. Thus, the amino group of glutamate is derived from BCAAs. Glutamate is either amidated with ammonia by GS to form glutamine or transaminated with pyruvate and α-KG to yield alanine and aspartate, respectively (Figure 7.25). Glucose is the major source of the carbon skeletons of glutamine, glutamate, alanine, and aspartate. The NH_4^+ for glutamine synthesis is supplied from the blood. In skeletal muscle, an additional source of ammonia for glutamine synthesis is inosine monophosphate through the purine nucleotide cycle, particularly during exercise (Figure 7.26). During fasting, both alanine and glutamine are major precursors for the renal synthesis of glucose (Watford et al. 1980).

In mammals and birds, skeletal muscle, heart, adipose tissue, and lungs, as well as the placenta in gestating dams and the mammary gland in lactating mothers, have high BCAA transaminase activities and therefore play an important role in initiating BCAA catabolism in the body (Harper

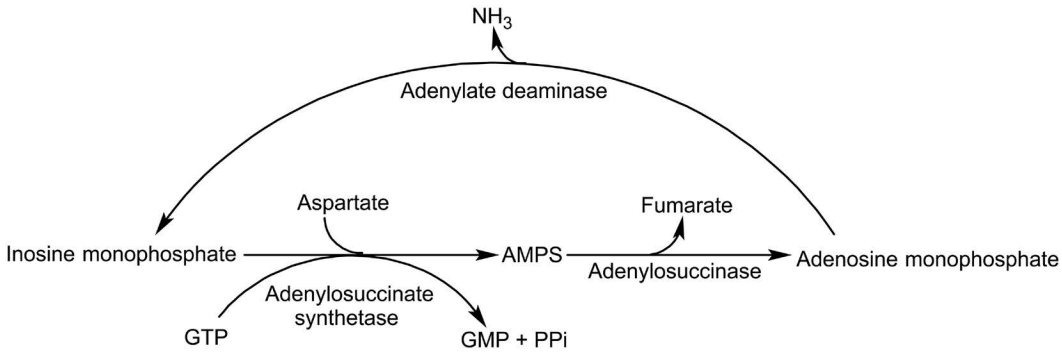


FIGURE 7.26 The purine nucleotide cycle for ammonia production in skeletal muscle. This metabolic pathway is enhanced in response to muscular contraction. AMP, adenosine monophosphate; AMPS, adenosine monophosphate succinate; GTP, guanosine triphosphate; GMP, guanosine monophosphate; and IMP, inosine monophosphate.

et al. 1984). However, these extrahepatic tissues only partially decarboxylate BCKAs due to the relatively low activity of BCKA dehydrogenase, releasing most of the BCKAs into the blood. The BCKAs are taken up primarily by the liver for (a) oxidation to CO_2 and water, (b) gluconeogenesis (except for the ketoacid of leucine), (c) ketogenesis, and (d) synthesis of lipids. At present, little is known about BCAA metabolism in various tissues of fish and crustaceans.

Endogenous synthesis of glutamine plays an important role in animal growth and development. This is because (a) glutamine is essential for the synthesis of protein (including glycoproteins), nucleic acids, amino sugars, and nicotinamide adenine dinucleotide (phosphate) [NAD(P)]; and (b) these roles of glutamine cannot be replaced by any other amino acid. For example, both glycoproteins and nucleic acids are critical for the growth and development of embryos and fetuses. This is consistent with the fact that glutamine is the most abundant amino acid in the plasma of most postnatal animals (including ruminants, rats, and chickens) and is one of the two most abundant amino acids in the plasma of pigs and sheep (with the other amino acid being glycine; Wu 2022).

The supply of alanine from diets is inadequate to meet the requirements of milk-fed mammals such as piglets. This necessitates endogenous synthesis of alanine from BCAAs by the skeletal muscle of neonates. For example, based on alanine content in sow's milk, milk meets at most 66% of the need for protein synthesis in 14-day-old piglets, which must daily synthesize at least 0.18 g alanine/kg BW (Wu 2010). Hou et al. (2016a) estimated that at least 30% of Ala in tissue proteins is provided from endogenous synthesis. In all animals, under the conditions of food deprivation or when food intake is reduced, BCAA-derived alanine makes a major contribution to glucose production by the liver and kidneys and, therefore, the whole-body homeostasis of glucose (see Chapter 5). The use of glucose for the formation of pyruvate (the alanine carbon skeleton) in skeletal muscle, the release of alanine from the muscle, and the uptake of alanine by the liver for gluconeogenesis constitute the glucose–alanine cycle in animals (Figure 7.27).

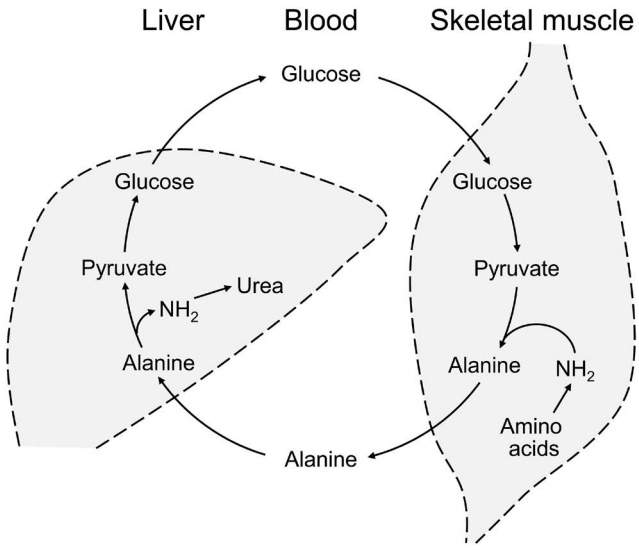


FIGURE 7.27 The glucose–alanine cycle in animals. The liver releases glucose into the blood circulation. Skeletal muscle takes up arterial glucose for generation of pyruvate, which is used for alanine synthesis via transamination. Skeletal muscle releases alanine, which is converted into urea and glucose in the liver. There is no net synthesis of glucose by the glucose–alanine cycle when the source of pyruvate in the muscle is glucose.

Conversion of Proline to Glycine through 4-Hydroxyproline

The milk of all mammals studied (including pigs, cattle, sheep, goats, and humans) is severely deficient in glycine. For example, in sow-reared piglets, milk provides at most only 23% of glycine for tissue protein synthesis and other metabolic pathways. Of interest, sow's milk contains high concentrations of peptide-bound 4-hydroxyproline (Hu et al. 2022). There is evidence that 4-hydroxyproline is converted into glycine in the kidneys, providing endogenous glycine to support neonatal growth, development, and health (Wu et al. 2013a). Through glycine synthesis, both the carbons and N of the proline molecule are effectively spared in milk-fed neonates. This pathway likely plays an important role in maintaining glycine homeostasis in animals, particularly in milk-fed young mammals. Additionally, through the formation of 4-hydroxy-L-proline and subsequently glycine and serine, proline and its precursors (e.g., arginine and glutamine) serve as methyl donors in the one-carbon metabolism of animals.

NO-Dependent Blood Flow

Utilization of dietary amino acids requires cooperation among many different organs and therefore blood flow through them. Rates of blood flow, which affect the transport of amino acids and other nutrients across tissues, are regulated by NO. This free radical is synthesized from arginine by BH_4- and NADPH-dependent NO synthase in virtually all cell types (Blachier et al. 2011). In endothelial cells and placentae, arginine stimulates BH_4 synthesis by enhancing the expression of GTP cyclohydrolase I (Wu et al. 2009). NO is a major vasodilator released by endothelial cells of blood vessels to control blood flow to tissues such as the small intestine, utero-placental unit, liver, skeletal muscle, and mammary gland. Without dietary arginine or glutamate- glutamine- and proline-derived endogenous arginine, there is insufficient blood flow and insufficient interorgan metabolism of amino acids in animals (Wu et al. 2021).

Regulation of Amino Acid Oxidation to Ammonia and CO_2

In animals, the oxidation of amino acids to ammonia and CO_2 is regulated by many factors: (a) allosteric activators or inhibitors, (b) reversible phosphorylation and dephosphorylation of enzymes, (c) concentrations of substrates

and cofactors, (d) concentrations of activators and inhibitors of enzymes, (e) signal transduction, (f) changes in cell volume, and (g) other forms of regulation of enzyme activity (Wu 2022). Some of these factors related to the oxidation of amino acid-derived pyruvate and α-KG have been discussed in Chapter 5. The following well-documented examples substantiate these principles.

First, GDH, which is a protein in the inner mitochondrial matrix, catalyzes the interconversion of glutamate ⇌ α-KG + NH_4^+. In the liver and possibly other tissues, the chemical equilibrium of this enzyme favors the production of ammonia from glutamate under physiological conditions. GDH is allosterically activated by ADP and leucine but inhibited by GTP and ATP (Li et al. 2012). This has nutritional and physiological importance. For example, when extracellular concentrations of leucine are elevated through high protein intake or leucine supplementation, GDH in pancreatic β-cells is activated to enhance energy metabolism, ATP-sensitive K^+ channels, and membrane depolarization, leading to an increase in insulin release. Likewise, mutations in GDH that abrogate allosteric GTP inhibition result in high levels of insulin and ammonia in plasma (Li et al. 2012). Therefore, allosteric regulation of GDH plays an important role in insulin homeostasis and whole-body nutrient metabolism.

Second, some enzymes for amino acid metabolism are modified by protein phosphorylation and dephosphorylation. Whether phosphorylation of an enzyme increases or decreases its catalytic activity varies with individual enzymes. For example, BCKA dehydrogenase is inactivated by phosphorylation and activated by dephosphorylation. This enzyme is present mainly in the phosphorylated (inactive) form in the heart and skeletal muscle but in the dephosphorylated (active) form in the liver and kidneys (Brosnan and Brosnan 2006). Consumption of a high-protein diet by animals increases BCKA dehydrogenase activity in the heart and kidneys by increasing the conversion of the enzyme from the phosphorylated into the dephosphorylated form. Similarly, cortisol and glucagon enhance BCAA degradation in bovine mammary epithelial cells, whereas insulin and growth hormone stimulate the phosphorylation of BCKA dehydrogenase in the cells to reduce BCAA oxidative decarboxylation (Flynn et al. 2009; Lei et al. 2013). In contrast to BCKA dehydrogenase, the activities of phenylalanine hydroxylase, tryptophan hydroxylase, tyrosine hydroxylase, and tyrosine aminotransferase are enhanced by protein phosphorylation but inhibited by protein dephosphorylation. This kind of metabolic control does not involve a change in the amount of the enzyme protein but allows for rapid changes (within seconds or minutes) in product formation in a tissue- and substrate-specific manner.

Third, concentrations of substrates and cofactors play important roles in regulating amino acid metabolism, as illustrated by BCAA catabolism in skeletal muscle exposed to ketone bodies. Both acetoacetate and β-hydroxybutyrate inhibit BCAA transamination by suppressing the conversion of glucose into pyruvate and then α-KG (Wu and Thompson 1988). Conversely, the inhibitory effect of ketone bodies on BCAA transamination is prevented by the addition of pyruvate, which generates α-KG. Ketone bodies also inhibit the oxidative decarboxylation of BCKA in extrahepatic tissues (including skeletal muscle, mammary tissue, kidneys, and the small intestine) in the absence or presence of pyruvate. The oxidation of acetoacetate and β-hydroxybutyrate to CO_2 and water in these tissues requires both CoA-SH and NAD^+, thereby decreasing the availability of these cofactors for BCKA dehydrogenase. Thus, competition for the cofactors of enzymes is a major mechanism responsible for the effect of ketone bodies on inhibiting BCAA catabolism in skeletal muscle.

Fourth, the availability of activators and inhibitors can affect metabolic fluxes, respectively. For example, chronic high concentrations of lactate (e.g., 10–20 mM) in plasma are associated with severe hypocitrullinemia and hypoargininemia but hyperprolinemia in infants. Kinetics analysis of enzymatic activity revealed noncompetitive inhibition of enterocyte proline oxidase by lactate (decreased maximal velocity and unaltered K_M; Dillon et al. 1999). This provides a biochemical basis for the treatment of lactate-induced hyperammonemia in neonates. Another example of enzyme inhibition is the competitive inhibition (Ki = 1.0–1.6 μM) of all NOS isoforms by N^G-monomethy-L-arginine (NMMA) and asymmetric dimethylarginine (ADMA). The concentrations of these methylarginines in plasma are elevated in patients with obesity, diabetes, cardiovascular disease, and renal dysfunction. Reductions in NMMA and ADMA can improve their metabolic profiles and health.

Fifth, signal transduction in cells, which is brought about by their responses to extracellular chemicals (including nutrients, hormones, cytokines, drugs, toxins, or phytochemicals), can regulate amino acid catabolism in animals. For example, glucagon stimulates glycine oxidation in the mitochondria of hepatocytes (Jois et al. 1989), whereas cortisol promotes the catabolism of arginine, glutamine, ornithine, and proline in enterocytes (Flynn et al. 2009). The responses of amino acid degradation to cell signaling may be cell-specific, as cAMP increases the synthesis of tyrosine aminotransferase in the liver and kidneys but not in the small intestine or skeletal muscle. Defects in signal transduction can cause (a) impairments in growth, development, and homeostasis; (b) increased susceptibility to infectious diseases; and (c) obesity, diabetes, cardiovascular disorders, DNA mutation, cancer, and other diseases.

Sixth, changes in cell volume occur in response to alterations in uptake of amino acids or extracellular osmolarity. For example, upon ingestion of a meal and fluids, amino acids, glucose, ions, and water are transported into the cell, leading to cell swelling (Haüssinger 1990). Conversely, a loss of cellular water in dehydration leads to cell shrinkage. The maintenance of adequate cell volume is a major prerequisite for cell survival, growth, and development. Cell swelling induced by amino acids or hypotonicity stimulates glutamine degradation, but cell shrinkage increases

the net synthesis of amino acids in the perfused rat liver (Lang et al. 1998). Furthermore, cell swelling decreases, but cell shrinkage increases, BCAA catabolism as well as the release of glutamine and alanine from the rat skeletal muscle (Parry-Billings et al. 1991). Finally, an increase in cell volume (as occurring in response to immunological challenges) promotes glutamine catabolism in both lymphocytes and macrophages (Wu and Flynn 1995). This may have physiological and immunological importance, as the volume of immunocytes increases in response to mitogenic stimulation and immunological activation.

Detoxification of Ammonia as Urea Via the Urea Cycle in Mammals and Some Fish

The Urea Cycle for Disposal of Ammonia in Mammals and Some Fish

Oxidation of amino acids in all animals produces ammonia. In aqueous solution, free ammonia is at equilibrium with the ammonium ion. In physiological fluids of terrestrial animals, 1.6% and 98.4% of ammonia exist as free NH_3 and NH_4^+, respectively, at blood pH 7.4 and 37°C (e.g., in pigs), and the corresponding values are 2.0% and 98.0%, respectively, at blood pH 7.4 and 40°C (e.g., in chickens). Here, free NH_3 and NH_4^+ are collectively referred to as ammonia.

$$NH_3 + H^+ \rightleftharpoons NH_4^+ \left(NH_3 = 1.6\%;\ NH_4^+ = 98.4\%;\ pK_a \text{of } NH_4^+ = 9.2 \text{ at pH 7.4 and } 37°C\right)$$

$$NH_3 + H^+ \rightleftharpoons NH_4^+ \left(NH_3 = 2.0\%;\ NH_4^+ = 98.0\%;\ pK_a \text{of } NH_4^+ = 9.1 \text{ at pH 7.4 and } 40°C\right)$$

Physiological concentrations of ammonia are essential to life because they participate in many reactions to (a) synthesize carbamoylphosphate in the cytosol by CPS-II, (b) bridge amino acid metabolism with glucose metabolism, and (c) regulate acid-base balance in the kidneys. However, excess ammonia in blood is particularly toxic to the brain, as well as embryos and fetuses, and must therefore be removed through the formation of urea (ureagenesis) primarily via the urea cycle (Figure 7.28). The last step of the urea cycle (the hydrolysis of arginine) is catalyzed by arginase. This enzyme uses both L-arginine and L-homoarginine as substrates.

$$\text{L-Arginine} + H_2O \rightarrow \text{Urea} + \text{L-Ornithine} \quad (\text{Liver arginase})$$

$$\text{L-Homoarginine} + H_2O \rightarrow \text{Urea} + \text{L-Lysine} \quad (\text{Liver arginase})$$

In mammals, the conversion of extracellular (blood) and intracellularly generated ammonia into urea occurs primarily in hepatocytes (Meijer et al. 1990) and, to a much lesser extent, in post-weaning enterocytes (Wu 1995). In growing pigs, nearly all (95%) of the urea-N released from the liver is derived from the hepatic uptake of ammonia from the portal vein and the hepatic artery, as noted previously. This indicates a low rate of hepatic amino acid degradation in the fed state under physiological conditions. Interestingly, urea is synthesized from glutamine and bicarbonate in the liver and/or skeletal muscle of certain teleost fish (e.g., the Lake Magadi tilapia, the marine gulf toadfish, and Indian freshwater air-breathing teleost), as well as marine and freshwater elasmobranchs (e.g., sharks, rays, and skates; Anderson 2001; Saha and Ratha 2007). Note that in both mammals and fish, the aspartate used for the reaction of ASS is ultimately derived from ammonia and OAA via GDH and glutamate-OAA transaminase (Wu 2022).

Urea, which is highly water-soluble and nontoxic, is a desirable form to dispose of ammonia in mammals, which periodically excrete water as the major component of urine. Urea elimination via the kidneys involves (a) glomerular filtration, (b) reabsorption in the proximal renal tubules into blood through facilitated or carrier-mediated transporters, and (c) the movement of the remaining urea through the collecting duct as part of urine.

Energy Requirement of Urea Synthesis

Urea synthesis (a) starts with NH_3 (rather than NH_4^+) and HCO_3^- (rather than CO_2) as the substrates for carbamoylphosphate synthase-I, (b) involves the cytosol and mitochondria, as well as substrate channeling in both compartments, and (c) requires 6.5 mol of ATP per 2 mol of ammonia. Specifically, the formation of 1 mol of urea from its substrates (i.e., 1 mol each of ammonia, aspartate, and HCO_3^-) requires 4 mol of ATP for urea-cycle reactions. In addition, 2.5 mol of ATP is required for the incorporation of 1 mol of ammonia into 1 mol of aspartate via the following reactions.

$$NH_4^+ + \alpha\text{-Ketoglutarate} + \text{NADH} + H^+ \rightarrow \text{Glutamate} + NAD^+ \left(\text{equivalent to 2.5 mol of ATP}\right)$$

$$\text{Glutamate} + \text{Oxaloacetate} \rightleftharpoons \alpha\text{-Ketoglutarate} + \text{Aspartate}$$

Because the detoxification of protein-derived ammonia as urea consumes approximately 10–15% of ATP produced from the oxidation of amino acids, optimizing the quality and quantity of dietary amino acid requirements by mammals can reduce urea production, thereby improving the efficiency of protein and energy utilization. When the rate of formation of carbamoylphosphate from ammonia, CO_2, and ATP exceeds the rate of conversion of carbamoylphosphate plus ornithine into citrulline in mitochondria, carbamoylphosphate exits into the cytoplasm for synthesis of orotate (Wu 2022). This can occur when there is (a) a deficiency of ornithine carbamoyltransferase or arginine, (b) excess intake of dietary protein, or (c) a high concentration of ammonia in plasma.

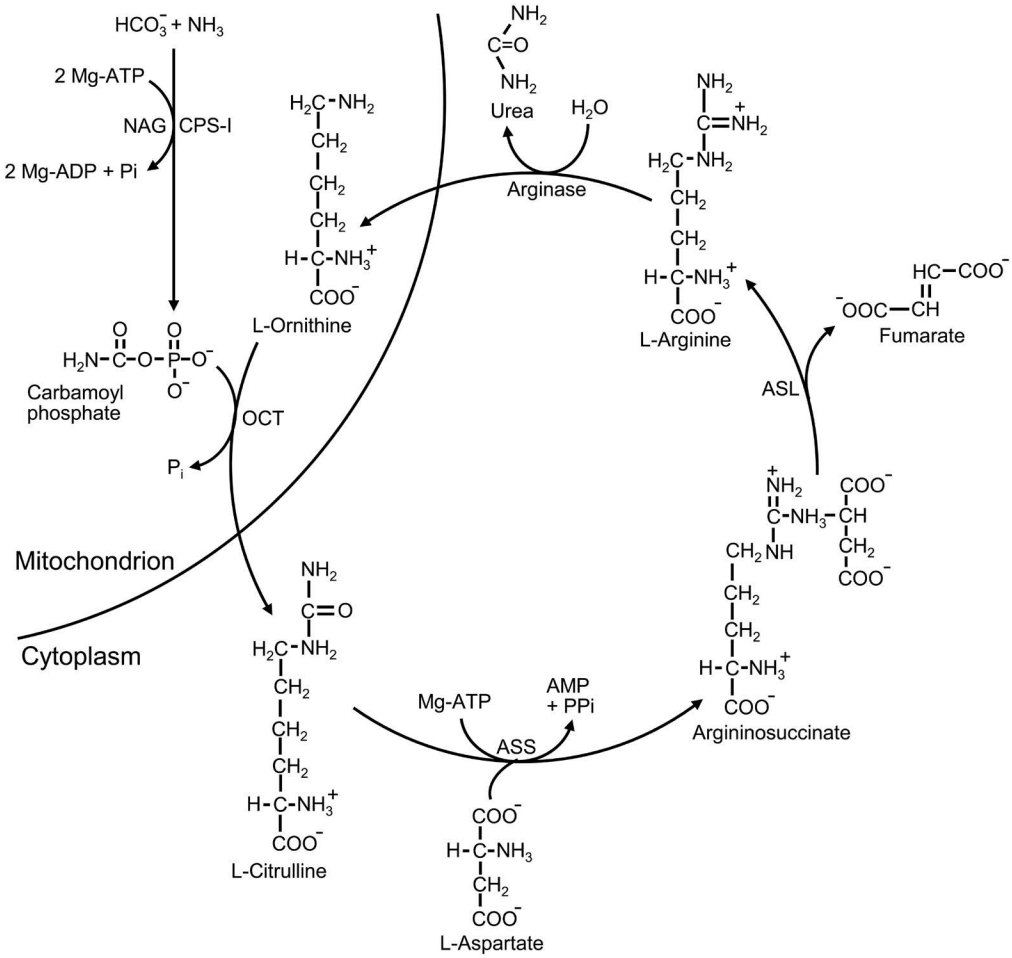


FIGURE 7.28 Synthesis of urea from ammonia and bicarbonate in mammals. This is the major metabolic pathway for ammonia detoxification in mammalian species. The synthesis of urea from ammonia and bicarbonate involves both the mitochondria and the cytoplasm. Citrulline exits the mitochondrion into the cytoplasm, where it is converted into arginine, which is rapidly hydrolyzed by arginase into urea plus ornithine. Ornithine is then reused for another turnover of the cycle. ASL, argininosuccinate lyase; ASS, argininosuccinate synthase; CPS-I, carbamoylphosphate synthase-I; and NAG, *N*-acetylglutamate.

Regulation of the Urea Cycle

The activity of the urea cycle is controlled primarily by the availability of substrates (e.g., amino acid and ammonia), cofactors (e.g., Mn^{2+} and Mg^{2+}), and arginine or ornithine in plasma. Thus, in animals with low protein intake, hepatic urea synthesis is reduced to conserve both energy and N. Conversely, increasing dietary intake of protein beyond the optimal amino acid requirements of the animal progressively increases ureagenesis in the mammalian liver. Second, changes in the activities of urea cycle enzymes also influence hepatic ureagenesis. For example, the expression and activities of hepatic urea cycle enzymes are enhanced by high intake of dietary protein and amino acids to facilitate the removal of amino acid-derived ammonia from the body. In contrast, hepatic expression of urea cycle enzymes is markedly reduced in response to low intake of dietary protein or amino acids as an adaptation mechanism. Thus, sudden consumption of a large amount of dietary protein immediately after chronic protein malnutrition can result in hyperammonemia and even death. Likewise, in mammalian neonates with low activities of one or more urea cycle enzymes, high protein intake may exceed the ability of the liver to remove ammonia and cause death. This may be more severe for IUGR or low-birth-weight neonates than for normal-birth-weight neonates. Carbamoyl phosphate synthetase I (CPS-I) and ASS are two key regulatory enzymes in hepatic urea synthesis. Third, the urea cycle is regulated by the allosteric activators such as arginine (on NAG synthase) and NAG (on CPS-I). Thus, when arginine is deficient, ammonia exits mitochondria into the cytoplasm, where ammonia is utilized for the synthesis of purines and then orotate and uric acid. High levels of orotate and uric acid potentially result in fatty liver and gout, respectively. Hyperammonemia due to a deficiency of arginine is a common factor for deaths in neonatal mammals with low birth weights (including those with IUGR and preterm infants). Importantly, increasing exogenous arginine provision can save the lives of these compromised neonates. For example, the oral administration of 0.2, 0.4, and 0.8 g arginine (as arginine-HCl)/kg BW/day beginning immediately after

birth increased the survival rates of sow-reared IUGR piglets from 50% in the isonitrogenous control group to 75%, 89%, and 89%, respectively (Long et al. 2025). Fourth, reducing extracellular pH reduces hepatic ureagenesis by inhibiting amino acid transport. Thus, in metabolic acidosis, glutamine is channeled to the kidneys for ammoniagenesis. In contrast, increasing extracellular pH from 7.4 to 7.6 enhances glutaminase activity and urea synthesis in the liver.

Finally, urea synthesis is also regulated by some hormones (Morris 2002). For example, growth hormone reduces the expression and activities of CPS-I, ASS, ASL, arginase, and glutaminase in the liver, thereby contributing to the conservation of amino acids for protein synthesis. Growth hormone and insulin reduce the concentrations of amino acids in plasma by increasing net protein synthesis in skeletal muscle, thereby reducing the availability of the amino acid substrates for urea synthesis (Bush et al. 2002). In contrast, high levels of glucocorticoids upregulate expression of amino acid-degrading enzymes and of urea cycle enzymes through a cAMP-dependent mechanism, thereby promoting the production of ammonia and urea under catabolic conditions. At present, little is known about the hormonal regulation of the urea cycle in enterocytes of the small intestine.

Detoxification of Ammonia as Uric Acid in Birds

Uric Acid Synthesis for Disposal of Ammonia in Birds

Uric acid is a weak organic acid with a pK_a of 5.75. At physiological pH values, uric acid is present primarily as monosodium urate (the chemically stable form of uric acid). In birds, uric acid is synthesized from ammonia as the major product of amino acid catabolism, with the liver being the most active site, followed by the kidneys and pancreas (Figure 7.29). This is true for some animals (e.g., lizards and snakes) and perhaps most species of fish, which lack the urea cycle (Singer 2003). Uric acid synthesis also exists in the livers and other tissues of mammals. Like the urea cycle in mammals, the synthesis of uric acid also starts with ammonia and HCO_3^- and requires mitochondria and the cytosol. Specifically, ammonia is first incorporated into glutamine (mitochondria), aspartate (mitochondria and cytosol), and glycine (mitochondria and cytosol). These amino acids then react with HCO_3^- and N^{10}-formyltetrahydrofolate in the cytosol to form purines (adenosine and guanosine) via a series of enzyme-catalyzed steps. Finally, adenosine and guanosine are oxidized to xanthine and then uric acid in the cytosol. In many mammalian species (excluding primates) and various insects, uric acid can be oxidized by uricase to allantoin.

Energy Requirement of Uric Acid Synthesis

The ATP requirements for uric acid synthesis are determined by three events: (a) formation of adenosine or guanosine from glutamine, aspartate, glycine, ribose-5-phosphate, bicarbonate, and N^{10}-formyltetrahydrofolate; (b) the incorporation of ammonia into glutamine, aspartate, and glycine; and (c) formation of N^{10}-formyl-tetrahydrofolate from formate and tetrahydrofolate. One mole of ammonia is generated from 1 mol of adenosine by adenosine deaminase in the metabolic pathway for uric acid synthesis from adenosine. This ammonia molecule subsequently participates in another round of purine and uric acid synthesis. The incorporation of 4 mol of NH_4^+ into 1 mol of uric acid requires 19.5 mol of ATP if adenosine is an intermediate or 18.5 mol of ATP if guanosine is an intermediate (Wu 2022). Assuming that equal amounts of ammonia are converted into uric acid through the adenosine and guanosine pathways, the entire process for uric acid synthesis from 4 mol of NH_4^+ requires, on average, 19 mol of ATP.

Despite the high energy requirement for uric acid synthesis, this pathway is of physiological importance for birds. First, uric acid synthesis in birds contributes to their higher body temperature (e.g., 40°C in chickens and ducks) than in mammals (e.g., 37°C in humans and pigs). Second, uric acid is relatively insoluble in water (solubility = 0.2 mM in plasma) and is excreted as a concentrated salt, therefore allowing birds to conserve water and maintain a low BW during long-distance flights. Second, at physiological concentrations, uric acid is a scavenger of oxygen free radicals and can protect cells and tissues from oxidative damage. This may counteract any potential adverse effects of hyperglycemia (e.g., 12–15 mM in plasma) occurring in birds under normal feeding conditions.

Species Differences in Uric Acid Degradation

There are species differences in uric acid degradation (Table 7.20). In the livers of most mammals (e.g., cattle, dogs, horses, mice, pigs, rabbits, sheep, rats), except for humans

TABLE 7.20
Species Differences in the Excretion of Major Nitrogenous Metabolites by Animals

Metabolite	Animal Species
Urea	Mammals, terrestrial amphibians, teleost fish, elasmobranchii (sharks, rays, and skates), some aquatic reptiles, and some aquatic turtles (chelonians; but absent from most of them)
Uric acid	Birds, land reptiles (including squamate *reptiles such as* lizards and snakes; lacking the hepatic urea cycle), Dalmatian dogs, mollusks, some terrestrial crustaceans, and various insects
Allantoin	Most mammals except for primates, earthworms, lizards, aquatic turtles (chelonians), and various insects
Allantoic acid	Various insects
Ammonia	Aquatic amphibians, most fish, aquatic turtles (chelonians), earthworms, crustaceans, dipnoans, and some insects

Source: Wu (2022).

and higher primates, uric acid is oxidized to allantoin (a diureide of glyoxylic acid) by the copper-containing uricase (urate oxidase) in peroxisomes (Singer 2003). Allantoin-producing mammals do not express allantoinase or allantoicase and therefore contain allantoin in plasma and release this purine metabolite in urine. The conversion of uric acid to allantoin also occurs in the kidneys of amphibia. In contrast, uricase is absent from the tissues of humans and other primates, and therefore they excrete uric acid in urine as the end product of purine catabolism. Likewise, uricotelic species (e.g., chickens, ducks, and geese) do not express uricase and therefore excrete large amounts of uric acid in urine as the major end product of amino acid catabolism. In the liver and kidneys of amphibia and teleost fish, allantoin is hydrolyzed by allantoinase (a mitochondrial enzyme in amphibia but a cytosolic and peroxisomal enzyme in fish) to form allantoic acid, which is further hydrolyzed by allantoicase (a peroxisomal enzyme) to urea and glyoxylic acid. Uric acid and its metabolites are excreted by the kidneys through urate/anion exchangers.

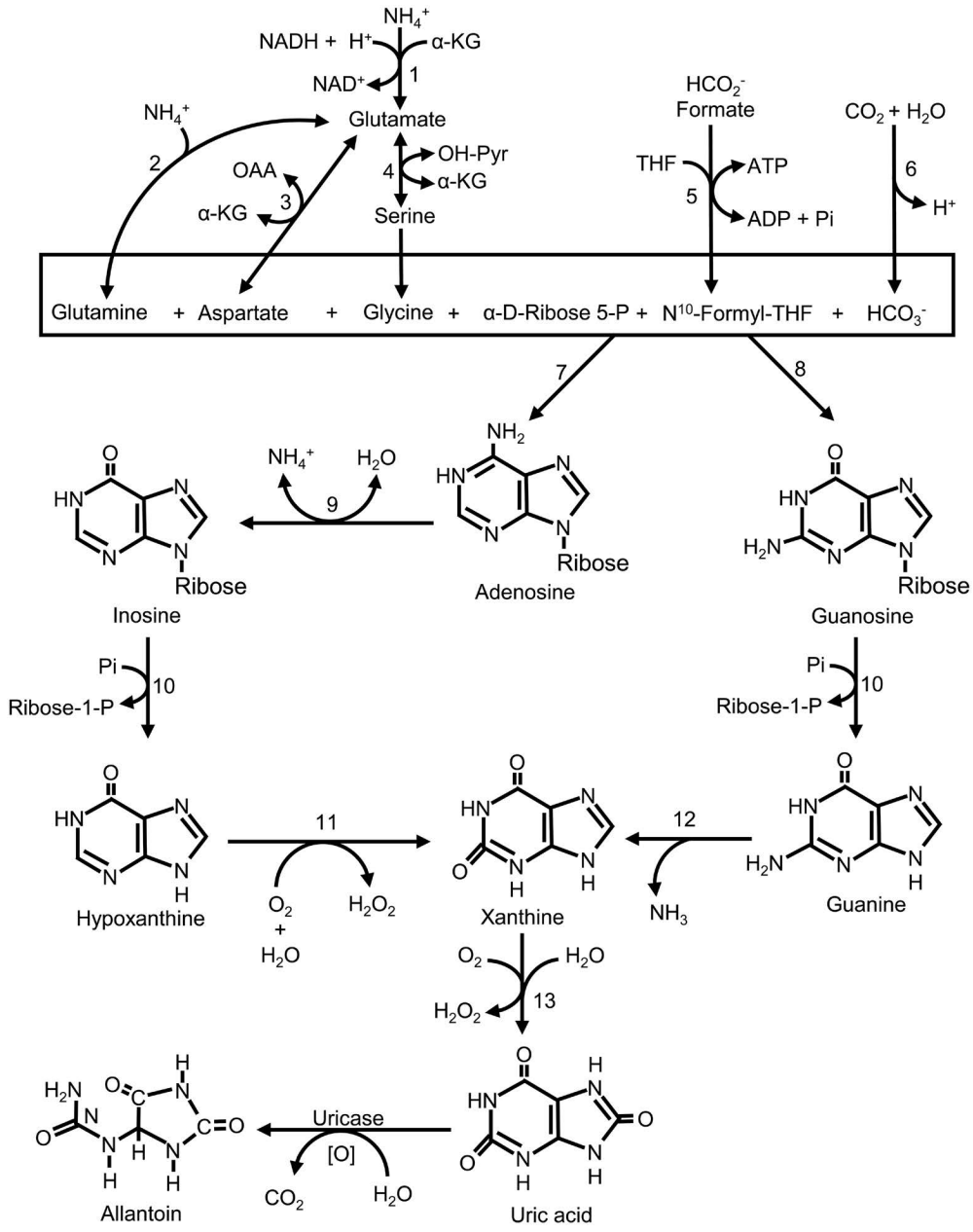


FIGURE 7.29 Synthesis of uric acid from ammonia and bicarbonate in birds and mammals. This is the major metabolic pathway for ammonia detoxification in birds. The enzymes that catalyze the indicated pathways are (1) glutamate dehydrogenase, (2) glutamine synthetase, (3) glutamate-oxaloacetate transaminase, (4) glutamate-hydroxypyruvate transaminase, (5) N^{10}-formyl-tetrahydrofolate synthetase, (6) carbonic anhydrase, (7) a series of enzymes for adenosine synthesis, (8) a series of enzymes for guanosine synthesis, (9) adenosine deaminase, (10) purine nucleoside phosphorylase, (11) xanthine oxidase, (12) guanine deaminase (guanase), and (13) xanthine oxidase.

Regulation of Uric Acid Synthesis

Uric acid synthesis is regulated by the same nutritional and hormonal factors as noted for urea synthesis. In addition, the conversion of ammonia and bicarbonate into adenosine and guanosine is affected by the concentration of tetrahydrofolate as well as the activities of adenosine deaminase, purine nucleoside phosphorylase, and xanthine oxidase/xanthine dehydrogenase in cells. Thus, one-carbon-unit metabolism modulates the production of uric acid via the synthesis of purine nucleosides. Of note, N^{10}-formyltetrahydrofolate synthetase is a folate enzyme that catalyzes the formylation of tetrahydrofolate in an ATP-dependent manner. This enzyme is part of a trifunctional protein called C_1-tetrahydrofolate synthase. In addition to its N^{10}-formyltetrahydrofolate synthetase activity, C_1-tetrahydrofolate synthase also contains both $N^{5,10}$-methenyltetrahydrofolate cyclohydrolase and $N^{5,10}$-methylenetetrahydrofolate dehydrogenase activities. Cytokines (e.g., tumor necrosis factor-α, interferon-γ, interleukin-1, and interleukin-6) and glucocorticoids all increase expression of xanthine dehydrogenase/xanthine oxidase in many cell types, thereby stimulating uric acid production under inflammatory and stressful conditions.

Comparison between Urea and Uric Acid Synthesis

Different animal species develop different strategies for ammonia disposal to meet their metabolic and physiological needs. Urea and uric acid syntheses share some common features: (a) the use of the same raw materials (ammonia, aspartate, bicarbonate, and ATP); (b) the formation of a neutral amino acid (citrulline vs. glutamine) in the mitochondria and a basic substance (arginine vs. xanthine) in the cytosol as intermediates; and (c) the production of a neutral end product (urea vs. sodium urate at physiological pH; Figure 7.30). However, there are many differences between uric acid and urea syntheses: (a) a requirement of 46% more ATP to detoxify 1 mol of ammonia via uric acid synthesis than through urea synthesis; (b) amino acids and purines, respectively, as intermediates in urea and uric acid syntheses; (c) the participation of bicarbonate in mitochondrial and cytosolic reactions, respectively, for urea and uric acid syntheses; (d) the need of tetrahydrofolate in uric acid, but not urea, synthesis; (e) the use of glycine as a direct substrate for uric acid synthesis, but only as a source of ammonia in urea synthesis; (f) the occurrence of urea synthesis from ammonia only in mammals and a limited species of fish, but the presence of uric acid synthesis in all animal species; and (g) ureagenesis being cell-specific but uric acid synthesis taking place in virtually all cells.

Species-Specific Degradation of Amino Acids and Polyamine Synthesis

Different animal species exhibit different pathways for the metabolism of some amino acids, possibly as an adaptation to diets and living environments, as well as physiological and anatomical changes during evolution (Davis and Austic 1997; Wright 1995; Wu 2022). Let us use arginine metabolism in sheep, pigs, chickens, and fish to illustrate this notion.

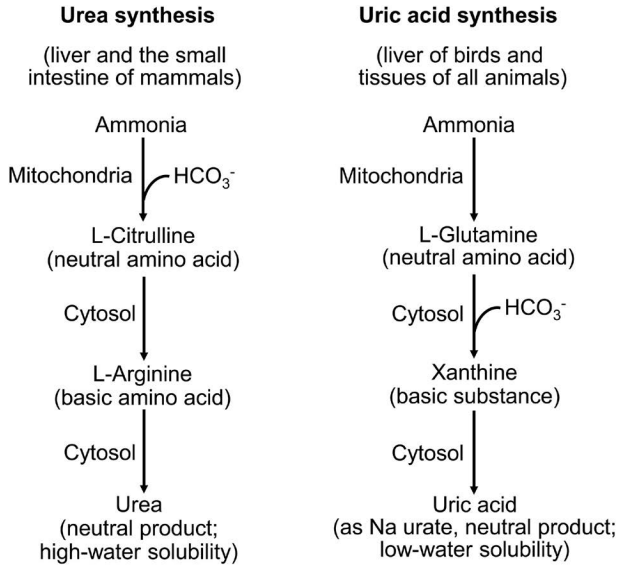


FIGURE 7.30 Comparison between urea and uric acid syntheses in animals. These two pathways span both the mitochondria and the cytosol, yielding nitrogen-rich intermediary metabolites and end products (urea and uric acid). Urea is highly soluble in water, but uric acid has a limited solubility in water at physiological pH.

Ovine placentomes express high arginase activity to hydrolyze arginine into ornithine and urea, so that ornithine is utilized for the synthesis of proline (present at relatively low content in forages) and polyamines (Kwon et al. 2003). In ruminants, urea can be recycled for amino acid and protein syntheses by ruminal bacteria. Thus, the presence of arginase in the placentomes of ruminants would not result in an irreversible loss of arginine N. Sheep can also utilize arginine and agmatine (a product of arginine decarboxylation by arginine decarboxylase in microbes and ovine conceptuses) to form polyamines via agmatinase (Figure 7.31). To further spare arginine, gestating sheep store a large amount of citrulline (e.g., 10 mM on day 60 of gestation) and a smaller quantity of arginine (e.g., 2 mM on day 60 of gestation) in allantoic fluid. Because citrulline is readily converted into arginine in fetal extrahepatic tissues, citrulline in the allantoic fluid serves as a reservoir of arginine in ovine fetuses. Additionally, in ovine placentomes and conceptuses, an alternative and physiologically significant source of polyamines is the decarboxylation of arginine to agmatine by arginine decarboxylase, followed by the hydrolysis of agmatine to putrescine (Wang et al. 2014c).

Pigs exhibit a very different picture of citrulline and arginine metabolism than sheep during the entire pregnancy (Wu et al. 2013a,b). Specifically, the porcine placenta does not contain arginase or arginine decarboxylase activity (Li et al. 2022; Wu et al. 2005b, 2021) and therefore effectively

transfers arginine from mother to fetus. Thus, gestating pigs have unusually high concentrations of arginine (e.g., 4–6 mM on day 40 of gestation) but only a low concentration of citrulline (e.g., 0.06–0.07 mM on day 40 of gestation) in allantoic fluid. To overcome the lack of arginase for polyamine synthesis from arginine in the pig placenta, this tissue expresses both proline oxidase and ornithine aminotransferase to convert proline into ornithine, which then serves as the substrate for ornithine decarboxylase to form putrescine (Figure 7.31). Because the extraintestinal tissues of the pig do not use urea for amino acid synthesis due to the lack of urease, the absence of arginase from its placenta helps conserve arginine, while allowing proline (a product of the catabolism of glutamate and glutamine, abundant amino acids in swine diets) to serve as the carbon skeleton for the synthesis of putrescine (Figure 7.32). To date, there is no evidence for polyamine synthesis from arginine via arginine decarboxylase and agmatinase in pigs, which is in sharp contrast to sheep (Wang et al. 2014a,b).

In contrast to mammals (e.g., pigs and sheep), the liver and small intestine of chickens lack arginase, arginine decarboxylase, and proline oxidase and therefore cannot form ornithine or polyamines from arginine or proline (Furukawa et al. 2021). Interestingly, the avian kidneys express all enzymes needed for the polyamine-synthetic pathways, readily convert arginine and proline into putrescine, spermidine, and spermine, and play a major role in the whole-body production of polyamines (Figure 7.33). Remarkably, the concentrations of putrescine, spermidine, and spermine in the plasma of chickens are about 20- to 100-fold greater than those for mammals (e.g., pigs, rats, and sheep) to support the rapid growth of broiler chickens (Furukawa et al. 2021). Thus, different animals utilize different mechanisms to regulate the homeostasis of amino acids and polyamines in the body.

LMB have particularly high arginase activities in the kidneys, intestine, and spleen, which are much greater than those in HSB (Table 7.21). The activity of arginase is relatively low in the skeletal muscles of LMB and HSB, as in terrestrial mammals and birds. Arginase, proline oxidase, and ornithine aminotransferase were mitochondrial enzymes in the LMB and HSB tissues studied. In contrast

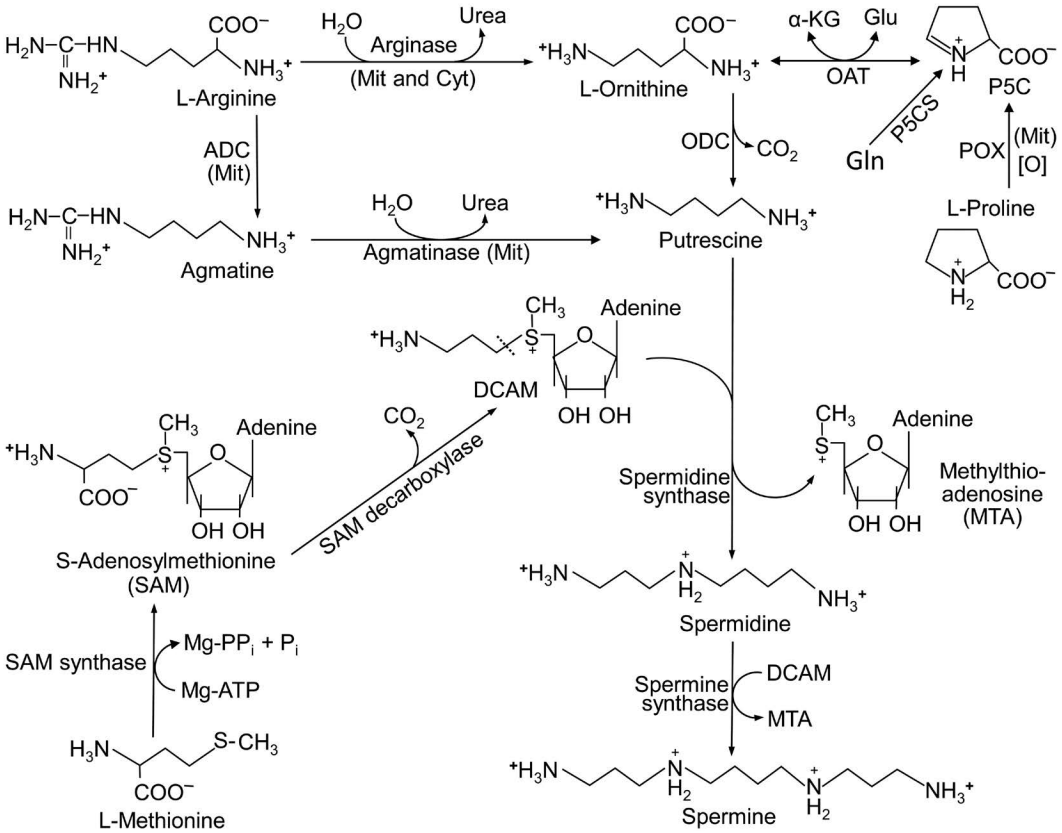


FIGURE 7.31 Synthesis of polyamines from arginine and proline in animal cells. This metabolic pathway is widespread in animals, with its activity being greater in digestive, reproductive, mammary, and malignant tissues than in other cell types. Through the formation of decarboxylated 5-adenosylmethionine (also known as *S*-adenosylmethioninamine, a product of *S*-adenosylmethionine decarboxylase), methionine donates the trimethyleneamine [$-(CH_2)_3-NH_3^+$] group for spermidine and spermine synthesis by spermidine synthase and spermine synthase, respectively. ADC, arginine decarboxylase; Cyt, cytosol; DCAM, decarboxylated 5-adenosylmethionine; α-KG, α-ketoglutarate; Mit, mitochondria; OAT, ornithine aminotransferase; ODC, ornithine decarboxylase; PAO, N^1-acetylpolyamine oxidase; P5C, pyrroline-5-carboxylate; P5CS, pyrroline-5-carboxylate synthase; POX, proline oxidase; SAM, S-adenosylmethionine; SAMD, S-adenosylmethionine decarboxylase.

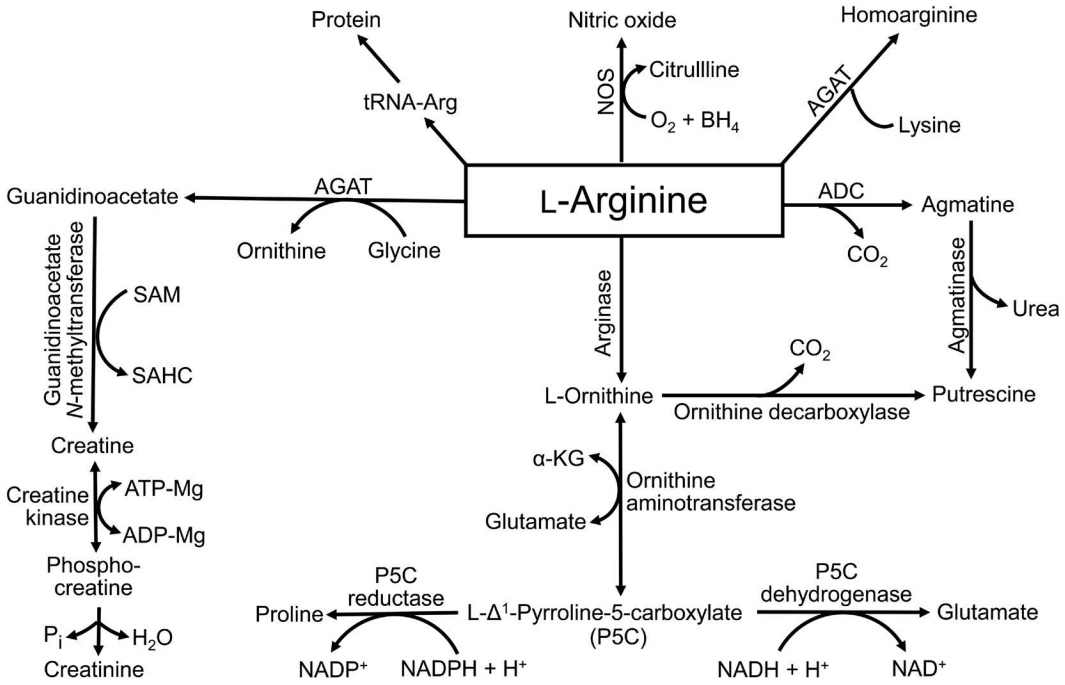


FIGURE 7.32 Arginine catabolism via multiple pathways in animals. Arginase is the major pathway to initiate arginine degradation in mammals, birds, and fish. Large amounts of creatine are synthesized in all animals. In some tissues (e.g., skeletal muscle and brain) of mammals and birds, phosphocreatine serves to store ATP. The spontaneous loss of creatine as creatinine is 1.7% per day. ADC, arginine decarboxylase; AGAT, arginine:glycine amidinotransferase; BH4, (6R)-5,6,7,8-tetrahydro-L-biopterin; α-KG, α-ketoglutarate; NOS, nitric oxide synthase; SAM, *S*-adenosylmethionine; SAHC, *S*-adenosylhomocysteine.

TABLE 7.21
Activities of Enzymes for Arginine Catabolism and Polyamine Synthesis in Largemouth Bass and Hybrid Striped Bass[a]

Variable	Location	Liver	Intestine	Kidney	Spleen	Skeletal Muscle
			Largemouth Bass			
Arginase	Mitochondria	106 ± 7.0^{c}	110 ± 5.7^{c}	149 ± 6.6^{b}	81.5 ± 3.5^{d}	2.71 ± 0.21^{e}
POX	Mitochondria	ND	ND	1.55 ± 0.09	ND	ND
OAT	Mitochondria	2.26 ± 0.21^{b}	0.50 ± 0.03^{c}	2.07 ± 0.18^{b}	0.48 ± 0.05^{c}	2.01 ± 0.16^{b}
ODC	Cytosol	2.18 ± 0.12^{b,c}	1.76 ± 0.13^{c}	2.23 ± 0.14^{b,c}	2.62 ± 0.11^{b}	1.80 ± 0.08^{c}
Agmatinase	Mitochondria	ND	ND	1.19 ± 0.08	ND	ND
			Hybrid Striped Bass			
Arginase	Mitochondria	42.6 ± 2.3^{b,†}	1.61 ± 0.17^{e,†}	13.6 ± 1.1^{c,†}	2.98 ± 0.41^{d,†}	0.31 ± 0.03^{f,†}
POX	Mitochondria	ND	ND	1.62 ± 0.10	ND	ND
OAT	Mitochondria	40.8 ± 6.8^{b,†}	4.35 ± 0.26^{d,†}	20.2 ± 2.5^{c,†}	1.92 ± 0.15^{e,†}	4.21 ± 0.30^{d,†}
ODC	Cytosol	2.03 ± 0.10^{b,c}	1.89 ± 0.08^{c}	2.14 ± 0.12^{b,c}	2.37 ± 0.13^{b}	1.76 ± 0.07^{c}
Agmatinase	Mitochondria	ND	ND	1.05 ± 0.06	ND	ND

[a] Data, expressed as nmol/mg protein/min, are means ± SEM, n = 6. Mitochondrial and cytosolic fractions were prepared from the tissues of 50-g largemouth bass (LMB) and 50-g hybrid striped bass (HSB) for the measurement of enzymatic activities, as described by Furukawa et al. (2021). Results were analyzed by one-way analysis of variance and the Student-Newman-Keuls multiple comparison (**Assaad** et al. 2014). Among all LMB and HSB tissues examined, arginase, proline oxidase (POX), ornithine aminotransferase (OAT), and agmatinase activities were absent from the cytosol, and ornithine decarboxylase (ODC) was absent from the mitochondria.

[b-f] Within a row, means not sharing the same superscript letter differ ($P < 0.05$).

[†] Different ($P < 0.05$) from the corresponding value for the LMB, as analyzed by the unpaired t-test.

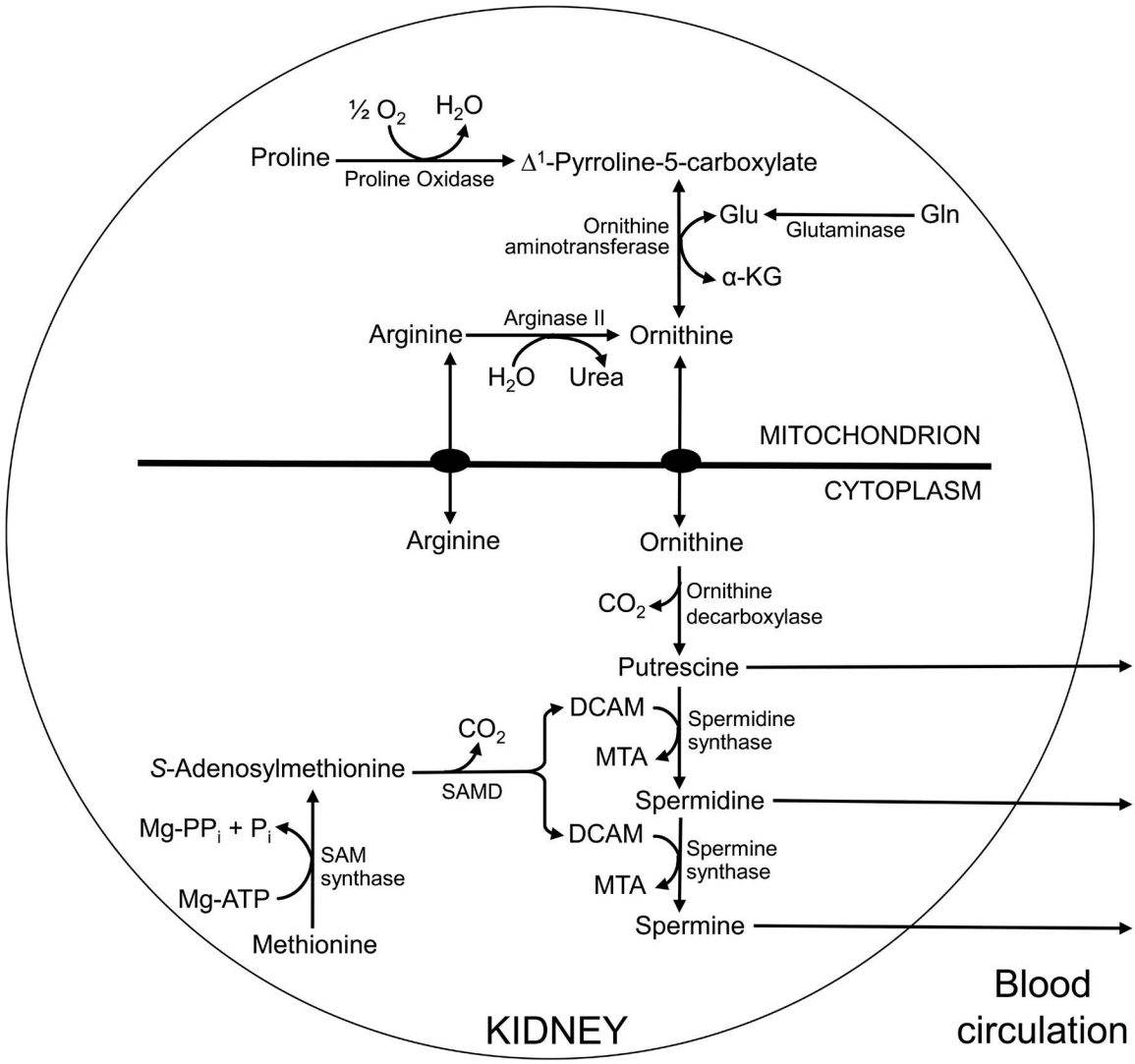


FIGURE 7.33 Synthesis of polyamines from arginine and proline in the kidneys of chickens. In renal mitochondria, arginine is hydrolyzed to ornithine plus urea by arginase II, and proline is converted into ornithine via proline oxidase and ornithine aminotransferase. The glutamate required for the ornithine aminotransferase-catalyzed reaction can be derived from glutamine. The mitochondria-derived ornithine is exported into the cytosol for the formation of putrescine by ornithine decarboxylase (ODC). The kidney releases putrescine, spermidine, and spermine into the blood. For comparison, the liver, small intestine, and skeletal muscle of chickens lack both arginase and proline oxidase; therefore, these avian tissues cannot convert arginine into polyamines. DCAM, decarboxylated 5-adenosylmethionine (also known as *S*-adenosylmethioninamine); Glu, glutamate; Gln, glutamine; α-KG, α-ketoglutarate; MTA, methylthioadenosine; SAM, *S*-adenosylmethionine; SAMD, *S*-adenosylmethionine decarboxylase. (Reproduced from Furukawa et al. 2021, with permission from Springer.)

to mammals, proline oxidase (a mitochondrial enzyme) is expressed in the kidneys but absent from the liver and intestine of both LMB and HSB. Both fish species contain agmatinase (mitochondrial enzymes) activity in the kidneys and, this enzyme is absent from their liver, intestine, skeletal muscle and spleen. Thus, arginine is a substrate for the synthesis of polyamines in all the LMB and HSB tissues studied, but proline is converted into polyamines only in their kidneys. In LBM, the unusually high activity of arginase in extrahepatic tissues (e.g., the gut, kidneys, and spleen) results in rapid arginine catabolism and therefore low circulating levels of arginine (e.g., 25–40 μM in serum; Li et al. 2021d,e), as compared with those in HSB (e.g., 140–160 μM in plasma; Li et al. 2023a; He et al. 2023b). It is possible that low arginine availability may limit growth in LMB.

Although enzymes for degrading amino acids share functional activities among animal species, the tissue-specific expression of these enzymes differs markedly between mammals and birds. This conclusion is supported by the following lines of evidence. First, glutamine is extensively degraded in enterocytes of mammals but occurs at a very low rate in enterocytes of chickens (Wu et al. 1995). This helps explain why the concentration of glutamine (~1 mM) in the plasma of chickens is twice that (~0.5 mM) in

postnatal mammals. Second, in the liver of chickens, GS is a mitochondrial enzyme and traps the mitochondrion-derived ammonia for the synthesis of glutamine, which is then used for uric acid production in the cytosol. In birds, a net synthesis of glutamine can occur in the mitochondrion of hepatocytes because these cells lack phosphate-activated glutaminase activity. In contrast, the liver of mammals contains a very high activity of phosphate-activated glutaminase, which would prevent net glutamine synthesis if GS was localized in the mitochondrion (Haüssinger 1990). Thus, the presence of GS in the cytosol of the mammalian liver makes it possible for hepatocytes to synthesize glutamine and aids in detoxifying blood-borne ammonia as a neutral amino acid. Third, as noted previously, arginase is absent from the hepatocytes of birds but is present at exceedingly high activity in the liver of mammals to initiate arginine catabolism. Fourth, intestinal ornithine aminotransferase is very abundant in pigs but is weakly expressed in chickens (Wu et al. 1995). Differences in amino acid catabolism among animal species are a metabolic basis for their different requirements of dietary amino acids (Baker 2005).

Other examples of species-specific catabolism of amino acids include (a) the limited conversion of cysteine into taurine in the feline liver and of glutamine into glutamate in chicken enterocytes, (b) the absence of arginase and proline oxidase from the chicken liver, (c) no formation of niacin from tryptophan in feline hepatocytes, and (d) no net synthesis of arginine from citrulline in the whole body of LMB. The liver of cats has all the enzymes for synthesizing niacin from tryptophan but also has a relatively high activity of picolinate decarboxylase, resulting in the diversion of 2-amino-3-carboxymuconate semialdehyde (an intermediate of the niacin-synthetic pathway) into the formation of α-ketoadipate rather than the production of nicotinate mononucleotide (Wu 2022). LMB express ASS and lyase for converting citrulline into arginine (Wang et al. 2025), but the exceedingly high activity of arginase in multiple tissues (e.g., the liver, intestine, kidneys, and skeletal muscle) rapidly hydrolyzes dietary arginine into urea plus ornithine. This is analogous to arginine degradation in the mammalian liver (Wu and Morris 1998).

Major Products of Amino Acid Catabolism in Animals

As alluded to previously, different amino acids have different catabolic pathways but share several common steps to generate some common intermediates: pyruvate, OAA, α-KG, fumarate, succinyl-CoA, and acetyl-CoA. Transamination is an important step to initiate the degradation of amino acids in animal cells, but the catabolism of many amino acids also starts with other reactions such as cleavage, deamination, decarboxylation, dioxygenation, hydrolysis, and hydroxylation. However, the catabolism of some amino acids [e.g., arginine (Figure 7.32) and lysine] is not initiated by transamination. Complete oxidation of many amino acids to CO_2, water, and ammonia requires interorgan cooperation, as noted previously for BCAA catabolism. The detailed pathways for amino acid metabolism in animals and microbes are illustrated elsewhere (Wu 2022).

The major end products of amino acid catabolism in animals are CO_2, ammonia, urea, uric acid, sulfate, creatinine, nitrite, and nitrate (Table 7.22). Other metabolites of amino acids with nutritional and physiological importance include

TABLE 7.22
Major Metabolites and Functions of Amino Acids (AAs) in Animals[a]

Amino Acid	Metabolite	Major Functions
Amino acids	Ammonia	Bridge AA and glucose metabolism; regulation of acid-base balance
	Urea	Removal of excess nitrogen from mammals
	Glucose	A major energy source for brain and red blood cells
	Ketone bodies	Energy substrates for extrahepatic tissues; signaling molecules
	Fatty acids	Membrane components; components of TAGs for energy storage
Alanine	D-Alanine	A component of peptidoglycan layer in bacterial cell walls
β-Alanine	Coenzyme A	Numerous metabolic reactions (e.g., pyruvate dehydrogenase)
	Pantothenic acid	Metabolic reactions (e.g., fatty acid synthesis)
	Antioxidant dipeptides	Carnosine (β-alanyl-L-histidine), carcinine (β-alanyl-histamine), anserine (β-alanyl-1-methyl-L-histidine), and balenine (β-alanyl-3-methyl-histidine)
Arginine	Nitric oxide	A signaling molecule; a major vasodilator; a neurotransmitter
	Agmatine	Inhibition of NOS; synthesis of putrescine in ruminant conceptuses
	Ornithine	Urea cycle; syntheses of proline, glutamate and polyamines (PA)
	Methylarginines	Competitive inhibition of NOS
Aspartate	Arginine	See above (Citrulline + Aspartate → → Arginine)
	D-Aspartate	Activation of NMDA receptors in brain
Cysteine	Taurine	Antioxidant; regulation of cellular redox state; osmolyte
	H_2S	A signaling molecule; a major vasodilator; a neurotransmitter

(*Continued*)

TABLE 7.22 (*Continued*)
Major Metabolites and Functions of Amino Acids (AAs) in Animals[a]

Amino Acid	Metabolite	Major Functions
Glutamate	NAG	Urea cycle; intestinal arginine synthesis
	γ-Aminobutyrate	Inhibitory or excitatory neurotransmitter in the mature or developing brain, respectively
Glutamine	Glu and Asp	Excitatory neurotransmitters; the malate shuttle; cell metabolism
	Glucosamine-6-P	Synthesis of amino sugars and glycoproteins; cell growth and development
	Ammonia	Renal regulation of acid-base balance
Glycine	Heme	Hemoproteins (e.g., hemoglobin, myoglobin, catalase, and cytochrome c)
	Bilirubin	Natural ligand of aryl hydrocarbon receptor in the cytoplasm
Histidine	Histamine	Allergic reaction; vasodilator; gastrointestinal tract function
	Imidazole acetate	Analgesic and narcotic actions
	Urocanate	Modulation of skin immunity; protecting the skin against ultraviolet radiation
Ile, Leu, Val	Gln and Ala	See above for "Glutamine" and "Alanine"
Leucine	HMB	Regulation of immune response and skeletal-muscle protein synthesis
Lysine	Hydroxylysine	Structure and function of collagen
Methionine	Homocysteine	Oxidant; inhibition of nitric-oxide synthesis
	Betaine	Methylation of homocysteine to methionine; one-carbon unit metabolism
	Cysteine	Cellular metabolism and nutrition
	SAM	Methylation of proteins and DNA; creatine, epinephrine and PA synthesis
	Taurine	Antioxidant; osmolytes; conjugation with bile acids
	Phospholipids	Synthesis of lecithin and phosphatidylcholine cell signaling
Phenylalanine	Tyrosine	See below for "Tyrosine"
Proline	H_2O_2	Killing pathogens; a signaling molecule; an oxidant
	P5C	Cellular redox state; ornithine, citrulline, arginine and PA synthesis
OH-Pro	Glycine	See above for "Glycine"
Serine	Glycine	See above for "Glycine"
	D-Serine	Activation of NMDA receptors in brain
Threonine	Mucins	Maintaining intestinal integrity and function
Tryptophan	Serotonin	Neurotransmitter; regulation of food intake
	N-Acetylserotonin	Antioxidant
	Melatonin	Antioxidant; circadian rhythms
	Anthranilic acid	Inhibiting production of proinflammatory T-helper-1 cytokines
	Niacin	A component of NAD(H) and NADP(H)
	Indoles	Natural ligands of aryl hydrocarbon receptor; regulation of immunity
Tyrosine	Dopamine	Neurotransmitter; regulation of immune response
	EPN & NEPN	Neurotransmitters; cell metabolism
	Melanin	Antioxidant; pigmentation of skin and hair
	T3 and T4	Regulation of energy and protein metabolism
	Coenzyme Q	A component of the mitochondrial electron transport system; an antioxidant
Arg & Met	Polyamines	Gene expression; DNA and protein synthesis; cell function
Arg, Met & Gly	Creatine	Antioxidant; antiviral; antitumor; energy metabolism in muscle and brain
Cys, Glu & Gly	Glutathione	Free radical scavenger; antioxidant; cell metabolism; gene expression
Gln, Asp & Gly	Nucleic acids	Components of RNA and DNA; protein synthesis
	Uric acid	Antioxidant; detoxification of ammonia in birds
Lys & Met	Carnitine	Transport of long-chain fatty acids into mitochondria for oxidation
Ser & Met	Choline	A component of acetylcholine and phosphatidylcholine; betaine synthesis

Source: Wu (2022).

[a] Except for Gly, taurine, and β-alanine, the amino acids mentioned herein are L-isomers.

Note: BCAA, branched-chain amino acids; SAM, S-adenosylmethionine; EPN, epinephrine; HMB, β-hydroxy-β-methylbutyrate; NMDA, N-methyl-D-aspartate receptor (a glutamate receptor and ion channel protein found in nerve cells); NEPN, norepinephrine; NOS, nitric oxide synthase; PA, polyamines; TAGs, triacylglycerols; T_3, triiodothyronine; and T_4, thyroxine.

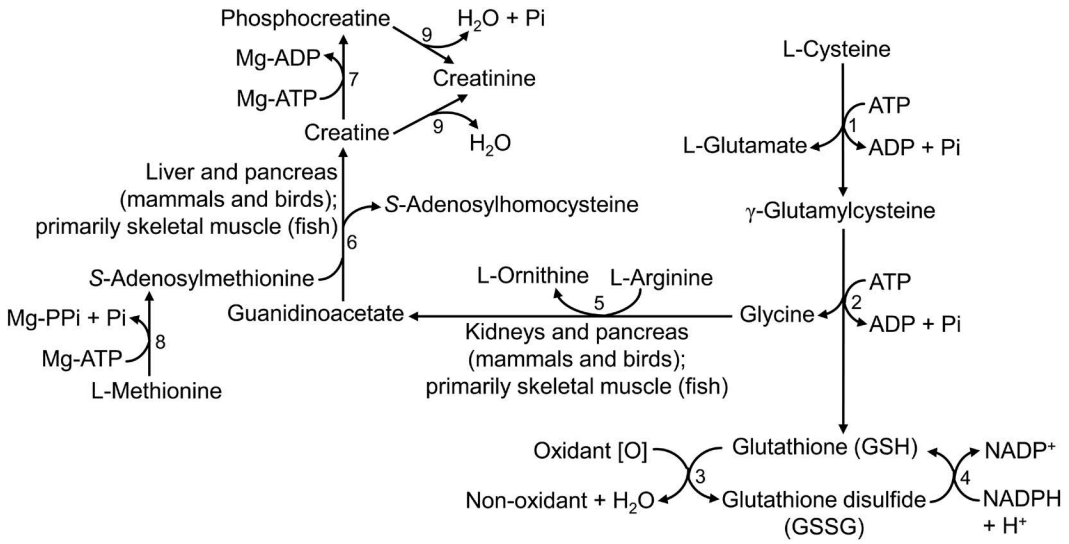


FIGURE 7.34 Synthesis of creatine and glutathione in animals. Creatine is produced from arginine, glycine, and methionine and is phosphorylated to phosphocreatine to store energy in tissues. Creatine synthesis requires the cooperation of the kidneys, pancreas, and liver in mammals but occurs primarily in skeletal muscle in fish. In all animals, creatine and phosphocreatine are spontaneously converted into creatinine at 1.7% per day. In mammals, birds, and fish, glutathione is formed from glutamate, cysteine, and glycine in most tissues, with the liver and small intestine being the major sites. In response to oxidants, glutathione (GSH) is oxidized to glutathione disulfide (GS-SG), which is enzymatically reduced to glutathione in the presence of NADPH + H^+. The enzymes that catalyze these reactions are (1) γ-glutamyl-cysteine synthetase (also known as glutamate-cysteine ligase), (2) glutathione synthetase, (3) a nonenzymatic reaction, (4) glutathione reductase, (5) arginine:glycine amidinotransferase, (6) guanidinoacetate methyltransferase, (7) creatine kinase, (8) *S*-adenosylmethionine synthetase, and (9) a nonenzymatic (spontaneous) reaction.

(a) glucose, galactose, and glucosamine; (b) short-, medium-, and long-chain fatty acids, cholesterol, and ketone bodies; (c) signaling gases (NO, CO and H_2S); (d) amino sugars, nucleotides, and heme; (e) antioxidants (e.g., GSH, creatine, carnitine, taurine, melanin, melatonin, indoles, urocanate, anthranilic acid, carnosine, anserine, and balenine); (f) nongaseous neurotransmitters (e.g., GABA, *N*-acetylcholine, serotonin, and dopamine); (g) hormones (e.g., thyroid hormones, epinephrine, and norepinephrine); (h) ethanolamine, histamine, polyamines, homoarginine, and agmatine; (i) D-amino acids (e.g., D-serine and D-aspartate); and (j) others (e.g., SAM, heme, bilirubin, niacin, and betaine).

The tissue-specific pathways for the syntheses of creatine from arginine, glycine, and methionine and of GSH from cysteine, glutamate, and glycine in animals are outlined in Figure 7.34. In mammals, birds, fish, and crustaceans, nearly all cell types can form GSH, but the liver is the most active organ for GSH synthesis, followed by the small intestine (He et al. 2024a). Interestingly, creatine synthesis requires interorgan cooperation involving the kidneys, pancreas, and liver in mammals but occurs mainly in skeletal muscle in fish such as HSB (He et al. 2024a). The generation of creatine is a major metabolic pathway for the use of dietary arginine, glycine, and methionine in fish and shrimp. About 50% of absorbed arginine, 27% of absorbed glycine, and 42% of absorbed methionine are used for creatine production daily in Atlantic salmon, and 27% of absorbed arginine, 14% of absorbed glycine, and 38% of absorbed methionine are used for creatine production daily in Pacific white shrimp (Gatlin and Wu 2025). Notably, creatine synthesis accounts for 94%, 88%, 87%, and 76% of arginine utilization by gilts (maternal plus fetal tissues) during days 0–30, 30–60, 60–90, and 90–114 of gestation, respectively (Table 7.23). In addition, creatine production requires 40% and 36% of the arginine utilized by nonpregnant and late pregnant ewes, respectively (Table 7.24). It must be recognized that creatine is not a source of cellular energy but rather helps store energy as phosphocreatine (also known as creatine phosphate) in animal tissues, particularly the brain, skeletal muscle, heart, and reproductive organs. Thus, many amino acids not only serve as the precursors of protein but also regulate key metabolic pathways to improve the health, survival, growth, development, lactation, and reproduction of animals. Those amino acids are called functional amino acids (Wu 2010). Glycine is one of those amino acids, as graphically illustrated in Figure 7.35.

Concentrations of taurine (a potent antioxidant) vary markedly among different tissues and breeds within the same species. For example, in male White Leghorn chickens, leg muscle contains a 25-fold greater amount of taurine than breast muscle per kilogram of tissue, but in male Cobb broilers, breast muscle has 60% more taurine than leg muscle per kilogram of tissue (Table 7.19). Research is warranted to determine whether such a divergent pattern of taurine distribution plays a role in avian muscle health.

Excessive accumulations of some amino acid metabolites are harmful and detrimental to animals. Examples

TABLE 7.23
Endogenous Synthesis of Arginine in Gestating Gilts for Fetal Growth and Development[a]

Variable	Amounts of Arginine (g/day)			
Days of Gestation	0–30	30–60	60–90	90–114
Body Weights of Sows (kg)	155–160	160–170	170–185	185–200
Arginine Requirements for maternal Tissues and Fetal Growth	≥8.62	≥9.61	≥10.5	≥13.0
Uptake of portal vein arginine by maternal liver	0.40	0.40	0.40	0.40
Placental growth	0.13	0.57	0.11	0.12
Embryonic/fetal growth	0.005	0.17	0.85	2.60
Allantoic fluid	0.005	0.005	0.000	0.000
Amniotic fluid	0.000	0.001	0.000	0.000
Arginine utilization for maternal creatine synthesis	8.08	8.46	9.11	9.88
Arginine Provision from the Diet to Extraintestinal Tissues	5.04	5.04	5.04	5.04
2 kg diet (0.7% arginine)	14.0	14.0	14.0	14.0
Indigestible arginine in the diet (15%)	2.10	2.10	2.10	2.10
Intestinal catabolism (40% of arginine in the lumen)	4.76	4.76	4.76	4.76
Arginine Provision from Synthesis in Mother Plus Fetus	≥3.58	≥4.57	≥5.46	≥7.96
(% of daily requirement)	(41%)	(48%)	(52%)	(61%)

Source: Wu et al. (2018).

[a] The gilt gestating 12 fetuses is fed daily 2 kg of a typical corn- and soybean meal-based diet.

TABLE 7.24
Synthesis of Creatine in Adult Pregnant and Nonpregnant Ewes[a]

Variable	Nonpregnant Ewes	Pregnant Ewes[b]	
		Mother	Fetus
Needs for Cr to Replenish the Urinary Excretion of Cr Plus Creatinine			
Urinary excretion of Cr + creatinine, mmol/kg BW/day	0.327	0.326	0.548
Arg required for creatine synthesis, mg/kg BW/day	57.0	56.8	95.5
Arg required for creatine synthesis, g/animal/day	3.42	3.41	0.305
Arg required for creatine synthesis in mother plus fetus, g/whole body/day	---	3.72	---
Feed intake (11.7% CP; as-fed basis), kg/day	1.20	1.33	---
CP intake, g/day	140	156	---
Microbial and feed proteins entering the duodenum, g/day	95.2	106	---
Nitrogen leaving the rumen as ammonia and reentering the rumen via N recycling for microbial protein synthesis, g/day	18.2	20.3	---
Total digestible microbial and feed proteins in the SI, g/day	113.4	126.3	---
Digestible microbial and feed proteins in the SI, g/day	96.4	107.4	---
Arg content in microbial and feed protein, %	5.12	5.12	---
Arg released from microbial and feed protein in the SI, g/day	4.94	5.50	---
Arg entering the portal vein (40% extraction by the SI), g/day	2.96	3.30	---
Endogenous synthesis of Arg, g/day	5.54[i]	6.93	---
Total amount of Arg utilized by the whole body, g/day	8.50	10.23	---
Contribution of Arg synthesis to Arg requirements, %	65.2	67.7	---
Percent of utilized Arg for creatine synthesis, %	40.2	36.4	---
Needs for Cr Accretion in the Body			
Skeletal muscle (wet mass), kg	27.0	27.0	1.28
Skeletal muscle (dry mass), kg	8.1	8.1	0.384
Content of Cr + PCr in skeletal muscle, mmol/kg dry mass	134.6	135.0	96.2

(*Continued*)

TABLE 7.24 (*Continued*)
Synthesis of Creatine in Adult Pregnant and Nonpregnant Ewes[a]

Variable	Nonpregnant Ewes	Pregnant Ewes[b]	
		Mother	Fetus
Cr + PCr in total skeletal muscles, mmol	1090	1094	36.94
Cr + PCr in the whole body,[c] mmol	1147	1152	38.9
Loss of Cr + PCr (1.7%/day) as creatinine, mmol/kg BW/day	0.325	0.326	0.206
Skeletal muscle (wet mass) gain (BW gain of 90 mg/day), g/day	0	0	36
Skeletal muscle (dry mass) gain (BW gain of 90 g/day), g/day	0	0	10.8
Cr + PCr accretion in skeletal muscle, mmol/kg BW/day	0	0	0.325
Cr + PCr accretion in the whole body, mmol/kg BW/day	0	0	0.342

Source: Wu et al. (2022).

[a] Body weights (BW) of both adult nonpregnant female sheep (ewes) and adult pregnant ewes at the start of gestation are 60 kg.

[b] Day 130 of gestation, when fetal body weight is 3.2 kg. The term of gestation is 147 days.

[c] About 95% of creatine plus creatine phosphate in the body is found in skeletal muscle.

Note: CP, crude protein; Cr, creatine; PCr, phosphocreatine; SI, the small intestine; "---", not applicable.

include (a) hyperammonemia and gout (noted previously); (b) hyperhomocysteinemia [elevation of homocysteine in the plasma due to nutritional factors (e.g., excessive intakes of methionine or cysteine; and deficiencies of folate, vitamin B_6, and vitamin B_{12})] or inherited defects in homocysteine metabolism (e.g., a deficiency of cystathionine β-synthase or $N^{5,10}$-methylenetetrahydrofolate reductase); (c) NO and H_2S toxicity; (d) porphyria [resulting from the excessive accumulation of porphyrins (metabolites of glycine and succinate) in tissues due to defects in their conversion into heme]; and (e) H_2SO_4-induced metabolic acidosis. In addition, melanosis (either inherited or acquired) results from the deposition of a brownish-black pigment [e.g., tyrosine-derived melanin or melanin-like polyphenols in tissues such as the skin, mammary gland, adipose tissue, and lymph nodes (Wu 2020)]. Melanosis is more common in pigs than in other livestock species. This phenomenon is a serious problem in farm-raised crustaceans and also occurs in young ruminants (e.g., calves and lambs). The pigmentation could negatively affect sensory characteristics of meat and its marketability.

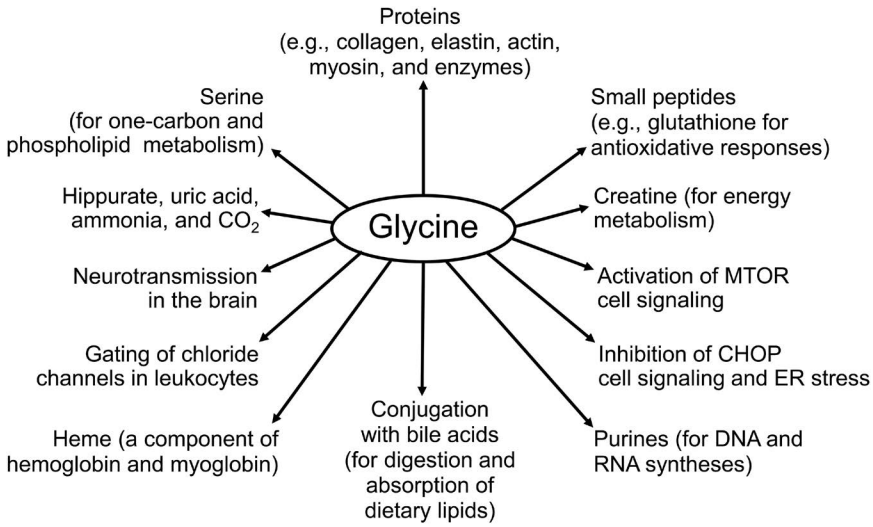


FIGURE 7.35 Physiological functions of glycine in animals. Glycine is a source of ammonia and bicarbonate in the kidneys for the regulation of acid-base balance. In addition, glycine is a substrate for the synthesis of not only proteins (including collagen) but also glutathione, heme, creatine, nucleic acids, and uric acid with physiological importance. Furthermore, glycine is required for the conjugation with bile acids to form bile salts that facilitate the digestion of dietary fat as well as the absorption of long-chain fatty acids and lipid-soluble vitamins. Finally, glycine regulates cell signaling pathways and therefore nutrient utilization. Thus, glycine plays vital roles in the metabolism, growth, development, and function of animals. CHOP, C/EBP-homologous protein (anti-oxidative response); ER, endoplasmic reticulum; MTOR, mechanistic target of rapamycin.

CONJUGATION OF AMINO ACIDS OR GLUTATHIONE WITH SUBSTANCES

In the peroxisomes and cytosol of hepatocytes, taurine or glycine conjugates with bile acid to form tauro- or glycocholate (bile salt), respectively, via bile acid-CoA:amino acid *N*-acyltransferase, in a species-specific manner (Table 7.25) due to differences in the isoform of the enzyme and the intracellular concentrations of taurine and glycine. For example, cats, dogs, birds, and some fish species generate almost exclusively taurocholate, but rabbits produce almost exclusively glycocholate (Hou et al. 2020). Some mammals (e.g., humans, cattle, and pigs) form both taurocholate and glycocholate in different proportions [e.g., ~30% taurocholate and ~70% glycocholate (mol/mol) in humans and cattle; ~15% taurocholate and ~85% glycocholate (mol/mol) in pigs] (Hafkenscheid and Hectors 1975). In guinea pigs, although bile acid-CoA:amino acid *N*-acyltransferase has a greater affinity for taurine than glycine, taurocholate accounts for only a very low proportion of total bile salts due to a much lesser concentration of taurine than glycine in the hepatocytes of these animals. Oral administration of taurine (200–300 mg daily) to guinea pigs for 10 days substantially increases the amount of taurine-conjugated bile acids and decreases the ratio of glycine- to taurine-conjugated bile acids (mol/mol) from 4.4 to 0.19 (Kibe et al. 1980). Bile salts are required for the digestion and absorption of lipid-soluble nutrients, as well as the health of the gut and liver.

Glycine also conjugates with benzoyl-CoA or phenylacetyl-CoA (a metabolite of phenylalanine) to form hippurate or phenylacetylglycine by glycine *N*-acyltransferase or glycine *N*-phenylacetyltransferase, respectively (Wu 2022). In addition, glutamine conjugates with phenylacetyl-CoA to generate phenylacetylglutamine by glutamine *N*-phenylacetyltransferase, respectively. All these reactions occur in the liver with their products being excreted in the urine and play a role in removing ammonia from animals.

GSH is the most abundant low-molecular-weight antioxidant in cells and bile (Hou et al. 2020). This tripeptide can conjugate with (a) free radicals and other reactive oxygen species (e.g., hydroxyl radical, lipid peroxyl radical, and peroxynitrite) for their removal; (b) various electrophiles, physiological metabolites (e.g., estrogen, melanins, prostaglandins, leukotrienes, and bilirubin), and xenobiotics (e.g., bromobenzene and acetaminophen) to form

TABLE 7.25
Ratios of Glycocholate to Taurocholate in the Bile of Animals

		Ratios of Glycocholate to Taurocholate in the Bile	
Animal Species	**Amino Acid for Conjugation with Bile Acid**	**mol/mol**	**gram/gram[f]**
Cats	Almost exclusively taurine (99.0%)	0.01 (0.01:0.99)	0.01 (0.01:0.99)
Dogs	Exclusively taurine	0.00[a]	0.00[a]
Poultry[b]	Exclusively taurine	0.00[a]	0.00[a]
Goats (fetus, adult)	Almost exclusively taurine (99.9%)	0.00	0.00
Goats (preruminant)	Almost exclusively taurine (98.0%)	0.02 (0.02:0.98)	0.02 (0.02:0.98)
Sheep (fetus)	Almost exclusively taurine (99.9%)	0.00	0.00
Sheep (adult)	Both taurine and glycine; mainly taurine	0.62 (0.38:0.62)	0.56 (0.36:0.64)
Rat	Both taurine and glycine; mainly taurine	0.14 (0.12:0.88)	0.13 (0.11:0.89)
Mouse	Both taurine and glycine; mainly taurine	0.05 (0.05:0.95)	0.05 (0.05:0.95)
Cattle (at birth)	Both taurine and glycine; mainly taurine	0.63 (0.39:0.61)	0.60 (0.36:0.64)
Cattle (adult)	Both taurine and glycine; mainly glycine	2.5 (0.71:0.29)	2.3 (0.69:0.31)
Rabbits	Almost exclusively glycine	---[c]	---[c]
Horses	Both taurine and glycine; mainly glycine	0.18 (0.15:0.85)	0.16 (0.14:0.86)
Humans	Both taurine and glycine; mainly glycine	2.0 (0.67:0.33)	1.8 (0.64:0.36)
Pig	Both taurine and glycine; mainly glycine	6.3 (0.86:0.14)	5.7 (0.85:0.15)
Guinea pigs	Both taurine and glycine; mainly glycine[d]	4.4 (0.81:0.19)	4.0 (0.80:0.20)
Nonhuman primate	Both taurine and glycine; mainly glycine	2.0 (0.67:0.33)	1.8 (0.64:0.36)
Other animals[e]	Possibly exclusively or mainly taurine	---	---

Source: Ghaffari et al. (2025), Hafkenscheid and Hectors (1975), Khatim and Gumaa (1982), Kibe et al. (1980), and Rabin et al. (1976).

[a] 100% as taurocholate and no glycocholate.

[b] Including chickens, ducks, geese, and turkeys.

[c] Almost 100% as glycocholate.

[d] Glycocholate is more abundant than taurocholate under normal feeding conditions due to low concentrations of taurine in hepatocytes.

[e] Including bears, ferrets, giant panda, hamsters, penguins, tigers, and some fish (e.g., hybrid striped bass).

[f] Calculated on the basis of the molecular weights of glycocholic acid (465.6 Da) and taurocholic acid (515.7 Da).

mercapturates via enzyme-catalyzed reactions; (c) NO to form an *S*-nitroso-glutathione adduct for NO storage and transport; and (d) formaldehyde (a metabolite of methionine, choline, methanol, sarcosine, and xenobiotics) to yield *S*-formyl-glutathione by formaldehyde dehydrogenase, thereby facilitating the removal of formaldehyde (a carcinogen) from the body (Wu 2022).

INTRACELLULAR PROTEIN TURNOVER

Dietary protein is converted into tissue protein in animals through complex digestive and metabolic processes. The tissue protein is not static, but undergoes continuous degradation and synthesis, which is collectively called intracellular protein turnover (Figure 7.36). The balance between the rates of these two processes is the determinant of protein accretion in cells and therefore tissue growth (Bergen et al. 1987; Buttery 1983). A gain of protein in animals results from a greater rate of protein synthesis than protein degradation, whereas a loss of protein in stressed animals under catabolic or disease conditions is caused by a greater rate of protein degradation than protein synthesis. When the rate of protein synthesis is equal to that of proteolysis, there is no net change in protein mass, as in healthy adults. However, postprandial protein balance in tissues is not zero due to increased protein synthesis. In addition, the partitioning of energy for protein and fat syntheses affects not only the rates of animal growth but also meat quality (Liu et al. 2024). Thus, studies of intracellular protein turnover in tissues, particularly skeletal muscle (the major reservoir of protein in the body), have important implications for improving the growth performance and feed efficiency of livestock, poultry, fish, and crustaceans (Wu 2009; Li et al. 2021b,c).

Intracellular Protein Synthesis

The process of cytosolic protein synthesis in eukaryotic organisms includes five steps: (a) gene transcription to form mRNA, (b) initiation of mRNA translation to generate peptides at ribosomes, (c) peptide elongation to produce protein, (d) termination as recognized by a terminating codon on the mRNA, and (e) post-translational modifications of the newly synthesized protein (Figure 7.37). In bacteria, transcription in the nucleus is closely coupled with the subsequent translation in the rough endoplasmic reticulum. tRNA is necessary for translation because mRNA does not directly recognize any amino acid but can bind to a tRNA that carries a corresponding amino acid (Manning and Cooper 2017). In essence, the mRNA synthesized from DNA encodes the polypeptide with each amino acid designated by a specific codon (three nucleotides). Thus, tRNAs serve as the adaptors to translate genetic information from nucleic acids (e.g., DNAs) into proteins.

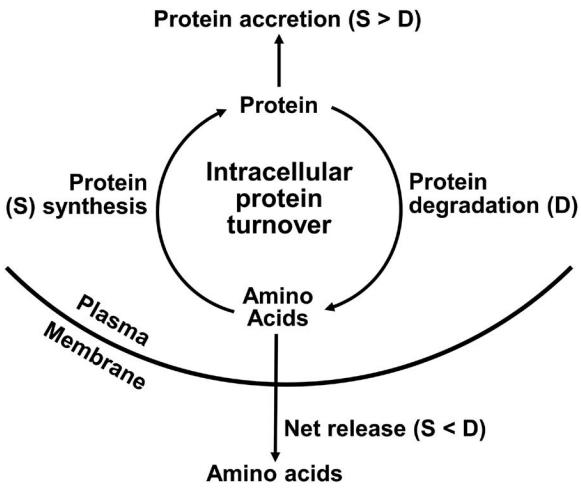


FIGURE 7.36 Intracellular protein turnover in animals. The balance between protein synthesis and protein degradation in the cell determines its protein accretion and release of amino acids.

Gene Transcription to Form mRNA

The first step in protein synthesis is the transcription of a DNA gene to form rRNA, mRNA, and tRNA in the nucleus by RNA polymerases I, II, and III, respectively. These processes are similar between eukaryotic and prokaryotic cells (Lackner and Bähler 2008). Each tRNA contains a trinucleotide sequence (collectively known as an anticodon), which is complementary to a codon on mRNA for a specific amino acid. The mRNA has a 5'-cap (methyl-guanosyl triphosphate), which is critical for protection from RNases in the nucleus. In eukaryotic organisms, most mRNA molecules are polyadenylated at the 3' end via the covalent linkage of a polyadenylyl moiety. The poly(A) tail and its associated proteins protect mRNA from degradation by exonucleases. Eukaryotic precursor mRNA requires extensive processing [including the methylation of guanosine by SAM-dependent guanine-N^7-methyltransferase, and the removal of introns (splicing) by the spliceosome (a ribozyme)] to become mature mRNA before transport through the nuclear pore to ribosomes in the cytoplasm (Manning and Cooper 2017).

Initiation of mRNA Translation to Generate Peptides at Ribosomes

Before protein synthesis is initiated, the 80S ribosome is formed from 60S and 40S subunits in eukaryotic cells. In prokaryotic cells, the 70S ribosome is formed from 50S and 30S subunits. The steps of this process are similar between eukaryotic and prokaryotic cells, but more initiation factors are required by eukaryotes than prokaryotes and the sizes of the ribosomes differ. For example, the 40S subunit in eukaryotic cells contains an 18S RNA that is homologous to the prokaryotic 16S RNA, whereas the 60S subunit in eukaryotic cells contains three RNAs: the 5S and 28S RNAs (which are the counterparts of the prokaryotic 5S and 23S molecules) and a 5.8S RNA (which is unique to eukaryotes; Kong and Lasko 2012).

Initiation of mRNA translation can be divided into four steps:

a. GTP-dependent dissociation of the 80S ribosome (e.g., in animals) or 70S ribosome (e.g., in bacteria) into its constituent subunits, which is controlled by initiation factors [e.g., eukaryotic initiation factors

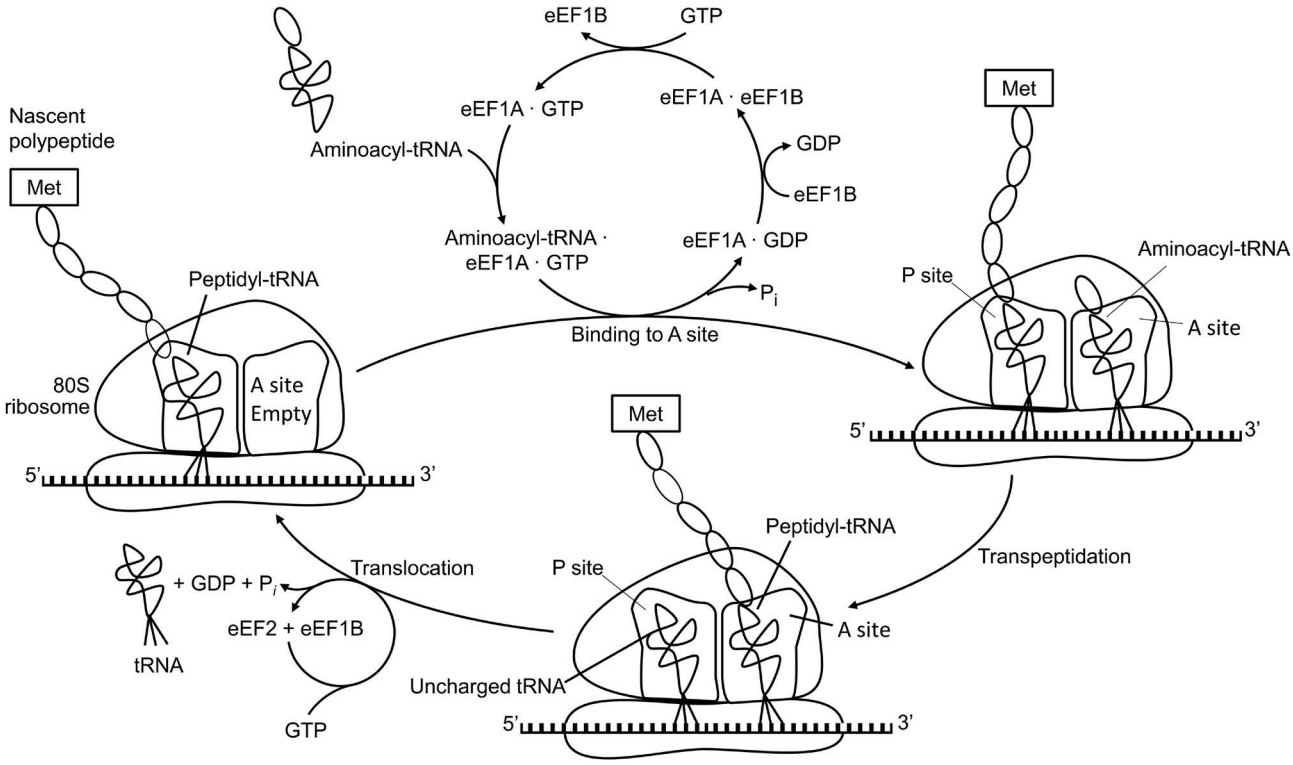


FIGURE 7.37 The pathway of protein synthesis in animal cells. The 80S ribosome is the functional site for the translation of mRNA into protein. These processes require enzymes (aminoacyl-tRNA synthetase and peptidyl transferase), eukaryotic initiation factors (eIFs), elongation factors (eEFs), and release factors (eRFs). eEF1A·GTP is required for the binding of an incoming aminoacyl-tRNA to the A site, and eEF1A·GDP is recycled into eEF1A·GTP under the action of eEF1B. Polypeptide elongation is terminated by a terminating codon on the mRNA template. Peptidyl transferase, together with eRF1, hydrolyzes peptidyl-tRNA to release the newly synthesized peptide.

(eIFs)] and elongation factors [eukaryotic elongation factors (eEFs)];

b. formation of the 43S preinitiation complex (ternary complex) from eIF2, GTP, Met-tRNAi (the tRNA for binding to the initial methionine) and the 40S subunit (eukaryotes; Figure 7.38) or of the 30S preinitiation complex from IF2, GTP, *N*-formylmethionine-tRNAi, mRNA, and the 30S subunit (prokaryotes; Laursen et al. 2005);
c. ATP-dependent formation of either the 48S initiation complex from eIF4E, eIF4G, eIF4A, eIF4G, mRNA, and the 43S preinitiation complex in eukaryotes (Figure 7.36) or the 30S initiation complex through codon-anticodon interaction and conformational change in prokaryotes (Laursen et al. 2005); and
d. formation of the translationally active 80S ribosome from the 48S initiation complex, the 60S ribosome, and the ribosomal protein S6 in eukaryotes (Figure 7.36) or the formation of the 70S ribosome from the 30S initiation complex and the 50S ribosome in prokaryotes (Laursen et al. 2005). The 80S ribosome contains three RNA binding sites: the aminoacyl site (also known as acceptor site; A site), which binds an aminoacyl-tRNA; the peptidyl site (P site), which binds the nascent polypeptide chain linked to the last aminoacyl-tRNA; and the exit site (E site, usually not recognized in eukaryotes), which allows for the release of deacylated tRNA after peptide-bond formation (Figure 7.39).

Peptide Elongation to Produce Protein

Active translation occurs on the functional 80S ribosome complex, where the ribosome reads mRNA in the 5' to 3' direction. During this process, each tRNA that carries the corresponding amino acid moves through the 80S ribosome from the A site to the P site and then exits the ribosome via the E site. Elongation factors are crucial for the addition of an amino acid to a polypeptide and for peptide elongation. Protein synthesis occurs at a high speed and accuracy. Eukaryotic and prokaryotic ribosomes can incorporate 6 and 18 amino acids per second, respectively. This rapid process of peptide elongation starts with the ATP-dependent activation of an amino acid to form aminoacyl-tRNA, which is catalyzed by each of 20 different aminoacyl-tRNA synthetases, in addition to Met-tRNAi. Such a reaction consumes 1 mol of ATP and generates 1 mol of AMP plus 1 mol of inorganic pyrophosphate (PPi). During elongation,

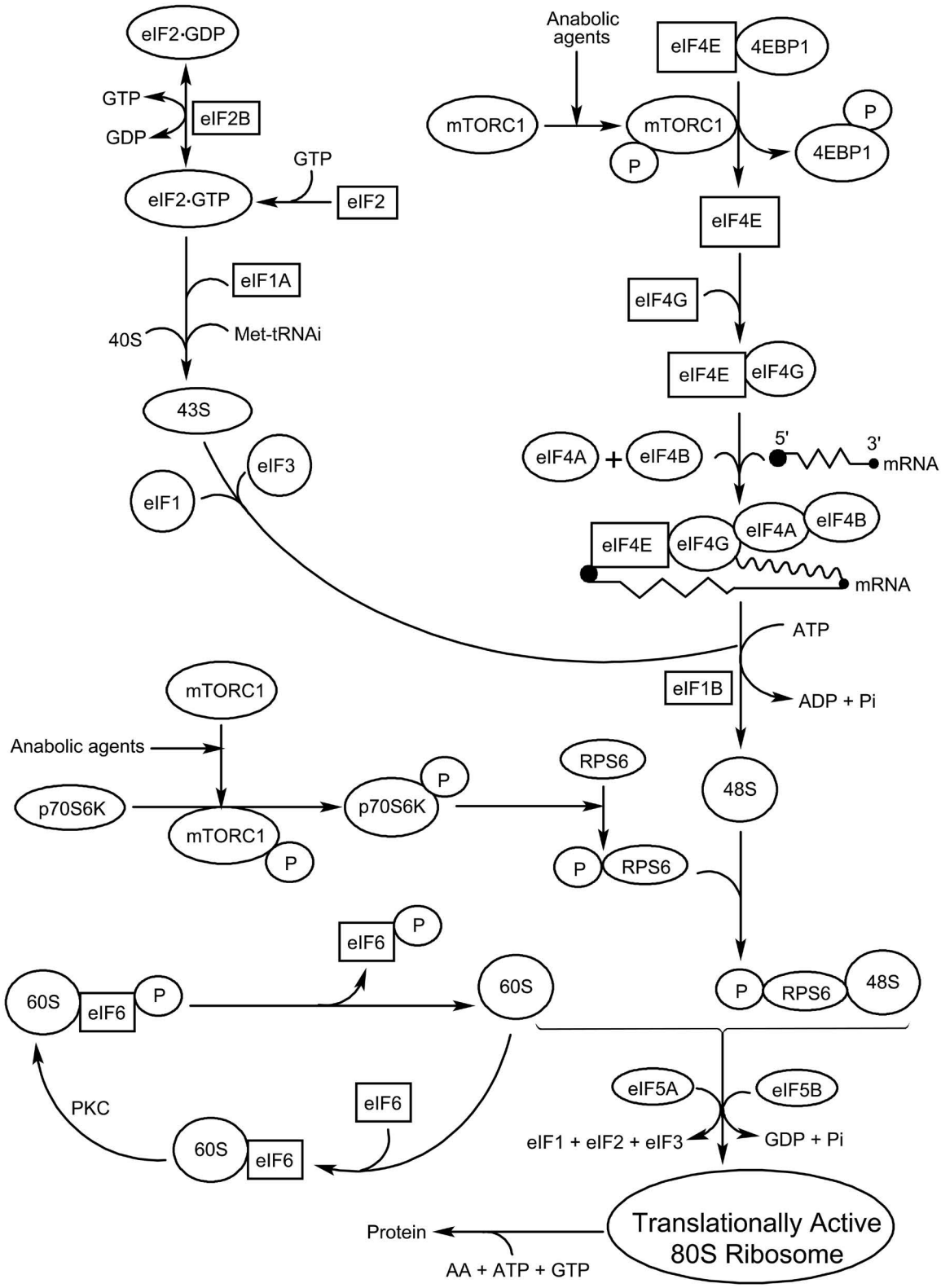


FIGURE 7.38 Initiation processes for protein synthesis in animal cells. In the presence of various initiation factors and the mRNA template, 40S and 60S ribosomes combine to form the translationally active 80S ribosome, where protein synthesis in the cytoplasm and mitochondria takes place. eIF, eukaryotic initiation factor; 4EBP1, eIF4E-binding protein 1; mTORC1, complex 1 of mammalian or mechanistic target of rapamycin; p70S6K, ribosomal protein S6 kinase 1 (a 70-kDa protein); PKC, protein kinase C; RPS6, ribosomal protein S6; S, Svedberg unit of flotation.

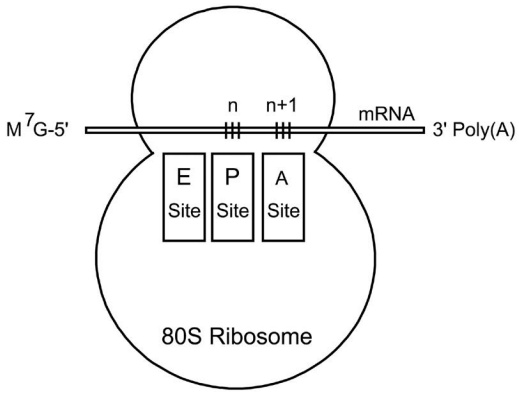


FIGURE 7.39 The 80S ribosome for protein synthesis in animal cells. The 80S ribosome contains three RNA-binding sites designated as A, P, and E. During peptide elongation, the peptidyl moiety from the AA-tRNA on the P site of the 80S ribosome is transferred to the acceptor end of the existing aminoacyl-tRNA on the A site of the ribosome. The discharged tRNA rapidly dissociates from the P site and is transferred to the E site before exiting the ribosome. AA, amino acid; M^7G, 7-methylguanylate cap; Poly(A), polyadenylyl tail.

amino acids are continually added to the peptide, eventually forming a polypeptide or protein chain bound together through peptide bonds.

$$\text{Amino acid} + \text{tRNA} + \text{ATP} \rightarrow \text{Amino acyl-tRNA} + \text{AMP} + (\text{PPi}) \quad (\text{Aminoacyl-tRNA synthetase})$$

Termination of Peptide Chain Elongation

After multiple cycles of elongation to polymerize amino acids into a protein molecule, the completion of polypeptide chain elongation is recognized in the A site by the terminating signal known as the nonsense or terminating codon (e.g., UGA, UAG, or UAA) on the mRNA. Except for the special Sec-$tRNA_{(Sec)}$, tRNA normally does not have an anticodon that can recognize such a termination codon. Rather, the recognition of a termination codon residing in the A site of the 80S ribosome is performed by eukaryotic protein release factors (eRFs), including eRF1, eRF2, and eRF3. The stop codon induces the binding of a release factor that prompts the disassembly of the entire ribosome-mRNA complex. eRF1 recognizes UAA and UAG, whereas eRF2 recognizes UAA and UGA. In complexes with GTP, eRF3 promotes the binding of eRF1 and eRF2 to the 80S ribosome, and the eRF then hydrolyzes the bond between the peptide and the terminal tRNA. GTP hydrolysis triggers the dissociation of the eRF from the ribosome, which dissociates into the 40S and 60S subunits for another cycle of protein synthesis.

Post-Translational Modifications of Newly Synthesized Proteins

Most of the newly synthesized proteins have no biological activities when released from the ribosome. The polypeptides must undergo appropriate modifications in the cytoplasm and/or on the rough endoplasmic reticulum in eukaryotes (equivalent to becoming mature proteins). These post-translational modifications include (a) removal of the initiating amino acid (e.g., Met or fMet) and of the C and N-terminal residues (e.g., signal peptide by signal peptidase); (b) limited proteolysis of proproteins or propeptides (inactive proteins or peptides); and (c) covalent modifications of certain amino acid residues in proteins or peptides, including acetylation (lysine), ADP-ribosylation, biotinylation, γ-carboxylation, disulfide linkage (cysteine), flavin attachment, glycosylation (lysine), heme attachment, hydroxylation (lysine, proline, serine, threonine, and tyrosine), methylation (arginine and lysine), myristoylation, palmitoylation, ubiquitination (lysine), and transglutamination. The release of modified amino acids (e.g., 3-methylhistidine and 4-hydroxyproline) from proteins can be used to estimate the degradation of myofibrillar proteins in skeletal muscle and connective tissue, respectively.

Protein Synthesis in Mitochondria

Most mitochondrial proteins are synthesized on ribosomes in the cytoplasm. The newly synthesized proteins for subsequent transport into the mitochondrion contain mitochondrion-targeting sequences and are taken up into this organelle by binding to its surface receptor proteins that can recognize specific mitochondrion-targeting sequences. It is now known that the synthesis of a limited number of proteins (e.g., 8 in certain yeasts, 13 in mammals, and approximately 20 in plants) from amino acids occurs within mitochondria. Mitochondrially translated proteins are encoded by mitochondrial DNA. They are subunits of enzyme complexes located on the inner membrane that are involved in respiration and oxidative phosphorylation. Mitochondrial translation systems are more similar to those in prokaryotes than in eukaryotes and include several unusual features such as some different codon assignments from the "universal" genetic code, the use of a restricted number of tRNAs, and unusual structural features of mitochondrial ribosomes.

Energy Requirement of Protein Synthesis

One mole of protein consists of various quantities of different amino acids that collectively amount to 1 mol of amino acids. Intracellular protein synthesis requires substantial amounts of energy, primarily in the forms of ATP and GTP. The energy-dependent reactions include (a) amino acid activation to form tRNA-amino acid, (b) the entry of tRNA-amino acid into the A site on the ribosome (the initiation of peptide chain formation) that requires the cleavage of GTP to GDP, (c) the translocation of the newly formed peptidyl-tRNA from the A site to the P site on the ribosome (chain elongation), and (d) the termination of polypeptide synthesis.

1. Amino acid activation: ATP → AMP + PPi (2 high-energy phosphate bonds)
2. Initiation of peptide chain formation: GTP → GDP + Pi (1 high-energy phosphate bond)

3. Peptide chain elongation: GTP → GDP + Pi (1 high-energy phosphate bond)
4. Termination of polypeptide synthesis: GTP → GDP + Pi (1 high-energy phosphate bond)

Thus, an equivalent of 5 mol of ATP (5 ATP → 5 ADP + 5 P_i) is required to synthesize 1 mol of protein. In addition, the transport of some amino acids into cells requires ATP (e.g., 0.5 mol of ATP/mol of amino acid on average). This consideration is nutritionally relevant because dietary (exogenous) amino acids are required for protein maintenance and accretion in animals. Thus, 5.5 mol of ATP are needed to synthesize 1 mol (100 g) of protein ($C_{4.3}H_7O_{1.4}N_{1.2}S_{0.069}$) from extracellular amino acids, which amount to 118 g of amino acids based on the composition of amino acids in the pig body (Chapter 4). When amino acids are derived within the cell through synthesis or intracellular protein degradation, the energetic cost of protein synthesis is 5 mol of ATP/mol of protein. The energy released during protein synthesis contributes to "heat increment" after an animal eats a meal containing protein.

Let us use the energetic cost of protein synthesis at 5.25 mol of ATP/mol of protein to calculate the amount of metabolizable energy (ME) needed for protein synthesis in animals. The ME of 85.4 kJ/mol is required to synthesize 1 mol of ATP from 1 mol of ADP + 1 mol of Pi (McDonald et al. 2021), or 448.4 kJ ME for 5.25 mol of ATP (i.e., 5.25 mol of ATP × 85.4 kJ/mol = 448.4 kJ). This is equivalent to 448.4 kJ/100 g of protein (or 4.484 kJ/g of protein) synthesized in tissues. However, much more ME is needed to deposit 1 g of protein in the body because some of the synthesized protein undergoes continuous degradation.

The energetic cost of protein retention in growing animals depends on the rates of intracellular protein synthesis and degradation in the whole body and is affected by a plethora of factors such as species, breeds, age, environment, and nutrition. For example, in 30-, 60-, and 90-kg pigs that gain 93, 96, and 54 g of protein/day, respectively, and have various rates of whole-body protein turnover, the ME values for protein retention in the bodies are 26.2, 38.9, and 78.0 kJ/g of protein, respectively (Table 7.26). These values do not include the combustion energy of retained protein (22.6 kJ/g). Clearly, the energetic costs of protein retention in the same animal are not constant during its life cycle. The energetic costs for protein synthesis and protein deposition in unspecified animals have previously been estimated to be 3.6 kJ ME/g of protein (20% lower than the current value) and 48 kJ ME/g of retained protein, respectively (Chwalibog 1991). The latter value would be for growing pigs that are slightly heavier than 60 kg.

Measurement of Protein Synthesis

Labeled amino acid tracers (nonmetabolizable amino acids; e.g., leucine for liver and phenylalanine for skeletal muscle) are used to measure protein synthesis in isolated tissues or incubated cells (Bergen et al. 1987; Klasing et al. 1987; Wu and Thompson 1990). For example, with the use of ^{14}C-phenylalanine as a tracer plus 1 mM phenylalanine in a medium to study protein synthesis in skeletal muscle, the rate of protein synthesis (measured by the incorporation of ^{14}C-phenylalanine into protein) within a 2-h period is calculated as

$$\text{Amount of } {}^{14}\text{C-Phe radioactivity in protein (dpm)} / \text{Specific activity of } {}^{14}\text{C-Phe in the precursor pool (dpm/nmol)} = \text{nmol Phe/2 h.}$$

The fractional rate of protein synthesis (a key indicator of efficiency in the translation of mRNAs into proteins) is calculated as

$$\text{Specific activity of } {}^{14}\text{C-Phe in protein (dpm/nmol)} / \text{Specific activity of } {}^{14}\text{C-Phe in the precursor pool (dpm/nmol)} \times 100 = \%/2 \text{ h.}$$

The absolute amount of protein synthesis is calculated as

$$\text{Amount of protein in tissue (mg)} \times \text{Fractional rate of protein synthesis } [\%/(2 \text{ h} \times 100)] = \text{mg/2 h.}$$

TABLE 7.26
Energetic Costs of Protein Retention in the Whole Bodies of Growing Pigs

BW (kg)	Protein Synthesis[a]		Protein Degradation[a]		Protein Retention		ME Requirements		
BW day	g/kg BW per day	g/day	g/kg BW per day	g/day	g/kg BW per day	per day	Protein Synthesis[b] (kJ/day)	Protein Degradation[c] (kJ/day)	Protein Retention[d] (kJ/g/day)
30	13.5	405	10.4	312	3.1	93	1816	624	26.2
60	10.1	606	8.5	510	1.6	96	2717	1020	38.9
90	7.4	666	6.8	612	0.6	54	2986	1224	78.0

[a] Wu (2022).
[b] Calculated based on the energetic cost of protein synthesis at 4.484 kJ/g protein.
[c] Calculated based on the energetic cost of protein degradation at 4.484 kJ/g protein.
[d] Calculated based on the energetic cost of protein retention (i.e., the sum for protein synthesis and degradation) divided by the amount of retained protein.

In all of these calculations, the amount of tissue used for incubation must be taken into consideration.

Many methods involving tracers have also been developed to measure in vivo protein synthesis in tissues of animals, which include (a) a single administration of a tracer amino acid, (b) the flooding dose technique (e.g., young animals; 10–30 min labeling of protein), and (c) the constant infusion of a tracer amino acid (larger animals; 4–6 h labeling of protein) (Davis and Reeds 2001; Latham et al. 2019; Southorn et al. 1992; Watford and Wu 2005; Zhang et al. 2024). Measurement of whole-body protein synthesis based on models with two or more compartments has also been validated for use in animals (Waterlow et al. 1978). The most common methods are the flooding dose technique and the constant infusion of a tracer. However, the experimental approach chosen for measuring tissue protein synthesis in vivo should be dictated by the scientific question being addressed. For example, the short-term flooding dose technique is useful for tissues (e.g., liver and small intestine) with high rates of protein synthesis to minimize the recycling of the tracer and for tissues (skeletal muscle and heart in young animals) with lower rates of protein synthesis. In contrast, the long-term constant infusion technique is appropriate for tissues with lower rates of protein synthesis in large animals to reduce the cost of tracer and achieve a steady state of precursor's specific radioactivity or isotope enrichment (Bergen et al. 1987).

Intracellular Protein Degradation

Proteases for Intracellular Protein Degradation

Protein degradation is catalyzed by proteases, with the resultant peptides being hydrolyzed by oligopeptidases, including tri- and dipeptidases (Baracos et al. 1986; Wu 2022). Most proteases are hydrolases (also known as peptidases), but some (e.g., asparagine peptide lyases) are not. Therefore, the terms "proteases" and "peptidases" should not be treated as synonymous. Proteases are classified according to (a) the reaction type (e.g., exopeptidases and endopeptidases); (b) the chemical nature of the enzyme's catalytic site (e.g., serine-, cysteine-, aspartate-, threonine-, glutamate-, asparagine-, and metallo-proteases); and (c) their evolutionary relationship (e.g., families), with families with related structures being grouped into clans (Rawlings et al. 2012). The classification and naming of enzymes based on the type of reaction is the primary principle of the enzyme nomenclature of the International Union of Biochemistry and Molecular Biology.

Intracellular Proteolytic Pathways

Intracellular proteins are degraded via highly selective pathways to maintain a dynamic state of protein turnover (Bergen 2008). Proteases are present in the cytoplasm, plasma membrane, and many organelles of the cell (Goldberg 2003). In addition to the cytoplasm and the lysosome, peptidases are present in the plasma membrane, mitochondria, nucleus, and rough endoplasmic reticulum. Thus, intracellular proteolytic pathways are classified according to the location of proteases: lysosomal and nonlysosomal systems (Figure 7.40).

1. *The lysosomal proteolytic pathway.* Proteins are delivered from the cytoplasm to the lysosome via the endocytic pathway mediated by endocytosis or autophagy (self-eating; Shen et al. 2021). Once cytosolic proteins are inside the lysosome, they are denatured due to the low pH and then hydrolyzed by proteases to release amino acids. These

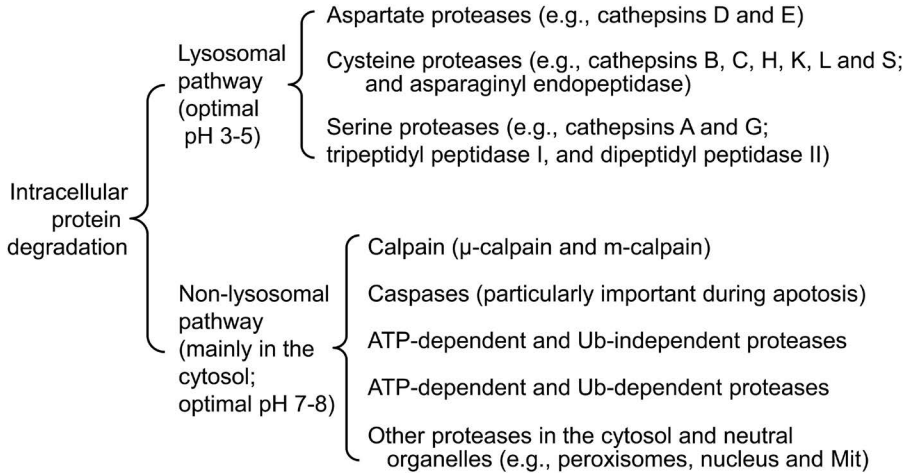


FIGURE 7.40 Intracellular pathways for protein degradation in animal cells. Proteins enter the lysosome from the cytosol via autophagy, endocytosis, and crinophagy. The lysosomal pathway is responsible for the degradation of endocytosed proteins and nonmyofibrillar proteins under conditions of nutritional deprivation (20–30% of total cellular proteins). The nonlysosomal pathway is responsible for the degradation of normal short-lived proteins; abnormal, denatured, and aged proteins under basal metabolic conditions; and myofibrillar and nonmyofibrillar proteins under conditions of nutritional deprivation (70–80% of total cellular proteins). Mit, mitochondria; Ub, ubiquitin.

proteases include (a) cathepsin B (cysteine protease) with both endopeptidase and exopeptidase (C-terminus) activities, (b) cathepsin H (cysteine protease; a glycoprotein) with both endopeptidase and exopeptidase (N-terminus) activities, (c) cathepsin L (a major lysosomal cysteine protease; endopeptidase), (d) cathepsin D (aspartate protease; endopeptidase), (e) cathepsin K (cysteine protease in osteoclasts and bronchial epithelium), and (f) other proteases such as cathepsins C (myeloid cells), F (macrophages), O (widespread), V (thymic epithelium), W ($CD8^+$ T-lymphocytes), and Z (widespread) (Ciechanover 2012; Goldberg 2003). The optimal pH for lysosomal proteases is 3–5. Thus, some weak bases [e.g., ammonia, methylamine, chloroquine, or monensin (an ionophore)] inhibit lysosomal protein degradation by increasing intralysosomal pH above 5.0. The lysosomal proteolytic system participates in the intracellular degradation of (a) endocytosed proteins and (b) nonmyofibrillar proteins under conditions of nutritional deprivation. Studies involving the use of inhibitors of lysosomal enzymes indicate that, in the presence of physiological concentrations of insulin, glucose, and amino acids, the lysosomal proteolytic system contributes to the degradation of 30–35% and 20–25% of intracellular proteins in enterocytes and skeletal muscle, respectively (Wu 2022). The lysosomal system is not involved to a significant extent in the degradation of myofibrillar proteins in skeletal, cardiac, or smooth muscles.

2. *Nonlysosomal proteolytic pathways.* A variety of low- and high-molecular-mass proteases are found outside the lysosome. Based on catalytic mechanisms, the nonlysosomal pathway for proteolysis can be divided into (a) the Ca^{2+}-dependent proteolytic system (calpain), (b) the caspases (e.g., caspases 1, 3, and 9; cysteine proteases), (c) the ATP-dependent, ubiquitin-independent proteolytic system (e.g., the 20S proteasome), and (d) the ATP- and ubiquitin-dependent proteolytic system (e.g., the 26S proteasome; Goll et al. 2008). All of these proteolysis pathways are present in the cytoplasm and may also be expressed in certain organelles (e.g., peroxisomes, nuclei, and mitochondria). The optimal pH for nonlysosomal proteases is 7–8. The nonlysosomal system is responsible for the degradation of (a) normal short-lived proteins, (b) abnormal, denatured, and aged proteins under basal metabolic conditions, and (c) both myofibrillar and nonmyofibrillar proteins under conditions of nutritional deprivation (Wu 2022). Depending on cell type, the nonlysosomal system can contribute to the degradation of 70–80% of intracellular proteins in the presence of physiological concentrations of insulin, glucose, and amino acids.

Biological Half-Lives of Proteins

Because of their different amino acid sequences and structures, different proteins are degraded at different rates in animal cells. Thus, the half-lives of intracellular proteins range from several minutes for short-lived proteins to days for long-lived proteins and to months for very long-lived proteins (Wu 2022). Abnormal proteins are more rapidly degraded than normal proteins. The rates of degradation of normal proteins vary widely with their functions. Enzymes at key metabolic control points usually have a much shorter half-life than those enzymes that catalyze near-equilibrium reactions. For example, ornithine decarboxylase (which catalyzes a rate-controlling step of polyamine synthesis) has a half-life of 11 minutes, whereas actin and myosin (cytoskeletal proteins) in skeletal muscle have a half-life of ≥30 days.

Energy Requirement for Intracellular Protein Degradation

The energy requirement for peptide bond cleavage cannot be explained by thermodynamic considerations because the hydrolysis of peptide bonds is an exergonic process (Goll et al. 2008). However, experimental evidence shows that ATP is required for protein breakdown by (a) ATP-dependent but ubiquitin-independent proteases (2 ATP molecules/peptide bond) and (b) the ATP- and ubiquitin-dependent 26S proteasome (equivalent to 3 ATP molecules/peptide bond). Combining all proteolytic pathways in cells, approximately 2 ATP molecules are used for the cleavage of one peptide bond in proteins.

Let us use the energetic cost of protein degradation at 2 mol of ATP/mol of protein to calculate the amount of ME needed for protein degradation in animals. This is equivalent to 170.8 kJ ME for 1 mol (or 100 g) of degraded protein (i.e., 2 mol of ATP × 85.4 kJ/mol = 170.8 kJ) or 1.708 kJ/g of degraded protein.

Measurements of Intracellular Protein Degradation

Intracellular protein degradation releases free amino acids. Thus, the rate of protein degradation is also measured by the release of a nonmetabolizable amino acid (labeled or nonlabeled amino acid) from intracellular proteins by cells or tissues (Bergen 2008). Such a measurement is affected by the re-incorporation of amino acids into newly synthesized proteins. In incubated cells or tissues whose pathway of protein synthesis is not inhibited, the release of an amino acid is an indicator of only net protein degradation, and protein synthesis should be determined simultaneously to calculate the rate of protein degradation. In contrast, when an inhibitor of protein synthesis (e.g., 0.5 mM cycloheximide) is included in the incubation medium, the release of an amino acid is an indicator of total protein degradation (which can simply be called protein degradation) in cells or tissues (Wu and Thompson 1990). In studies involving the release of a labeled amino acid from labeled proteins, inclusion of a high concentration of the unlabeled amino acid in the incubation medium (e.g., 1 mM phenylalanine in a

medium for skeletal muscle and intestinal cells) is sufficient to minimize the reincorporation of the labeled tracer amino acid into the protein pool.

$$\text{Net protein degradation} = \text{Protein degradation} - \text{Protein synthesis}$$

$$\text{Protein degradation} = \text{Net protein degradation} + \text{Protein synthesis}$$

Measurements of intracellular protein degradation in vivo also involve the use of a labeled amino acid (Waterlow et al. 1978). Stable isotope tracers are often used in humans and large farm animals because they are ethically acceptable and safe for studies. In contrast, radioactive tracers are good choices for studying in vivo protein degradation in rodents and small farm animals because of their high sensitivity, easy analysis, and low costs. In rapidly growing animals, assessment of intracellular protein degradation can be based on fractional rates of protein synthesis and protein growth, as follows:

$$K_d = K_s - K_g,$$

where K_d (%/day) is the fractional rate of protein degradation, K_s (%/day) is the fractional rate of protein synthesis, and K_g (%/day) is the fractional rate of protein growth.

Urinary excretion of 3-methylhistidine is often used as a noninvasive method to estimate whole-body muscle proteolysis in certain species of animals (Young and Munro 1978). As noted previously, post-translational methylation of some histidine residues in actin and myosin proteins generates 3-methylhistidine residues. After these proteins are hydrolyzed by intracellular proteases, 3-methylhistidine is produced by skeletal, smooth, and cardiac muscles and is not reincorporated into proteins (Bergen et al. 1987). 3-Methylhistidine is quantitatively excreted in the urine in some species (including cats, cattle, chickens, deer, frogs, humans, rats, and rabbits) and can therefore be a useful noninvasive technique for measuring in vivo protein degradation in these animals. In some species (e.g., dogs, goats, mice, pigs, and sheep), 3-methylhistidine cannot be quantitatively excreted in the urine and thus is not a useful indicator of muscle protein degradation. In pigs, sheep, and goats, 3-methylhistidine reacts with β-alanine to form β-alanyl-L-3-methylhistidine. In dogs, 3-methylhistidine undergoes decarboxylation to form 3-methylhistamine, and a large amount of 3-methylhistidine is excreted in feces. In mice, 3-methylhistidine is also decarboxylated to form 3-methylhistamine, followed by oxidative deamination to yield 1-methylimidazole-4-acetic acid, whereas 3-methylhistidine can be acetylated to *N*-acetyl-3-methylhistidine.

Nutritional and Physiological Significance of Protein Turnover

Both protein synthesis and protein degradation require significant amounts of energy. In maintenance, the intracellular protein turnover requires a total of 7 mol of ATP/mol protein (5 mol of ATP for protein synthesis and 2 mol of ATP for protein degradation). This does not appear to have the advantage of efficient utilization of dietary energy. Then the question is, why do cells continuously degrade the proteins that have been synthesized? Also, during fasting in animals, including humans, a decrease in protein loss would be advantageous to their survival. Why does protein breakdown continue even under catabolic conditions? These questions can be answered on the basis of the nutritional and physiological significance of protein turnover.

Protein Turnover Regulates Cellular Levels of Proteins (Including Enzymes) and Cell Growth

Animal growth depends on the balance between the rates of protein synthesis and protein degradation in their tissues, particularly skeletal muscle (Table 7.27). The rate of protein synthesis is much greater than the rate of protein deposition in growing animals (Davis et al. 2004). For example, in 30-kg pigs, an increase in the deposition of 1 g of protein

TABLE 7.27
Effects of Dietary Protein Quality and Energy Intake on Protein Deposition in Growing Pigs (30–40 kg Body Weight)

	Change (g protein/day)		
Dietary Factor	**Protein Deposition**	**Protein Synthesis**	**Protein Degradation**
Feed intake (3 times vs. 2 times maintenance requirement)[a]	62	97	35
Protein quantity (29% vs. 15% CP in diet)	23	127	104
Protein quality (sufficient vs. insufficient lysine)	75	40	–35
Dietary energy (with or without added carbohydrate)	43	20	–23
Dietary energy (with or without added fats)	60	20	–40

Source: Reeds (1989).

[a] Maintenance requirements were 7.4 MJ metabolizable energy/day and 8.1 g digestible CP/day/kg of body weight$^{0.75}$.

in the body requires the synthesis of 1.5 to 2 g of protein coinciding with the degradation of 0.5 to 1 g protein (Reeds 1989). In general, the deposition of 1 g of protein in tissues is associated with the retention of 3 g of water. Thus, protein accretion is the major determinant of BW gain in animals. The efficiency of protein gain in the body decreases with age, primarily because the fractional rate of protein synthesis is reduced with advanced age, while the fractional rate of protein degradation remains largely unchanged, as reported for 30- to 90-kg pigs (Davis et al. 2004; Reeds et al. 1980).

$$\text{Cellular protein} = \text{Protein synthesis} - \text{Protein degradation}$$

Changes in cellular protein concentrations are also important for the metabolic control of enzymes in various pathways such as amino acid oxidation, the urea cycle, gluconeogenesis, ketogenesis, and the Krebs cycle. Maintenance of intracellular and extracellular homeostasis of proteins is consistent with their essential functions in (a) cell structure (integral and peripheral membrane proteins); (b) extracellular matrix (collagen, elastin, and proteoglycans); (c) enzyme-catalyzed reactions; (d) gene expression (e.g., histones and methylases); (e) hormone-mediated effects (e.g., insulin and growth hormone, and placental lactogen); (f) muscle contraction (actin, myosin, tubulin, tropomyosin, and troponin); (g) osmotic regulation (e.g., proteins in plasma); (h) protection (e.g., blood clotting factors, antibodies, and interferons); (i) regulation of metabolism (e.g., calmodulin, leptin, and osteopontin); (j) storage of nutrients and O_2 (e.g., ferritin, metallothionein, and myoglobin); and (k) transport of nutrients and O_2 (e.g., albumin, hemoglobin, and plasma lipoproteins). Thus, a loss of more than 50% of the protein from the body is not compatible with the survival of animals.

Protein Turnover Is Required for the On-going Production of Rapidly Growing Cells

These cells include epithelial cells of the GIT, mammary glands, skin, and placental cells; reticulocytes; lymphocytes, macrophages, and other immunocytes; and eggs and sperm of the female and male reproductive systems, respectively. For example, the rapid growth of neonatal enterocytes requires high rates of net protein synthesis. In addition, when an animal is immunologically challenged, T-lymphocytes and B-lymphocytes rapidly proliferate so that large amounts of lymphokines and antibodies are generated to mount a successful response. Furthermore, successful wound healing, which is critical to the recovery of patients from injury, requires proliferation of endothelial cells, angiogenesis, collagen synthesis, granulation tissue formation, epithelialization, and wound contraction by the action of myofibroblasts. Increased provision of amino acids, particularly arginine, proline, and glycine, can promote protein synthesis and accelerate wound healing in injured animals.

Protein Degradation Is Required to Remove Aged, Abnormal, and Denatured Proteins

These proteins occur due to changes in extracellular and intracellular environments (e.g., pollution, heat stress, and the oxidative stress induced by free radicals and other oxidants). The presence of abnormal proteins within cells may interfere with the normal cellular metabolism or result in changes in cell volume, osmolarity, and function. This issue can be illustrated by the onset of amyloidosis, which results from the deposition and misfolding of amyloid fibrils (firm and solid extracellular substances, e.g., extracellular proteins) into tissues such as the spleen, liver, kidney, brain, lymph nodes, adrenal glands, and joints. In addition, protein degradation plays an important role in adaptation to nutritional deprivation (e.g., fasting, insufficient energy consumption, and the lack of dietary protein intake) and pathological conditions (e.g., burn, cancer, infection, inflammation, and injury) by supplying amino acids (e.g., alanine, glutamine, and arginine) for gluconeogenesis, ammoniagenesis, ATP production, the synthesis of essential proteins, the production of neurotransmitters, the generation of gaseous signaling molecules, and immunity.

Nutritional and Hormonal Regulation of Intracellular Protein Turnover

Dietary Provision of Amino Acids and Energy

The synthesis of proteins, including potent anabolic hormones (e.g., insulin, growth hormone, and insulin-like growth factor-I), requires all building blocks (i.e., amino acids), as well as sufficient energy, enzymes, and cofactors (Wu 2022). In addition, amino acids stimulate the release of these hormones from the endocrine organs. Thus, the availability of the amino acids, which are provided ultimately in diets, is crucial for protein synthesis in all animals (Buttery 1983; Posey and Davis 2023; Zhang et al. 2015, 2021). For example, in 30-kg pigs, the rate of whole-body protein synthesis is 270 g/day at energy equilibrium and is increased to 406 and 512 g/day, respectively, when their ration supplies twice and three times their maintenance (7.4 MJ ME/day and 8.1 g digestible CP/day per kilogram of $BW^{0.75}$) requirements (Reeds et al. 1980). Compared with carbohydrate supplementation, adding an isocaloric amount of fats to the diet of growing pigs does not affect protein synthesis but reduces protein degradation, resulting in greater protein deposition in the whole body (Table 7.27). It is likely that the metabolism of fats is more effective at sparing amino acids for tissue protein synthesis than carbohydrates.

All proteinogenic amino acids must be present in the cytosol at the same time and in the proper ratios to ensure optimal synthesis of proteins in cells. A deficiency of any amino acid (including an NEAA) impairs protein synthesis from other amino acids. This nutritional principle is the basis for feeding animals with diets containing adequate amounts of high-quality protein, which provides optimal ratios and sufficient concentrations of amino acids in the

plasma for protein synthesis in the whole body. Thus, as reported for young pigs (Reeds 1989), an improvement in dietary protein quality can result in a greater rate of protein deposition in the body than a simple increase in the amount of low-quality protein intake (Table 7.27).

MTOR Cell Signaling

Initiation of polypeptide formation is upregulated by the activation of the mechanistic target of rapamycin (MTOR; a highly conserved serine/threonine protein kinase), which integrates diverse signals from nutrients, hormones, and growth factors to upregulate protein synthesis in tissues (e.g., skeletal muscle, small intestine, mammary glands, and placenta) of animals including cattle, cats, chickens, dogs, fish, goats, horses, pigs, rats, and sheep (Bazer et al. 2014; Hu et al. 2025; Suryawan et al. 2011; Wagner and Urschel 2012). The MTOR system consists of two structurally and functionally distinct components: MTOR complex 1 (MTORC1) and MTOR complex 2 (MTORC2; Figure 7.41). The enzymatic activity of MTOR depends on its phosphorylation by an upstream kinase, which is activated by growth hormone, insulin, insulin-like growth factor-1, a variety of other growth factors, glucose, amino acids (e.g., arginine, glutamine, isoleucine, leucine, glycine, proline, tryptophan, and valine), and physiological levels of NO (Columbus et al. 2015; Dai et al. 2013b; He et al. 2024b; Rezaei and Wu 2022; Sun et al. 2016; Xi et al. 2012). The activated MTOR phosphorylates two downstream target proteins: eukaryotic initiation factor 4E-binding protein-1 (4EBP1, a translational repressor protein) and ribosomal protein S6 kinase-1 (S6K1).

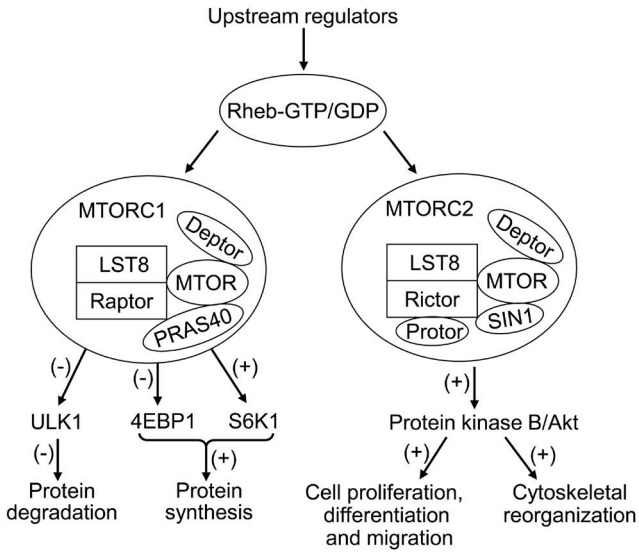


FIGURE 7.41 The MTOR complexes in animal cells to regulate intracellular protein turnover. 4EBP1, eukaryotic initiation factor 4E-binding protein-1; LST8, lethal with SEC13 protein 8 (also known as GβL); MTORC1, mechanistic target of rapamycin complex 1; MTORC2, mechanistic target of rapamycin complex 2; PRAS40, proline-rich Akt substrate of 40 kDa; SIN1, stress-activated MAP kinase interacting protein 1; S6K1, ribosomal protein S6 kinase-1; ULK1, unc 51-like kinase 1.

In a nonphosphorylated state, 4EBP1 binds tightly to eIF4E (an initiation factor) with high affinity, such that free eIF4E is not available to bind eIF4G (another initiation factor) to form a translationally active complex. When 4EBP1 is phosphorylated, eIF4E is dissociated from eIF4E-BP1 to bind with eIF4G, generating the eIF4F-eIF4G complex. In contrast, S6K1 phosphorylation results in the phosphorylation of ribosomal protein S6 (RPS6) to yield the RPS6-48S complex and finally the translationally active 80S ribosome for the initiation of polypeptide synthesis. In addition to the regulation of protein synthesis, the activation of MTORC1 also inhibits ULK1 (unc 51-like autophagy activating kinase) to suppress autophagy and lysosomal protein degradation in cells. Activation of MTORC2 stimulates cell proliferation, differentiation, and migration, as well as cytoskeletal reorganization (Figure 7.39). Thus, the combined actions of MTORC1 and MTORC2 play important roles in the growth and development of cells, particularly at fetal and neonatal stages (Bazer et al. 2015; Paudel et al. 2021).

Dietary supplementation with functional amino acids (e.g., arginine, BCAAs, glutamine, glycine, proline, and tryptophan) that activate MTOR has become an effective means to improve the growth performance (including BW gain and feed efficiency), productivity (e.g., pregnancy and lactation), and health of animals (Wu 2022). For example, oral administration of 0.2, 0.4, and 0.8 g of glycine/kg BW/day to IUGR piglets for 14 days did not affect milk consumption (Hu et al. 2025) but increased plasma concentrations of glycine and BW gain, as compared with the control group (Table 7.28). In addition, glycine supplementation stimulated the MTOR cell signaling (namely, the phosphorylation of the MTOR, 4EBP1, and S6K1 proteins) and protein synthesis in the skeletal muscle of IUGR piglets (Hu et al. 2025). Adequate intake of dietary amino acids is also useful to mitigate sarcopenia (losses of skeletal muscle mass and function) in aging humans (He et al. 2025) and animals (Li and Wu 2023).

Phosphorylation of Eukaryotic Translation Initiation Factor 2α (eIF2α)

In addition to MTOR, eIF2α [the α subunit of eukaryotic initiation factor 2 (eIF2, a GTPase)] is another important regulator of protein synthesis in animal cells. eIF2 forms a ternary complex with GTP and the initiator tRNA (Met-tRNA$_i$) and brings the Met-tRNA$_i$ to the 40S ribosome to promote the formation of the 43S subunit. Phosphorylation of eIF2α reduces its activity and general mRNA translation (Ryoo 2024). This occurs under the conditions of starvation or a deficiency of amino acids. Interestingly, excess NO induces eIF2α phosphorylation in mammalian cells (e.g., hepatocytes, macrophages, pancreatic β-cells, and

TABLE 7.28
Effects of Oral Administration of Glycine (Gly) on Growth, Plasma Amino Acid Concentrations [AAs], and Muscle MTOR Cell Signaling in Piglets with Intrauterine Growth Restriction (IUGR)[a]

Oral Gly Dose (g/kg BW/day)	Body Weight (kg; n = 10)			Plasma [AAs] (μM; n = 6)			Ratio of P-Protein to GAPDH (n = 6)		
	Day 0	Day 7	Day 14	Glycine	Serine	Threonine	P-MTOR	P-4EBP1	P-S6K1
0	0.86 ± 0.07	1.63 ± 0.08[c]	2.30 ± 0.12[d]	586 ± 10[e]	278 ± 13[c]	154 ± 7.7[c]	0.17 ± 0.03	0.40 ± 0.08	0.20 ± 0.06
0.2	0.88 ± 0.02	1.88 ± 0.06[b]	2.76 ± 0.08[c]	890 ± 17[d]	312 ± 8.6[b,c]	171 ± 6.1[b,c]	---	---	---
0.4	0.85 ± 0.04	2.07 ± 0.05[b]	3.16 ± 0.14[b]	1134 ± 19[c]	328 ± 12[b]	189 ± 11[b]	0.73 ± 0.07[†]	1.08 ± 0.05[†]	0.56 ± 0.05[†]
0.8	0.88 ± 0.04	2.01 ± 0.08[b]	3.07 ± 0.07[b]	1382 ± 22[b]	347 ± 6.8[b]	194 ± 11[b]	---	---	---

[a] Adapted from Hu et al. (2025). Data are means ± SEM, with the number (n) of piglets/group given in the parentheses. Piglets were the offspring of Yorkshire × Landrace sows bred to Duroc boars.

[b-e] Within a column, means not sharing the same superscript letter differ ($P < 0.05$).

[†] Different ($P < 0.05$) from the isonitrogenous control group (0 g glycine/kg BW/day).

Note: P-4EBP1, the phosphorylated protein of eukaryotic initiation factor 4E-binding protein-1; GAPDH, glyceraldehyde 3-phosphate dehydrogenase protein (a house-keeping protein as the loading control); P-MTOR, the phosphorylated protein of mechanistic target of rapamycin; P-S6K1, the phosphorylated protein of ribosomal protein S6 kinase-1 (S6K1).

tumors) and inhibits the rates of their protein synthesis (Kim et al. 1998). This may explain, in part, why the oral administration of a relatively high dose of arginine is not effective in improving animal growth. For example, beginning immediately after birth, the oral administration of 0.2 and 0.4 g of arginine (as arginine-HCl)/kg BW/day for 14 days increased the weight gains of sow-reared IUGR piglets by 24% and 35%, respectively, but a higher dose of arginine (i.e., 0.8 g/kg BW/day) had no effect compared with the isonitrogenous control group without arginine supplementation (Long et al. 2025).

Physiological and Pathological Stresses

Under stress conditions (e.g., heat, cold, transportation, weaning, and social mixing stresses) where the circulating levels of glucocorticoids and the production of oxidants are markedly elevated, muscle protein synthesis is depressed and muscle proteolysis is augmented, leading to no or even a negative weight gain in animals (including mammals, birds, and fish; McAllister et al. 2000; Samuels and Baracos 1995; Southorn et al. 1990). An effective approach to ameliorate the stress-induced negative protein balance may be dietary supplementation with Yucca extracts (120–180 ppm), a structural analogue of glucocorticoids (Chapter 3). By antagonizing glucocorticoids, *Yucca Schidigera* extracts can improve protein deposition and growth performance in animals (e.g., chickens reared at high ambient temperatures; Rezaei et al. 2017). Likewise, in diseases (including sepsis), inflammatory cytokines stimulate muscle protein degradation and inhibit muscle proteolysis, leading to the loss of body protein (Baracos et al. 1987; Bergen 2008; Orellana et al. 2011). This is even more severe when the subjects eat no or very little food.

Efficiencies in the Use of Dietary Amino Acids for Protein Deposition in Animals

Factors affecting protein digestion and intracellular protein turnover also influence the efficiencies in the use of dietary amino acids for protein deposition in mammals, birds, fish, and crustaceans. These factors include intakes of energy and nutrients (e.g., particularly amino acids), animal species, physiological state (e.g., age, pregnancy, lactation, and exercise), disease, sex, and environment. In young, growing pigs and chickens, about 42% and 40% of dietary protein is converted into tissue protein in the body (Table 7.29). The ratios of total amino acids deposited in the bodies of juvenile fish to total ingested dietary amino acids vary from 28% for Nile tilapia to 39% for rainbow trout, with a value of ~30% for most of the fish species studied, including carnivores, herbivores, and omnivores. The values for shrimp vary greatly among species such as 34% for Indian white shrimp, 46% for Pacific white shrimp (46%), and only 12% for black tiger shrimp. Among proteinogenic amino acids, proline has the greatest rates of deposition in HSB, whereas glycine has the greatest rates of deposition in LMB, Atlantic salmon, red drum, Nile tilapia, goldfish, and Indian white shrimp, and the second-greatest rates of deposition in rainbow trout. Most fish have lower rates of retention of absorbed amino acids in their bodies (31–43%) than Pacific white shrimp (51%) but greater rates than black tiger shrimp (12%). The rates of production of CO_2 and ammonia from amino acids and the rates of use of dietary amino acids for creatine synthesis also differ markedly among different species of aquatic animals or between aquatic and terrestrial animals (Table 7.29). Further research is warranted to enhance the conversion of dietary amino acids into protein and creatine in farm animals while reducing the excretion of amino acids and ammonia.

TABLE 7.29
Metabolic Transformation of Absorbed Dietary Amino Acids (AAs) in Fishes, Shrimp, Pigs, and Chickens[a]

	Carnivores					Herbivores		Omnivores			Shrimp			Land Animals	
AA	HSB	LMB	Atlantic Salmon	Rainbow Trout	Red Drum	Grass Carp	Silver Carp	Channel Catfish	Nile Tilapia	Gold-Fish	IWS	PWS	BTS	Pig	Chicken
Percentage (%) of Absorbed Dietary AAs Deposited in the Whole Body															
Ala	45.5	35.8	39.2	40.5	48.9	42.1	32.0	48.3	35.5	60.6	43.6	50.1	12.3	60.5	58.5
Arg	32.2	31.2	41.6	50.1	42.1	40.0	31.4	48.2	26.7	50.0	26.8	46.7	11.8	49.5	47.0
Asx	24.2	32.2	44.1	50.4	43.8	33.9	40.0	49.6	33.7	38.5	50.3	47.1	12.6	35.0	33.1
Cys	33.3	42.3	29.4	24.9	29.4	43.9	22.5	74.1	24.4	58.3	22.6	68.1	13.3	37.6	41.9
Glx	28.6	31.0	26.6	34.1	17.6	30.6	23.2	24.3	24.9	21.6	30.9	42.5	12.9	36.0	33.5
Gly	38.4	50.1	57.7	55.6	64.0	35.1	34.2	30.8	44.2	95.6	53.2	67.5	11.4	122	107
His	32.7	35.0	50.3	52.9	33.2	41.9	29.5	27.9	31.2	28.5	44.5	37.5	15.8	36.9	38.3
Ile	29.2	29.9	35.2	44.3	31.8	36.3	32.2	31.5	30.2	24.6	38.4	46.4	14.6	38.5	36.7
Leu	30.3	30.5	26.9	32.8	29.8	36.0	30.5	29.8	31.7	25.9	45.2	45.5	11.9	36.5	35.4
Lys	31.0	28.4	46.4	64.7	36.9	35.4	45.7	38.6	37.7	33.8	37.2	50.2	13.9	54.7	51.8
Met	35.1	23.7	30.9	50.5	41.5	29.4	40.5	38.5	34.7	33.5	49.0	56.4	11.8	54.0	55.4
Phe	28.6	32.3	31.9	37.3	28.4	33.8	32.4	29.9	29.1	25.7	31.4	45.9	12.2	33.7	31.0
Pro	52.5	40.7	34.4	39.0	34.6	23.6	24.6	19.2	32.8	26.4	40.3	70.5	6.0	91.7	81.0
Ser	35.0	35.6	34.3	42.5	34.3	34.1	28.4	31.5	29.8	28.8	29.9	58.2	14.2	44.0	41.8
Thr	33.7	33.5	51.3	48.9	37.7	40.8	37.7	34.0	34.7	33.5	37.5	47.6	12.3	42.5	44.0
Trp	28.3	33.4	35.5	28.5	29.1	24.0	35.7	23.0	20.0	22.9	35.9	67.3	11.1	42.6	42.6
Tyr	26.9	29.6	38.2	34.3	25.1	30.5	34.1	23.6	29.0	19.3	36.1	70.5	15.5	31.7	30.1
Val	32.2	31.6	40.6	43.8	33.6	36.5	33.8	30.2	31.1	22.7	34.1	49.6	13.3	43.3	39.6
Tau	22.0	28.5	71.9	65.0	47.6	---	46.5	---	35.8	121	121	120	288	---	---
TAA	32.4	33.3	36.8	42.7	32.2	34.1	31.5	31.4	31.4	31.1	38.0	51.0	12.2	48.2	45.4
Production of CO_2 and Ammonia from Total Absorbed Dietary AAs by the Whole Body (μmol/g body weight/day)															
CO_2	250	260	69	136	364	217	70	179	281	161	184	160	704	116	763
NH_3	71	74	18	35	96	61	20	42	79	38	52	47	202	30	195
Percentage (%) of Absorbed Dietary AAs Used for Creatine (Cr) Synthesis in the Whole Body															
Arg	20.8	18.6	50.0	37.7	24.4	28.5	47.8	39.6	18.9	44.0	26.4	27.4	8.0	21.9	13.5
Gly	8.0	9.4	27.3	21.2	13.4	18.2	33.0	17.3	9.9	28.8	14.4	14.4	4.4	13.2	7.7
Met	47.4	42.0	38.1	24.9	13.2	13.2	36.1	11.3	52.5	55.8	40.7	37.8	10.6	34.3	8.1
Ratios of Absorbed Dietary AAs Used for Cr Synthesis to Absorbed Dietary AAs Used via Nonprotein Synthesis Pathways (g/100 g)															
Arg	30.8	27.1	85.5	75.7	42.1	47.5	69.6	76.3	25.8	87.9	36.1	51.4	9.0	43.3	25.6
Gly	13.0	18.8	42.1	38.1	19.6	28.5	48.7	25.3	17.7	652	30.8	44.3	4.9	–61.3	–119
Met	73.0	55.1	58.1	40.8	20.2	17.3	47.9	14.0	80.4	83.8	79.9	86.8	12.0	74.6	18.1

Source: Gatlin and Wu (2025).

[a] The ranges of body weight (g) were: HSB, 5.6–45.7; LMB, 4.87–26.4; Atlantic salmon, 110–246; RBT, 34.8–175; red drum, 3.4–20.7; grass carp, 3.8–21.0; silver carp, 25.3–44.2; channel catfish, 9.6–25.8; Nile tilapia, 5.5–42.1; goldfish, 1.67–3.78; IWS, 0.34–8.44; PWS, 0.36–5.95; BTS, 2.4–5.2; pig, 9420–40200; and chicken, 118–297. Calculations are based on (a) the content of creatine plus creatine phosphate (21.6, 13.5, and 14.2 μmol/g fresh weight in fish, pigs, and chickens, respectively); (b) the spontaneous loss of creatine plus creatine phosphate via conversion into creatinine in the body (1.7%/day); and (c) 95% of the methionine-derived *S*-adenosylhomocysteine is recycled to methionine in the body. A value less than 0 means that the diet does not provide sufficient amounts of the AA for creatine synthesis in the body.

Note: Asx, aspartate plus asparagine; BTS, black tiger shrimp; Glx, glutamate plus glutamine; HSB, hybrid striped bass; IWS, Indian white shrimp; IWS, Indian white shrimp; LMB, largemouth bass; NH_3, total ammonia (NH_3 gas plus NH_4^+); RBT, rainbow trout; Tau, taurine; TAA, total amino acids.

DIETARY REQUIREMENTS OF ANIMALS FOR AMINO ACIDS

Need for Formulating Dietary Amino Acid Requirements of Animals

Livestock, poultry, and fish of agricultural importance are raised under various production conditions (e.g., intensive and extensive systems), depending on species, region, and season. For example, pigs, chickens, and dairy cows are commonly housed in pens and fed their formulated rations (an intensive system), whereas waterfowl (e.g., ducks and geese) often graze pastures and watersheds in developing countries. In contrast, many ruminants (e.g., cattle, sheep, goats, and deer) and horses usually graze pasture in rangeland, consume forages (an extensive system), and/or

receive feedlot diets containing various supplemental levels of energy, protein, vitamins, and minerals. Knowledge of amino acid metabolism is the foundation for the development of protein or amino acid supplements for the diets of farm animals to optimize their survival, growth, development, and reproduction. Many countries have established committees to recommend amino acid requirements for animals (e.g., the NRC in the United States, Agricultural Research Council (ARC) in the United Kingdom, and Animal Nutrition Research Council in China). These recommendations serve only as guides for producers and researchers because animal metabolism is a dynamic process and its rate can change markedly in response to alterations in many nutritional, physiological, and environmental factors.

General Considerations of Dietary Requirements of Amino Acids

In determining dietary requirements of amino acids by animals, oxidation of amino acids in the body should be taken into consideration (Figure 7.42). First, regardless of amino acid balance in the diet, when the dietary intake of a given amino acid is augmented, its oxidation is increased according to the substrate-enzyme relationship of Michaelis-Menton kinetics. Thus, when the dietary intake of a given amino acid (e.g., lysine) is below the animal's requirement, its oxidation is reduced to spare it for protein synthesis. As noted previously, the tRNA-amino acid synthases have much lower K_M values for amino acid substrates than the enzymes that degrade amino acids. Only a small fraction of dietary amino acids is available for oxidation in animals fed an amino acid-balanced diet. Excessive amino acids are usually not stored in tissues and must be oxidized to CO_2, water, ammonia, and urea. Second, when the dietary intake of a given amino acid is optimal for protein synthesis, its oxidation is at a minimum level compared with its excess. In an amino acid-balanced diet, an excess of an amino acid would increase its oxidation, but not necessarily the oxidation of other amino acids. In contrast, in an amino acid-imbalanced diet, when one of the amino acids whose carbon skeleton is not synthesized by animal cells (e.g., lysine) is deficient in the diet, the oxidation of other amino acids (e.g., phenylalanine) is increased gradually with increasing dietary intake of other amino acids. This is because the short supply of the limiting amino acid restricts the utilization of other amino acids for protein synthesis, and all the nonlimiting amino acids are degraded in a tissue-specific manner.

Dietary requirements of amino acids can be classified as qualitative and quantitative. Qualitative requirements refer to the question, "What is needed by animals for maintenance and production (e.g., growth, lactation, reproduction, and sports competition)?" Quantitative requirements refer to the question, "How much is required by animals for maintenance and production?" Both terms are practically important in animal feeding practices. Traditionally, growth or N balance experiments are employed to determine both qualitative and quantitative requirements of dietary amino acids by animals.

Qualitative Requirements of Dietary Amino Acids

As noted preciously, amino acids were traditionally classified as EAAs, conditionally essential amino acids (CEAAs), and NEAAs. The term EAA was coined in 1912, and these amino acids must be provided to animals to maintain their growth and N balance. The EAAs are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine in all animals, and this list also includes arginine in birds and most fish species. CEAAs are amino acids that normally can be synthesized in adequate amounts by animals but must be provided in the diet to meet optimal needs under certain conditions [e.g., weaning and infection

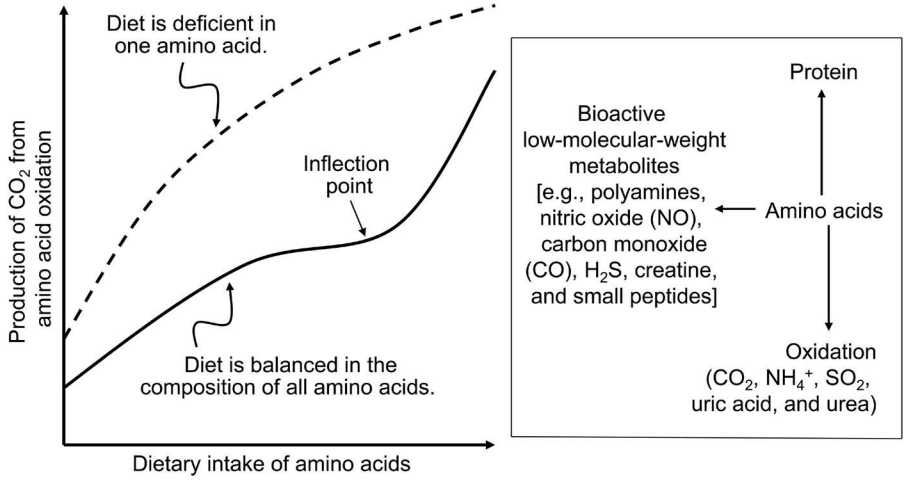


FIGURE 7.42 The relationship between the oxidation of amino acids (AAs) and protein synthesis in animals. The interrelationships between AA oxidation and the dietary intake of AAs in animals. In animals fed an AA-adequate diet, an excess of a specific AA (indicated by the inflection point) results in an increase in its oxidation but not necessarily the oxidation of other AAs. In contrast, when an AA (particularly an AA not synthesized by animals) is deficient in a diet, protein synthesis is impaired and the oxidation of other AAs is increased with increasing dietary intake of AAs.

(Ewaschuk et al. 2011; Wu 2010)], wherein the rates of utilization are greater than rates of synthesis. The CEAAs are (a) arginine, glutamine, glutamate, glycine, and proline for most mammals and (b) glutamine, glutamate, glycine, and proline for birds, fish, and crustaceans (Li et al. 2021b,c). In contrast, NEAAs were considered to be dispensable in diets. In most mammals (e.g., humans, rats, and pigs), the traditionally classified NEAAs are alanine, arginine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, proline, serine, and tyrosine. Although the EAA and NEAA concepts have been used for over a century, growing evidence from studies with pigs, poultry, and fish has shown that animals do have dietary requirements of NEAAs to fulfill their genetic potential for maximum growth, reproduction, lactation, and production performance, as well as optimal health and well-being (Hou et al. 2015a; Yoshida et al. 2016). Thus, the term NEAAs should no longer be used in nutritional sciences. Hou and Wu (2017) recommended that NEAAs be replaced by a new term, AASAs, i.e., amino acids that are synthesizable de novo in animal cells. Because an animal has dietary requirements for AASAs for maximal growth and optimal health, amino acids should not be classified as EAAs or NEAAs.

The ability of animals to synthesize AASAs de novo has nutritional and physiological significance in ensuring their constant provision to the cell for maintaining its homeostasis (Liao et al. 2018). One can argue that the selective conservation of pathways for the syntheses of AASAs indicates that they are indispensable for the metabolic needs and survival of animals (Wu 2022). Conversely, the lack of EAA synthesis can help (a) spare phosphoenolpyruvate, D-erythrose-4-phosphate, and acetyl-CoA for the formation of glucose, nucleotides, and fatty acids, respectively; (b) reduce energy expenditure; and (c) minimize the numbers of proteins and intermediary metabolites, metabolic complexity, and cell size in animals. Although protein biosynthesis requires all proteinogenic amino acids, AASAs confer many functions that cannot be fulfilled by EAAs. In addition, AASAs are more abundant in the body than EAAs, and the needs for AASAs for growth, lactation, and egg production are greater than those for EAAs (Hou et al. 2016a).

Another concept often used in protein nutrition is the "limiting amino acid", which is defined as an amino acid that is in the shortest supply from the diet relative to its requirements by an animal for maintenance, growth, and health. Thus, nutritional and physiological needs of animals for amino acids, as well as the amount and bioavailability of amino acids in food ingredients, are determinants of limiting amino acids, which can vary with the different stages of the life cycle and the different sources of foods (Table 7.30). Limiting amino acids are usually EAAs and may also be functional amino acids (e.g., arginine, glycine, glutamate, and glutamine) under certain production conditions (e.g., weaning, pregnancy, lactation, and recovery from illness). The first limiting amino acid is often an EAA that is present in the diet in the least amount relative to the animal's daily requirement (Kim et al. 2005; Liao et al. 2015; Trottier et al. 1997). Lysine, tryptophan, threonine, and methionine are the 1st, 2nd, 3rd, and 4th limiting amino acids, respectively, in corn- and SBM-based diets for growing swine (Benevenga and Blemings 2007; Le Floc'h et al. 2011; Russell et al. 1998), whereas methionine, threonine, lysine, and valine are the 1st, 2nd, 3rd, and 4th limiting amino acids, respectively, in these diets for growing chickens (Baker and Han 1994; Fernandez et al. 1994). In addition, methionine, lysine, threonine, and tryptophan in ruminal microbes are the 1st, 2nd, 3rd, and 4th limiting amino acids, respectively, for the growth of calves and lambs, whereas lysine, methionine, histidine, and arginine are the 1st, 2nd, 3rd, and 4th limiting amino acids, respectively, for the lactation of cows fed corn- and forage-based diets (Hanigan et al. 1998; Räisänen et al. 2023; Schwab and Broderick 2017). Knowledge of the order of limiting amino acids in feedstuffs can facilitate precise dietary formulation and ensure that requirements of animals for amino acids are met cost-effectively. It should be borne in mind that an emphasis on limiting amino acids in nutrition should not negate the important roles of adequate dietary provision of AASAs or functional amino acids as both substrates and regulators of metabolic pathways to improve the growth, productivity, health, and survival of livestock, poultry, fish, crustaceans, and other animal species (Andersen et al. 2016; Le Floc'h et al. 2018; Liao et al. 2024; Luise et al. 2023; McCoard 2016; van Keulen et al. 2020; Wu 2010). For example, dietary supplementation with 0.4% or 0.8% arginine reduces embryonic/fetal mortality in gestating swine (Wu et al. 2018), dietary supplementation with 1–2% monosodium glutamate improves milk production by lactating sows (Li and Kim 2024; Rezaei et al. 2022a). Likewise, supplementing 0.4% and 0.5% citrulline to diets for gestating gilts (days 14–25; Li et al. 2023b) and beef cows (days 1–60; Gilbreath et al. 2025) increases embryonic survival, respectively. Furthermore, dietary supplementation with 1% glycine enhances postweaning growth and meat quality of pigs, particularly those with IUGR (He et al. 2023a).

Quantitative Requirements of Dietary Amino Acids

The classic method to assess amino acid requirements of animals involves growth or N balance studies during a period of time (e.g., days or weeks). Minimal requirements of amino acids can also be estimated by so-called factorial analysis. Namely, the loss of N in animals fed a protein-free diet (maintenance) + N deposited in animals + N excreted as animal products (e.g., milk, eggs, wool, and fetuses). Modern tracer techniques for assessing dietary amino acid requirements by animals have been developed. Recommended requirements of dietary EAAs are available for domestic and laboratory animals (e.g., NRC 2012), and those for dietary AASAs for pigs and chickens have recently been published (Wu 2014). Generally, the requirements of dietary amino acids by animals decrease with advanced age. Of note, dietary requirements of amino acids are much greater in growing fish (e.g., 30–60% CP) than in growing (5- to 100-kg BW) pigs (20–14% CP) and growing (0.05- to 3-kg BW) broilers (20–16% CP). Because the composition

TABLE 7.30
Limiting Amino Acids (AAs) in Foodstuffs for Pigs, Chickens (Broilers or Hens), and Ruminants

			Limiting Amino Acid					
Animal	**Stage**	**Feedstuff or Diet**	**First**	**Second**	**Third**	**Fourth**	**Fifth**	**Sixth**
Pigs	Growth	Barley	Lys	Thr	His	---	---	---
		Corn grain	Lys/Trp	Thr	Met	Ile	Val	Gly?
		DDG or DDGS	Lys	Trp	Thr	Arg	Met	His
		Milk protein	Gly	Arg	Cys	---	---	---
		Sorghum	Lys	Thr	Trp	Met	Val	Ile
		Soybean meal (SBM)[a]	Met	Lys	Trp	Val	Gly?	Arg?
		Wheat	Lys	Thr	Trp	---	---	---
		Corn- and SBM-based diet	Lys	Trp	Thr	Met	Val/Ile	Gly?
		Corn-, SBM-, and whey-based diet	Lys	Trp	Thr	Met	Val	Gly?
	Lactation	Corn- and SBM-based diet; no TM	Lys	Val	Thr	Ile	His	Arg?
		Corn- and SBM-based diet; TM	Thr	Lys	Val	Phe	Ile	Arg?
	Gestation	Corn- and SBM-based diet	Lys	Trp	Thr	Met	Arg?	Gly?
Broilers	Growth	Corn grain	Lys	Trp/Arg	Ile	Thr	Val	Gly?
		SBM[a]	Met	Thr	Lys	Val	Ile	Arg
		Corn- and SBM-based diet	Met	Thr	Lys	Val	Arg	Trp
Hens	Laying	Corn- and SBM-based diet	Met	Lys	Thr	Ile	Val	Arg
		Wheat-Sorghum-SBM-CM-based diet[b]	Lys	Met	Thr	Val	Mix[c]	Gly?
Cattle	Growth	Ruminal microbes	Met	Lys	Thr	Trp	His	Gly?
		Corn- and SBM-based diet	Met	Lys	Trp	Arg?	Gln?	Gly?
		Corn and its products (all or primary)[d]	Lys	Met	Trp	Arg?	Gln?	Gly?
		Forage-based diet	Met	Lys	His	Arg?	Gln?	Gly?
		High forage- and soyhull-based diet	Met	Lys	His	Arg?	Gln?	Gly?
Cows	Lactation	Ruminal microbes	Met	Lys	His	Arg?	Gln?	Gly?
		Corn- and forage-based diet	Lys	Met	His	Arg?	Gln?	Gly?
		Corn- and its products (all or primary)[d]	Lys	Met	His	Arg?	Gln?	Gly?
		Corn silage-based diet (little or no PS)	Lys	Met	His	Arg?	Gln?	Gly?
		Grass silage-based diet (little or no PS)	Lys	Met	His	Arg?	Gln?	Gly?
		High forage- and soyhull-based diet	Met	Lys	His	Arg?	Gln?	Gly?
		SBM as the major source of RUP	Met	Lys	His	Arg?	Gln?	Gly?
Sheep	Growth	Ruminal microbes	Met	Lys	His	Arg?	Gln?	Gly?
		Corn or its products (all or primary)[d]	Lys	Met	His	Gln?	Glu?	Gly?
		Forage-based diet	Met	Lys	His	Arg?	Gln?	Gly?
		High forage- and soyhull-based diet	Met	Lys	His	Arg?	Gln?	Gly?

Source: Cohen and Tanksley (1976), Fernandez et al. (1994), Fuller et al. (1979), Jahan et al. (2023), Kim et al. (2001), Liang et al. (2003), Macelline et al. (2021), Mavromichalis et al. (1998), Richardson et al. (1978), Räisänen et al. (2023), Russell et al. (1998), Schwab (2011), Storm and Ørskov (1984), van Keulen et al. (2020), Wu et al. (2022), and Wu et al. (2024).

[a] At inclusion levels found in typical swine or poultry diets.

[b] Reduced-CP diet (15% CP).

[c] Ile, Arg, His, Ile, and Trp are the co-fifth limiting AAs.

[d] Providing all or most of rumen-degraded protein in the diet.

CM, canola meal; DDGS, dried distillers grains with solubles; DDG, dried distillers grains; PS, protein supplement; TM, tissue mobilization

Note: RUP, rumen-undegraded protein; "?", possible but experimental evidence is needed to support the proposed classification.

of amino acids in the carcass is similar among different animal species and the relative rates of growth in fish are not necessarily greater than those for mammals and birds, fish may have low rates of AASA syntheses and require sufficient provision of these amino acids in diets to compensate for their inadequate endogenous synthesis.

Growth or N Balance Studies for Determining Dietary Requirements of Amino Acids

Young animals (e.g., pigs, cattle, sheep, goats, chicks, rats, and fish) are very sensitive to dietary provision of amino acids and therefore their growth performance is a useful indicator of their needs for dietary amino acids

(Baker 2005; Kim and Wu 2004; Mai et al. 2022). To further assess protein deposition in the body, N balance studies are often conducted with growing animals that require dietary amino acids for maintenance and growth. When there is no N accumulation in the body (zero N equilibrium), N intake from the diet should be equal to N excretion in various forms, including (a) urea, ammonia, nitrite, nitrate, amino acids, and other nitrogenous substances in urine; (b) NO gas, (c) fecal nitrogenous substances (N output); and (d) N losses through the hair and skin (Young and Borgonha 2000). When dietary provision of an amino acid is deficient, N balance in animals is not optimal and can even be negative. To date, with the availability of improved techniques for rapid and accurate amino acid analysis, amino acid but not N balance should be measured in animal research or production.

Factorial Method for Determining Amino Acid Requirements

Dietary requirements for amino acids by the whole body or a tissue of interest (e.g., the small intestine) can be estimated based on factorial analysis, namely, the sum of fecal and urinary N in response to a protein-free diet (maintenance), amino acids deposited in the body, and amino acids excreted as important products (e.g., milk and fetal growth). For certain amino acids, the factorial method can also be based on the sum of the needs for the amino acids for metabolic pathways and obligatory losses of the amino acids via secretions from the body. The obligatory loss of amino acids occurs through hair, sweat, nasal secretions, and seminal fluid (males) when an animal is fed an essentially N-free diet that meets energy requirements (Wu 2014). The factorial method is particularly useful for determining dietary requirements of amino acids that are synthesized in mammalian cells.

Tracer Studies for Determining Dietary Requirements of Amino Acids and Protein

The rate of oxidation of an amino acid depends on its concentration in the free pools (e.g., plasma and intracellular fluids), the nutritional status, the physiological needs of the subject, and environmental factors. As noted previously, excessive amounts of amino acids are generally not stored in the body and will be disposed of primarily via oxidation. A marked increase in the oxidation of an amino acid is usually an indicator of its excessive availability in the body, provided there are no significant changes in the concentrations of regulatory hormones, coenzymes, cofactors, or metabolites. In other words, if the supply of an amino acid exceeds its needs by animals, the rate of its oxidation to CO_2, water, ammonia, and urea is increased (Figure 7.42). In the direct amino acid oxidation method, the production of CO_2 from a test amino acid (e.g., L-[1-^{13}C]lysine) is measured to estimate its dietary requirement by the animal. In the indicator (indirect) amino acid oxidation method, the production of CO_2 from an amino acid (e.g., L-[1-^{13}C] phenylalanine) other than the test amino acid (e.g., proline) is determined to estimate the dietary requirement of the test amino acid (Ball et al. 1986). Since the early 1980s, direct and indirect amino acid oxidation techniques have been used to determine dietary requirements of many EAAs by swine and poultry (Elango et al. 2012), as well as companion animals (Singh et al. 2024).

Each method for determining dietary requirements of amino acids has its own strengths and weaknesses. The N balance study is a simple and relatively inexpensive approach to estimate dietary requirements of amino acids by animals, but is insensitive to the metabolic needs of amino acids within a short period and is beset with large variations among animals (Table 7.31). The common advantages of both the direct and the indicator amino acid oxidation methods over the N balance technique are that (a) dietary requirements of amino acids can be estimated within a few hours after several days of adaptation to experimental diets, and (b) changes in whole-body oxidation of a specific amino acid in response to different intakes of dietary amino acids can be assessed. However, N balance and isotope studies do not consider the functional needs of amino acids beyond protein synthesis in animals.

Physiological, Metabolic, and Anatomical Criteria for Assessing Dietary Requirements of Amino Acids

Determination of dietary requirements of EAAs for animals is simpler than that for AASAs because the organisms

TABLE 7.31
Limitations of Nitrogen Balance Studies in Assessing Dietary Requirements of AAs by Animals

1. Failure to account for all the losses of N from the body, resulting in underestimation of AA requirements;
2. High variability in daily N balance because small changes in N deposition may not be detected;
3. Failure to detect functional changes in cells and organs or to fully evaluate dietary requirements of AAs;
4. Not being sufficiently sensitive to dietary intake of certain AAs (e.g., Arg and His) particularly within a short experimental period;
5. An inability to provide information about the cellular processes of intermediary metabolism of AAs or protein;
6. Difficulty in interpreting the experimental data either when dietary protein intake is high or when dietary protein intake is low, because enzyme systems adapt and AA oxidation can be altered;
7. N balance being strongly influenced by dietary energy intake, environmental conditions, and changes in endocrine status;
8. Requirement of a relatively long period of adaptation to an experimental diet (e.g., 5-7 days for rats and pigs);
9. Possible inconsistency between animal growth and N balance because an animal can lose weight and still be in a positive nitrogen balance; and
10. Like all bioassays, the response per increment of intake declines as the maximum attainable response is approached.

Source: Wu (2022).

respond more sensitively and more rapidly to a deficiency of an EAA in the diet. The end points for determining dietary EAA requirements by animals (e.g., mortality, morbidity, food intake, growth, lactation, and reproductive performance) can be used to estimate their dietary requirements for AASAs (Wu 2022). Additional criteria can also be helpful and include assessments of physiological parameters (e.g., concentrations of hormones, hemoglobins, ammonia, urea, amino acids, nitrogenous metabolites, lipids, and glucose in plasma; concentrations of neurotransmitters, GSH, creatine, and polyamines in tissues; and cellular signaling; Halloran et al. 2022; Wu et al. 2014a). Furthermore, dietary requirements for AASAs can be based on any abnormal values for anatomical, physiological, biochemical, and immunological indices, including (a) abnormalities in small-intestinal morphology, mass, absorptive capacity, and integrity; (b) imbalances among amino acids and high concentrations of ammonia in plasma and urine; (c) dysfunctional regulation of nutrient metabolism to reduce muscle protein gain, promote white-fat accretion, and cause metabolic syndrome in the body; (d) impaired response of peripheral lymphocytes to stimulation by mitogens; (e) abnormal blood chemistry; and (f) abnormal organ function (e.g., impaired vision, skin lesions, and skeletal muscular weakness). Thus, multiple variables can be used to define dietary requirements of AASAs by animals, and the choices should depend on species and goals.

Factors Affecting Dietary Requirements of Amino Acids

Dietary requirements of amino acids by animals are highly dynamic and are affected by a variety of factors (Field et al. 2002; Remus et al. 2020; Wu 2022). These factors include (a) dietary composition (e.g., amino acid content and proportions, energy intake, presence or absence of other substances, and food processing); (b) physiological characteristics of animals (e.g., age, sex, genetic backgrounds, circadian clock, hormones, pregnancy, lactation, and physical activity); (c) pathological states (e.g., injury, infection, trauma, neoplasia, diabetes, obesity, cardiovascular disease, and fetal growth restriction); and (d) the living environment (e.g., hot or cold ambient temperatures, toxic agents, air pollution, sanitation, noise, and social grouping). Thus, caution should be exercised when one uses any recommended values of dietary amino acid requirements for animals. Feeding practice is the best way to test a nutritional theory.

The "Ideal Protein" Concept

The "ideal protein" concept, which was proposed for diets of chickens in the late 1950s, refers to optimal proportions and amounts of EAAs (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) without the consideration of any NEAAs (Fisher and Scott 1954). It was thought that the composition of EAAs in diets should be the same as that in eggs or casein. However, approaches based on this proposition were largely unsuccessful. An improvement in chicken growth performance was achieved when the profile of EAAs in the chick carcass was used to formulate dietary EAAs, but the result remained unsatisfactory (Klain et al. 1960). Subsequent studies showed that inclusion of both EAAs and several NEAAs (cysteine, glycine, proline, and glutamate) in diets yielded better results on chicken growth performance than EAAs alone (Baker et al. 1968; Graber and Baker 1973). This extensive research culminated in several versions of the "chick amino acid requirement standard" for the first three weeks post-hatching (e.g., Sasse and Baker 1973; Baker and Han 1994). However, the observed growth rate and feed efficiency of broiler chicks remained largely unsatisfactory due to incomplete knowledge of the composition and tissue-specific metabolism of amino acids in animals. For example, the published data on the composition of glycine, proline, and 4-hydroxyproline in the chicken carcass were highly inconsistent. In addition, the inclusion of a large amount of glutamate (e.g., 13 times the lysine value) in diets as an isonitrogenous control may interfere with the transport, metabolism, and utilization of other dietary amino acids. Thus, the NRC (1994) and Baker (1997) did not include glutamate, glycine, or proline in an ideal protein for diets of 0- to 56-day-old chickens. Omission of these three and other proteinogenic AASAs from the ideal protein is not desirable due to their important nutritional and physiological roles in poultry (Wu et al. 2014a). Note that AASAs are much more abundant than EAAs in the bodies of birds, with the ratio of AASAs/EAAs being 60.0:40.0 (g/g).

Research on the dietary ideal protein for chickens laid a foundation for studies with growing pigs. Cole (1980) suggested that swine diets could be formulated to contain ideal ratios of isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (with lysine as the reference value) based on their content in the pig carcass. This idea was adopted by the ARC (1981) and NRC (2012). Of note, histidine and all AASAs (including arginine) were not included in the ideal protein concept of the ARC for growing pigs or by Wang and Fuller (1989) for 25- to 50-kg gilts. This oversight was partially corrected by Chung and Baker (1992) in their work involving 10- to 20-kg swine through the addition of arginine, glycine, histidine, and proline (42%, 100%, 32%, and 33% of lysine, respectively) to the basal diet containing 1.2% true digestible lysine. However, due to incomplete knowledge of amino acid biochemistry and nutrition, the NRC (2012) did not recognize the needs of pigs for dietary proline or glycine. Consequently, proline and glycine were removed from the ideal protein in swine diets (Baker 2000; Kim et al. 2005). This conceptual foundation is flawed because (a) the pattern of amino acids in the diet does not match that in the animal body due to their extensive catabolism at different rates in the small intestine (Wu et al. 2014a), and (b) with the exception of arginine, all AASAs were not included in the ideal protein (Kim et al. 2001). Much evidence shows that animals have dietary

requirements for both EAAs and AASAs in proper proportions and adequate amounts (Hou et al. 2015a; Hou and Wu 2017). As in poultry, AASAs are also much more abundant than EAAs in the swine body, with the ratio of AASAs/EAAs being 60.6:39.4 (g/g). Wu's research group has recently proposed optimum patterns of dietary amino acid requirements for pigs (Wu and Li 2022) and chickens (He et al. 2021a), as well as companion (Li and Wu 2023) and zoo (Herring et al. 2021) animals.

Biochemical Basis for the Use of Low-Protein Diets to Feed Animals

In nonruminant animals fed conventional plant-based diets, the exogenous provision of all EAAs and most AASAs exceeds the needs for protein deposition in the bodies, albeit at suboptimal rates (Wu et al. 2014a). For example, in 30-day-old pigs that are weaned at 21 days of age to a corn- and SBM-based diet (21.5%-CP), the amounts of all EAAs and most AASAs (except aspartate, glutamate, and glycine) that enter the portal circulation are much greater than those in extraintestinal tissue proteins (Hou et al. 2016). Similar results are obtained for 14-day-old broilers fed a corn- and SBM-based diet containing 21.5% CP (He et al. 2021b). Any excessive amino acids are oxidized to CO_2 and ammonia in a tissue-specific manner. Thus, it is possible to reduce dietary CP levels by 15–25% without compromising the supply of most EAAs and AASAs or the growth performance of animals if small amounts of deficient amino acids [including lysine, methionine, threonine, tryptophan, and AASAs (i.e., glutamate, glutamine, and glycine)] are added to the low-CP diets (He et al. 2022; Hou and Wu 2018b). Furthermore, based on Michaelis-Menten kinetics for enzyme-catalyzed reactions (Chapter 1), reducing dietary CP intake can decrease the rates of whole-body amino acid oxidation, thereby minimizing the release of CO_2 and nitrogenous wastes. When a large amount of plant-sourced protein ingredient(s) is removed from diets, potentially adverse effects of this practice on the supply of other nutrients must be considered. For example, if the dietary level of SBM (which is a good source of potassium) is reduced, supplementation with this mineral (e.g., in the form of KCl) can prevent potassium deficiency. Collectively, there are both nutritional and biochemical bases for the use of amino acid-supplemented low-protein diets to satisfactorily feed animals without compromising their growth performance or productivity.

EVALUATION OF THE QUALITY OF DIETARY PROTEIN

The quality of any dietary protein depends on the following factors: (a) the amounts and profile of its amino acids, and (b) the digestibility and availability of its amino acids to the animal relative to nutritional requirements. A protein that has a balanced composition of all amino acids but cannot be hydrolyzed by proteases in the GIT cannot be a high-quality protein for animals. Thus, although the analysis of the amino acid content in feedstuffs is necessary to predict which amino acid is deficient or excessive in the diet, this chemical method should not be used as the sole means of evaluating the nutritive quality of feed proteins. Therefore, biological studies to determine protein digestibility in vivo and the animal's growth performance must be carried out concurrently with the determination of dietary amino acid composition (Chapter 4). In human nutrition, various amino acid scoring systems have been used over the past three decades, including the protein digestibility corrected amino acid score (PDCAAS, recommended in 1989) and the digestible indispensable amino acid score (DIAAS, recommended in 2011) (FAO 2013).

In the PDCAAS system, a single value of apparent (e.g., fecal) CP digestibility is used as the digestibility of each EAA in the dietary protein, the ratio of the amount (mg) of an apparently digestible EAA in 1 g of the dietary protein to the amount (mg) of the same EAA in 1 g of the reference protein is calculated for each EAA, and the lowest value (usually for the first limiting EAA) is designated as the PDCAAS; growing rats were recommended as test animals. In the newer DIAAS system, the ratio of the amount (mg) of a truly digestible EAA in 1 g of the dietary protein to the amount (mg) of the same EAA in 1 g of the reference protein is calculated for each EAA, and the lowest value is designated as the DIAAS; growing pigs are recommended as test animals (Hodgkinson et al. 2020). Both PDCAAS and DIAAS methods focus on only one EAA, but ignore the composition and balance of all other amino acids in test proteins.

$$\text{PDCAAS} = \frac{\text{Amount (mg) of a nutritionally essential amino acid (EAA) in 1 g of the dietary protein} \times \text{Apparent digestibility of the dietary crude protein}}{\text{Amount (mg) of the same EAA in 1 g of the reference protein}}$$

$$\text{DIAAS (\%)} = \frac{\text{Amount (mg) of a nutritionally essential amino acid (EAA) in 1 g of the dietary protein} \times \text{True digestibility of the amino acid in the dietary protein}}{\text{Amount (mg) of the same EAA in 1 g of the reference protein}} \times 100$$

Analysis of Amino Acids in Diets and Feed Ingredients

Proteins in complete diets or feed ingredients must be hydrolyzed to individual amino acids before their analysis. This is usually done by (a) acid hydrolysis for the analysis of amino

acids other than glutamate/glutamine, aspartate/asparagine, and tryptophan; and (b) alkaline hydrolysis solely for the quantification of tryptophan (Chapter 4). When feedstuffs are finely ground, their proteins can be hydrolyzed with a mixture of known proteases and peptidases of microbial, plant, and animal origin for the determination of glutamate, glutamine, aspartate, and asparagine (Li and Wu 2020). Alternatively, feed can be incubated with fluids obtained from the stomach and small intestine of postabsorptive animals to mimic the in vivo digestion process. Free amino acids can be measured by high-performance liquid chromatography, gas chromatography, or enzymic methods (Dai et al. 2014). Care must be taken in the analysis of proline and cysteine because their content in food and animal tissues has been substantially underestimated in some studies (see Li and Wu 2022, 2023 for review).

Values from chemical analysis can tell us which amino acid(s) in dietary proteins might be deficient, adequate, or excessive, but give little information on their availability to the animal. A good-quality protein contains high amounts of arginine, cysteine, lysine, methionine, threonine, and tryptophan. Glutamate, glutamine, asparagine, aspartate, plus BCAAs represent most of the amino acid content in both plant and animal proteins (Li et al. 2021a). Certain plant (e.g., peanut meal and cottonseed meal)- and animal (e.g., fish meal and gelatin)-sourced products provide high levels of arginine. However, feedstuffs of animal origin usually contain higher percentages of cysteine, lysine, methionine, threonine, and tryptophan than feedstuffs of plant origin. Unlike other animal products, the content of isoleucine is relatively low in red blood cells or blood meal (only 22% of leucine). Overall, feedstuffs of animal origin (except for gelatin, which is abundant in proline and 4-hydroxyproline but deficient in most EAAs) are excellent sources of all amino acids for livestock, avian, and aquatic species (Li and Wu 2022; Li et al. 2021a).

Determination of Protein Digestibility

Apparent vs. True or Standardized Ileal Digestibility of Dietary Protein

Technically, protein digestibility refers to the disappearance of amino acids throughout the total or part of the digestive tract. The total tract (or fecal) digestibility (TTD) of protein is relatively easy to determine in terms of sample collection and may provide useful information on the daily losses of dietary plus endogenous amino acids. This noninvasive method is preferred for studies of zoo or large-size animals (e.g., giant pandas and elephants) because of ethical concerns and limited resources. A higher TTD of protein may indicate a greater proportion of its total amino acids available for utilization by the body, but not necessarily a higher digestibility of protein in the small intestine. Based on studies of postweaning pigs, the TTD technique potentially underestimates the true digestibility of protein due to microbial N metabolism in the large intestine to add or remove amino acids (Sauer and Ozimek 1986).

As noted previously, the ileum is the last segment of the small intestine for the digestion of dietary protein to release amino acids. Before the 1980s, the content of amino acids in dietary protein and in the lumen of the distal ileum was analyzed to determine the digestibility of dietary protein. However, such values should only be considered as "apparent digestibility" (Moughan and Rutherfurd 2012) due to the large flow of endogenous amino acids into the lumen of the small intestine, as shown in growing pigs (Table 7.32) and lactating cows (Table 7.33). The sources of the endogenous amino acids include saliva, bile, gastric plus pancreatic

TABLE 7.32
Dietary and Endogenous Sources of Nitrogen in the Lumen of the Gastrointestinal Tract of Growing Pigs and Rats

Source	Growing Pigs[a] (mg N/kg BW)	Growing Rats[b] (% of Total N along the GIT)
Sources of N in the small intestine		
Diet	1530	34.2
Endogenous source	500	65.8
Saliva	14	---
Stomach	180	9.5[c]
Bile	50	ND
Pancreatic secretions	56	59.9[d]
Small-intestinal secretions	100	43.3
Microbes in the lumen	100	24.7
Absorption of N in the small intestine	1600	84.3 (diet + endogenous)
Secretions of N into the lumen of the large intestine	65	77.3 (endogenous)
Absorption of N in the large intestine	320	8.2 (diet + endogenous)
Fecal N excretion	175	3.4 (diet + endogenous)[e]

Source: Bergen and Wu (2009), Deglaire et al. (2008), and Fuller and Reeds (1998).

[a] Values are for the 30–50 kg growing pig consuming an 18%-crude protein diet. Its dietary dry matter intake was 5.3% of body weight per day.

[b] Values are for the 220- to 240-g growing rat consuming a casein-based diet, which provided 16.6 g dry matter/day (17.7 mg N/g dry matter). The dietary N intake of the rat was 294 mg/day. Endogenous secretions from the saliva + stomach, the bile + pancreas, the small intestine, and the large intestine (cecum + colon) were 31, 439, 17.5, and 77.2 mg N/day, respectively, totaling 859 mg N/day. The endogenous N absorbed by the small intestine was 453 mg/day (i.e., 31 + 439 − 17.5 = 453). Endogenous N flows in the digestive tract (% of total N along the GIT) were as follows: saliva plus stomach, 9.5; the proximal intestine, 59.9; the medial intestine, 49.4; the terminal ileum, 43.3; and cecum + colon, 77.3.

[c] Including the secretions from saliva and stomach.

[d] Including the secretions from the bile and pancreas.

[e] Assuming that the apparent fecal digestibility of dietary protein was 90% and that the amount of N in the excreted feces was 29.4 mg/day.

Note: BW, body weight; GIT, gastrointestinal tract; ND, not determined.

TABLE 7.33
Dietary and Endogenous Sources of Crude Protein (CP) in the Lumen of the Small Intestine of Lactating Cows and Growing Sheep

Source	Lactating Cows[a] (kg CP/day/cow)	Growing Sheep[b] (g CP/day/sheep)
Diet	2.26	110.6
Rumen	2.71	130
Diet	2.26	110.6
Endogenous sources (saliva, blood, and microbes)	0.45[c]	19.4
Duodenum	2.58	95.0
Diet	2.17	61.3
Endogenous sources (the bile and pancreas)	0.41	33.7
Ileum (apparent digestible CP)	1.84	23.7
Diet	1.63	16.4
Endogenous sources	0.21	7.3
Ileum (true digestible CP)	1.94	27.0
Diet	1.63	18.6
Endogenous sources	0.31	8.3
Feces	0.74	23.1
Diet	0.61	12.5
Endogenous	0.13[d]	10.6

[a] Lapierre et al. 2006. Lactating cows consumed a diet (74% forage and 26% concentrate) containing 17.5% CP (on a dry-matter basis).
[b] Krawielitzki et al. 1999. Growing Merino male sheep of ~25 kg body weight consumed daily 800 g dry matter of a diet (40% hay and 60% concentrates) containing 15.4% CP (on a dry matter basis).
[c] Taken from Ouellet et al. 2002. Secretions into the forestomachs represent 20% of dietary N intake. Urea accounts for 55% of endogenous N secretions.
[d] Calculated on the basis of the report that 18% of the fecal N is of the endogenous origin in lactating cows (Ouellet et al. 2002).

plus intestinal secretions, epithelial cells sloughed off the digestive tract, and possibly intestinal bacteria. Sauer and de Lange (1992) reported that endogenous CP in the small intestine of growing pigs fed SBM-, canola meal-, wheat-, and barley-based diets was 25.5, 30.5, 27.4, and 27.7 g/kg of DM intake, respectively. In all animals, there is usually a net flow of endogenous amino acids into the lumen of the ileum, leading to substantial underestimation (up to 30%) of the true digestibility of dietary protein in the small intestine (Reynolds and Kristensen 2008; Sauer et al. 2000). A cornstarch-based N-free diet is often fed to nonruminants for estimating basal endogenous amino acid flow (AA_{EIb}) in their small intestine, but such a technique is not applicable to ruminants because ruminal fermentation capacity would be greatly reduced. Rather, labeled amino acids (e.g., ^{15}N-leucine, ^{15}N-phenylalanine, or ^{15}N-lysine) are often used in intravenous infusion to determine endogenous amino acid flow in the small intestine of ruminants (e.g., sheep and cattle) and nonruminants (e.g., pigs and humans; Bandyopadhyay et al. 2022). In addition, the isotopic labeling of either body or dietary proteins can be employed to estimate amino acid digestibilities in mammals (including humans) and birds (Moughan 2023). Formulas for the calculation of apparent and true ileal amino acid digestibilities are provided in the following paragraphs.

a. When all digesta are collected at the terminal ileum, the apparent or true ileal digestibility of an amino acid can be calculated as follows (Knabe et al. 1989; Liao et al. 2005):

$$\text{Apparent ileal digestibility of AA} = \frac{\text{AA intake (g)} - \text{AA in ileal digesta (g)}}{\text{AA intake (g)}} = 1 - \frac{\text{AA in ileal digesta (g)}}{\text{AA intake (g)}}$$

$$\text{True ileal digestibility of AA} = \frac{\text{AA intake (g)} - [\text{AA in ileal digesta (g)} - AA_{EI}\ \text{(g)}]}{\text{AA intake (g)}} = \text{Apparent ileal digestibility of AA} + \frac{AA_{EI}\ \text{(g)}}{\text{AA intake (g)}}$$

AA_{EI} is the total flow of the endogenous amino acid into the lumen of the ileum (i.e., the total amount of the endogenous amino acid present in ileal digesta), expressed in grams. AA_{EI} can be measured by using the intravenous infusion of an ^{15}N-labeled amino acid (e.g., ^{15}N-leucine) or measuring the appearance of dietary homoarginine in the ileal digesta (Yin et al. 2015). In the tracer method, the presence of the ^{15}N-labeled amino acid at the end of the ileum reflects its entry from the blood into the small intestine. The homoarginine approach is based on the assumption that there is little or no endogenous secretion of homoarginine into the lumen of the small intestine and therefore homoarginine in the ileal digesta is of dietary origin only and reflects the true ileal digestibility of lysine. However, the validity of the homoarginine method should be re-evaluated in light of the recent evidence that tissues (including liver, kidneys, and brain) of pigs and rats can form homoarginine from arginine and lysine (Hou et al. 2015b, 2016b).

Because the measurement of AA_{EIb} in animals (especially large mammals) is time-consuming, resource-intensive, and expensive, the concept of standardized ileal digestibility (SID) has been developed over the past two decades

(Stein et al. 2007). When the published AA_{EIb} data for the same animal species replace the measured AA_{EI} in calculations using the above equation, SID values are obtained. Ideally, the experimental conditions (including the age, production stage, and physiological conditions of animals, as well as their diets and housing facilities) under which the reported AA_{EIb} values were determined should be the same as those for the calculation of the SID. Thus, caution should be taken in the use of the SID to ensure that its values can more accurately reflect the true ileal digestibilities of amino acids.

b. When an indigestible marker (e.g., 0.3% Cr_2O_3) is added to the diet to eliminate the need for total collection of ileal digesta (Mavromichalis et al. 2001), the apparent or true ileal digestibility of an amino acid can be calculated as follows, with all the measured variables being expressed as g/kg DM. Note that the term "Indicator content in diet/Indicator content in ileal digesta" is used to correct for the rate of the recovery of Cr_2O_3 from the ileal digesta.

$$\text{Apparent ileal digestibility of AA} = \frac{\text{AA content in diet} - \text{AA contant in ileal digesta} \times \dfrac{\text{Indicator content in diet}}{\text{Indicator content in ileal digesta}}}{\text{AA content in diet}}$$

$$= 1 - \frac{\text{AA content in ileal digesta}}{\text{AA content in diet}} \times \frac{\text{Indicator content in diet}}{\text{Indicator content in ileal digesta}}$$

$$\text{True ileal digestibility of AA} = \frac{\text{AA content in diet} - \left(\text{AA content in ileal digesta} - AA_{EI}\right) \times \dfrac{\text{Indicator content in diet}}{\text{Indicator content in ileal digesta}}}{\text{AA content in diet}}$$

$$= \text{Apparent ileal digestibility of AA} + \frac{AA_{EI}}{\text{AA content in diet}} \times \frac{\text{Indicator content in diet}}{\text{Indicator content in ileal digesta}}$$

AA_{EI} is the total flow of the endogenous amino acid into the lumen of the ileum (i.e., the total amount of the endogenous amino acid present in ileal digesta), expressed as g/kg of dietary DM.

Measurement of AA_{EIb} in the Small Intestine with the Use of an Indicator Technique

When an indigestible marker (e.g., 0.3% Cr_2O_3) is added to a cornstarch-based N-free diet, AA_{EIb} in the small intestine of an animal can be measured. All the measured variables are expressed as g/kg of DM. Accordingly, the unit of AA_{EIb} is g/kg of DM.

$$AA_{EIb} = \text{Endogenous AA in ileal digesta} \times \frac{\text{Indicator content in diet}}{\text{Indicator content in ileal digesta}}$$

Measurement of Protein Digestibility of a Feed Ingredient Added to a Basal Diet

If it is desirable to measure protein digestibility of a feed ingredient added to a basal diet, the principles of the calculations of protein digestibility are the same as those for the basal diet, except that changes in ileal amino acid content due to the added test ingredient should be considered.

1. When all digesta are collected at the terminal ileum, the formula for calculating the apparent or true digestibility of a protein-bound amino acid in the test ingredient is as follows:

$$\text{Apparent ileal digestibility of AA} = \frac{\text{AA intake from test ingredient (g)} - [\text{AA in ileal digesta}_{\text{Basal+Test}} \text{ minus AA in ileal digesta}_{\text{Basal}} \text{ (g)}]}{\text{AA intake from test ingredient (g)}}$$

$$\text{Ture ileal digestibility of AA} = \text{Apparent ileal digestibility of AA} + \frac{AA_{EI} \text{ resulting from test ingredient (g)}}{\text{AA intake from test ingredient (g)}}$$

The AA in ileal $\text{digesta}_{\text{Basal + Test}}$ is the amount of amino acid in the ileal digesta of the animal fed the basal diet plus the test ingredient, and the AA in ileal $\text{digesta}_{\text{Basal}}$ is the amount of amino acid in the ileal digesta of the animal fed the basal diet.

2. When an indigestible marker (e.g., 0.3% and 0.2% Cr_2O_3 in growing pigs and lactating cows, respectively) is added to the diet to eliminate the need for total collection of ileal digesta, the apparent or

true ileal digestibility of an amino acid can be calculated as follows, with all the measured variables being expressed as g/kg of DM.

$$\text{Apparent ileal digestibility of AA} = 1 - \frac{[\text{AA content in ileal digesta}_{\text{Basal+Test}} \text{ minus AA content in ileal digesta}_{\text{Basal}}]}{\text{AA content in test ingredient}} \times \frac{\text{Indicator content in diet}}{\text{Indicator content in ileal digesta}}$$

$$\text{True ileal digestibility of AA} = \text{Apparent ileal digestibility of AA} + \frac{\text{AA}_{\text{EI}} \text{ resulting from test ingredient}}{\text{AA intake from test ingredient}} \times \frac{\text{Indicator content in diet}}{\text{Indicator content in ileal digesta}}$$

The AA in ileal digesta$_{\text{Basal + Test}}$ is amino acid content in the ileal digesta of the animal fed the basal diet plus test ingredient, whereas the AA in ileal digesta$_{\text{Basal}}$ is amino acid content in the ileal digesta of the animal fed the basal diet.

Note that an indigestible marker can also be used to estimate the flow of DM or a specific nutrient along the length of the GIT in an animal fitted with a cannula. Under this experimental condition, digesta samples are collected, through the cannula, from a segment (e.g., duodenum) of the GIT for chemical analyses. For example, the flow of DM within the duodenum of a pig or cow fed a diet containing 0.3% Cr_2O_3 can be calculated as follows:

$$\text{DM flow within the duodenum (g/day)} = \frac{Cr_2O_3 \text{ content in feed (mg/g of DM)} \times \text{DM intake (g/day)}}{Cr_2O_3 \text{ content in duodenal digesta (mg/g of DM)}}$$

Animal Feeding Experiments to Determine the Quality of Dietary Protein

The quality of dietary protein is best evaluated by determining its bioavailability to animals (Table 7.34). The test animals are usually chicks and rats based on practical convenience and low costs. Ideally, this is performed using multiple levels of a test protein in the diet that provides adequate caloric intake. Traditional concepts for protein utilization include (a) protein digestibility, which is a measure of the dietary amino acid that is made available to the animal after digestion and absorption; and (b) biological value, which is a measure of how efficiently the absorbed amino acid is utilized to synthesize proteins in the animal. Overall protein utilization, as indicated by net protein utilization, reflects both protein digestibility and biological value. This method has been most widely used in animal growth studies involving the utilization of dietary protein that can be either in a fixed amount or at varying levels in the diet. Thus, feeding experiments can give meaningful information on the availability of dietary amino acids to animals. Such studies normally involve small animals such as rats and chicks because of both practical convenience and low costs.

The methods to evaluate the quality of dietary protein include (a) the biological value (the fraction of absorbed N retained in the body for maintenance and growth of the animal), (b) the protein efficiency ratio (the gain in BW per gram of dietary protein or N consumed), (c) the net protein ratio (similar to the protein efficiency ratio method but with the consideration of maintenance requirement by the animal), (d) the net protein utilization (similar to the biological

TABLE 7.34
Evaluation of Dietary Protein Quality for Animals

Method	Equation or Comment
Chemical Analysis	
Chemical score	e.g., Lys/Lys$_{egg}$ or Met/Met$_{egg}$, with the lowest ratio being the chemical score
EAA index	Geometric mean of ratios of EAAs in a test protein to those in a reference protein
Dye-binding	Reaction of Lys ε-NH_2 in protein with fluoro-2,4-dinitrobenzene
Mutual AA ratios	Retaining EAAs whose ratios in the test protein are similar to those in a reference protein
Animal Experiments	
Biological value	= [NI – (FN – MFN) – (UN – EUN)] ÷ [NI – (FN – MFN)] × 100
Protein efficiency ratio	= weight gain/total protein intake
Net protein ratio	= [weight gain (test diet) + weight loss (N-free diet)] ÷ total protein intake (test diet)
Relative N utilization	= [weight gain (test diet) + 0.1 (initial + final weight)] ÷ total N intake
Net protein utilization	= [NI – (FN – MFN) – (UN – EUN)] ÷ NI × 100
Nitrogen growth index	= slope of the regression of weight gain (Y-axis) on N intake (X-axis)

Note: EUN, endogenous urinary N; FN, fecal N; MFN, metabolic fecal N; NI, dietary N intake; FN – MFN, the amount of N from the diet that is excreted in the feces; UN – EUN, the amount of N from the diet that is excreted in the urine; NI – (FN – MFN) – (UN – EUN), the amount of dietary N retained by a test animal; NI – (FN – MFN), the amount of dietary N that is absorbed by a test animal.

value method but with the use of dietary N absorbed by the test animal rather than total N intake), (e) the slope ratio (also called N growth index) [i.e., the slope of the regression of weight gain (Y-axis) on dietary N intake (X-axis)], and (f) relative N utilization (inclusion of a factor for the protein utilized for maintenance; Table 7.33). Common weaknesses of these methods are that (a) the components of weight gains in animals are not known, and weight gains may not necessarily result from protein deposition, (b) fecal N and urinary N in animals fed a protein-free diet may not reflect their true endogenous values under normal feeding conditions, and (c) factors that impair the function of the GIT will reduce the utilization of dietary protein independent of its quality (Wu 2022). Thus, test feed-ingredients should be free of anti-nutritional factors, the digestive physiology of animals be considered, and protein accretion in test animals measured during the experimental period.

SUMMARY

Amino acids are the building blocks of protein in feedstuffs and animals. In the stomach of nonruminants, dietary protein is denatured by HCl and then broken down by proteases (e.g., pepsins). The stimulatory or inhibitory factors for the control of gastric acid secretion act primarily through affecting the production of gastrin (stimulatory) or somatostatin (inhibitory) in the mucosa of the stomach and duodenum. In ruminants, dietary protein is degraded by microbial proteases, peptidases, and deaminases to generate ammonia and free amino acids for microbial protein synthesis in the rumen. Ruminal microbes flow into the abomasum (the true stomach), where their membranes are lysed by lysozymes to release protein, which then undergoes hydrolysis by proteases. The most common strategy to increase the escape of high-quality dietary protein from the rumen aims at minimizing proteolysis, peptidolysis, and deamination while maximizing the digestibility of the escaped protein. Optimal protein supply to the host ruminant requires adequate RDP and energy to maximize the capture of dietary OM in microbial protein. In both nonruminants and ruminants, the gastric digesta enters the duodenum, where it is neutralized by pancreatic and duodenal secretions. The terminal digestion of protein and polypeptides occurs primarily in the jejunum under the combined actions of the pancreas- and small intestine-derived proteases and oligo-, tri-, and dipeptidases to release tripeptides and dipeptides (~80%) and free amino acids (~20%). Bacteria present in the lumen of the small intestine can degrade large amounts of the protein digestion products. The remaining small peptides and amino acids are absorbed into enterocytes of the small intestine by PepT1 and amino acid transporters, respectively, with the rate of peptide transport being greater than that of free amino acids. More than one amino acid transporter can transport a certain amino acid, and a group of amino acids with similar chemical structures compete for the same transporter. Within the enterocytes of all animals, peptides are hydrolyzed into amino acids. Of note, aspartate, glutamate, and glutamine are extensively oxidized as metabolic fuels in the intestinal mucosa of mammals and serve as substrates for citrulline and arginine syntheses in the enterocytes of most mammals (e.g., pigs and ruminants). The amino acids that are not degraded by the enterocytes are transported through the basolateral membrane into the lamina propria of the small intestine.

The amino acids that enter the portal vein participate in interorgan metabolism. BCAAs largely bypass the liver and are taken up by the skeletal muscle, heart, adipose tissue, mammary gland, and placenta. In the extrahepatic tissues, BCAAs are transaminated to form glutamate, which is either amidated with NH_4^+ to yield glutamine or transaminated with pyruvate (or OAA) to generate alanine (or aspartate). Alanine and glutamine are substrates for hepatic and renal gluconeogenesis, whereas glutamine is extracted by the small intestine and kidneys to produce citrulline and ammonia, respectively. Citrulline is also converted into arginine by the kidneys. In animals, amino acids are used for the production of peptides, proteins, and low-molecular-weight bioactive metabolites (e.g., gaseous signaling molecules, neurotransmitters, and antioxidant small peptides). Intracellular protein undergoes continuous synthesis and degradation in an energy-dependent manner to constitute protein turnover, which regulates protein deposition, cell growth, removal of oxidized protein, and whole-body homeostasis. As components of this metabolic cycle, amino acids are catabolized and synthesized via compartment-, cell-, and tissue-specific reactions. Some amino acids or their metabolites activate cell signaling pathways and regulate nutrient metabolism and therefore the accretion of protein, fat, and glycogen in the body; these amino acids are called functional amino acids. The ammonia generated from amino acid catabolism is detoxified primarily as urea in the liver of mammals and as uric acid in birds and is directly released by most fish into their living environment. In postweaning mammals, enterocytes are also capable of ureagenesis as the first line of defense against dietary and gut-derived ammonia. Although animals can synthesize many amino acids from the traditionally classified EAAs, these pathways cannot provide sufficient AASAs for maximal survival, growth, fertility, or lactation in farm animals. Thus, livestock, poultry, and fish have dietary requirements for proteinogenic EAAs and AASAs and, in the case of carnivores, also taurine. Nutritionists must move beyond the "ideal protein concept" in formulating animal diets. This is a new paradigm shift in our knowledge of amino acid nutrition. To meet animal needs, diets must provide adequate amounts of amino acids in proper proportions. Major amino acid-related metabolic disorders in ruminants and nonruminants are summarized in Table 7.35. The quality of dietary protein is assessed by both quantifying its amino acid composition and determining its ability to support the growth of young animals (e.g., chicks, rats, and piglets). Optimizing amino acid nutrition through dietary supplementation with high-quality protein or amino acids is an efficient and feasible way to enhance the yields and

TABLE 7.35
Amino Acid-Related Metabolic Disorders (Including Metabolic Diseases) in Ruminants and Nonruminants

Metabolic Disorder	Cause	Major Symptoms
Amyloidosis[a]	Abnormal amount and folding of amyloid	Abnormal brain structure; neurological dysfunction
Ascites syndrome	Pulmonary arterial hypertension due to ↓ NO	Accumulation of fluid and hypertension in broilers
Black skin syndrome (in largemouth bass)	Deficiency of methionine	Skin darkening; retinal degeneration; intestinal and liver atrophies and structural abnormalities
Cardiomyopathy	Taurine deficiency	Abnormal cardiac structure and function, and death
Gout[b]	Uric acid crystals in joints and tendons	Red, tender, hot, and swollen joints; pain
Hyperammonemia	Arg or UCE deficiency in mammals; excess AAs	Brain damage, coma, and death
Hyper-HCys[c]	Impaired catabolism of methionine	Cardiovascular disease, neural tube defect, and death
Kwashiorkor	Deficiency of dietary protein	Poor health, stunting, anemia, edema, muscle wasting
Marasmus	Deficiencies of both protein and energy	Severe wasting, stunting, muscle wasting, and death
Melanosis	Deposition of a brownish-black pigment in tissue	Negatively affect sensory characteristics of meat
Systemic metabolic acidosis	Excessive accumulation of sulfuric acid	Low blood bicarbonate concentration, low blood pH, and multi-organ failure
Porphyria	Defects in heme synthesis; excessive porphyrins	Abdominal pain, vomiting, seizures, and paralysis

[a] Amyloid is a protein produced by reticuloendothelial cells, histiocytes, and plasma cells, and contains less than 5% carbohydrates.

[b] Excess ammonia increases the production of uric acid due to a deficiency of either arginine (impairing the urea cycle) or urea-cycle enzymes.

[c] Vitamins (folate, vitamin B_6, and vitamin B_{12}) and betaine play important roles in one-carbon metabolism and in the methylation reaction that is required to recycle methionine-derived homocysteine back to methionine.

Note: AAs, amino acids; Hyper-HCys, hyperhomocysteinemia; NO, nitric oxide; UCE, urea-cycle enzymes; ↓, decreased bioavailability.

efficiency of livestock, poultry, and fish production, while ensuring the optimal health of animals and reducing the generation of animal waste on farms.

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8 Energy Metabolism

Energy is not matter but is contained in matter. Carbohydrates, lipids, protein, and amino acids are the primary sources of dietary energy for animals (Jobgen et al. 2006; van Milgen et al. 2000), with the proportions of these nutrients in diets differing among carnivores, herbivores, and omnivores (Stevens and Hume 2004). An animal must survive before it can grow and produce food (e.g., muscle, milk, or eggs) for human consumption (Pond et al. 2005). Animals will not grow and will lose body weight (BW) if their dietary intake of energy is insufficient because metabolic processes and tissue accretions require adequate energy (Milligan 1970; Verstegen and Henken 1987). A major cost of animal production is maintaining the basal energy requirement of the body (e.g., maintaining a stable body temperature in mammals and birds, muscular activity, and essential physiological function; Kleiber 1961). The animal requires additional energy for production, including tissue growth (and wool growth), fattening, lactation, draft power, transport, pregnancy, or egg formation, depending on the environment, species, breeds, sex, and age (McDonald et al. 2021). Thus, the efficiency of utilization of dietary energy is of great importance for productivity, economic returns, and sustainability in animal agriculture.

Adenosine triphosphate (ATP) is the major currency of energy in organisms because it is used by all cells for biochemical reactions and physiological processes (Dai et al. 2013; Newsholme and Leech 2009). There are more than 8,700 biochemical reactions and more than 16,000 metabolites in a mammal, which have been organized as integrated metabolic networks in the Kyoto Encyclopedia of Genes and Genomes (KEGG), MetaCyc, and Rhea pathway databases (Alcántara et al. 2012; Altman et al. 2013; Van Milgen 2024). As highlighted in Chapters 5–7, metabolic pathways are cell-, tissue-, species- and time-specific. All animals obtain their energy from the oxidation of fatty acids, glucose, and amino acids that are derived, respectively, from triacylglycerols (TAGs), starch/glycogen, and protein/amino acids in diets (Jobgen et al. 2006; Wu 2022). In addition, fructose is a metabolic fuel for sperm, conceptuses, neonatal ruminants and pigs, and cancers (Bazer et al. 2024; Kurz and Willett 1992).

The conversion of food energy into biological energy requires complex pathways for the metabolism of the macronutrients to CO_2 and H_2O, and coupled redox reactions, as discussed in Chapters 1 and 5–7. Because all forms of energy are convertible into heat, heat production is measured by direct or indirect calorimetry studies of animal energy metabolism (Blaxter 1971). Energy metabolism is the foundation of life. This chapter aims to highlight the partitioning of food energy in animals and the energy systems used in feedstuff evaluation and feeding.

BASIC CONCEPTS IN ENERGY

Definition of Energy

Energy may be defined as the capacity to do work (Newsholme and Leech 2009). There are various forms of energy, including chemical, thermal, electrical, and radiant energy, all of which can be interconvertible by suitable means. Chemical energy is the energy stored in bonds between atoms within a molecule. Energy can be added or released from a molecule by rearranging its chemical bonds (Brown et al. 2003). Both in foods and animals, energy is stored primarily in carbohydrates, TAGs, fatty acids, proteins, and amino acids. The energy released from the oxidation of fatty acids, glucose, glycerol, and amino acids is used for the synthesis of ATP, guanosine triphosphate (GTP), cytidine triphosphate (CTP), and uridine triphosphate (UTP), as well as NADH and NADPH (Newsholme and Leech 2009). Among these nucleotides, ATP is the most abundant and important for energy metabolism in animal cells, but GTP, UTP, and CTP participate in some special reactions (e.g., G-protein signaling mechanisms, as well as the synthesis of protein, lactose, and glycogen; Lodish et al. 2000). The reduced form of nicotinamide adenine dinucleotide (NADH) and the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) are required for gluconeogenesis and fatty acid synthesis, respectively. The metabolism of energy in the whole body may lead to anabolism (energy storage) or catabolism (energy mobilization; Verstegen and Henken 1987).

According to the first law of thermodynamics, energy can neither be created nor destroyed and can only be converted from one form into another (Brown et al. 2003). Thus, there is no energy production in organisms, and an inadequate intake of dietary energy will always result in energy deficits (Blaxter 1989). In the ecosystem, the radiant energy of the sun is converted into chemical energy in green plants. The latter are consumed by animals for conversion into macromolecules (e.g., protein, fat, and glycogen), biological energy (mainly in the form of ATP), and heat (Figure 8.1). In all organisms, when chemical reactions release energy, it goes into the surroundings (Lodish et al. 2000). Energy metabolism in cells or animals can be defined as biochemical reactions involved in the transformation of various forms of chemical energy. Because the efficiency of trapping energy released from substrate oxidation is less than 60%, these processes generate heat.

Units of Energy Used in Animal Nutrition

The thermochemical calorie (cal), derived from the Latin word "calor" meaning heat, was first introduced in 1824 by Nicolas Clément (a French physicist and chemist) as

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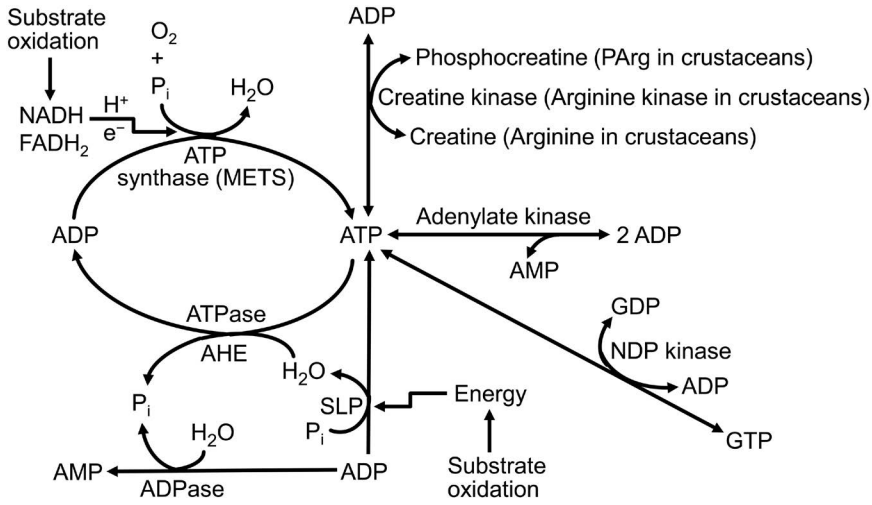


FIGURE 8.1 ATP synthesis in animals. Energy is released from substrates (glucose, glycerol, lactate, fatty acids, and amino acids) during their oxidation in animal cells. This energy is subsequently used to drive the synthesis of ATP from ADP through substrate level phosphorylation and mitochondrial oxidative phosphorylation. In vertebrates (e.g., mammals, birds, and fish), energy is stored primarily as phosphocreatine in skeletal muscle, brain, reproductive organs, and other extrahepatic tissues. In crustaceans (e.g., crabs and shrimp), energy is stored primarily as phosphoarginine (PArg). The rate of ATP utilization is matched by the rate of ATP production. GTP is converted into ATP by nucleoside-diphosphate (NDP) kinase. AHE, transmembrane ATPase (e.g., Na^+/K^+-ATPase and H^+/K^+-ATPase); METS, mitochondrial electron transport system; SLP, substrate-level phosphorylation via glycolysis and the Krebs cycle.

a unit of heat energy. The word *calorie* did not enter the English language until 1863. The calorie is based on the combustion value of benzoic acid as the reference standard. Traditionally, 1 cal is defined as the heat required to increase the temperature of 1 g H_2O in an insulated tank by 1°C (usually from 14.5°C to 15.5°C) at a pressure of 1 atmosphere. The U.S. public began using the term "calorie" after it was introduced by W.O. Atwater in 1887 as an energy unit for foods. Since then, the calorie has been used as the basic energy unit in animal and human nutrition in North America. Because the calorie is inconveniently small, the kilocalorie (kcal; 1 kcal = 1000 cal) or the megacalorie (Mcal; 1 Mcal = 10^3 kcal = 10^6 cal) is often used. The term "large calorie," written as Calorie (Cal), sometimes appears in the literature, with 1 Cal = 10^3 cal.

In 1882, Carl Wilhelm Siemens (a German-British electrical engineer and a businessman) first proposed the *joule* (J) as a unit of heat in honor of James Prescott Joule (an English physicist; 1818–1889), who was a pioneer in the development of the thermodynamical theory. In physics, one joule is defined as the energy transferred to (or work done on) an object when a force of one newton acts on that object in the direction of its motion through a distance of one meter (i.e., 1 newton meter or N·m; IBWM 2006). One joule is also equivalent to the energy dissipated as heat when an electric current of one ampere passes through a resistance of one ohm for one second. The work of moving 1 kg by 1 m against gravitational acceleration at the sea level equals 9.807 (IBWM 2006); namely, 1 kg·m = 9.807 J or 1 kg·km = 9.807 kJ. For unit conversions, 1 cal = 4.1842 J. At the ninth General Conference on Weights and Measures in 1948, the joule was adopted as the unit of heat to deprecate the use of the calorie. In 1960, the joule was declared by The International Bureau of Weights and Measures as the international unit of energy (IBWM 2006). In 1969, the International Union of Nutritional Sciences recommended the joule as the energy unit for use in nutritional and physiological studies. This suggestion has been almost universally adopted (Hargrove 2007). However, because of convenience, the unit of calories (e.g., kcal) is still widely used in the formulation of animal diets on many farms and the practice of human nutrition.

$$1 \text{ cal} = 4.1842 \text{ J} = 0.4267 \text{ kg·m};$$
$$1 \text{ kcal} = 4.1842 \text{ kJ} = 0.4267 \text{ kg·km}$$

Gibbs Free Energy

Basic Concepts in Gibbs Free Energy Change (ΔG)

A chemical reaction either absorbs or releases energy and always involves breaking the bonds of reactants and making bonds in products (Brown et al. 2003). A thermodynamic system includes the reactants and products of a reaction, as well as any associated energy. The second law of thermodynamics states that energy transfers increase the entropy or disorder of the system (e.g., an insulated tank, a test tube, a cell, a tissue, or an animal; Brown et al. 2003). This principle is crucial for understanding the spontaneity, direction, and energy requirements of chemical reactions under given conditions. Combining the first and second laws of thermodynamics, the American mathematician Josiah Willard Gibbs (1839–1903) proposed the concept

of Gibbs free energy (G), which is defined as a portion of the energy that is available to do work. In a thermodynamically closed system, where there is no exchange between its energy and the surrounding environment, work may be a change in the state or motion of molecules. For example, the spontaneous dissolution of NaOH (a solid structure) in H_2O placed within an insulated chamber tube leads to the formation of the constituent ions (Na^+ and OH^-) that can move freely in the solution (a condition of greater disorder) and the release of heat. Another example is the oxidation of glucose (a highly organized molecule) into H_2O and CO_2 (less organized and smaller molecules) to release heat within a bomb calorimeter. In a thermodynamically open system (e.g., an animal), where there are exchanges between its energy and the surrounding environment, work may be any activity that requires energy expenditure such as nutrient absorption, muscle contraction, the formation of complex molecules [e.g., the accretion of white adipose tissue (WAT) to store energy and the accumulation of collagen in connective tissue], and urinary excretion of metabolites. As the organisms grow via energy-dependent reactions, the release of heat (expressed as kcal per animal) increases, and the entropy of the surroundings (the overall system) increases (Lodish et al. 2000). Thus, the open system of an animal does not defy the laws of thermodynamics.

The Gibbs free energy change (ΔG) is a difference in the G of a system between its final and initial states. The value of ΔG indicates the maximum energy released (a negative sign) or absorbed (a positive sign) during a chemical or biochemical reaction. ΔG takes into consideration the alterations in the enthalpy (heat) and entropy (degree of disorder) of a reaction (Brown et al. 2003). At constant temperature, ΔG can be expressed as follows.

$$\Delta G = \Delta H - T\Delta S$$

ΔG (kJ/mol) = change in Gibbs free energy of a system; $\Delta G = G_{final} - G_{initial}$. The value of ΔG indicates the maximum energy released (a negative sign) or absorbed (a positive sign) during a chemical reaction.

ΔS [kJ/T (in Kelvin) per mol] = $\Delta Q/T$, where ΔQ is the amount of heat transferred to the system and T is the system's temperature in Kelvin. ΔS is a measure of change in the entropy (disorder) of a system. The value of ΔS signifies either a decrease (a negative sign) or an increase (a positive sign) in the system's order. In other words, if ΔS is a positive value, the system becomes more disordered during a reaction (e.g., the breakdown of protein into amino acids that releases energy). A larger increase in disorder within a system (i.e., a greater positive ΔS) makes a chemical reaction more likely to occur. In contrast, if ΔS is a negative value, the system becomes more ordered during a reaction (e.g., the formation of protein from amino acids that requires added energy).

ΔH (kJ/mol) = change in the enthalpy (heat) of a system, with a negative or positive sign indicating energy release or absorption, respectively, during a chemical reaction. Enthalpy refers to energy stored in the chemical bonds of substances (e.g., nutrients and metabolites).

Both ΔG and ΔH are independent of the pathway of transformation and unaffected by its mechanism but depend on only the substrates and products (Lodish et al. 2000). When an organic substrate (e.g., glucose) is oxidized to CO_2 and H_2O in either an animal or a bomb calorimeter, the ΔH is the same. However, when the oxidation of an organic substrate (e.g., protein) in an animal generates products (e.g., CO_2, H_2O, ammonia, and urea) that are different from those in a bomb calorimeter (e.g., CO_2, H_2O, and N_2), the resulting ΔH values are not the same (Wu 2022). This becomes important when heat production is calculated according to data on O_2 consumption and CO_2 production generated from studies involving indirect calorimetry. ΔH and ΔS contribute to the overall sign (i.e., negative or positive) and value of ΔG in a chemical or biological system. The value of ΔG can indicate whether a chemical reaction can occur spontaneously. Let us consider the following three situations.

a. When $\Delta G < 0$, the reaction takes place spontaneously (exergonic). The reaction releases energy (heat or other forms of energy) to the surroundings, e.g., for heat production or mechanical energy, and is characterized by a decrease in the Gibbs free energy. An exergonic reaction results in products that have lower amounts of energy and are more stable than the reactants. Examples of this type of reaction are the dissolution of salts (e.g., NaCl and KCl) to their constituent ions in H_2O, the hydrolysis of ATP by ATPase to ADP + Pi (inorganic phosphate; HPO_4^{2-}) + H^+, and mineralization (i.e., the deposition of minerals in bone) in growing animals. A chemical reaction that releases specifically heat is called an exothermic reaction ($\Delta H < 0$) and is a type of exergonic reaction.
b. When $\Delta G = 0$, the reaction is said to be at equilibrium (no net formation of products). An example of this reaction is the transamination of an amino acid with an α-ketoacid to form a new amino acid and a new α-ketoacid (Wu 2022). Cells or animals with $\Delta G = 0$ are dead. The system has no exchanges with its surroundings.
c. When $\Delta G > 0$, the reaction does not take place spontaneously (endergonic). Energy must be fed into the system for the reaction to occur. An endergonic reaction absorbs energy (heat or other forms of energy) from its surroundings to break bonds in reactants and generate products with a higher energy level (Newsholme and Leech 2009). Examples of endergonic reactions include the phosphorylation of glucose by Pi to form glucose-6-P and the evaporation of H_2O from animals that requires the input of energy and heat, respectively,

into the system. A chemical reaction that absorbs specifically heat from its surroundings is called an endothermic reaction ($\Delta H > 0$) and is a type of endergonic reaction.

In animal cells, endergonic (energetically unfavorable) reactions can be coupled with exergonic (energetically favorable) reactions, as shown in the following equations. The phosphorylation of glucose by ATP is enzymatically catalyzed by hexokinase (Chapter 5). The amount of energy released from this reaction depends on not only its $\Delta G^{o\prime}$ (standard free-energy change) but also the concentrations of its substrates and products.

Endergonic reaction (1): Glucose + Pi $\rightleftharpoons$ Glucose-6-P + H_2O ($\Delta G^{o\prime} = 13.8$ kJ/mol)

Exergonic reaction (2): ATP + H_2O $\rightleftharpoons$ ADP + Pi + H^+ ($\Delta G^{o\prime} = -30.5$ kJ/mol)

Overall reaction [(1)+(2)]: Glucose + ATP $\rightleftharpoons$ ADP + Glucose-6-P + H^+ ($\Delta G^{o\prime} = -16.7$ kJ/mol)

Computation of $\Delta G^{o\prime}$ and ΔG for Biochemical Reactions Based on Equilibrium Constants

Because it is difficult to quantify ΔS, the ΔG of a biochemical reaction can be calculated according to the concentrations of its reactants and products at equilibrium.

$$\Delta G = \Delta G^{o\prime} + 2.3RT \times \log K_{eq}$$

$\Delta G^{o\prime}$: Standard free-energy change. $\Delta G^{o\prime}$ is determined for each biochemical reaction under standard conditions. The latter are defined as an initial concentration of 1 M for all reactants and all products (except for H^+), 25°C, 1 atm pressure, and pH 7.0 (approximately cytosolic pH in animal cells). In biochemical reactions, 1 M [H^+] (i.e., pH 0) is usually not adopted because enzymes are denatured and have no activity under such a condition. $\Delta G^{o\prime}$ can generally indicate whether a biochemical reaction releases (a negative sign) or absorbs (a positive sign) energy under standard conditions but cannot accurately predict the spontaneity of a biochemical reaction under nonstandard conditions (e.g., those in animals). [Note that for a chemical reaction at a given pH (not necessarily pH 7.0), standard free-energy change is designated as ΔG^{o}.]

R: The gas constant, i.e., 1.987 cal/(K × mol) or 8.314 J/(K × mol).

T: Kelvin temperature (K) (K = °C + 273. e.g., 25°C = 298 K).

K_{eq}: The equilibrium constant of a reaction under nonstandard conditions (e.g., those in animals).

$\log K_{eq}$: The logarithm of K_{eq} to the base of 10 (e.g., $\log_{10} K_{eq}$), with the subscript number "10" being omitted.

For $A \rightarrow B$, $K_{eq} = [B]/[A]$; For $A \rightarrow 2B$, $K_{eq} = [B]^2/[A]$.

For $A + C \rightarrow B + D$, $K_{eq} = ([B] \times [D])/([A] \times [C])$; For $2A + C \rightarrow 3B + D$, $K_{eq} = ([B]^3 \times [D])/([A]^2 \times [C])$.

T: Kelvin temperature (K). [K = °C + 273. e.g., 0°C = 273 K; 25°C = 298 K; 37°C = 310 K; 40°C = 313 K]

Determination of K'_{eq} and $\Delta G^{o\prime}$ for Biochemical Reactions under Standard Conditions: When a biochemical reaction under standard conditions (see above) is at equilibrium, $\Delta G = 0$. Then, $\Delta G^{o\prime} = -2.3RT \times \log K'_{eq}$.

$\Delta G^{o\prime}$: Standard free-energy change at pH 7.0, as defined above.

K'_{eq}: The equilibrium constant of a biochemical reaction at pH 7.0 under standard conditions (defined above), where there is no net gain or loss in reactants and products. K'_{eq} is calculated according to the concentrations (at equilibrium) of all reactants and products, except for H_2O and H^+. Each reactant or product includes all of its ionic forms (if any) as one entity in the equation. The concentration of H^+ as a reactant or product is omitted from the calculation of K'_{eq} because the pH of the solution (i.e., pH 7.0) is constant during the reaction. In addition, the concentration of H_2O as a reactant or product, which does not change significantly during the reaction, is set to unity (i.e., 1 M) for the determination of K'_{eq} despite its actual concentration of 55.6 M, so that the calculations of both $\Delta G^{o\prime}$ and ΔG can be simplified (Segel 1976).

Computation of K_{eq} and ΔG for Biochemical Reactions under Nonstandard Conditions: $\Delta G^{o\prime}$ is an intrinsic property of a biochemical reaction and therefore can be used to calculate ΔG under nonstandard conditions (e.g., those in animals) at different concentrations of substrates and different temperatures. As described above for the relationship between ΔG and $\Delta G^{o\prime}$, the K_{eq} of a biochemical reaction under nonstandard conditions is determined at equilibrium, where there is no net gain or loss in reactants and products. In addition, as explained above, the concentration of H^+ as a reactant or product is omitted from the calculation of K_{eq} and the concentration of H_2O as a reactant or a product is set to unity (i.e., 1 M). Note that the temperature for a biochemical reaction may not be 298 K (i.e., 25°C) in a biochemical reaction, and thus the RT value in the equation for the calculation of ΔG must be computed accordingly.

At 25°C (i.e., 298 K), RT = 2.478 kJ/mol. Thus, $\Delta G(\text{kJ/mol}) = \Delta G^{o\prime}(\text{kJ/mol}) + 2.3 \times 2.478$ kJ/mol $\times \log K_{eq}$.

At 37°C (i.e., 310 K), RT = 2.577 kJ/mol. Thus, $\Delta G(\text{kJ/mol}) = \Delta G^{o\prime}(\text{kJ/mol}) + 2.3 \times 2.577$ kJ/mol $\times \log K_{eq}$.

At 40°C (i.e., 313 K), RT = 2.602 kJ/mol. Thus, $\Delta G(\text{kJ/mol}) = \Delta G^{o\prime}(\text{kJ/mol}) + 2.3 \times 2.602$ kJ/mol $\times \log K_{eq}$.

$\Delta G^{o\prime}$ and ΔG for the Hydrolysis and Synthesis of ATP in Animal Cells

Under physiological conditions, ATP, GTP, and UTP are hydrolyzed to provide energy for a plethora of metabolic needs such as driving energy-dependent biochemical reactions, nutrient transport, and muscular contractions (Chapters 5–7), with the heat being released into the surroundings. ΔG and $\Delta G^{o\prime}$ values for the hydrolysis of ATP to ADP + Pi, AMP + PPi, and AMP + 2Pi under both standard and physiological conditions are provided in Table 8.1. The concentrations of these metabolites used for the ΔG calculation are based on those in human skeletal muscle (Doyle et al. 1997; Hellsten et al. 1999). ΔG values for the hydrolysis of ATP to ADP + Pi at 25°C, 37°C, and 40°C are –49.6, –51.6, and –52.1 kJ/mol, respectively. Conversely, ΔG values for the synthesis of ATP from ADP + Pi at 25°C, 37°C, and 40°C are +49.6, +51.6, and +52.1 kJ/mol, respectively. The amount of dietary ME required to synthesize 1 mol of ATP from 1 mol of ADP + 1 mol of Pi in animals at 37°C is 85.4 kJ/mol (McDonald et al. 2021). Thus, the overall efficiency of oxidative phosphorylation and substrate-level phosphorylation for capturing energy to produce ATP from ADP + Pi in skeletal muscle is 60.4% [i.e., (51.6/85.4) × 100]. Notably, this value is similar to the estimate of ~60% in biochemistry textbooks (Garrett and Grisham 2013).

$$ATP + H_2O \rightleftharpoons ADP + Pi + H^+ \left[\text{ATP synthase}\right]$$

$$ATP + H_2O \rightarrow AMP + PPi \left[\text{A coupled enzymatic reaction; e.g., aminoacyl-tRNA synthetase}\right]$$

$$PPi + H_2O \rightarrow 2Pi \left[\text{Inorganic pyrophosphatase}\right]$$

$$ADP + H_2O \rightarrow AMP + Pi \left[\text{Adenosine diphosphatase}\right]$$

ATP Synthesis in Animal Cells and Microbes

There are two mechanisms for the synthesis of ATP from ADP plus Pi in animal cells and microbes: (a) substrate-level phosphorylation and (b) oxidative phosphorylation (Chapter 1). Substrate-level phosphorylation refers to the direct synthesis of ATP from ADP plus Pi during a biochemical reaction, whereas electron transport systems use the energy of electrochemical gradients across the inner mitochondrial membrane (eukaryotes) or the cell membrane (prokaryotes) to convert ADP plus Pi into ATP. The relative contributions of substrate-level phosphorylation and oxidative phosphorylation to ATP production depend on organisms, cell types, and physiological states, as well as nutrient and O_2 supplies.

TABLE 8.1
$\Delta G^{o\prime}$ for ATP Hydrolysis and Synthesis under Standard Conditions (Including 1 M Reactants and Products), as well as K_{eq} and ΔG for the Biochemical Reactions in Animal Cells under Physiological Conditions[a]

Biochemical reaction	$\Delta G^{o\prime}$ (kJ/mol)[b]	K_{eq}	$\log K_{eq}$	ΔG (kJ/mol) 25°C	37°C	40°C
ATP or PPi hydrolysis						
$ATP + H_2O \rightarrow ADP + Pi + H^+$	–30.5	4.454×10^{-4}	–3.351	–49.6[c]	–51.6	–52.1
$ADP + H_2O \rightarrow AMP + Pi + H^+$	–30.0	3.600×10^{-4}	–3.444	–49.6	–51.6	–52.1
$ATP + H_2O \rightarrow AMP + PPi + 2H^+$	–45.6	2.474×10^{-7}	–6.607	–83.3	–86.6	–87.4
$PPi + H_2O \rightarrow 2Pi$	–19.4	0.6480	–0.1884	–20.5	–21.3	–21.5
$ATP + 2H_2O \rightarrow AMP + 2Pi + 2H^+$	–65.0	1.6033×10^{-7}	–6.795	–103.7	–107.9	–108.9
$ATP + AMP \rightarrow 2ADP$	–0.50	1.237	0.0924	0.027	0.028	0.028
ATP synthesis						
$ADP + Pi + H^+ \rightarrow ATP + H_2O$	30.5	2245	3.351	49.6	51.6	52.1
$2ADP \rightarrow ATP + AMP$	0.50	0.8083	–0.0924	–0.027	–0.028	–0.028

[a] The calculations of K_{eq} and ΔG values under physiological conditions are based on 9.7 mM ATP, 1.2 mM ADP, 0.12 mM AMP, and 3.6 mM Pi in human skeletal muscle (Doyle et al. 1997; Hellsten et al. 1999). The concentration of PPi in the tissue is assumed to be 0.02 mM (Williamson and Brosnan 1974).

[b] Adopted from Devlin (2006), as well as Garrett and Grisham (2013).

[c] Calculated as $\Delta G = \Delta G^{o\prime} + 2.3 \times 2.478 \times \log K_{eq} = -30.5 + [2.3 \times 2.478 \times (-3.351)] = -49.6$ kJ/mol.

Note: $\Delta G^{o\prime}$, standard Gibbs free energy change for the indicated biochemical reaction at pH 7.0; ΔG, Gibbs free energy change for the indicated biochemical reaction in animal cells under physiological conditions; K_{eq}, equilibrium constant for the indicated biochemical reaction in animal cells under physiological conditions; Pi, inorganic phosphate; PPi, pyrophosphate.

For example, substrate-level phosphorylation provides the bulk of energy in the brain and tumors and is the only means for ATP formation in cells containing no mitochondria (e.g., mammalian erythrocytes). In contrast, oxidative phosphorylation is the major process for ATP generation in terrestrial and aquatic animals (Figure 8.1). Additionally, in the sprinting of human athletes, approximately 85%, 10%, and 5% of the energy need is provided by phosphocreatine, glycolysis, and oxidative phosphorylation, respectively (Turnbull 2024). Furthermore, both substrate-level phosphorylation and oxidative phosphorylation (the membrane-bound electron transport system) play important roles in the production of ATP by anaerobic microbes (e.g., those present in the rumen; Ungerfeld and Hackmann 2020).

Substrate-level Phosphorylation

Examples of substrate-level phosphorylation can be seen in glycolysis, fructolysis, and glutaminolysis (Chapter 7) in both eukaryotes and prokaryotes, as well as some reactions of mitochondrial and cytosolic Krebs-cycle enzymes (e.g., succinyl-CoA + Pi + ADP → succinate + CoA + ATP) in eukaryotes and prokaryotes, respectively. Additionally, in microbes, ATP is produced via substrate-level phosphorylation through the production of SCFAs (Figure 8.2). As reported for *Thermotoga maritima*, the use of cytosolic electron-bifurcating hydrogenases for the formation of H_2 from $2H^+ + 2e^-$ enables the anaerobic bacterium to produce 4 mol of ATP from the fermentation of 1 mol of glucose to 2 mol of acetate at substrate-level phosphorylation, i.e. 2 mol ATP/mol of acetate (Buckel and Thauer 2018). Through electron bifurcation or electron confurcation, an energetically unfavorable (exergonic) reaction is coupled with an energetically unfavorable (endergonic) reaction within the same enzyme complex to drive the energetically unfavorable transformation with ATP hydrolysis (Chapter 1). In contrast, the catabolism of 1 mol of glucose to 2 mol of lactate in anaerobic microbes without electron bifurcation or electron confurcation reactions yields only 2 mol of ATP, i.e., 1 mol of ATP/mol of lactate. In ruminal microbes, ATP yields for the production of propionate and butyrate (mol/mol) from glucose (via both substrate-level phosphorylation and electrochemical gradients across the cell membrane) are 3.5 and 4.5, respectively (Ungerfeld and Hackmann 2020). Thus, anaerobic microbes develop an effective strategy to capture food energy as biological energy for growth and survival and to conserve energy.

This indicates the importance of dietary carbohydrates (e.g., plant-sourced starch and fiber) for ATP supply to ruminal microbes.

$$\text{D-Glucose} + 4\ \text{ADP} + 4\ \text{Pi} \rightarrow 2\ \text{Acetate} + 2\ \text{CO}_2 + 4\ \text{H}_2 + 4\ \text{ATP}\ (\text{Cytosolic electron bifurcation})$$

$$\text{D-Glucose} + 2\ \text{ADP} + 2\ \text{Pi} \rightarrow 2\ \text{L-Lactate} + 2\ \text{ATP} + 2\ \text{H}_2\text{O}\ (\text{Without electron bifurcation})$$

Oxidative Phosphorylation

Examples of oxidative phosphorylation are the electron transport systems in either the mitochondrial membrane of eukaryotes or the plasma (cell) membranes of prokaryotes for the oxidation of NADH, NADPH, and the reduced form of flavin adenine dinucleotide ($FADH_2$), as noted previously. Electrochemical gradients are formed from the movement of H^+ across the inner mitochondria in eukaryotes or the cell (plasma) membrane in prokaryotes and are used for the production of ATP from ADP and Pi by ATP synthase (Hackmann 2024). The aforementioned reducing equivalents are generated from the catabolism of nutrients (e.g., glucose, fructose, fatty acids, and amino acids) in both eukaryotes and prokaryotes. In the presence of adequate O_2, mitochondria-containing animal cells oxidize NADH,

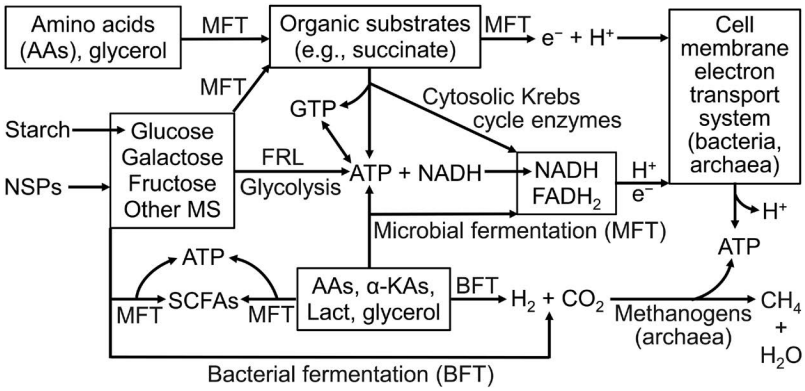


FIGURE 8.2 Production of ATP by microbes in the rumen of ruminants. Bacterial fermentation of starch, nonstarch polysaccharides (NSPs), amino acids, fatty acids, and other organic substances generates ATP, GTP, NADH, FADH2, short-chain fatty acids (SCFAs), electrons (e^-), H^+ and CO_2 via a plethora of metabolic pathways and reactions, including glycolysis, fructolysis (FRL), amino acid and fatty acid degradation, and cytosolic Krebs cycle enzymes. Bacterial hydrogenases (electron-bifurcating enzymes) reduce H^+ to H_2 via a biochemical reaction called electron bifurcation. Methanogens use electrons derived from H_2 to reduce CO_2 into methane (CH_4), generating ATP during the process of methanogenesis. The relative proportions of the metabolic pathways to ATP production by ruminal microbes remain to be determined. α-KAs, α-ketoacids (such as pyruvate, branched-chain α-ketoacids, and α-ketoglutarate); Lact, lactate.

NADPH, and $FADH_2$ to H_2O, NAD^+, $NADP^+$, and FAD, respectively, for ATP production. This is an important process for converting food energy into biological energy in animals. Under physiological conditions, the efficiency of oxidative phosphorylation for capturing energy to produce ATP from ADP + Pi in animal cells is approximately 60% (Garrett and Grisham 2013).

As in animal cells, microbes [primarily anaerobic bacteria and archaea (as those in the rumen)] also synthesize ATP from ADP and Pi by ATP synthase via the cell-membrane electron transport systems when NADH and $FADH_2$ are oxidized (Chapter 1). In addition, methanogenesis results in the production of 1.5 and 0.5 mol of ATP/mol of methane by methanogens with and without cytochromes, respectively, through electrochemical gradients across the cell membrane that are generated by H^+ and Na^+ pumping (Thauer et al. 2008). Methanogens without cytochromes (i.e., *Methanobrevibacter*, *Methanosphaera*, and *Methanomassiliicoccales*) account for about 90% of total methanogens in the rumen of ruminants (Khairunisa et al. 2023); thus, ATP yield per methane production (mol/mol) in the rumen is 0.6. Hackmann (2024) has estimated that oxidative phosphorylation can provide up to 50% additional ATP beyond substrate-level phosphorylation in anaerobic prokaryotes.

P/O Ratios in Eukaryotic and Prokaryotic Electron Transport Systems

The production of ATP or its equivalents (e.g., GTP and UTP) in cells is not 100% efficient because not all chemical energy in nutrients is fully captured in biological energy and also because there is a phenomenon called the mitochondrial or membrane leakage of protons and superoxide anion (O_2^-). The theoretical P/O ratios (mol of ATP produced per mol of [O] consumed) are 3 and 2 for the oxidation of NADH and $FADH_2$, respectively, via the mitochondrial electron transport system in animals, as indicated in older biochemistry textbooks (e.g., Lehninger 1975). However, due to mitochondrial leakage under normal physiological conditions, it is now generally accepted that the P/O ratios are 2.5 and 1.5 for the oxidation of NADH and $FADH_2$, respectively (Nelson and Cox 2021). Brand et al. (1994) estimated that mitochondrial proton leakage could account for as much as 26% of O_2 consumption by various species of animals, including reptiles, ruminants, and nonruminant mammals. Assuming that NADH and $FADH_2$ represent 70% and 30% of the reducing equivalents entering the mitochondrial respiratory chain, the P/O ratio is approximately 2.2 in animal cells.

By definition, P/O ratios do not apply to anaerobic microbes in the absence of O_2 but are possibly 2.5 and 1.5 for the oxidation of NADH and $FADH_2$ via the cell membrane respiratory chain, respectively, in obligately aerobic bacteria exposed to sufficient O_2 (Kalnenieks et al. 2019). However, P/O ratios in facultative anaerobic bacteria cultured in the presence of O_2 appear to vary substantially with extracellular conditions such as the theoretical lower and upper limits of 0.67 and 2.67, respectively, in *E. coli* (Kalnenieks et al. 2019). The measured P/O ratios of 0.6–1.0 have been reported for *Proteus mirabilis* (a facultatively anaerobic bacterium) in the presence of O_2 (van der Beek and Stouthamer 1973), which are much lower than those for eukaryotic cells, possibly due to the production of large amounts of SCFAs as electron sinks. Increasing P/O ratios may provide a useful method to improve the ATP yields and growth of aerobic or facultative anaerobic microbes in the presence of O_2.

Phosphocreatine and Phosphoarginine as Energy Stores in Animals

Creatine plays a critical role in storing energy by forming phosphocreatine from ATP and creatine via creatine kinase in the nerves, skeletal muscle, and reproductive organs of mammals and birds. Concentrations of creatine plus phosphocreatine in the skeletal muscles of mammals and fish are about 35–45 mM [e.g., 37, 43, and 130 mM in humans (Wu 2020), pigs (Posey et al. 2025), and hybrid striped bass (He et al. 2024), respectively]. Interestingly, phosphoarginine, which is synthesized from ATP and L-arginine via arginine kinase, is highly abundant (up to 120 mM in skeletal muscle) and serves as the major form of energy storage in aquatic invertebrates such as crustaceans (Clark et al. 2012). In both phosphocreatine and phosphoarginine, chemical bonds form after the valence electrons of two or more atoms interact to make them more stable. In response to physiological needs, phosphocreatine and phosphoarginine are hydrolyzed by enzymes (creatine kinase and arginine kinase, respectively) to provide energy, which is used for biochemical reactions and physiological events (Newsholme and Leech 2009).

Interconversion of Nucleotides in Eukaryotes and Prokaryotes

In eukaryotes and prokaryotes, purine and pyrimidine nucleotides can be interconverted by specific kinases. Thus, CTP, GTP, and UTP can be formed from ATP in living organisms. These reactions are summarized below. Note that nucleoside monophosphate kinase is specific for each purine (adenosine and guanosine) or pyrimidine (cytidine and uridine) nucleotide. The interconversion of nucleotides plays an important role in balancing the concentrations of purine and pyrimidine nucleotides in cells, while helping recycle nucleotides that are released from metabolic pathways.

$$\mathrm{ATP} + \mathrm{AMP} \rightleftharpoons 2\mathrm{ADP}\ \left[\text{Adenylate kinase}\right]$$

$$\mathrm{ATP} + \mathrm{CDP} \rightleftharpoons \mathrm{ADP} + \mathrm{CTP}\ \left(\text{Cytidine triphosphate}\right)\ \left[\text{Nucleoside diphosphate kinase}\right]$$

$$\mathrm{ATP} + \mathrm{GDP} \rightleftharpoons \mathrm{ADP} + \mathrm{GTP}\ \left(\text{Guanosine triphosphate}\right)\ \left[\text{Nucleoside diphosphate kinase}\right]$$

$$\text{ATP} + \text{UDP} \rightleftharpoons \text{ADP} + \text{UTP}\ (\text{Uridine triphosphate})$$
$$[\text{Nucleoside diphosphate kinase}]$$

$$\text{ATP} + \text{Nucleoside monophosphate}\ (\text{NMP}) \rightleftharpoons \text{ADP} + \text{Nucleoside diphosphate}\ [\text{NMP kinase}]$$

$$\text{GTP} + \text{UDP} \rightleftharpoons \text{GDP} + \text{UTP}$$
$$[\text{Nucleoside diphosphate kinase}]$$

PARTITION OF FOOD ENERGY IN ANIMALS

Food energy is partitioned into various fractions on the basis of nutrient digestion and metabolism in animals (Baldwin and Bywater 1984). The scheme is shown in Figure 8.3. Different energy systems are used to formulate the diets of different animal species because of differences in their digestive physiology and anatomy (Wu et al. 2007). Factors that affect digestion, absorption, and metabolism of dietary nutrients can also influence the efficiency of their utilization by animals (Moughan et al. 2000; Noblet et al. 1994). Gross, digestible, metabolizable, and net energy values of individua feedstuffs or diets can be determined through animal studies or predicted, based on their chemical composition, from mathematical equations for convenience (Table 8.2). Note that these equations are used only for reference because the digestion and metabolism of dietary energy in animals are affected by a plethora of physiological, pathological, and environmental factors.

Gross Energy

Gross energy (GE) is defined as the energy released as heat (called heat of combustion) when a substance is fully oxidized in a bomb calorimeter, which contains a strong metal chamber resting in an insulated tank of H_2O (a thermodynamically closed system; McDonald et al. 2021). The heat produced from substrate oxidation is calculated from the rise in temperature of the surrounding H_2O.

The heat of combustion is often referred to as the change in the enthalpy of the reaction (ΔH). ΔH is independent of the chemical pathway by which a process is carried out and depends only on the differences in chemical energy before and after the process has occurred (i.e., the substrates and products; Newsholme and Leech 2009). For example, the ΔH of fat is the same when it is completely oxidized to CO_2 and H_2O in a bomb calorimeter or an animal. This is also true for starch and glucose. In contrast, protein is completely oxidized to CO_2, H_2O and nitrogen (N) oxides (NO_x) in a bomb calorimeter but incompletely oxidized to CO_2, H_2O, ammonia, urea, uric acid, nitrate, creatinine, and other nitrogenous metabolites in an animal. Thus, the physiological ΔH of protein is lower than its value of combustion energy (i.e., GE) obtained from a bomb calorimeter.

Oxidation of fat and starch in a bomb calorimeter and an animal:

$$\text{Fat} + O_2 \rightarrow CO_2 + H_2O$$
$$\text{Starch} + O_2 \rightarrow CO_2 + H_2O$$

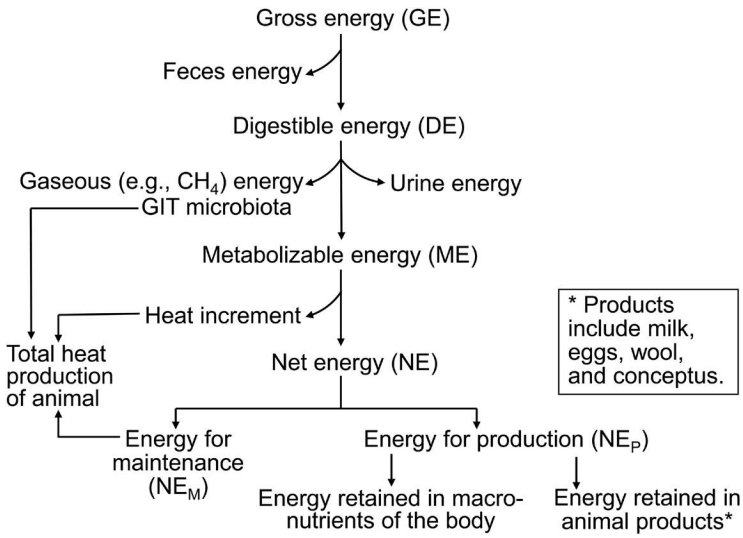


FIGURE 8.3 Partitioning of food energy into various fractions in animals. When feces energy is determined, apparent digestible energy (DE) is obtained. True DE can be calculated on the basis of the true digestibility of macronutrients in diets. Metabolizable energy (ME) values of feedstuffs may vary greatly within the same animal and among animal species, depending on the dietary content of fiber, fat, carbohydrate, and protein. Heat is produced by an animal via basal metabolism (maintenance); the gastrointestinal tract (GIT) bacteria; and the heat increment of feeding associated with the motility of the GIT and the digestion, assimilation, and metabolism of dietary nutrients. The heat that is released after feeding from metabolic processes (e.g., the synthesis and catabolism of nutrients) is a part of heat increment. Net energy (NE) is the energy retained in the body of the animal (e.g., protein, fats, and glycogen) and its products (e.g., milk, eggs, wool, and conceptuses). NE can better predict the efficiency of dietary energy for maintenance and production than GE, DE, and ME. NE can be partitioned into NE for maintenance (NE_m) and production (NE_p).

TABLE 8.2
Equations for Predicting or Estimating the Digestible, Metabolizable, and Net Energy Values of Conventional Diets for Ruminants and Nonruminants (MJ/kg of Dietary Dry Matter)

Animals	Equation
Ruminants[a]	DE = GE × (OMd – 3.94 + 0.104 CP + 0.149 EE + 0.022 NDF – 0.244 Ash)
	ME = DE (in MJ/kg of DM) × [(86.38 – 0.099 CF_o – 0.196 CP_o)/100]
	NE for lactation = ME × [0.60 + 0.24 (q – 0.57)]
	NE for fattening (meat production) = ME × (0.78q + 0.006)
	NE for maintenance = ME × (0.278q + 0.554)
Growing pigs[a]	DE = 0.2247 CP + 0.3171 EE + 0.1720 Starch + 0.0318 NDF + 0.1632 Residue
	ME = DE – (0.19 + 0.031 N_{urine}) – (0.67 DRes)
	NE for growth = 0.730 ME + 0.055 EE + 0.015 Starch – 0.028 CP – 0.041 CF
Adult sows[a]	DE = 4.37 + 0.742 × (17.69 + 0.146 EE + 0.071 CP – 0.132 NDF – 0.341 Ash)
	ME = DE – (0.22 + 0.031 N_{urine}) – (1.34 DRes)
Poultry[b]	AME = 0.227 CP + 0.325 EE + 0.160 Starch – 0.065 NDF + 0.07 Residue
	AMEn = 0.207 CP + 0.322 EE + 0.157 Starch – 0.063 NDF + 0.068 Residue
	NE for broiler growth = 0.781 ME – 0.028 CP + 0.029 EE
Cats[c]	DE = 47.24 CP + 32.52 EE − 81.27 Ash + 2532.76
	ME = 75 EE + 2766

[a] INRAE (2024).
[b] Noblet J. et al. (2021).
[c] Montes de Oca et al. (2017).

Note: AME, apparent metabolizable energy; AMEn, AME corrected for a zero nitrogen retention; Ash, the content of minerals in diet (% of DM); CF_o, the content of crude fiber in diet expressed as % of OM; CP, the content of crude protein in diet (% of DM); CP_o, the content of crude protein in diet expressed as % of OM; DE, digestible energy; DM, dry matter; DRes, digestible residue (g of DM; see below for the definition of residue); EE, the content of ether extract in diet (% of DM); GE, the content of gross energy in diet; ME, metabolizable energy; N_{urine}, quantity of nitrogen in urine (g/kg of dietary DM); OM, organic matters; OMd, the digestibility of dietary organic matter (%); NDF, the content of neutral detergent fiber in diet (% of DM); NE, net energy; q = ME/DE; residue, in % of DM, the difference between the quantity of OM and the sum of the other constituents used in the equation, i.e., OM (in % of DM) – Sum of the other constituents used in the equation (in % of DM).

Oxidation of protein in a bomb calorimeter:

$$\text{Protein} + O_2 \rightarrow CO_2 + H_2O + NO_x$$

Oxidation of protein in an animal:

$$\text{Protein} + O_2 \rightarrow CO_2 + H_2O + \text{ammonia, urea, uric acid, nitrate, creatinine, and other metabolites.}$$

Because of different composition and metabolic fates, the GE values of amino acids vary substantially (Wu 2022). Expressed as kcal/g for proteinogenic amino acids, phenylalanine has the greatest combustion energy (6.72), followed by tryptophan (6.60), leucine and isoleucine (6.52), lysine (6.02), and valine (5.98), and aspartate has the lowest value (2.87). However, when expressed as 10^3×kJ/mol, tryptophan has the greatest ΔH value (5.63), followed by phenylalanine (4.65), tyrosine (4.43), arginine (3.74), lysine (3.68), and leucine and isoleucine (3.58), but glycine has the lowest value (0.97). Amino acids are the major metabolic fuels in the whole bodies of fish.

The GE of a diet can be calculated according to the content of dietary carbohydrates including neutral detergent fiber (NDF), TAG (fat), and protein. Their enthalpies (combustion energy values) are as follows:

Starch or NDF = 4.1 kcal/g; Glucose = 3.72 kcal/g.

Fat = 9.4 kcal/g; Palmitic acid (a long-chain fatty acid) = 9.13 kcal/g.

Protein = 5.4 kcal/g; Based on their intact molecular weights, constituent amino acids in pig-tissue and soybean proteins = 4.75 and 4.80 kcal/g, respectively.

Organic polymers contain more hydrogen atoms and greater energy values than their monomers. For example, starch is more reduced than glucose because 1 H_2O molecule is removed when 2 glucose molecules form a glycosidic bond. Likewise, protein is more reduced than its constituent amino acids because 1 H_2O molecule is removed when 2 amino-acid molecules form a peptide bond. More O_2 is needed to oxidize starch, fat, and protein for releasing energy than glucose, constituent fatty acids (i.e., medium- or long-chain), and constituent amino acids, respectively.

Thus, the GE content of starch, fat, and protein is greater than that of glucose, long-chain fatty acids, and constituent amino acids.

$$\text{GE (kcal/kg of diet)} = (\%\ \text{fat} \times 9.4 + \%\ \text{starch} \times 4.1 + \%\ \text{protein} \times 5.4) \times 10$$

(for a diet containing fat, starch/glycogen, and protein; e.g., % fat = 10, % starch = 65, and % protein = 20).

$$\text{GE (kcal/kg of diet)} = (\%\ \text{fat} \times 9.4 + \%\ \text{glucose} \times 3.72 + \%\ \text{protein} \times 5.4) \times 10$$

(for a diet containing fat, glucose, and protein; e.g., % fat = 10, % glucose = 65, and % protein = 20).

Table 8.3 lists the ΔH values of protein, fats, and carbohydrates in bomb combustion and animal oxidation. On the gram basis, the GE of fat is 74% and 129% greater than

TABLE 8.3
ΔH Values of Organic Substances in Bomb Combustion and Animal Oxidation[a]

Nutrient [molecular weight (mw), Da]	Bomb Calorimeter			Animal Oxidation			ATP Yield (mol/mol)	Efficiency (%) of ATP Production from Substrate Oxidation[b]
	kcal/g	kJ/g	kJ/mol	kcal/g	kJ/g	kJ/mol		
Fat[c] (mw 806)	9.39	39.3	31676	9.39	39.3	31676	336.5	54.8
Palmitic acid (mw 256)	9.35	39.1	10,031	9.35	39.1	10,031	106	54.5
Protein[d] (mw 100)	5.40	22.6	2260	4.10	17.2	1720	20	45.7 (mammal)
Protein[d] (mw 100)	5.40	22.6	2260	3.74	15.7	1566	18.2	41.6 (bird)
Protein[d] (mw 100)	5.40	22.6	2260	4.91	20.5	2054	23.9	54.6 (fish)
Starch[e] (mw 162)	4.11	17.49	2836	4.11	17.2	2786	30	55.7
Lactose (mw 342)	3.92	16.4	5630	3.92	16.4	5630	60	55.0
Sucrose (mw 342)	3.94	16.5	5640	3.94	16.5	5640	60	55.0
Glucose (mw 180)	3.72	15.6	2803	3.72	15.6	2803	30	55.2
Galactose (mw 180)	3.72	15.6	2803	3.72	15.6	2803	30	55.2
Glycerol (mw 92)	4.30	18.0	1655	4.30	18.0	1655	18.5	57.7
Lactic acid (mw 90)	3.56	14.9	1344	3.56	14.9	1344	15	57.6
Ethanol (mw 46)	7.10	29.7	1366	7.10	29.7	1366	15	56.7
Acetic acid (mw 60)	3.25	13.6	816	3.25	13.6	816	8	50.6
Propionic acid (mw 74)	4.97	20.8	1536	4.97	20.8	1536	17.5	58.8
Butyric acid (mw 88)	5.95	24.9	2193	5.95	24.9	2193	22	51.8
Glutamate (mw 147)	3.66	15.3	2244	1.72	7.21	1060	20.5	47.2 (mammal)
Glutamate (mw 147)	3.66	15.3	2244	1.60	6.69	984	18.7	43.9 (bird)
Glutamate (mw 147)	3.66	15.3	2244	1.85	7.75	1139	24.4	50.8 (fish)
Proline (Pro; mw 115)	5.66	23.7	2730	2.47	10.3	1188	23.0	43.5 (mammal)
4-Hydroxy-Pro (mw 131)	4.73	19.8	2593	2.21	9.25	1213	23.5	46.8 (mammal)
Creatine (mw 131)	4.23	17.7	2323	0.0[f]	0.0	0.0	---	---
Creatinine (mw 113)	4.94	20.7	2336	0.0[f]	0.0	0.0	---	---
Urea (mw 60)	2.51	10.5	632	0.0[f]	0.0	0.0	---	---
Uric acid (mw 168)	2.73	11.4	1920	0.0[f]	0.0	0.0	---	---
Ammonia (NH_3, mw 17)	5.37	22.5	382	0.0[f]	0.0	0.0	---	---
Allantoin (mw 158)	2.59	10.8	1714	0.0[f]	0.0	0.0	---	---
Methane (mw 16)	13.3	55.6	891	0.0[f]	0.0	0.0	---	---

Source: Cox and Pilcher 1970); and Wu 2022.

[a] The calculation does not involve substrate oxidation via the formation of glucose or ketone bodies. If propionate is first converted into glucose, which is subsequently oxidized into CO_2 and H_2O, the net production of ATP is 11.5 mol per 1 mol of propionate. If butyrate is first converted into D-β-hydroxybutyrate, which is subsequently oxidized into CO_2 and H_2O, the net production of ATP is 20 mol per 1 mol of butyrate. When expressing per gram (g), values are on a dry matter basis. The approximate molecular weights of substances are given.

[b] Calculated on the basis of 51.6 kJ/mol for one high-energy bond in ATP. Namely, mol of net ATP production per 1 mol of substrate × 51.6 kJ/mol ÷ the combustion energy of kJ/mol substrate × 100%.

[c] Tripalmitoylglycerol (tripalmitin).

[d] Based on the MWs of animal protein (98.8 Da; Kleiber 1961) and the whole-body protein (102.2 Da) of pigs (Chapter 4). For convenience, the MW of 100 Da is used for animal protein.

[e] The MW of glucose residue in starch = the MW of intact glucose – the MW of H_2O = 180.16 – 18.02 = 162.14.

[f] For nonruminants. Except for methane, the listed substances can be energy sources in ruminants with a functional rumen.

that of protein and starch, respectively, whereas the GE of protein is 32% greater than that of starch. This is because fat and protein are more reduced than carbohydrates, as noted previously. The more reduced an organic compound (i.e., the higher the hydrogen atom content), the more O_2 is required for its oxidation to H_2O.

Digestible Energy

Definition of Digestible Energy

Digestible energy (DE) is the GE minus the energy of the feces (FE).

$$DE = GE - FE$$

This is apparent DE, because the energy of the feces includes the energy of undigested feed, the endogenous substances from the gastrointestinal tract (GIT), and microbial fermentation products. Apparent DE values can be estimated from the apparent digestibilities and enthalpies (i.e., GE) of individual types of organic matter (OM) in diets. A digestion marker (e.g., 0.3% chromic oxide) that is neither absorbed nor metabolized by the small and large intestines must be included in the diet when only part of the intestinal digesta or feces is collected from animals. When the true digestibilities of individual OM are determined, the true DE can be predicted.

$$\begin{aligned}\text{Apparent DE} = {} & \text{Apparent digestibility of protein} \times \text{its GE} \\ & + \text{Apparent digestibility of starch} \times \text{its} \\ & \text{GE} + \text{Apparent digestibility of NDF} \times \text{its GE} \\ & + \text{Apparent digestibility of fat} \times \text{its GE.}\end{aligned}$$

$$\begin{aligned}\text{True DE} = {} & \text{True digestibility of protein} \times \text{its GE} \\ & + \text{True digestibility of starch} \times \text{its GE} + \text{True} \\ & \text{digestibility of NDF} \times \text{its GE} + \text{True digestibility} \\ & \text{of fat} \times \text{its GE.}\end{aligned}$$

Fiber is not a single type of complex carbohydrate in animal feeds (Chapter 2). The digestibility of NDF varies with the dietary content and digestion of cellulose, hemicellulose, and lignin that have different combustion energy values. Thus, alterations in their proportions in the NDF can impact its combustion calories. The enthalpy of fiber in feeds is a bit lower than that in feces, mainly because of concentrated lignin in the former. Recently, Stypinski et al. (2024) found that increasing dietary lignin content from 9.6% to 13.3% of NDF-reduced DE by 5.4% in lactating Jersey cows and that energy supplied from digestible NDF in their diets may be less than that predicted by some nutritional models.

Losses of Fecal Energy in Various Animals

The energy in the feces represents a single large loss of the ingested energy. In ruminants, the heat produced during fermentation is positively correlated with digestible OM content of the diet; the higher the fiber intake, the more heat loss occurs in the GIT. In these animals, fecal energy accounts for 20–30% and 40–50% of GE, respectively, when concentrates and roughage are fed.

Fecal energy represents approximately 40%, 20%, 20%, and 25% of the GE in horses, pigs, poultry, and fish, respectively (Pond et al. 2005). The higher fecal energy value in horses (herbivores) consuming their habitual fibrous diets compared to that in nonherbivorous nonruminants ingesting little forage can be explained by plant cell-wall material in the equine diets that is not digested in the small intestine and enters the large intestine, where it is fermented. The enlarged colon and cecum of an adult horse have the capacity of 60 and 25–35 L, respectively; the combined capacity in the hindgut contributes to the digestion of 30% protein, 15–30% soluble nonstarch polysaccharides, and 75–85% of plant cell-wall polysaccharides in diets (Ralston 1984). As indicated in Chapter 1, horses do not have the same retention capacity in their stomach as the rumen of ruminants but, through eating more of the forage, can still obtain the same amount of NDF as do ruminants. When fed a mixture of hay and concentrates, the digestibility of OM in horses is 85% of that in ruminants, indicating a high ability of horses to utilize forages among nonruminants (Hintz 1975). Because of the complexity of hindgut fermentation in horses, dietary DE values vary greatly among their feedstuffs.

When pigs are fed a diet containing 5% fiber, their large intestine can degrade as much as 50% of cellulose and hemicellulose in cereal grains and their products (McDonald et al. 2021). In contrast, the rates of degradation of nonstarch polysaccharides by the hindgut in poultry (Qaisrani et al. 2015), fish, and shrimp (Stevens and Hume 2004) are much lower than those for horses. Because dogs and cats have a small hindgut where microbial capacity is limited (Stevens and Hume 2004), dietary DE values will be low if these carnivores are fed a fibrous diet.

In all animals, the true DE values should be calculated by adding, to the apparent DE, the energy of endogenous (gastrointestinal) secretions and the endogenous microbial mass that are present in the feces. In other words, the true DE is always greater than the apparent DE. Like the apparent DE, the true DE can be calculated or predicted according to the content and true digestibility of individual macronutrients in diets (Table 8.4).

Measurements of the Digestibility of Feeds

A digestion experiment involves the collection of ileal digesta or feces from an animal that is kept in a specially designed crate (for facilitating the separation of feces from urine) and fed a constant amount of a test diet. The time required for feed to pass through the GIT differs markedly between ruminants and nonruminants (Chapter 1). A pretest period (e.g., 4–7 days for pigs and horses; 10–14 days for ruminants) is necessary for the GIT to be free of residues from previous feeds and for an animal to adapt to the test diet. The period of ileal digesta or feces collection

TABLE 8.4
Calculation of Gross Energy and Digestible Energy of Diets for Animals[a]

	Starch		Lipids		Protein		Gross Energy[d]	Digestible Energy[e]	
Animal	Content in Diet (%)	True Digestibility	Content in Diet (%)	True Digestibility	Content in Diet (%)	True Digestibility	(GE) (kcal/kg diet)	Amount (kcal/kg diet)	% of GE
Pigs[b]	50	0.95	10	0.92	18	0.87	3967	3663	92.3
Chickens[c]	50	0.95	6	0.92	20	0.90	3699	3443	93.1
Ruminants	65	0.40–0.95	5 (2–8)	0.72	14 (10–18)	0.65	3898	2633	67.5
Horses	10	0.95	6	0.92	14	0.85	1731	1552	89.7
Fish	20	0.85	10	0.90	40	0.85	3922	3381	86.2

[a] Dietary dry matter content is 90%. Within the same species, optimal nutrient contents in diets vary with productive purposes and ages. In this table, averaged values are used for calculation.
[b] Pigs (2 months of age) are fed cereal grains.
[c] Chickens (4 weeks of age) are fed cereal grains.
[d] Gross energy (kcal/kg of diet) = (% starch × 10 × 4.1 kcal/g) + (% lipids × 10 × 9.4 kcal/g) + (% protein × 10 × 5.4 kcal/g). Use the diet for pigs as an example, gross energy (kcal/kg of diet) = (50 × 10 × 4.1) + (10 × 10 × 9.4) + (18 × 10 × 5.4) = 3967 kcal/kg of diet.
[e] Digestible energy (kcal/kg of diet) = (% starch × digestibility × 10 × 4.1 kcal/g) + (% lipids × digestibility × 10 × 9.4 kcal/g) + (% protein × digestibility × 10 × 5.4 kcal/g). Use the diet for pigs as an example, gross energy (kcal/kg of diet) = (50 × 0.95 × 10 × 4.1) + (10 × 0.92 × 10 × 9.4) + (18 × 0.87 × 10 × 5.4) = 3663 kcal/kg of diet.

varies with animal species (e.g., 4–7 days for pigs and horses or 4–10 days for ruminants; Bondi 1987; Kirk et al. 1994; Ray et al. 2013). The digestibility of a concentrate feed for ruminants is generally determined as the difference in the digestibility of roughage (the basal diet) and that of the roughage plus concentrate to ensure sufficient provision of dietary fiber to the rumen. There are likely interactive effects between forages and concentrate feeds on feed intake and digestion in ruminants (Niderkorn and Baumont 2009). In poultry, which normally excrete both feces and urine from a single orifice, the determination of fecal digestibility requires that urinary flow be diverted from the cloaca to a collecting device. However, this procedure is not needed when the ileal digesta is obtained.

Metabolizable Energy

Metabolizable energy (ME) is DE minus the energy in urine (UE) and the energy in combustible gases (E_{gases}) leaving the GIT. Feces and urine are collected from individual animals placed in metabolism cages or stalls, whereas specialized facilities (e.g., respiratory chambers and the GreenFeed system) are required to measure the production of methane by individual ruminants (Tedeschi et al. 2022).

$$ME = DE - UE - E_{gases}$$

The gases are mainly methane (which contains 13.3 kcal/g; Cox and Pilcher 1970), and, to a lesser extent, ammonia, H_2, H_2S, and CO_2. These gases are produced by the GIT microbiota (including bacteria, protozoa, and fungi). The carbon atom in CO_2 is already in its highest oxidation state (+4) and cannot gain an additional oxygen atom or undergo further oxidation. Thus, there is no energy in CO_2 and its production by the GIT does not represent a loss of feed energy.

In the rumen of ruminants, methane (CH_4) is generated by methanogens, but protozoa and fungi can contribute significantly to the production of H_2 (a precursor for CH_4). There are not many protozoa in the hindgut of the standard agricultural animals (and if there are, they are pathogen-like coccidia or crypto), and little information is available about fungi in their hindgut. However, horses can have non-pathogenic protozoa in their hindgut. Loss of combustible gases through intestinal digestion is negligible in monogastric animals but is nutritionally and quantitatively significant in ruminants and hind gut fermenters (Stevens and Hume 2004). Fermentation by microbes in the rumen and the hindgut produces large amounts of gases, which can represent 7–8% of the dietary ME intake (McDonald et al. 2021). In addition, when dietary carbohydrates, proteins, and fats are fermented to short-chain fatty acids (SCFAs) in the rumen, the energy released from the reactions is not fully trapped in the products or by the bacteria (Chapter 5). Thus, enhancing the fermentation of degraded fiber to SCFAs in the rumen and the subsequent utilization of the SCFAs by the host tissue will substantially reduce the gaseous energy per unit of production and improve feed efficiency in ruminants.

Methane can account for 6–12% and 1–4% of the dietary GE intake in cattle and horses, respectively. There are reports that dietary nitrate mitigated ruminal methanogenesis by serving as an H_2 sink (Chapter 5) but reduced dry matter (DM) intake and milk protein yield in lactating cows (Feng et al. 2020; Meller et al. 2019). Likewise, 3-nitrooxypropanol (NOP) may be an effective inhibitor of methane production by both beef and dairy cattle. For example, dietary supplementation with 150 mg NOP/kg

of DM to growing beef cattle decreased CH_4 production (g/day) and yield (g/kg of DM intake) by 30.6% and 27.2%, respectively, without affecting daily DM intake or BW gain, during indoor feeding and when fed a forage plus concentrate (50:50) diet (Kirwan et al. 2024). In addition, the inclusion of NOP in diets for lactating cows at a dose of 70.5 mg/kg of DM reduced CH_4 production (g/day), yield (g/kg of DM intake), and intensity (g/kg of energy-corrected milk) by 32.7%, 30.9%, and 32.6%, respectively (Kebreab et al. 2023). NOP acts by directly inhibiting methyl-coenzyme M reductase, which catalyzes the final step of methane synthesis by anaerobic archaea in the rumen of ruminants. Interestingly, elevated DM or NDF intake attenuated the effect of dietary nitrate or NOP supplementation on CH_4 production by beef and dairy cattle (Feng et al. 2020), likely due to increased concentrations of CO_2 in ruminal fluid for competing with nitrate for H_2. Furthermore, cattle type influences methane production by the GIT in response to NOP supplementation because the methane-mitigating effect of NOP is greater in dairy cattle than in beef cattle (−39.0% vs. −22.2%) when adjusted for NOP dose and NDF content (Dijkstra et al. 2018). In pigs, the E_{gas} represents only a small fraction (ranging from 0.1 to 3%) of DE, depending on the diet and environmental temperature (Velayudhan et al. 2015). Because of the different rates of fermentation in the GIT of animal species, the ME values of feedstuffs may differ widely among nonruminants and ruminants, particularly when they are fed diets containing high percentages of fibrous byproducts or nonstarch polysaccharides (Table 8.5). Of note, the use of these ingredients for animal feeding is increasing globally. Thus, because of large variations in their ME values, the National Research Council (NRC 2012) no longer recommends ME as the energy system for formulating swine diets.

Urine energy results primarily from the excretion of incompletely oxidized nitrogenous compounds (e.g., urea, uric acid, ammonia, creatinine, amino acids, and nitrate) under normal conditions (Morris et al. 2021; Wu 2022). However, products of the oxidation of fatty acids and ketogenic amino acids (e.g., ketone bodies) are also present in the urine of most animals, and the amounts are substantially increased in animals with type-I diabetes mellitus. Indeed, tissue-specific catabolism of amino acids occurs in the whole body regardless of their dietary intake. Dietary intake of amino acids affects not only the amounts of total nitrogenous metabolites but also the ratio of urea to nonurea N-containing substances in urine (Meller et al. 2019). Urine energy accounts for 2–3%, 4–5%, and 4–4.5% of the dietary GE intake in pigs, cattle, and horses, respectively (Bondi 1987; Velayudhan et al. 2015). Urea, uric acid, creatinine, and ammonia contain 2.51, 2.73, 4.94, and 5.37 kcal/g, respectively (Cox and Pilcher 1970). Because they are the major end-products of amino acid oxidation

TABLE 8.5
Metabolizable Energy Values of Feed Ingredients for Animals[a]

Animal	Feed	Gross Energy (GE)	Energy in			Metabolizable Energy	
			Feces	Urine	Methane	Amount	% of GE
Fowl	Corn	18.4	2.2[b]	---	---	16.2	88.0
	Wheat	18.1	2.8[b]	---	---	15.3	84.5
	Barley	18.2	4.9[b]	---	---	13.3	73.1
Pig	Corn	18.9	1.6	0.4	---	16.9	89.4
	Oats	19.4	5.5	0.6	---	13.3	68.6
	Barley	17.5	2.8	0.5	---	14.2	81.1
	Coconut cake meal	19.0	6.4	2.6	---	10.0	52.6
Sheep	Barley	18.5	3.0	0.6	2.0	12.9	69.7
	Dried ryegrass (young)	19.5	3.4	1.5	1.6	13.0	66.7
	Dried ryegrass (mature)	19.0	7.1	0.6	1.4	9.9	52.1
	Grass hay (young)	18.0	5.4	0.9	1.5	10.2	56.7
	Grass hay (mature)	17.9	7.6	0.5	1.4	8.4	46.9
	Grass silage	19.0	5.0	0.9	1.5	11.6	61.1
Cattle	Corn	18.9	2.8	0.8	1.3	14.0	74.1
	Barley	18.3	4.1	0.8	1.1	12.3	67.2
	Wheat bran	19.0	6.0	1.0	1.4	10.6	55.8
	Lucerne hay	18.3	8.2	1.0	1.3	7.8	42.6

Source: Adapted from McDonald et al.2021.

[a] Values for energy content are MJ/kg of dry matter, except for the % of GE. Different sources of corn and barley were used for studies with various animal species.

[b] Energy in feces plus urine.

in animals, the physiological energy of dietary protein is much lower than its GE, as noted previously. Thus, the high intake of dietary protein is associated with low ME in all animals, when compared with the isocaloric intake of fats and starch (Verstegen and Henken 1987).

ME is the portion of the food energy that is available for metabolic processes in an animal (Blaxter 1989). The efficiency of ME utilization for maintenance and production is affected by energy substrates, their metabolic pathways, the physiological states of the animal, and the ambient environment. Dietary the conversion of ME into NE is more efficient when animals are fed diets below maintenance requirements, compared to diets above maintenance requirements (i.e., fat and protein deposition; Table 8.6). This is because (a) the relative rates of absorption of dietary nutrients are greater but the motility and activity of the GIT (e.g., gas production) are lower under the former than the latter conditions, and (b) the metabolic transformation for the synthesis of fat and protein (that is less active under the former than the latter conditions) in all animals is always less than 100% (see the sections below). These factors are independent of the need for the mobilization of energy reserves from tissues when animals do not consume sufficient energy for maintenance. The dietary ME from fat is utilized more efficiently when animals are fed diets either below or above maintenance requirements in comparison with the dietary ME from protein because the energetic efficiency of the synthesis of fat is higher than that of protein (Verstegen and Henken 1987). Because of differences in digestion and metabolism, the values of dietary ME (Table 8.7) and the efficiency of its utilization differ greatly among animals. For example, ME is 82–87% of DE in ruminants (beef cattle, sheep, and goats); 84–88% of DE in horses consuming forages; 94–97% of DE in pigs, and 95–97% of DE in chickens, depending on diets and breeds (McDonald et al. 2021; Pond et al. 2005). The rate of conversion of DE into ME is estimated to be 87% in dairy cows (NRC 2001), which is greater than that in beef (82%; Galyean et al. 2016). This is because of a higher energetic efficiency in the use of dietary nutrients for milk production by lactating cows than muscle growth in beef cattle, thereby reducing the production of (a) nitrogenous compounds (e.g.,

TABLE 8.6
Efficiency of Utilization of Metabolizable Energy (ME) below Maintenance and for Fat and Protein Deposition above Maintenance in Animals[a]

		Efficiency (% of ME)		Heat Increment (% of ME)	
Nutrient	Animal	Below Maintenance	Above Maintenance[b]	Below Maintenance	Above Maintenance[b]
Carbohydrate	Nonruminants	94	78	6	22
	Ruminants	80	54	20	46
	Other herbivores	90	64	10	36
	Birds	95	77	5	23
Fat	Nonruminants	98	85	2	15
	Ruminants	92[c]	79	8[c]	21
	Other herbivores	92[c]	79	8[c]	21
	Birds	95	78	5	22
Protein	Nonruminants	77	64	23	36
	Ruminants	70	45	30	55
	Other herbivores	76	50	24	50
	Birds	80	55	20	45
Regular diets	Man	90	75	10	25
	Rat	90	75	10	25
	Dog	85	70	15	30
	Pig	85	70	15	30
	Rabbit	80	65	20	35
	Horse	75	60	25	40
	Cattle	70	50	30	50
	Sheep	70	50	30	50
	Chicken	90	75	10	25

Source: Blaxter 1989.

[a] Different animals consume their habitual diets. The corresponding heat increment for utilization of dietary ME increases as the amount of energy for maintenance or production decreases.

[b] Used for deposition of fat and protein.

[c] Tedeschi and Fox 2020.

TABLE 8.7
Energy Values of Conventional Feedstuffs for Animals[a]

Animal	Gross Energy (GE)	Digestible Energy (DE, % of ME)	Metabolizable Energy (ME, % of DE)	Net Energy (NE, % of ME)
Chicken	100	92–93	96	80
Fish	100	85–88	97	90–95
Horse	100	80–90	90–95 for cereals	80
			84–88 for forages	80
Pig	100	90–92	96	82
Ruminants	100	66–71	82 (e.g., beef) to 87 (e.g., dairy)	80–82

[a] These are estimated values, which vary with a plethora of factors, including feedstuffs, nutrient intakes, animals (e.g., breeds and physiological states), production (e.g., muscle growth and lactation), and environmental conditions. NE is the sum of NE for maintenance plus NE for production. The aforementioned factors that affect the utilization of GE also influence the efficiency in the use of NE for maintenance and production.

ammonia, urea, uric acid, and creatinine) found in the urine and (b) methane by the GIT in the former.

Over the past 40 years, the ME system has been used widely in the swine and poultry industries (de Lange and Birkett 2005; NRC 2012). Thus, data on the dietary ME values of conventional feedstuffs for nonruminants are available in the literature (de Lange and Birkett 2005; Le Goff and Noblet 2001). Of note, the use of the ME system is convenient for poultry feeding because poultry void both urine and feces into the same opening and are conveniently collected together (Sibbald 1982). Currently, apparent ME (AME) corrected for zero N retention, AMEn, is used in poultry feeding with the goal of providing a more accurate measure of the energy available from feed or diet by adjusting for the energy needed to metabolize protein (forming nitrogenous metabolites), so that a more precise comparison of energy values can be made among different feed ingredients or diets regardless of their protein content. The ME values of swine and poultry diets can be readily determined through standard metabolism and digestion experiments (McDonald et al. 2021).

$$\mathrm{AME_{diet}} = \left[\left(\text{Feed intake} \times \mathrm{GE_{diet}}\right) - \left(\text{Excreta output} \times \mathrm{GE_{excreta}}\right)\right]/\text{Feed intake},$$

where $\mathrm{AME_{diet}}$ is the AME value of diet (MJ/kg of DM); $\mathrm{GE_{diet}}$ and $\mathrm{GE_{excreta}}$ are the GE values (MJ/kg of DM) of diet and excreta (urine plus feces), respectively; and feed intake and excreta output are expressed as kg of DM.

$$\mathrm{AMEn_{diet}} = \mathrm{AME_{diet}} - \left[\text{N retention}\left(\text{g/kg of dietary DM intake}\right) \times \text{GE of N in urine}\left(\text{MJ/g of N}\right)\right],$$

where $\mathrm{AME_{diet}}$ (the AME of diet) and $\mathrm{AMEn_{diet}}$ (dietary AME corrected for N retention in the body) are expressed as (MJ/kg of DM), N retention (the retention of N in the body) is expressed as g of N/kg of dietary DM intake, and the GE of N in urine has been taken to be 0.034 or 0.037 MJ/g of N in the literature (Abdollahi et al. 2021), which is the GE of uric acid or total nitrogenous metabolites in urine, respectively.

$$\mathrm{AMEn_{feed}} = \left(\mathrm{AMEn_{test}} - \mathrm{AMEn_{ref}} \times \mathrm{a}\right)/\mathrm{b},$$

$\mathrm{AMEn_{feed}}$, the AMEn of a feed ingredient; $\mathrm{AMEn_{ref}}$, the AMEn of the reference diet; $\mathrm{AMEn_{test}}$, the AMEn of the test diet; a, the percentage (%) of energy ingredients of the reference diet that are present in the test diet; b, the inclusion level (%) of the feed ingredient of interest in the test diet.

Because protein catabolism in poultry generates not only uric acid (the major metabolite) but also ammonia, creatine, creatinine, and urea (a product of arginine hydrolysis), the GE value of 0.037 MJ/g of N for N in urine is more appropriate than the GE value of 0.034 MJ/g of N for N in urine for the determination of $\mathrm{AMEn_{diet}}$. There is a concern that correcting AME values to zero N retention for modern fast-growing broilers may penalize the energy value of protein feedstuffs, particularly those with high protein quantity and quality (Abdollahi et al. 2021). This issue should receive attention from nutritionists because dietary glutamate and aspartate are now known to be the major energy sources in chicken enterocytes that have high energy requirements for maintenance, health, and growth (He et al. 2022).

Net Energy and Heat Increment

Net energy (NE) is ME minus the heat increment (HI) associated with feeding. HI is part of the whole-body heat production in animals that results from the activity of feeding, food digestion, and nutrient metabolism (Bondi 1987). NE consists of the energy for maintenance (e.g., sustaining the life processes; $\mathrm{NE_m}$) and the energy for production ($\mathrm{NE_p}$) such as growth ($\mathrm{NE_g}$), fattening ($\mathrm{NE_f}$), lactation ($\mathrm{NE_L}$), pregnancy ($\mathrm{NE_{preg}}$), and egg formation ($\mathrm{NE_{egg}}$). Here, $\mathrm{NE_g}$ and $\mathrm{NE_L}$ are the amount of energy in a feed or diet that is available for growth and milk production, respectively.

Other minor NE losses, such as scurf and sweat, can be considered as part of NE_m. NE is a better indicator of the energy value of feedstuffs than ME if the efficiency of their ME utilization for maintenance and production differs substantially. This situation likely arises from the feeding of fibrous diets, which stimulate the activities of gastrointestinal bacteria and mucosal cells, as well as intestinal motility (Chapter 5). Of note, NE is much more difficult to determine and more complex than ME (Blaxter 1989). Thus, accurate equations for predicting or estimating NE are particularly useful for animal feeding.

The rates of conversion of ME to NE_g and NE_L have been estimated to be 0.33 and 0.66 in growing beef cattle (NASEM 2016) and lactating cows (NASEM 2021), respectively. The ratios of NE_m to NE_g and of NE_m to NE_L in typical diets for growing beef cattle and lactating cows are approximately 60:40 and 0.20:0.80, respectively. The following aspects of energy metabolism in animals are noteworthy. First, energy needs for maintenance must first be met before the animal can use any dietary energy for growth, lactation, or gestation. Second, the NE_m, NE_g, or NE_L of individual feedstuffs in a diet is additive for animals. Third, feed energy is used more efficiently for maintenance than for growth (Table 8.8) due to <100% energetic efficiency in metabolic transformations associated with the retention of protein, TAGs, and glycogen in animals. Fourth, feed energy is used more efficiently for milk production than for maintenance because protein, fats, and lactose synthesized by mammary epithelial cells undergo little or no intracellular turnover (ATP-dependent processes) and instead are rapidly exported into milk. Lactating cows need 0.74 Mcal of NE_L to produce 1 kg of milk (containing 4% fat and 136 g of DM; NASEM 2021), whereas growing beef cattle require 3.83 Mcal of NE_g to gain 1 kg of tissue (containing ~350 g of DM; NASEM 2016).

$$NE = ME - HI$$

Definition of HI

HI is also called the specific dynamic effect of food. The major causes of the HI include (a) nutrient digestion and absorption [e.g., chewing and mastication of feeds, degradation and fermentation in the GIT (e.g., the rumen of ruminants and the large intestine of all animals), active transport of nutrients by enterocytes and other cell types]; (b) the movement of the GIT; (c) the physiological activities of extra-GIT organs (e.g., kidneys, skeletal muscle, and heart) associated with feeding; (d) the intracellular turnover of macro- and other biomolecules (e.g., protein, TAGs, glycogen, and in the case of lactation, lactose) in animals; and (e) nonproduction metabolism for the removal of toxic substances in an animal such as the synthesis of urea and uric acid from ammonia (Johnson et al. 1990; Lobley 1990; Noblet and Etienne 1987). In growing, gestating, or lactating animals, the heat released after feeding from metabolic processes (e.g., the synthesis and catabolism of fatty acids, fats, amino acids, peptides, protein, glucose, glycogen, and in the case of lactation, lactose) is also a part of the HI. The values of HI in pigs, sheep, cattle, chickens, and salmons

TABLE 8.8
Net Energy of Feedstuffs for Maintenance and Growth in Growing Beef Cattle

Energy or Nutrient	Alfalfa[a]	Barley Grain	Corn Grain	Corn Silage	Field Peas	Oat Grain	Sorghum Grain	Soybean Meal[b]	Wheat Grain
Moisture, %	6.17	10.3	12.8	66.9	11.8	10.1	12.6	10.2	11.1
TDN, % DM	62.4	84.1	87.6	67.7	75.8	83.0	96.0	79.3	86.8
DE, Mcal/kg DM	2.74[c]	3.71	3.86	2.98	3.34[c]	3.66	3.82	3.50	3.83
ME, Mcal/kg DM	2.25	3.04	3.17	2.45	2.74	3.00	3.23	2.87	3.14
ME/DE	0.82	0.82	0.82	0.82	0.82	0.82	0.85	0.82	0.82
NE_m, Mcal/kg DM	1.39	2.06	2.17	1.56	1.81	2.03	2.12	1.92	2.15
NE_g, Mcal/kg DM	0.81	1.40	1.49	0.96	1.19	1.37	1.45	1.28	1.47
NE_m/NE	63:37	60:40	59:41	62:38	60:40	60:40	59:41	60:40	59:41
CP, % DM	18.5	12.8	8.79	8.24	25.2	12.6	10.8	48.85	13.8
Starch, % DM	0.93	56.7	72.1	32.6	43.9	44.1	74.5	1.32	62.4
Crude fat, % DM	3.99	2.20	3.81	3.25	0.95	6.16	3.4	8.27	1.89
NDF, % DM	40.4	18.3	9.72	43.0	13.1	26.7	11.0	22.67	12.4
ADF, % DM	31.2	7.09	3.56	25.5	7.16	13.3	4.3	10.9	4.15

Source: Data for sorghum grain were obtained from INRA (2024). Data for other feedstuffs were taken from NASEM (2016).

[a] Dehydrated alfalfa.

[b] Heated soybean meal.

[c] Estimated accoriding to a ME/DE ratio of 0.82.

Note: ADF, acid detergent fiber; CP, crude protein; NDF, neutral detergent fiber; NE_g, net energy for growth; NE_m, net energy for maintenance; TDN, total digestible nutrients.

TABLE 8.9
Heat Increment of Feeding (% of ME)

Nutrient	Pig	Sheep	Cattle	Chickens	Salmonids	Rainbow trout
Fat	9	29	35	10	---	1.4
Starch	17	32	37	18	---	3.5
Protein	26	54	52	44	---	3.3
Mixed rations	10–40	35–70	35–70	25–30	2.5	1.6

Source: Bondi (1987) for pigs, ruminants and chickens; and Smith et al. (1978) for salmonids (Atlantic salmon) and rainbow trout. ---, data are not available.

are summarized in Table 8.9. HI increases when the intake of food or dietary fiber is enhanced (Blaxter 1989). The underlying reasons are that (a) activities of the GIT, musculature, and omentum are increased to move the food or undegradable fiber down the GIT, and (b) more nutrients are available for digestion and absorption in the small intestine, oxidation in the whole body, as well as degradation and fermentation in the large intestine.

Differences in HI among Energy Substrates

The HI of energy substrates fed to mammals and birds is protein > carbohydrates > fat. The greater HI value for protein than that of carbohydrates or fats can be explained by the following biochemical reactions (Wu 2022). First, intracellular protein turnover (i.e., protein synthesis and degradation) requires large amounts of energy. Second, the disposal of ammonia as urea in mammals and as uric acid in birds requires large amounts of energy (Chapter 7). Van Milgen (2021) has estimated that the energy cost is at least 40.3, 60.7, 64.7, and 65.4 kJ/g of excreted N for the synthesis of urea, uric acid, allantoin, and creatinine, respectively, of which 56%, 56%, 47%, and 85% are retained in the excretion product. Third, energy is required to concentrate and excrete nitrogenous compounds by the kidneys. Finally, the oxidation of amino acids produces heat, particularly in cases of amino acid imbalance. Protein has a lower HI when used for protein synthesis than when used for oxidation to provide energy because the latter is always associated with the conversion of amino acid-derived ammonia into urea or uric acid.

The intestinal absorption of lipids requires virtually no ATP, and the transport of fatty acids through the plasma and organelle (e.g., mitochondria) membranes of all cells is largely energy-independent (Chapter 6). In contrast, the intestinal and renal absorption of amino acids and glucose into the blood requires ATP, and the transport of the carboxylic acids (e.g., pyruvate) by all cell types is energy-dependent (Chapter 5). All these factors explain why the HI associated with the feeding of fat is much lower than that for glucose or protein in nonruminants. Among all macronutrients, protein has the highest HI in mammals and birds, as noted previously. Thus, during heat stress, protein is not an ideal source of energy for the whole bodies of land animals. However, certain cell types in mammals as well as possibly birds and fish have specific requirements for some amino acids as major metabolic fuels (e.g., glutamate, glutamine and aspartate for enterocytes in pigs, and glutamine for lymphocytes in pigs and rats) (Jobgen et al. 2006).

In nonruminant mammals except for carnivores and in birds, the HI of starch is greater than that of fat as noted previously, but either starch or glucose should be provided in diets as a major source of energy, particularly under stress (e.g., heat stress) and disease conditions. This is because (a) the oxidation of fatty acids in animals produces more oxidants (e.g., H_2O_2 and superoxide anion) than that of equal molar glucose (Chapters 5 and 6); (b) saturated fatty acids increase risk for insulin resistance and metabolic syndrome, whereas under physiological conditions the concentration of glucose in the plasma is constant and not elevated; and (c) fatty acids, particularly polyunsaturated fatty acids, are more prone to chemical oxidation than glucose at high ambient temperatures. Whether formulated diets for fishes should contain high levels of carbohydrates depends on both animal species and production goals because even a moderate content of starch (e.g., >10% on a DM basis) impairs hepatic functions and reduces growth in some teleost such as for the feeding of largemouth bass (Li et al. 2022).

Species Differences in HI

The HI value is greater in ruminants than in monogastric animals (Blaxter 1989) for the following reasons. First, greater amounts of energy are required for eating (including chewing and swallowing) and ruminating in ruminants, with the energy costs being 3–6% and 0.3% of the dietary ME intake, respectively. Second, the active degradation and fermentation of dietary substances by the rumen of ruminants generate a large amount of heat. Third, a greater movement of the GIT (including the rumen and the hindgut) in ruminants produces a larger amount of heat than in nonruminants. The rates of conversion of ME into NE in ruminants under various physiological conditions are as follows: maintenance, 70–80%; lactation, 60–70%; growth, 40–60%; and pregnancy, 10–25% (Bondi 1987; McDonald et al. 2021). These values are lower than those for nonruminants, as noted previously.

The disposal of nitrogenous wastes and the pathways of fat utilization in fish differ from those of mammals and birds (Chapter 7). For example, most fish lack the hepatic

urea cycle and directly excrete ammonia into the surrounding H_2O. Thus, the oxidation of amino acids to provide ATP is energetically more efficient in fish than in mammals and birds (He et al. 2021; Li et al. 2021; Zhang et al. 2021). In addition, most fish have a lower capacity to oxidize glucose and fatty acids than amino acids. Smith et al. (1978) measured the HI in salmonids fed a complete diet or a purified macronutrient (fat, protein, or carbohydrate). These authors observed that heat production increased about 30 min after feeding and the increases remained elevated for 1–5 h, depending on the amount and type of the ingested material. Of interest, the HI of protein in the fish was 3–10% of the dietary ME (depending on species) and was much lower than the HI for mammals or birds (Smith et al. 1978). This further supports the notion that high rates of urea and uric acid syntheses are the major cause of the HI associated with protein feeding to mammals and birds, respectively. Moreover, the HI of starch in fish did not differ from that of protein, but was also higher than the HI of fat, as in land animals. Overall, the HI of complete diets in salmonids was <3% of the dietary ME, which is 10% of that in nonruminants and 5–10% of the HI in ruminants. Thus, the NE of protein is higher for fish than for mammals or birds.

NE is the portion of the feed energy that is used for maintenance and retained in the animal (Baldwin and Bywater 1984). The NE_m is fully converted into heat. NE_p is the energy retained in macro- and other molecules (e.g., TAGs, fatty acids, protein, amino acids, glycogen, glucose, and lactose) of tissues (e.g., skeletal muscle and WAT) and products (e.g., milk, eggs, and wool). The efficiencies of the conversion of dietary fat, protein, and starch/glucose into fat, protein, and glycogen in animals are always less than 100% (Table 8.10). NE represents 80–82% of the ME (or 43–45% of the GE) in ruminants fed high-quality concentrates; ~80% of the ME (or 55–62% of the GE) in horses; ~82% of the ME (or 71–73% of GE) in pigs; 80% of the ME (or 70–72% of GE) in laying hens; and 90–95% of the ME (or 76–80% of GE) in fish (Table 8.7). In fish, a large amount of NE is used for constant swimming. In all animals, lean tissue growth generally increases with increasing the dietary intake of NE up to a point where the rate of protein deposition in skeletal muscle reaches a maximum as determined by the genetic capacity, nutrition, and environment (Campbell 1988; van Milgen et al. 2001). Any additional provision of energy beyond this point will result in a marked increase in lipid deposition with only a modest increase in lean tissue gain (Campbell 1988).

TABLE 8.10
Energetic Efficiencies of Biosynthetic Processes in Animals

Substrate	Product in Animal	Efficiency of Energy Transfer (%)
	Fat synthesis	
3 mol palmitic acid plus ½ mol glucose	Fat[a]	98.1
8 mol acetate plus ½ mol glucose	Fat[a]	86.4
Glucose	Fat[a]	81.4
Protein	Fat[a]	59.0
Dietary fat	Fat[a]	98.9
	Carbohydrate synthesis	
Lactate	Glucose	87.8
Protein	Glucose	62.3
Glucose	Lactose	97.5
Glucose	Glycogen	93.7
	Protein synthesis	
Amino acids	Protein	80.8

[a] Tripalmitoylglycerol (tripalmitin).

Factors Affecting Dietary HI and Net Energy

Factors that affect the ME and HI value of a diet also influence its NE. Thus, the NE value of a feedstuff is not fixed, but varies with diets, ages, or phases of animal production, and the environment (Kil et al. 2013; Tedeschi and Fox 2020). For example, for all animals, feeds with high percentages of protein and nonstarch polysaccharides usually have lower NE values than feeds with high percentages of starch and fats (Baldwin and Bywater 1984). The NE of diets for swine and ruminants is generally in the following order: growth > lactation > gestation (Bondi 1987; Pond et al. 2005). Because of a higher energetic efficiency of fat synthesis than protein synthesis, NE is used more efficiently for fat deposition than for protein accretion in mammals, birds, fish, and shrimp.

Measurement of HI

HI, like total whole-body heat production, is often measured using direct or indirect calorimetry methods (Blaxter 1971). Direct calorimetry is simple in theory but difficult in practice. In direct calorimetry, the animal calorimeter is an airtight insulated chamber, in which O_2 is supplied by the flow of air, and the heat produced by the animal increases the temperature of a surrounding medium. Sensible heat is lost from the body surface to the environment by radiation, conduction, and convection (Verstegen and Henken 1987). Evaporative heat is lost from the surface of the skin and the respiratory tract. In indirect calorimetry, the magnitude of energy metabolism is estimated on the basis of the volume of O_2 consumed by an animal, the volume of CO_2 produced by the animal, and a respiratory quotient ($RQ = CO_2/O_2$; Table 8.11). Calculation of heat production from the measurement of gaseous exchange is based on the principle that ΔH is independent of the pathway but depends only on the difference in energy between substrates and products, as explained later in this chapter.

TABLE 8.11
Measurement of Heat Production by Animals Based on Respiratory Quotients (RQ)

Substrate	RQ (CO_2/O_2, vol/vol)	Heat of Combustion		Heat Produced from Substrate Oxidation per L of O_2 Consumed[a]		Percentage (%) of Total Heat Produced from the Oxidation of Substrate	
		kJ/g	kcal/g	kJ/L	kcal/L	CH_2O[e]	Fat[f]
Carbohydrate	1.00	16.74	4.0	21.12	5.047	100	0
Fat	0.707	37.66	9.0	19.61	4.686	0	100
Protein	0.831	22.59	5.4	18.62	4.450	---	---
Mixture of carbohydrate:fat (g/g)							
15:85	0.751[b]	34.52[c]	8.25	19.84[d]	4.742[e]	16.0[f]	84.3[g]
30:70	0.795	31.38	7.50	20.06	4.794	31.6	68.4
40:60	0.824	29.29	7.00	20.21	4.830	47.7	58.3
50:50	0.854	27.20	6.50	20.36	4.866	52.0	48.0
60:40	0.883	25.11	6.00	20.52	4.904	61.8	38.2
70:30	0.912	23.02	5.50	20.67	4.940	71.5	28.5
85:15	0.956	19.88	4.75	20.89	4.993	85.9	14.1

Source: Kleiber1961; and Lusk 1924. Carbohydrate (CH_2O) consists of 35.7% starch and 64.3% sucrose. Fat is 1-palmitoleoyl-2-oleoyl3-stearoyl-glycerol. Heat production from protein oxidation is not included in the calculations.

[a] Heat production is measured under standard conditions.

[b] Calculated as follows: (0.15 × 1.00) + (0.85 × 0.707) = 0.751. Values of RQ for other mixtures of carbohydrate:fat were calculated in the same manner [i.e., proportion of carbohydrate × 1.00) + (proportion of fat × 0.707)].

[c] Calculated as follows: (0.15 × 16.74) + (0.85 × 37.66) = 34.52. Values of the heat of combustion for other mixtures of carbohydrate:fat were calculated in the same manner [i.e., proportion of carbohydrate × 16.74) + (proportion of fat × 37.66)].

[d] Calculated as follows: (0.15 × 21.12) + (0.85 × 19.61) = 19.84. Values of the heat produced from substrate oxidation per L of O_2 consumed for other mixtures of carbohydrate:fat were calculated in the same manner [i.e., proportion of carbohydrate × 21.12) + (proportion of fat × 19.61)].

[e] 1 kJ = 4.1842 cal. The value (kcal/L) can also be calculated as follows: 4.686 + 0.361 × (RQ – 0.707)/0.293.

[f] Calculated as follows: [504.7 × (RQ – 0.707)]/[5.047 × (RQ – 0.707) + 4.686 × (1 – RQ)].

[g] Calculated as follows: [468.6 × (1 – RQ)]/[5.047 × (RQ – 0.707) + 4.686 × (1 – RQ)].

Energy Requirements for Maintenance and Production

Dietary energy is required for the maintenance and production of all animals. Maintenance energy requirement includes the needs for all physiological functions and moderate physical activity. Growing, gestating, lactating, laying, or exercising animals requires additional energy so that they can gain tissues, support fetal growth, produce milk, form eggs, or generate muscular power, respectively. The rates of energy requirements for maintenance and production vary among animal species and their life cycles. For example, in a 50-kg pig that consumes 2.22 kg of diet (providing 7248 kcal ME)/day and gains 690 g BW/day, the energy requirements for maintenance and growth are 1993 and 5255 kcal ME/day, respectively. Thus, 27.5% of dietary ME is used for maintenance in the growing pig. In contrast, 60–80% of dietary ME, depending on parity, is used for maintenance in gestating swine (NRC 1988). Because of the high proportion of maintenance resulting from restricted feeding, enhancing embryonic/fetal survival is an effective means to reduce the energy and feed costs of reproduction in swine operations.

The efficiency of dietary energy for production varies with products. For example, Tess et al. (1984) estimated that the energy costs of protein and fat deposition in growing pigs are 10.6 and 12.5 Mcal of ME/kg, respectively. Because skeletal muscle contains ~20% protein and ~70% H_2O but WAT possesses ~90% fat and ~5% H_2O, the production of 1 kg of lean tissue requires much less energy than 1 kg of WAT. In a sow that gains 22.8 kg of maternal tissues (in uteri and mammary glands) plus conceptuses during the entire pregnancy, the total energy deposition (19.94 Mcal NE; NRC 1988) is only 2.7% of the total dietary ME intake (i.e., 744 Mcal of ME/114 days). This is because constituents (e.g., protein and fat) of maternal tissues plus conceptuses undergo continuous turnover during gestation. Thus, the efficiency of conversion of dietary ME to NE in conceptuses decreases with increasing gestational length. Although the maintenance energy requirement of conceptuses is unknown, most of the dietary energy consumed by the mother is used for maternal tissues to support fetal growth and development. The NRC (1988) estimated that 20–40% of dietary ME is used for production (i.e., conceptus growth plus maternal BW gain) in gestating swine, depending on parity. In lactating sows

consuming adequate energy and nutrients, the efficiency of conversion of dietary GE into milk energy is 72% (Noblet and Etienne 1987). If dietary energy intake is inadequate, the efficiency of conversion of tissue energy (including protein, amino acids, TAGs, and fatty acids) into milk energy is 88% (Noblet and Etienne 1987).

The ME not retained by the animal is lost as heat. Thus, when the supply of energy exceeds its expenditure for maintenance, storage (e.g., as glycogen and TAGs), tissue growth, or production (e.g., lactation, pregnancy, and egg laying), excess energy is dissipated as heat from cells (including microbes and cancers). This phenomenon is termed "energy spilling" and has also been called "uncoupling", "spillage", and "overflow metabolism" (Hackmann et al. 2013a; Russell 2007). Energy spilling occurs mainly through membrane ATPase activity and a futile cycle of protons through the cell membrane. This is potentially a waste in ruminants because it is favored by some ruminal microbes (e.g., *Streptococcus bovis*) to reduce the use of energy for protein synthesis. About 40% of heat production resulted from energy spilling in bovine ruminal microbes exposed to elevated concentrations of glucose or starch (Hackmann et al. 2013b), which can occur in lactating cows and feedlot cattle fed grain or concentrate-based diets. Glycogen synthesis (an ATP-dependent pathway) and cycling appear to play a quantitatively more important role in using excessive glucose by ruminal protozoa than ruminal bacteria (Denton et al. 2015). Increasing the provision of rumen-degradable protein, but not nonprotein N (e.g., urea and ammonia), can reduce energy spilling by bovine ruminal microbes (Hackmann et al. 2013b). Likewise, partially replacing urea with casein increases microbial efficiency by 36% in ruminants (Hume 1970). Delineation of complex metabolic pathways in ruminal microbes can provide specific targets to alleviate or eliminate energy spilling in the rumen (Hackmann et al. 2017). This strategy may also apply to immunocytes (e.g., lymphocytes and macrophages) and cancers.

CELL-, TISSUE-, BREED-, SPECIES-, AND AGE-SPECIFIC DIFFERENCES IN ENERGY METABOLISM

The rates of energy expenditure per kilogram of BW differ among various cells (e.g., perihepatic vs. perivenous), tissues (e.g., liver vs. skeletal muscle), breeds (slow- vs. fast-growing chickens), species (pigs vs. rats), and age (young vs. adult) of animals. Within the same species, basal metabolic rate (BMR) per whole body increases with BW, but BMR per kilogram of BW decreases with increasing age or BW during the growth period. For example, results from 138 studies involving 69 species of teleost fish (representing 28 families and 12 orders) indicate that the rates of resting O_2 consumption (mmol/h) are positively correlated with BW and environmental temperature (Clarke and Johnston 1999). In addition, as the BW of beef cattle increases from 50 to 1000 kg, BMR gradually increases when expressed per whole body but gradually decreases when expressed per kilogram of BW (Figure 8.4). Furthermore, small-size endothermic animals generally have a higher BMR per kilogram of BW than large-size ones because the former have a larger surface area, lose heat faster, and require more energy to maintain a constant internal temperature (Blaxter 1989; Speakman 2005). Likewise, small-size mammals (e.g., rats) have greater BMRs than large-size mammals (e.g., pigs) when expressed per kilogram of BW. The following reasons may explain this biological phenomenon. First, within the same species (e.g., pigs or rats), although heavier animals usually eat more food than smaller ones during the growth period when expressed per whole body, food intake per kilogram of BW is greater in small than in heavier animals. Between species, food intake per kilogram of BW is greater in small-size than in large-size animals (e.g., rats vs. pigs). Second, the rates of oxidation of energy substrates (e.g., glucose, fatty acids, and amino acids) in tissues per kilogram of mass are generally greater in small than in large animals. Third, the rate of mitochondrial

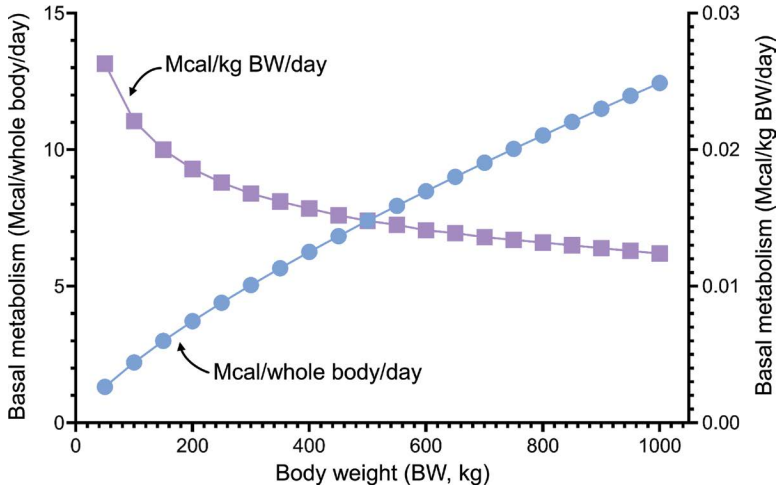


FIGURE 8.4 Relationships between basal metabolic rate and body weight in beef cattle. As the body weight of beef cattle increases from 50 to 1000 kg, the daily basal metabolic rate increases when expressed per whole body but decreases when expressed per kilogram of body weight. (Adapted from Tedeschi 2019 and Tedeschi and Fox 2020. Courtesy of Dr. Luis O. Tedeschi.)

proton leakage per gram of mitochondrial protein is greater in small than in large animals (Brand et al. 1994). This difference in the conversion of food energy into productive energy has important implications for the health, life span, and efficiency of animal production systems.

In the same animal species, different tissues have diverse rates of energy metabolism to meet their physiological needs, and the type of energy substrate varies with tissue and developmental stage (Milligan and Summers 1986). For example, in growing pigs, rats, and fish (e.g., hybrid striped bass and largemouth bass), the small intestine utilizes glutamate, glutamine, and aspartate as its major metabolic fuels, whereas fatty acids provide the bulk of ATP to the liver and skeletal muscle (He and Wu 2022; He et al. 2022; Jia et al. 2017; Jobgen et al. 2006; Li et al. 2020). In contrast, in growing chickens, the small intestine utilizes glutamate and aspartate as primary energy substrates but has a limited ability to hydrolyze or oxidize glutamine (He and Wu 2022). Interestingly, in birds, breast (glycolytic) muscle actively degrades glutamine for substantial ATP production (Wu et al. 1991), whereas both leg (oxidative) muscles and liver oxidize primarily fatty acids to provide the bulk of ATP (He et al. 2021).

ENERGETIC EFFICIENCY OF METABOLIC TRANSFORMATIONS IN ANIMALS

Under physiological conditions, each of the two terminal phosphate bonds of ATP contains 51.6 kJ/mol of potential energy, but a greater amount of energy (i.e., 85.4 kJ/mol) is required to form one high-energy phosphate bond (McDonald et al. 2021). In other words, the energy conserved through the synthesis of ATP from ADP plus Pi is 51.6 kJ/mol, and the efficiency of ATP synthesis is only 60.4% (i.e., 51.6/85.4 = 0.604). Based on these values, the energetic efficiency of substrate oxidation in cells and animals can be calculated. Examples are given for the oxidation of nutrients in mammals (Table 8.6). In general, the efficiency of metabolic transformation can be calculated as energy trapped in the product/energy value of the precursor (Bottje and Carstens 2009). Of note, the efficiencies of energy transfer from the oxidation of protein to ATP in mammals and birds are about 16% and 24%, respectively, which are lower than those of glucose, starch, palmitic acid, and fat. As noted in Chapter 7, there is a greater requirement of ATP for uric acid synthesis than urea synthesis, and most fish directly release almost exclusively ammonia into the surrounding H_2O. Thus, the energetic efficiency of the oxidation of amino acids in vertebrates is fish > mammals > birds. Note that $\Delta G^{o\prime}$ alone should not be used to calculate the energetic efficiencies of metabolic transformations in animals.

Gatlin and Wu (2025) estimated efficiencies in the use of dietary GE for protein deposition in juvenile fish, growing shrimp, young pigs, and young chickens (Table 8.12). These values are provided only for the purpose of reference because the various species of animals were not at the same stage of growth or development. Despite the use of amino acids as the major energy sources in carnivorous fish, these animals have 1.7- to 3.5-fold greater efficiencies in utilizing

TABLE 8.12
Estimated Efficiencies in the Use of Dietary Gross Energy for Protein Deposition in Juvenile Fish, Growing Shrimp, Young Pigs, and Young Chickens[a]

	Carnivorous Fish					Herbivorous Fish		Omnivorous Fish			Shrimp			Land Animals	
Variable	HSB	LMB	ATS	RBT	Red Drum	Grass Carp	Silver Carp	Channel Catfish	Nile Tilapia	Gold-Fish	IWS	PWS	BTS	Pig	Chicken
IGE[b]	0.447	0.412	0.231	0.419	0.683	0.448	0.171	0.369	0.960	0.236	0.501	0.537	1.34	0.520	3.05
ITP[c]	10.3	10.6	3.03	6.80	14.7	7.72	2.59	6.75	11.2	5.91	7.84	8.87	19.6	6.29	38.2
DTP[d]	3.00	3.21	1.06	2.60	4.21	2.54	0.734	2.00	3.15	1.72	2.64	4.03	3.42	2.62	15.1
EEP[e]	6.72	7.79	4.58	6.20	6.16	5.61	4.30	5.41	3.29	7.27	5.28	7.49	1.71	5.03	4.94

Source: Gatlin and Wu 2025.

[a] The ranges of body weight (g) were: HSB, 5.6–45.7; LMB, 4.87–26.4; Atlantic salmon, 110–246; RBT, 34.8–175; red drum, 3.4–20.7; grass carp, 3.8–21.0; silver carp, 25.3–44.2; channel catfish, 9.6–25.8; Nile tilapia, 5.5–42.1; goldfish, 1.67–3.78; IWS, 0.34–8.44; PWS, 0.36–5.95; BTS, 2.4–5.2; pig, 9,420-40,200; and chicken, 118–297.

[b] Intake of gross energy (kJ/g of body weight/day) during the experimental period.

[c] Intake of true protein (the sum of amino acid residues in diet; mg/g of body weight/day) during the experimental period.

[d] Deposition of true proteins (the sum of amino acid residues in proteins of the whole body; mg/g of body weight/day) during the experimental period.

[e] Efficiency of the use of dietary gross energy for protein deposition in the body, mg protein/kJ of gross energy.

Note: ATS, Atlantic salmon; BTS, black tiger shrimp; DTP, deposition of true proteins; EEP, Efficiency in the use of dietary gross energy for protein deposition in the body; HSB, hybrid striped bass; IGE, Intake of gross energy; ITP, Intake of true protein; IWS, Indian white shrimp; IWS, Indian white shrimp; LMB, largemouth bass; RBT, rainbow trout

dietary GE for protein deposition in the body than black tiger shrimp (carnivores).

$$\text{Efficiency of energy transer (\%)} = \frac{\text{Energy trapped in product}}{\text{Energy value of precursor}} \times 100\%$$

$$\text{Efficiency of energy transfer from substrate to ATP via oxidation (\%)} = \frac{\text{Mole of net ATP produced per mole of substrate oxidized} \times 51.6 \text{ kJ/mol}}{\text{Combustion energy of substrate (kJ/mole)}} \times 100\%$$

$$\text{Efficiency of energy transfer from amino acids to protein (\%)} = \frac{\text{Energy of protein}}{\text{Energy of amino acid} + \text{Energy of ATP or GTP needed for protein synthesis}} \times 100\%$$

As noted previously, the amount of dietary ME required for the production of ATP from ADP and Pi is 85.4 kJ/mol of ATP (McDonald et al. 2021). This constant is required for the calculation of energetic efficiencies of biosynthetic processes, which differ among different metabolic pathways (Wester 1979). The values of energetic efficiencies for the syntheses of fats, glucose, lactose, glycogen, and protein from their respective precursors are summarized in Table 8.10. The details of the calculations are provided in the following sections. Note that based on the composition of amino acids in the piglet body, the average molecular weight of protein is 100 (Wu 2022). This value can be used to calculate the energetic efficiency of protein synthesis from amino acids.

1. 8 mol of acetate plus ½ mol of glucose to 1 mol of fat (tripalmitin) in the cytosol

8 mol acetate	6,528 kJ
8 mol acetate → 8 acetyl-CoA	1,366 kJ (16 mol ATP)
7 mol acetyl-CoA → 7 mol malonyl-CoA	598 kJ (7 mol ATP)
7 mol malonyl-CoA to 1 mol palmitic acid	2989 kJ (14 NADPH or 35 ATP)
1 mol palmitic acid → 1 mol palmitoyl-CoA	171 kJ (2 mol ATP)
Energy for the synthesis of 1 mol palmitoyl-CoA	11,652 kJ
Energy for the synthesis of 3 mol palmitoyl-CoA	34,956 kJ
½ mol glucose	1,402 kJ
½ mol glucose → 1 mol dihydroxyacetone-P	85.4 kJ (1 mol ATP)
1 mol dihydroxyacetone → 1 mol glycerol-3-P	214 kJ (1 mol NADH or 2.5 mol ATP)
Energy for the synthesis of 1 mol glycerol-3-P	1,701 kJ (=1,402 + 85 + 214)
Total energy for the synthesis of 1 mol tripalmitin	36,657 kJ (= 34,956 + 1,701)
Energy in 1 mol tripalmitin	31,676 kJ
Efficiency of synthesis = (31,676/36,657) × 100% = 86.4%	

2. 3 mol of palmitic acid plus ½ mol of glucose to 1 mol of fat (tripalmitin) in the cytosol

3 mol palmitic acid	30,093 kJ (10,031 kJ/mol)
3 mol palmitic acid → 3 mol palmitoyl-CoA	512 kJ (6 mol ATP)
Energy for the synthesis of 3 mol palmitoyl-CoA	30,605 kJ
½ mol glucose	1,402 kJ
½ mol glucose → 1 mol dihydroxyacetone-P	85.4 kJ (1 mol ATP)
1 mol dihydroxyacetone → 1 mol glycerol-3-P	214 kJ (1 mol NADH or 2.5 mol ATP)
Energy for the synthesis of 1 mol glycerol-3-P	1,701 kJ (=1,402 + 85.4 + 214)
Total energy for the synthesis of 1 mol tripalmitin	32,306 kJ (= 30,605 + 1,701)
Energy in 1 mol tripalmitin	31,676 kJ
Efficiency of synthesis = (31,676/32,306) × 100% = 98.1%	

3. Glucose to fat (tripalmitin) [involving both the cytosol and mitochondria (Mit)]

4 mol glucose	11,212 kJ
4 mol glucose → 8 mol pyruvate	(–8 mol ATP and –4 mol NADH)
8 mol pyruvate → 8 mol acetyl-CoA (Mit)	(–8 mol NADH)
8 mol acetyl-CoA (Mit) → cytosolic 8 mol acetyl-CoA	(+8 mol ATP)
7 mol acetyl-CoA → 7 mol malonyl-CoA (cytosol)	(+7 mol ATP)
7 mol malonyl-CoA to 1 mol palmitic acid	(+14 mol NADPH)
1 mol palmitic acid → 1 mol palmitoyl-CoA	(+2 mol ATP)
Net ATP required (9 ATP)	769 kJ (9 mol ATP)
Net NAD(P)H required (2 mol)	427 kJ (2 mol NAD(P)H or 5 mol ATP)
Energy for the synthesis of 1 mol palmitoyl-CoA	12,408 kJ (= 11,212 + 769 + 427)
Energy for the synthesis of 3 mol palmitoyl-CoA	37,224 kJ
½ mol glucose	1,402
½ mol glucose → 1 mol dihydroxyacetone-P	85.4 kJ (1 mol ATP)
1 mol dihydroxyacetone → 1 mol glycerol-3-P	214 kJ (1 mol NADH or 2.5 mol ATP)
Energy for the synthesis of 1 mol glycerol-3-P	1,701 kJ
Total energy for the synthesis of 1 mol tripalmitin	38,925 kJ (=37,224 + 1,701)
Energy in 1 mol tripalmitin	31,676 kJ
Efficiency of synthesis = (31,676/38,925) × 100% = 81.4%	

4. Protein (amino acids) to fat (tripalmitin)

$C_{4.3}H_7O_{1.4}N_{1.2}$ (protein without the sulfur atom) – $C_{0.6}H_{2.4}O_{0.6}N_{1.2}$ (i.e., 0.6 urea) → $C_{3.7}H_{4.6}O_{0.8}$	
To form 8 mol pyruvate (C_{24}), 6.5 mol protein is required. (1 mol protein → 3.7 mol C or 1.23 mol pyruvate)	
6.5 mol protein	14,690 kJ (2,260 kJ/mol protein)
6.5 mol protein → 3.9 mol urea (= 0.6 × 6.5)	(+25.4 mol ATP; 6.5 mol ATP/mol urea)
6.5 mol protein → 8 mol pyruvate	(–30 mol ATP)
8 mol pyruvate → 8 mol acetyl-CoA (Mit)	(–20 mol ATP)
8 mol acetyl-CoA (Mit) → cytosolic 8 mol acetyl-CoA	(+8 mol ATP)
7 mol acetyl-CoA → 7 mol malonyl-CoA (cytosol)	(+7 mol ATP)
7 mol additional malonyl-CoA to 1 mol palmitic acid	(+14 mol NADPH)
1 mol palmitic acid → 1 mol palmitoyl-CoA	(+2 mol ATP)
Net ATP requirement (–7.6 mol ATP)	–649 kJ (–7.6 mol ATP)
NADPH required (14 mol)	2,989 kJ (14 mol NADPH or 35 mol ATP)
Energy for the synthesis of 1 mol palmitoyl-CoA	17,030 kJ (= 14,690 – 649 + 2,989)
Energy for the synthesis of 3 mol palmitoyl-CoA	51,090 kJ
0.811 mol protein ($C_3/C_{3.7}$ = 0.811)	1,833 kJ
0.811 mol protein → 0.49 mol urea	(+3.2 mol ATP; 6.5 mol ATP/mol urea)
0.811 mol protein → 1 mol pyruvate	(–3.7 mol ATP; 4.6 mol ATP/mol protein)
1 mol pyruvate → ½ mol glucose	(+5.5 mol ATP)
½ mol glucose → 1 mol dihydroxyacetone-P	(+1 mol ATP)
1 mol dihydroxyacetone → 1 mol glycerol-3-P	(+1 mol NADH)
Net ATP requirement (6 mol ATP)	512 kJ (6 mol ATP)
Net NADH required (1 mol)	214 kJ (1 mol NADH or 2.5 mol ATP)
Energy for the synthesis of 1 mol glycerol-3-P	2,559 kJ (= 1,833 + 512 + 214)
Total energy for the synthesis of 1 mol tripalmitin	53,649 kJ (= 51,090 + 2,559)
Energy in 1 mol tripalmitin	31,676 kJ
Efficiency of synthesis = (31,676/53,649) × 100% = 59.0%	

5. Dietary fat to body fat (tripalmitin)

1 mol fat (tripalmitin)	31,676 kJ
1 mol fat → 2 mol palmitic acid + 1 mol MAG	0 kJ
2 mol palmitic acid → 2 mol palmitoyl-CoA	342 kJ (4 ATP)
2 mol palmitoyl-CoA + 1 mol MAG → 1 mol tripalmitin	0 kJ
Energy for the synthesis of 1 mol tripalmitin	32,018 kJ
Energy in 1 mol tripalmitin	31,676 kJ
Efficiency of synthesis = (31,676/32,018) × 100% = 98.9% (MAG = monoacylglycerol)	

6. Lactate to glucose

2 mol lactate	2,688 kJ
2 mol pyruvate → 2 mol oxaloacetate	171 kJ (2 mol ATP)
2 mol oxaloacetate → 2 mol phosphoenolpyruvate	171 kJ (2 mol GTP)
2 mol 3-phosphoglycerate → 2 mol BPG	171 kJ (2 mol ATP)
Energy for the synthesis of 1 mol glucose	3,201 kJ
Energy in 1 mol glucose	2,803 kJ
Efficiency of synthesis = (2,803/3,201) × 100% = 87.8% (BPG = 1,3-bisphosphoglycerate)	

7. Protein to glucose

$C_{4.3}H_7O_{1.4}N_{1.2}$ (protein without the sulfur atom) – $C_{0.6}H_{2.4}O_{0.6}N_{1.2}$ (i.e., 0.6 urea) → $C_{3.7}H_{4.6}O_{0.8}$	
To form 2 mol pyruvate (C_6), 1.62 mol protein is required. (1 mol protein → 3.7 mol C)	
1.62 mol protein	3,661 kJ (2,260 kJ/mol protein)
1.62 mol protein → 0.97 mol urea (= 0.6 × 1.62)	(+6.3 mol ATP; 6.5 mol ATP/mol urea)
1.62 mol protein → 2 mol pyruvate	(–7.5 mol ATP)
2 mol pyruvate → 2 mol oxaloacetate	(+2 mol ATP)
2 mol oxaloacetate → 2 mol phosphoenolpyruvate	(+2 mol GTP)
2 mol 3-phosphoglycerate → 2 mol BPG	(+2 mol ATP)
2 mol BPG → 2 mol Glyceraldehyde 3-P	(+2 mol NADH)
Net ATP requirement (4.8 mol ATP)	410 kJ (4.8 mol ATP)
Net NADH required (2 mol)	427 kJ (2 mol NADH or 5 mol ATP)
Energy for the synthesis of 1 mol glucose	4,498 kJ (= 3,661 + 410 + 427)
Energy in 1 mol glucose	2,803 kJ
Efficiency of synthesis = (2,803/4,498) × 100% = 62.3% (BPG = 1,3-bisphosphoglycerate)	

8. Glucose to lactose

2 mol glucose	5,606 kJ
1 mol glucose → 1 mol glucose-1-P	85.4 kJ (1 mol ATP)
1 mol glucose-1-P → 1 mol UDP-Glucose	85.4 kJ (1 mol ATP)
Energy for the synthesis of 1 mol lactose	5,777 kJ
Energy in 1 mol lactose	5,630 kJ
Efficiency of synthesis = (5,630/5,777) × 100% = 97.5%	

9. Glucose to glycogen

1 mol glucose	2,803 kJ
1 mol glucose → 1 mol glucose-1-P	85.4 kJ (1 mol ATP)
1 mol glucose-1-P → 1 mol UDP-Glucose	85.4 kJ (1 mol ATP)
Energy for the incorporation of 1 mol glucose	2,974 kJ
Energy in 1 mol glycogen	2,786 kJ
Efficiency of synthesis = (2,786/2,974) × 100% = 93.7%	

10. Amino acids (AAs) to protein

118 g AAs (100 g or 1 mol protein; based on AA composition in pigs)	2,326 kJ
0.5 mol ATP needed for the transport of 1 mol AAs by cells	43 kJ (0.5 mol ATP)
5 mol ATP/mol AAs (the synthesis of 1 mol protein)	427 (5 mol ATP; 85.4 kJ/mol)
Energy for the synthesis of 1 mol protein	2,796 kJ (= 2,326 + 43 + 427)
Energy in 1 mol protein	2,260 kJ
Efficiency of protein synthesis from AAs = (2,260/2,796) × 100% = 80.8%	

Energetic efficiency in the utilization of food energy by animals varies with species, age, physiological state, GIT structure, production type, physical activity, health status, the living environment (e.g., temperature, humidity, noise, toxins, and air pollution), and dietary composition. For example, pigs are always more efficient in utilizing dietary energy for both maintenance and production than cattle, because of lower HI in pigs than in cattle (Table 8.6) and a lack of significant degradation and fermentation of nutrients in the stomach of pigs. In the post-rumen metabolism, lactating cows are as efficient as lactating sows in synthesizing milk protein from amino acids but are less efficient in forming milk fat and lactose from SCFAs and glucogenic substrates (e.g., propionate and amino acids), respectively, than lactating sows that produce milk fat and lactose mainly from dietary lipids and glucose, respectively. Mammalian carnivores (mammals that have a limited intake of carbohydrates and depend primarily on gluconeogenesis for glucose provision) are less efficient in making milk lactose than nonruminant mammals (Li and Wu 2024).

Maintenance of an animal is essential for its survival and has a higher priority than production in the use of dietary energy. In the same animal species, food energy is utilized more efficiently for maintenance or lactation than for growth or fattening, whereas milk production is energetically almost as efficient as maintenance. This phenomenon can be explained by the following reasons. First, the rates of ATP-dependent processes (e.g., intracellular protein turnover, TAG synthesis, and ion transport across cell membranes) for maintenance are lower than those for production, and the heat generated from nutrient oxidation by animals is used efficiently to sustain their body temperatures at maintenance but is lost to the surroundings during the production-associated reactions such as the syntheses of proteins, fats, and glucose from precursors. Second, the efficiencies of any metabolic transformations for production are always less than 100%, i.e., 80.8% for the conversion of amino acids into proteins. As shown in Table 8.10, energetic efficiencies in the conversion of glucose and protein into fats are only 81% and 59%, respectively. Third, the retention of nutrients (e.g., protein, TAGs, and glycogen) in the body depends on the rates of their synthesis and catabolism, but these metabolic processes require energy, with only a fractional amount of the synthesized molecules being deposited in tissues. Fourth, in lactating mammals, immediately after being synthesized and processed, proteins, fats, and lactose are excreted from the mammary epithelial cells into the lumen of the lactiferous duct without being subject to intracellular or extracellular degradation under normal physiological conditions. In contrast, in growing or fattening animals, intracellular constituent proteins in tissues (e.g., skeletal muscle, intestine, and liver), which are major DM components of animal growth, undergo continuous synthesis and degradation that require a large amount of ATP; and the maintenance or accretion of TAGs in WAT also requires energy.

Environmental and pathological factors affect energetic efficiency in animals. For example, animals (including humans) with exposure to air pollution inhale toxins and oxidants from the surrounding environment and generate endogenous oxidants from metabolism (Lakey et al. 2016). All these harmful substances can reduce not only the integrity of plasma and mitochondrial membranes but also nutrient absorption, digestion, and utilization (including the oxidation of NADH and $FADH_2$ via the mitochondrial electron transport system for ATP production). In addition, fever and infection result in increased whole-body heat production (maintenance energy requirement) and, therefore, a decreased efficiency in the utilization of dietary energy for production (Baracos et al. 1987). This catabolic response and reduced food intake contribute to negative energy balance in the affected host and constitutes a large metabolic cost.

All animals can adjust to sub-maintenance dietary energy levels with a higher energetic efficiency than above-maintenance requirements (Table 8.6). Some animals can adapt well to elevated environmental temperatures by lowering metabolic needs to stabilize their BW and physiological conditions, as reported for Hereford × Boran steers in Kenya (Ledger and Sayers 1977) and Brahman (*Bos indicus*) cattle in South Asia, the United States, and Australia (Butterworth, 1985). The physiological adaptation of animals to nutrient availability and other environmental factors has important implications for livestock (e.g., cattle, sheep, and swine) and poultry (e.g., chicken and goose) production in tropical and subtropical regions of the world.

DETERMINATION OF HEAT PRODUCTION AS AN INDICATOR OF ENERGY EXPENDITURE BY ANIMALS

Total Heat Production by Animals

An animal produces heat via (a) basal metabolism (for maintenance), (b) microbes (e.g., bacteria, protozoa, fungi, and archaea) in the GIT, and (c) the HI of feeding, including the heat released from the metabolism of nutrients. Fasting heat production by a food-deprived animal is a measure of its basal metabolism (Chwalibog 1991). In a fed animal, heat is produced from basal metabolism plus feeding-associated activities, including the digestion, absorption, and metabolism of dietary nutrients. Under either fasting or fed conditions, animals should have only a minimum of physical activity. Methods used to measure heat production by animals include direct calorimetry, indirect calorimetry, comparative slaughter, and the dilution of doubly labeled H_2O. These techniques generally yield similar results when they are rigorously applied, a sufficient number of independent observations is obtained, and experimental errors are minimized (Reynolds 2000).

Small animals have a larger surface area than larger ones, and have a higher metabolic rate, and produce more heat than larger animals per kilogram of BW, as noted previously. Expressed per metabolic BW (e.g., $BW^{0.75}$), the rate of heat production is relatively constant throughout the animal kingdom (Baldwin 1995; Blaxter 1989). This has been an intensive interest of biologists over the past two centuries (White and Marshall 2023). The earliest attempts to link energy expenditure and body mass were based on the physical principles that govern heat exchange. In 1839, F. Sarrus and J. Rameaux indicated that the heat produced by an animal was dissipated through its body surface and that the rate of heat loss was proportional to its surface area, namely, metabolic rate $\propto BW^{\alpha}$. In 1883, M. Rubner suggested an α value of 2/3 for dogs. This two-thirds power scaling of the metabolic rate was known as Rubner's surface law. Brody (1932) and Kleiber (1932) independently reported an α value of 0.734 for endotherms (birds and mammals). Subsequently, Kleiber (1947) recommended that an α value of ¾ be adopted, partly for simplified calculations. Evidence gained over recent decades shows that the 3/4 scaling may not be precise or universal for all animals (Sharma and McNeill 2009).

Within the same animal, different tissues have different metabolic rates and, therefore, exhibit different rates of heat production. This notion is exemplified by studies with rats (Table 8.13) and ruminants (Table 8.14). The relative

TABLE 8.13
Rates of Resting Oxygen Consumption by Major Tissues of Fasted Adult Rats[a]

		O_2 consumption in the Fasting State			Heat Production in the Fasting State		
Whole-Body or Specific Tissue	Body Weight or Tissue Weight in a 300-g adult rat (g)	Rate (ml/100 g of BW per min)	Total Amount (ml/300 g rat per min)	% of Whole-Body Fasting O_2 Consumption	Rate (kcal/100 g of BW per h)	Total Amount (kcal/300 g rat per h)	% of Whole Body Fasting Heat Production
Whole body	300	2.0	6.0	100	0.576	1.73	100
BAT	0.61	48	0.293	4.9	13.8	0.084	4.9
Heart	1.53	19.4	0.297	5.0	5.59	0.086	5.0
Kidneys	2.63	19.1	0.502	8.3	5.50	0.145	8.4
Liver	10.8	15.9	1.72	28.7	4.58	0.495	28.6
Brain	1.61	10.3	0.166	2.8	2.97	0.048	2.8
Small intestine	6.10	6.4	0.390	6.5	1.84	0.112	6.5
Spleen	0.77	2.2	0.017	0.28	0.63	0.0049	0.28
Testes	3.58	1.7	0.061	1.0	0.49	0.018	1.0
Lungs	1.63	1.0	0.016	0.27	0.29	0.0047	0.27
Skeletal muscle	135	0.92	1.24	20.7	0.27	0.358	20.7
WAT	33.9	0.18	0.061	1.01	0.052	0.018	1.01

Source: Assaad et al. 2014.

[a] The mean body weight of male Sprague-Dawley rats was 300 g (275 – 325 g). Rats were fasted for 16 h before rates of O_2 consumption by the whole body, kidneys, liver, and other tissues were made.

Note: BAT, brown adipose tissue; BW, body weight; WAT, white adipose tissue.

TABLE 8.14
Contributions of Tissues to Fasting Heat Production in Ruminants

Tissue	Percentage of Empty Body Weight (%)	Percentage of Cardiac Blood Output (%)	Percentage of Fasting Heat Production (%)
Skeletal muscle	41	18	23.0
White adipose tissue	15	9.6	8.0
Gastrointestinal tract	6.5	23.0	9.0
Skin	6.3	8.0	2.7
Nervous tissue	2.0	10	12
Liver	1.6	27	22.5
Heart	0.6	4.1	10
Kidneys	0.3	13.4	7.0
Other	27.7	9.9	5.8

Source: Baldwin 1995.

contribution of skeletal muscle to the whole-body fasting heat production is similar between rats and ruminants. In contrast, the percentage contribution of the GIT to the whole-body fasting heat production is much greater in ruminants than in rats because of greater mass and more active fermentation in the former than in the latter (Stevens and Hume 2004). Because blood circulation provides O_2 and nutrients to tissues, the rate of blood flow is positively correlated to heat production by the tissues. In addition, within the same animal, its tissues can develop differently during the period of growth and development. Thus, the scaling of BMR to BW is not necessarily proportional to $BW^{0.75}$ at all times during the life cycle, even within a species.

Energy needs of animals are met through the oxidation of fatty acids, glucose, and amino acids in a cell- and tissue-specific manner (Jobgen et al. 2006). The oxidation of 1 mol of NADH and 1 mol of $FADH_2$ yields 2.5 mol and 1.5 mol of ATP in mitochondria-containing cells, respectively. Thus, in all animals, approximately 55% of the chemical energy in glucose and fatty acids is conserved in energy-rich phosphate bonds (primarily ATP), with the remaining being converted into heat (Chapters 5 and 6). The yields of ATP production from protein oxidation are 46%, 42%, and 55% in mammals, birds, and fish, respectively, with the remaining energy being converted into heat (Chapter 1). This is because of the different metabolic fates of the ammonia generated from amino acid oxidation in these animal species. Energy-dependent biochemical reactions and physiological processes include intracellular protein turnover, the urea cycle, gluconeogenesis, nutrient transport, various "futile cycles," Na/K-ATPase activity and ion pumping (which may account for 10% of whole-body heat production in animals), renal reabsorption, intestinal motility and absorption, as well as cardiac and skeletal muscle contractions. These processes utilize approximately one-third of the energy released from ATP hydrolysis, with the remaining chemical energy being converted into heat (Wester 1979). In cells, rates of ATP utilization are precisely matched by rates of ATP production (Dai et al. 2013). In those mammals (e.g., cattle, sheep, rats, and humans) that possess brown adipose tissue, its unique uncoupling protein-1 inhibits ATP synthesis from ADP and Pi, thereby dissipating all the chemical energy from mitochondrial substrate oxidation into heat (Chapter 1). Thus, the energy released from the oxidation of macronutrients is ultimately transformed into heat in animals. In other words, heat production is a useful indicator of energy expenditure by animals.

Direct Calorimetry for the Measurement of Heat Production

Direct calorimetry is used to measure the amount of heat produced by an animal enclosed within an airtight and insulated chamber (known as a calorimeter; McDonald et al. 2021). This technique was first used by Antoine Lavoisier and coworkers in the late 1700s to measure the metabolic rates of animals. Specifically, this pioneer determined the heat production of a guinea pig through observing the amount of ice melted in an insulated chamber that contained both ice and the test animal. Direct calorimetry can accurately quantify the rate of heat production as an indicator of the metabolic rate in both metabolically normal and abnormal states (Kaiyala and Ramsay 2011). Heat is lost from an animal through four avenues: (a) conduction (heat transfer from the warmer animal to a cooler surface by direct contact), (b) convection (heat transfer by the bulk flow of a liquid or gas away from the animal), (c) radiation [heat transfer via long wave (3–100 μm) electromagnetic wave energy emitted by the animal], and (d) evaporation (heat transfer via the transformation of body H_2O from the liquid to the gas phase in the respiratory passages and on the skin) (Bondi 1987). The first three types of heat loss are collectively known as sensitive heat loss.

There are four types of direct calorimeters: isothermal, heat-sink, convection, and differential (Blaxter 1989). In an isothermal direct calorimeter, its wall temperature is kept constant by constant-temperature H_2O in a jacket or bath surrounding the animal chamber. The sensitive heat loss is taken up by the H_2O circulated through coils within the chamber and is computed on the basis of a difference in temperatures between entry and exit H_2O, as well as the rate of H_2O flow. Evaporative heat loss is calculated according to a difference in moisture content between entry and exit air and the volume of the air drawn through the chamber (Kaiyala and Ramsay 2011). In heat-sink direct calorimetry, the sensible heat released into the calorimeter chamber is removed via a H_2O-cooled heat exchanger, within which H_2O circulates through a jacket in the calorimeter walls surrounding the test animal. The transfer of dry heat from the animal is calculated on the basis of H_2O's specific heat, flow rate, and temperature increase, but evaporative heat loss is estimated by a different method (Mclean and Tobin 1987). In direct convection calorimetry, the sensitive heat loss from an animal is determined by measuring

the differences in both temperature and enthalpy between the entry and exit air, whereas evaporative heat loss is computed from the rate of gas flow and the change in the concentration of H_2O vapor. Finally, in direct differential calorimetry, two identical chambers are used to separately house an animal, and an electric heater is adjusted to yield identical temperature increases in both chambers, with the heat supplied to the heater equating to heat loss from the animal (Blaxter 1989). Because of the high cost to build and maintain direct-calorimetry instruments, heat production by animals is now often measured with the use of the indirect calorimetry methods described below.

Indirect Calorimetry for the Measurement of Heat Production

Indirect calorimetry is a technique that provides accurate estimates of heat production from measurements of CO_2 production and O_2 consumption by an animal, and in the case of ruminants, also methane release, thereby eliminating the need for using a complex, delicate, and costly instrument to directly determine heat production by direct calorimetry (Blaxter 1989; Carstens et al. 1987; Lusk 1924). Advances in gas exchange measurement have made indirect calorimetry readily available for animal research. This technique is based on the principles that (a) there are no considerable reserves of O_2 in animals and their uptake of O_2 reflects the oxidation of organic nutrients; (b) chemical energy in the body is derived from the oxidation of carbohydrates, fats, and proteins to CO_2 and H_2O; (c) the ratios of O_2 consumption to CO_2 production resulting from the oxidation of these macronutrients are constant in animals; (d) the oxidation of fatty acids, glucose and nonnitrogenous substances to CO_2 and H_2O in animals yields the same amounts of heat as those from combustion in a bomb calorimeter; (e) the oxidation of protein in animals generates urea (as the major nitrogenous end-product), CO_2 and H_2O; and (f) the interconversions of nutrients (e.g., glucose into TAGs). The quantitative requirements of animals for O_2 to produce ATP differ among macronutrients (Mclean and Tobin 1987). In mammals and birds, the consumption of 1 mol of O_2 by the animal produces 5.00, 4.61, and 4.49 mol of ATP, respectively, from the complete oxidation of 1 mol of glucose, fatty acid (palmitic acid), and protein; in other words, the amount of O_2 required for the synthesis of the same amount of ATP from energy substrates is protein > fats > glucose. In fish, which release ammonia into the surrounding H_2O, the energetic efficiency of ATP production from protein oxidation is ~55% (Table 8.3), which is essentially the same as that for fats and glucose.

Closed-Circuit Indirect Calorimetry

In the closed-circuit system, the animal breathes the same air from the chamber or prefilled container (spirometer), and CO_2 in expired air is absorbed by a canister of soda lime (potassium hydroxide) in the breathing circuit for measurement (Mclean and Tobin 1987). An oxygen analyzer attached to the chamber or spirometer records O_2 uptake from changes in the system's volume. Because O_2 is continuously used by the animal, the chamber or environment may eventually become hypoxic in a closed-circuit system, thus limiting the period of the RQ measurement.

Open-Circuit Indirect Calorimetry

In the open-circuit system, the animal inhales ambient air with a constant composition of O_2, CO_2, and N_2 (typically the same as the atmosphere: 20.93%, 0.04%, and 79.04%, respectively), and expired gases are then analyzed (Assaad et al. 2014). The changes in O_2 and CO_2 percentages in expired air, compared with those in inspired ambient air, indirectly reflect rates of energy metabolism in the body. Additionally, in the open-circuit system, air flows through the chamber at a rate that constantly replenishes the O_2 depleted by the animal while simultaneously removing the CO_2 and H_2O vapor produced by the animal (Mclean and Tobin 1987). Because of its convenience, computer-controlled open-circuit indirect calorimetry is now widely used for measuring energy expenditure in animals for hours or days (Assaad et al. 2014).

RQ is defined as the ratio of the volume of CO_2 produced (L/kg of BW/h) to the volume of O_2 consumed (L/kg of BW/h). The volumes of CO_2 and O_2 are indicated as VCO_2 and VO_2, respectively.

$$\text{Respiratory Quotient (RQ)} = \text{mol of } CO_2 \text{ produced/mol of } O_2 \text{ consumed}$$

$$1 \text{ mol of gas} = 22.41 \text{ L.}$$

The function of the lungs should be normal to obtain accurate values of O_2 consumption and CO_2 production by animals. Heat production is then calculated from the values of O_2 consumption and CO_2 production according to the Brouwer (1965) equation for nonruminants:

$$\text{Heat production (kcal)} = 3.82 \times VO_2\text{(in L)} + 1.15 \times VCO_2\text{(in L)}.$$

If the unit of VO_2 and VCO_2 is L/kg of BW/h, the unit of heat production should be kcal/kg of BW/h. When amino acid oxidation and the urinary excretion of N are considered, ΔH can be calculated using the following equation and the data are shown in Table 8.15.

$$\Delta H \text{ (kJ/mol)} = 16.34\ VO_2(L) + 4.50\ VCO_2(L) - 3.92\ N\ (g)$$

Methane contains energy. Thus, its production by the rumen represents the incomplete oxidation of dietary nutrients in ruminants. Brouwer (1965) proposed the following equation to calculate heat production by these animals.

$$\text{Heat production (MJ)} = 16.18 \times VO_2\text{(in L)} + 5.16 \times VCO_2\text{(in L)} - 5.90 \times UN\text{ (in g)} - 2.42 \times VCH_4\text{(in L)}$$

$$[UN = \text{urinary excretion of N; } VCH_4 = \text{volume of } CH_4]$$

TABLE 8.15
ΔH, O_2 Consumption, and CO_2 Production by Animals

Compound	ΔH (Animal) (kJ/mol)	O_2 Consumed (mol) [a]	CO_2 Produced (mol) [b]	N Excreted (mol) [c]	Equation
Glucose	2803	6	6	0	2803 = 6a + 6b + 0c
Palmitic acid	10,039	23	16	0	10,039 = 23a + 16b + 0c
Alanine	1296	3	2.5	1	1296 = 3a + 2.5b – 1c

Source: Blaxter 1989.
Solving the three equations gives: a = 366.3 kJ/mol of O_2 (or 16.34 kJ/L of O_2), b = 100.8 kJ/mol of CO_2 (or 4.50 kJ/L of CO_2), and c = –54.9 kJ/mol of N (or –3.92 kJ/g of N). Thus, ΔH (kJ/mol) = 16.34 VO_2 (L) + 4.50 VCO_2 (L) – 3.92 N (g). Note that when the oxidation of sucrose, oleic acid and glutamate is considered, a = 355.7 kJ/mol of O_2 (or 15.87 kJ/L of O_2), b = 114.8 kJ/mol of CO_2 (or 5.12 kJ/L of CO_2), and c = 107.3 kJ/mol of N (or 7.66 kJ/g of N). ΔH (kJ/mol) = 15.87 VO_2 (L) + 5.12 VCO_2 (L) – 7.66 N (g).

The coefficients for calculating heat production according to the metabolic data on O_2 consumption, CO_2 production, urinary N excretion, and methane production by various animals are summarized in Table 8.16. These values are useful for estimating whole-body energy expenditure. Note that the production of large amounts of methane by ruminants and horses should be taken into consideration in estimating whole-body heat production. These equations were derived under standard conditions (including temperature-controlled facilities); therefore, anyone using nonstandard conditions should account for varying pressure and housing temperature (Verstegen and Henken 1987).

Lean Tissues and Energy Expenditure

The metabolic rate of an animal is not linearly proportional to its BW or tissue mass (Campbell 1988). Additionally, relationships between metabolic rate and BW differ between small- and large-size mammals (Blaxter 1971). This complex picture is further complicated by different tissues having different rates of metabolism, heat production, or O_2 consumption per kilogram of tissue weight (e.g., heart > kidney > liver > small intestine > skeletal muscle > WAT; Table 8.13). Interestingly, the brain, liver, heart, kidneys, and small intestine account for 7.6% of the BW but 51.4% of whole-body O_2 consumption in nonruminants. In ruminants, the GIT and liver, which are highly active in motility, fermentation, and metabolism, can contribute to as much as 50% of total heat production of the whole body (Reynolds et al. 1991; Table 8.14). Although the rate of O_2 consumption or energy expenditure per kilogram of tissue in resting skeletal muscle is much lower than that for internal organs, this tissue contributes to 20.7% of the whole-body O_2 consumption due to its large mass. In contrast, WAT accounts for 12.4% of the BW but only 1% of the whole-body O_2 consumption or heat production due to its low metabolic rate. Thus, there is considerable debate about

TABLE 8.16
Coefficients for Estimating Heat Production Based on Metabolic Data in Animals[a]

Authors	O_2 Consumption (kJ/L)	CO_2 Production (kJ/L)	Urinary N Excretion (kJ/g)	Methane Production (kJ/L)	Heat Production (MJ/day)
			Nonruminant mammals (including man)		
Blaxter (1989)[b]	16.20	4.94	–5.80	---	9.99
Weir (1949)	16.50	4.63	– 9.08	---	9.96
Ben-Porat et al. (1983)	16.37	4.57	– 13.98	---	9.80
Brockway (1987)	16.57	4.50	– 5.90	---	10.00
			Ruminants (cattle and sheep)		
Blaxter (1989)	16.07	5.23	– 5.26	– 2.40	10.04
Brouwer (1965)	16.18	5.16	– 5.93	– 2.42	10.01
			Poultry		
Farrell (1974)	16.20	5.00	– 1.20	---	10.08

[a] The coefficients for the whole bodies of different animal species are derived from the equations in Table 8.15.
[b] The heat production was calculated for an O_2 consumption of 500 L/day, a CO_2 production of 400 L/day, and a urinary nitrogen (N) excretion of 15 g/day.

an appropriate way to express data on O_2 consumption, CO_2 production, and energy expenditure by animals (Even and Nadkarni 2012), but normalization of the data based on lean tissue weight may be nutritionally and physiologically more meaningful (Assaad et al. 2014).

The weights of internal organs (other than WAT) and skeletal muscle in healthy animals are relatively constant per kilogram of BW, but the weight of WAT is substantially increased in obese animals compared with lean animals (Jobgen et al. 2006). Thus, the expression of whole-body O_2 consumption or heat production data per kilogram of BW or metabolic weight (the BW raised to the power of 0.75) may lead to an underestimation of metabolic activities (including energy expenditure) in the fat-free tissues or lean body mass of obese animals (Assaad et al. 2014). Therefore, the simple division of metabolism variables by BW or metabolic weight does not provide accurate information to compare true metabolic rates between normal-weight and obese animals. A solution to solving this problem is to determine body composition, including the weights of WAT, brown adipose tissue, and other major O_2-consuming tissues in each animal. Then, data on the rates of energy metabolism in animals fed the low-fat or high-fat diet can be more accurately expressed per kilogram of nonfat mass. If the weights of nonwhite fat tissues (e.g., skeletal muscle, liver, heart, kidneys, small intestine, and brown adipose tissue) do not differ between low fat- and high fat-fed animals, data on energy metabolism (i.e., rates of O_2 consumption, CO_2 production, and heat production) can be expressed per animal to indicate its metabolic activity (Assaad et al. 2014). However, this is not the case because the nonfat masses of some tissues (e.g., skeletal muscle, heart, liver, and kidneys) are increased in high fat-fed animals than in low fat-fed animals. When the rates of whole-body O_2 consumption or energy expenditure are expressed per kilogram of BW, data should be interpreted with the above caveats in mind.

Usefulness of RQ Values in Assessing Substrate Oxidation by Animals

Due to the different composition of C, O, and H in glucose, fatty acids, and protein, the oxidation or synthesis of these nutrients gives different RQ values (Kleiber 1961). Thus, RQ can reveal information on the nature of oxidation of energy substrates or about anabolic pathways in animals (Table 8.17). For example, RQ is 1.00 and 0.70 for the oxidation of glucose and fatty acids, respectively. In addition, RQ values between 0.80 and 0.85 implicate oxidation of fatty acids, glucose, and amino acids to CO_2 and H_2O. Furthermore, RQ values between 0.67 and 0.70 suggest that animals mobilize fat stores, providing fatty acids for oxidation and glycerol for gluconeogenesis. In contrast, the RQ value for the synthesis of tripalmitoylglycerol (a fat) from glucose is 2.05. Of note, in growing pigs, the RQ is typically greater than 1, which is indicative of lipogenesis. In all animals, the oxidation and synthesis of glucose, fatty acids, and amino acids occurs simultaneously in a cell- and tissue-specific manner. The contribution of each substrate to the whole-body O_2 consumption can be calculated on the basis of RQ values (Table 10.6).

RQ values for the oxidation of glucose, fatty acids, fat, and protein can be calculated based on the established biochemical reactions. Examples are given as follows.

1. For glucose oxidation:

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$$
$$RQ = 6\ CO_2/6\ O_2 = 1.00$$

2. For fatty acid oxidation (e.g., palmitic acid):

$$C_{16}H_{32}O_2 + 23\ O_2 \rightarrow 16\ CO_2 + 16\ H_2O$$
$$RQ = 16\ CO_2/23\ O_2 = 0.696$$

TABLE 8.17
Respiratory Quotients (RQ) for Substrate Oxidation and for Synthetic Pathways in Animals

Oxidation of Substrate to CO_2 and H_2O	RQ Value	Synthetic Pathways	RQ Value
Glucose	1.00	Glucose to long-chain fatty acids[c]	8.00
Glycerol	0.857	Glucose to fat[b]	2.05
Protein[a]	0.831	Protein to fat[b]	1.26
Fat[b]	0.703–0.710	Protein to long-chain fatty acids[b]	1.22
Long-chain fatty acids[c]	0.696	Glycerol to glucose	0.667
Ethanol	0.667	Protein or amino acids to glucose	0.333

Source: Assaad et al. 2014.

[a] Animal protein ($C_{4.3}H_7O_{1.4}N_{1.2}S_{0.03}$; Kleiber 1961).

[b] RQ = 1 for tripalmitoylglycerol ($C_{51}O_6H_{98}$), and RQ = 0.710 for trioleoylglycerol.

[c] Palmitic acid as an example of long-chain fatty acid.

3. For fat (tripalmitoylglycerol) oxidation:
   Oxidation of tripalmitoylglycerol (tripalmitin, Chapter 3):

$$C_{51}H_{98}O_6 + 72.5\ O_2 \rightarrow 51\ CO_2 + 49\ H_2O$$
$$RQ = 51\ CO_2/72.5\ O_2 = 0.703$$

   Oxidation of 1-palmitoleoyl-2-oleoyl-3-stearoyl-glycerol (Chapter 3)

$$C_{55}H_{103}O_6 + 77.75\ O_2 \rightarrow 55\ CO_2 + 51.5\ H_2O$$
$$RQ = 55\ CO_2/77.75\ O_2 = 0.707$$

   Oxidation of trioleoylglycerol (triolein, Chapter 3):

$$C_{57}H_{105}O_6 + 80.25\ O_2 \rightarrow 57\ CO_2 + 52.5\ H_2O$$
$$RQ = 57\ CO_2/80.25\ O_2 = 0.710$$

4. For protein oxidation:
   $C_{4.3}H_7O_{1.4}N_{1.2}S_{0.03}$ (animal protein)
   Oxidation of 1.2 mol of N to 0.6 mol of urea (CH_4ON_2)

$$C_{4.3}H_7O_{1.4}N_{1.2}\,(\text{protein}) - C_{0.6}H_{2.4}O_{0.6}N_{1.2}\,(\text{i.e., 0.6 urea}) \rightarrow C_{3.7}H_{4.6}O_{0.8}$$
$$C_{3.7}H_{4.6}O_{0.8} + 4.45\ O_2 \rightarrow 3.7\ CO_2 + 2.3\ H_2O$$
$$RQ = 3.7\ CO_2/4.45\ O_2 = 0.831$$

RQ values for the syntheses of fatty acids, fat, glucose, and protein can also be calculated based on the established biochemical reactions (Table 8.9). Examples are given as follows.

1. Fatty acid synthesis from glucose:

$$4\ C_6H_{12}O_6 + O_2 \rightarrow C_{16}H_{32}O_2 + 8\ CO_2 + 8\ H_2O$$
$$RQ = 8\ CO_2/1\ O_2 = 8$$

   Fat (e.g., tripalmitoylglycerol; $C_{51}O_6H_{98}$) synthesis from glucose:

$$31\ C_6H_{12}O_6 + 41\ O_2 \rightarrow 2\ C_{51}O_6H_{98} + 84\ CO_2 + 88\ H_2O$$
$$RQ = 84\ CO_2/41\ O_2 = 2.05$$

2. Glucose synthesis from protein:
   After oxidation of protein N to urea, $C_{3.7}H_{4.6}O_{0.8}$ is available for glucose synthesis.

$$C_{3.7}H_{4.6}O_{0.8} + 2.1\ O_2 + 2\ H^+ \rightarrow 0.5\ C_6H_{12}O_6 + 0.7\ CO_2 + 0.6\ H_2O$$
$$RQ = 0.7\ CO_2/2.1\ O_2 = 0.333$$

3. Glucose synthesis from glycerol:

$$3\ C_3H_8O_3 + 4.5\ O_2 \rightarrow C_6H_{12}O_6 + 3\ CO_2 + 6\ H_2O$$
$$RQ = 3\ CO_2/4.5\ O_2 = 0.667$$

Caution in the Interpretation of RQ Values

In order for an RQ value to accurately indicate the metabolism of animals, they must have a healthy respiratory system to inhale O_2 and exhale CO_2 (Blaxter 1989; Kleiber 1961). The following issues should be borne in mind when using indirect calorimetry to determine heat production by animals. First, there may be differences in CO_2 produced and CO_2 exhaled by the animal during a short period of time (e.g., an animal or human with a mask) or under the conditions of pulmonary dysfunction (i.e., respiratory acidosis) or metabolic alkalosis. Second, in ruminants, the CO_2 produced through anaerobic fermentation by the rumen and hindgut should be distinguished from the CO_2 released from oxidative metabolism by the body tissues because the energetic efficiencies of nutrient metabolism differ between microbes and animal cells. This may also be a problem for obese animals, which may have a greater population of anaerobes in the GIT than their nonobese counterparts (Zhao 2013). Third, when large amounts of ketone bodies are synthesized from fatty acid-derived acetyl-CoA, O_2 is consumed but no CO_2 is produced; thus, RQ = 0 for fatty acid oxidation through ketogenesis. This can be a significant issue for animals with ketosis. Finally, there are diurnal changes in RQ among animals due to different metabolic activities at different times, as shown for rats (Figure 8.5). Thus, a short-term measurement of RQ may not be valid, depending on the time relative to the last feeding (Assaad et al. 2014).

$$\text{Fatty acids} + O_2 \rightarrow \text{Ketone bodies} + H_2O\ (\text{but no } CO_2)$$

Comparative Slaughter Technique for Estimating Heat Production

The comparative slaughter technique can be used to estimate whole-body heat production by animals, as well as their energy requirement for maintenance and growth (Tedeschi et al. 2002). Let us use cockerels (male chickens) as an example (Fuller et al. 1983). When the birds were fed a diet providing the ME of 2255 kJ over a period of 4 days, they gained 68 g BW (a final BW of 2823 g, an initial BW of 2755 g), which is equivalent to 679 kJ (i.e., NE for growth). Assuming that methane production is negligible, heat production (including HI, maintenance energy requirement, and intermediary metabolism) by the birds is calculated to be 1576 kJ (2255 – 679 = 1567) during 4 days. Analysis of gaseous exchange data from open-circuit indirect calorimetry indicated the heat production of 1548 kJ by the same animals. Thus, the efficiency of ME for growth in the 2.8-kg cockerels is 30%. Because of its ability to directly measure net tissue gains over an experimental period, the comparative slaughter method is considered the gold standard for determining the NE content of feeds for animals (Kil et al. 2011; McDonald et al. 2021). Based on the comparative slaughter approach, NE in the California Net Energy system includes NE for maintenance (NE_m) and NE for BW gain (NE_g), which allow for improved feeding strategies to maximize the growth performance of beef cattle

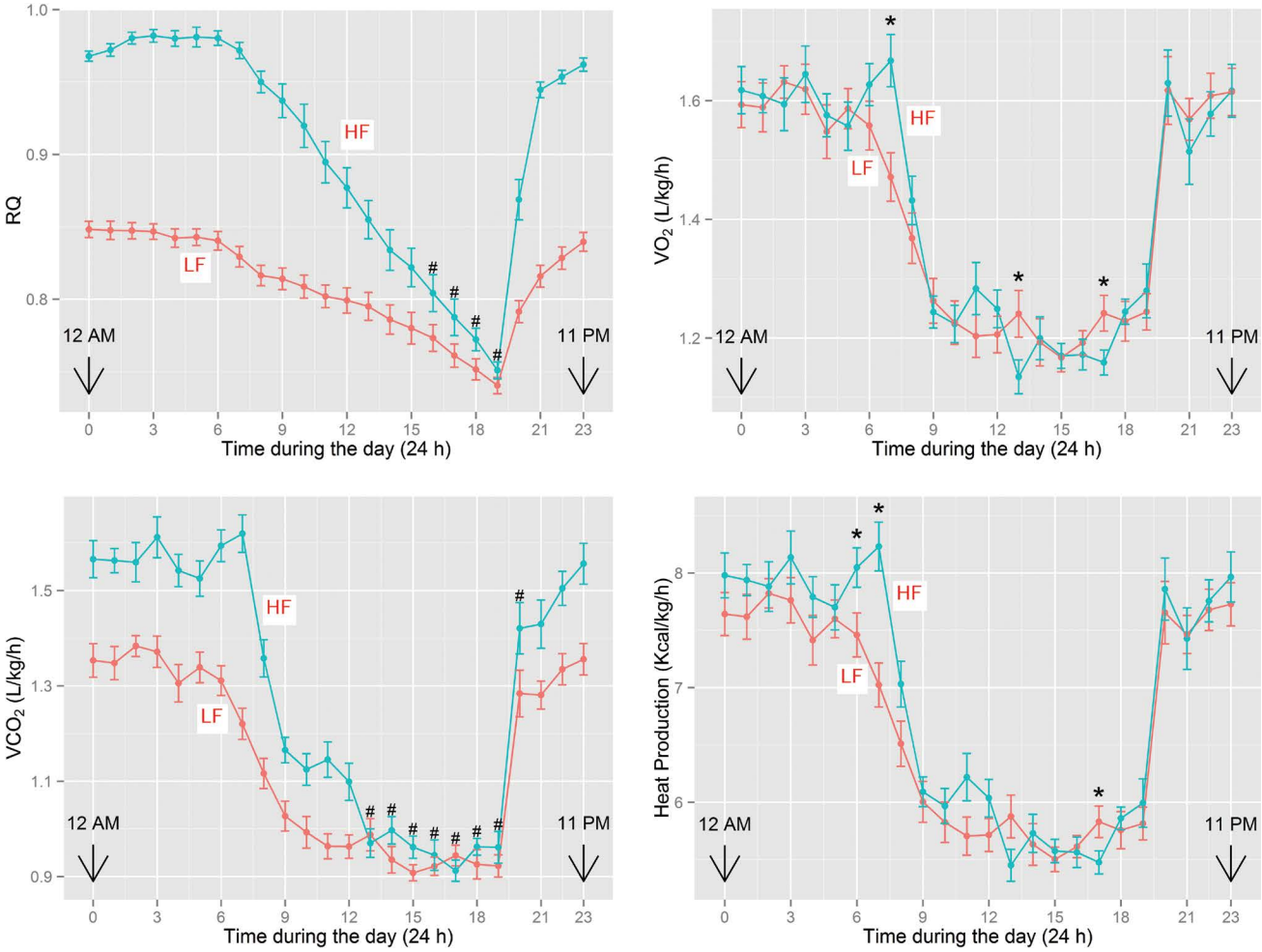


FIGURE 8.5 Energy metabolism of male Sprague-Dawley rats (Charles River Laboratories) fed a low-fat (LF) or high-fat (HF) diet. Rats were fed an LF or HF diet between 4 and 13 weeks of age. At 13 weeks of age, rats were placed in a computer-controlled Oxymax instrument (an open circuit calorimeter; Columbus Instruments, OH) to measure 24-h O_2 consumption, CO_2 production, RQ and heat production. Values are mean ± SEM during the day (24 h) at the end of the study. There were 16 rats per diet group, which consumed food at night. The feeding time was between 7:00 pm and 6:00 am. The sign "*" denotes a significant effect of diet at the indicated time point ($P < 0.05$). The sign "#" denotes no significant effect of diet at the indicated time point. (Courtesy of Assaad et al. 2014; an open-access article.)

(Tedeschi and Fox 2020). Disadvantages of the comparative slaughter method are the use of an animal only once, expensive and technically challenging for large animals (e.g., cattle and sows), and potentially unethical for some species (e.g., cats and dogs).

Use of Doubly Labeled Water (DLW) to Measure Energy Expenditure in Animals

The DLW method involves a single intravenous or oral administration of a known amount of H_2O (10 g/70 kg of BW) containing two stable isotopes [^{2}H] and [^{18}O], namely $^2H_2{}^{18}O$, into study participants (e.g., freely living humans and other animals). Thereafter, the isotopic enrichments of ^{18}O and ^{2}H in blood H_2O increase rapidly, and the isotopes equilibrate with total body H_2O. To assess these changes, samples (urine, saliva, and blood) are obtained from the study participants at two time points. The time between the initial and final samples depends on the size of animals, with as short as 24 h for small animals (e.g., rats) or as long as 14 days for large animals (e.g., adult humans). Through excretion, ^{18}O and ^{2}H are eliminated from the body at different rates as H_2O plus CO_2 and H_2O, respectively. The volume of total body H_2O is calculated on the basis of the isotope dilution. The difference in the rates of ^{18}O and ^{2}H disappearance from the body H_2O (calculated according to their isotopic enrichments in urine $^2H_2{}^{18}O$) is the rate of whole-body CO_2 production. O_2 consumption can be estimated from (a) the intakes and digestibilities of dietary carbohydrates, lipids, and protein; and (b) changes in body composition, and can then be used to calculate RQ and then total energy expenditure, as described previously. Thus, the DLW method is a form of indirect calorimetry. Note that

a noninvasive dual-energy X-ray absorptiometry (DEXA) scan is currently available to determine lean and fat masses for estimating protein and lipid composition in farm and laboratory animals as well as humans.

$$^{18}\text{O: Leaves the body as both } H_2O \text{ and } CO_2 \quad (1)$$

$$^{2}\text{H: Leaves the body as } H_2O \quad (2)$$

$$(1)-(2) = CO_2 \text{ production}$$

The DLW technique, which is convenient and noninvasive to estimate whole-body energy expenditure, was invented for small animals (e.g., birds, reptiles, and insects) by Lifson et al. (1955) and adopted for humans by Schoeller and van Santen (1982). This method can also measure simultaneously the H_2O turnover in the whole body. The ^{2}H and ^{18}O isotope elimination rates (K_H and K_O) can be determined by the two-point method using the initial (I) isotopic enrichment and the final (F) enrichments. Detailed calculations are described by Schoeller (1988) as follows:

$$K_H(\text{elimination rate of } ^{2}H \text{ as } H_2O) = (\ln IE_F - \ln IE_I)/\Delta t$$

$$K_O(\text{elimination rate of } ^{18}O \text{ as } H_2O + CO_2) = (\ln IE_F - \ln IE_I)/\Delta t,$$

where ln is the natural log, IE is the isotopic enrichment of ^{2}H or ^{18}O above the background enrichment, and Δt is the number of days between the initial and final sample.

The rate of CO_2 production (rCO_2) is calculated as described by Schoeller (1988):

$$rCO_2 = (N/2.078)\times(1.01K_O - 1.04\ K_H) - 0.0246\times rH_2O_F$$

where N is the total body H_2O calculated from the ^{18}O enrichment in the 4-hour saliva (or the average of initial and final 4-hour saliva samples if the total body H_2O is altered); and rH_2O_F is the fractional rate of H_2O loss, which is estimated according to the following equation.

$$\text{Fractional rate of } H_2O \text{ loss } (rH_2O_F) = 1.05N\times(1.01\times K_O - 1.04\times K_H)$$

NUTRITIONAL MEANS FOR THE REGULATION OF HEAT PRODUCTION AND ENERGY BALANCE IN ANIMALS

Impacts of Heat or Cold Stress on Animal Production and Nutritional Interventions

As discussed previously, the maintenance of body temperature depends on the balance between the rates of heat production and dissipation. At a constant body temperature, both rates are equal. Animals can be frequently challenged with extreme ambient temperatures in tropical nations and regions and in some temperate countries during the cold winter and the warm summer (Baumgard and Rhoads 2013). Except for their newborns, farm animals (e.g., livestock and birds) generally have a greater ability to tolerate low than high temperatures. Livestock and poultry are highly sensitive to heat stress due to the lack of functional sweat glands and skin insulation (by either a thick layer of subcutaneous fat in pigs and finishing cattle, feathers in poultry, and wool in sheep). In addition to physical cooling methods to ameliorate heat stress (e.g., sprinklers, H_2O baths, and the shade of trees), identifying effective nutritional solutions can alleviate the associated metabolic disorders and ensure their welfare (Baumgard and Rhoads 2013; Mayorga et al. 2019). Animals can adapt to changes in their living environments by increasing or decreasing food intakes and whole-body energy expenditures in response to ambient temperatures below or above their thermoneutral zones, respectively. However, there are limitations to such physiological adaptations and, therefore, countermeasures must be sought.

Elevated ambient temperatures result in reduced growth and productivity (e.g., lactation and gestation) of animals that are accounted for by both reduced food intake and abnormal nutrient metabolism in favor of catabolic effects (Rhoads et al. 2009). In lactating dairy cows subjected to cyclical heat stress (with temperatures ranging from 29.4°C to 38.9°C) for 7 consecutive days, rectal temperatures and respiration rates increased from 38.6°C to 40.4°C and from 44 to 89 breaths/min, respectively (Wheelock et al. 2010). Heat stress reduced the DM intake of the cows by 30%, and by design, cows in the pair-fed group had a similar decrease in feed intake (28%). Both heat-stressed cows and pair-fed cows were in negative energy balance, and their milk yields decreased by 28% and 14%, respectively (Wheelock et al. 2010). Thus, reduced feed intake accounted for only 50% of the decreased milk production. Similar results have been reported for lactating sows exposed to elevated ambient temperatures (e.g., 18°C vs. 28°C; 20°C vs. 32°C; 22°C vs. 32°C; or 22°C vs. 35°C; Black et al. 1993; Dourmad et al. 2022). Likewise, heat stress reduces the food intake and growth performance of pigs and poultry by 25–50%, as well as the production of eggs by hens (Chen et al. 2021). In all these farm animals, the energetic efficiencies of metabolic transformation for protein gain are substantially reduced due to impairments in the intestinal and renal absorption of nutrients, the enhanced catabolism of body protein as well as body and dietary amino acids, and the excess losses of body H_2O and minerals.

Severe heat stress is life-threatening in animals, and their survival depends on effective thermoregulation. First of all, adequate H_2O intake is critical for the dissipation of excessive heat from animals exposed to elevated ambient temperatures, and dehydration is the major cause of their mortality. Second, shade (which is almost as important as H_2O) should be provided. Third, under the conditions of elevated ambient temperatures, dietary supplementation

with fats within physiological ranges can partially offset a reduction in the food and energy intakes of an animal while generating metabolic H_2O in the body. In addition, any excessive intake of dietary protein, which has the highest HI value among macronutrients and also promotes the loss of H_2O via the urinary excretion of H_2O-soluble nitrogenous metabolites (e.g., urea, ammonia, and creatinine), should be avoided (Wu 2022). However, diets should contain sufficient amino acids, particularly arginine (the precursor of nitric oxide as the major vasodilator), as well as glycine, glutamate, and glutamine (for the synthesis of glutathione and protecting the small intestine from damage), for animals experiencing heat stress (Ríus 2019; Wu et al. 2014). This can facilitate the loss of heat via the flow of blood from internal organs to the skin (Laspiur and Trottier 2001), while ensuring an adequate flow of blood from mother to fetus in gestating dams (Wu et al. 2013) or through the mammary glands in lactating dams (Rezaei et al. 2016).

The ability of skeletal muscles to produce heat via ATP-dependent contraction in animals exposed to low ambient temperatures depends on adequate nutrition to provide amino acids, fatty acids, carbohydrates, vitamins, minerals, and H_2O. Additionally, in animals (e.g., sheep and cattle) containing brown adipose tissue (which produces a large amount of heat in the mitochondria due to the presence of the uncoupling protein), the mass and physiological activity of this tissue can be enhanced through dietary supplementation with citrulline or rumen-protected arginine (McCoard et al. 2014; McKnight et al. 2020). Furthermore, increasing the consumption of protein and fat (nutrients that have relatively high HI values) within physiological ranges can augment the availability of amino acids and fatty acids for heat production by the skeletal muscle, liver, heart, and kidneys of all animals under the conditions of cold stress. Notably, hypoglycemia is a life-threatening condition in neonates (particularly animals without brown adipose tissue such as pigs) exposed to a cold environment, and they should be provided with adequate nutrients (including fats, amino acids, and glucose or its sources) for effective thermoregulation.

Overweight and Obesity in Animals Due to Excessive Energy Imbalance

Obesity in mammals, birds, fish, and other animals results from the excess deposition of body fat due to a chronically greater intake of energy than its expenditure, namely an imbalance of energy metabolism. According to the World Health Organization (WHO 2022), people with a body mass index [BMI; BW (kg)/height $(cm)^2$] of $\geq$ 30.0 are generally classified as obese, and those with a BMI of 25.0–29.9 are defined as overweight. There are three classes of human obesity based on BMIs: class I (30.0–34.9), class II (35.0–39.9), and class III ($\geq$ 40.0). For comparison, individuals with BMIs of 18.5–24.9 or < 18.5 are considered to have a normal BW or be underweight, respectively. However, these BMIs may not apply to certain populations such as Asians with relatively high proportions of body fat and well-trained athletes with relatively high proportions of lean tissue mass. For example, Asian women with BMIs of 23.0–27.5 or > 27.5 are considered overweight or obese, respectively. Muscular men and women, such as bodybuilders, can have a BMI above the range for the general population but may not be obese. For farm, laboratory, companion, and zoo animals, BW may serve as a criterion for overweight or obesity classification: overweight if they weigh 10–20% above their ideal BW; obese if they weigh $\geq$ 20% above their ideal BW. Both genetic and environmental (including high fat intake and sedentary lifestyle) factors contribute to the development of obesity.

Obesity in humans and companion animals is a growing health problem in many regions of the world, including both developed and developing nations. The WHO reported that, in 2016, globally 39% of adults ($\geq$18 years of age; ~1.9 billion people) were overweight, with 13% of them being obese (i.e., ~650 million); and the prevalence of overweight or obese children and adolescents aged 5–19 years worldwide increased 3.5-times from 4% in 1975 to 18% in 2016 (340 million). According to the Centers for Disease Control and Prevention of the United States (CDC 2022), 41.9% of adults ($\geq$20 years of age) and 19.7% of children and adolescents (2–19 years of age) were obese as of March 2020, with the obesity rate for the whole population nearly tripling over the last 50 years. In North America, about 50% of domestic cats aged 5–11 years have a BW greater than their ideal weight, and about 30–35% of domestic cats are obese. In humans as well as farm, laboratory, companion, and zoo animals, obesity increases risks for hyperglycemia, type 2 diabetes, cardiovascular diseases (e.g., hypertension and stroke), many cancers (e.g., pancreatic and colon cancers), fatty liver disease, neurological disorders (e.g., dementia), kidney disease, gout, joint problems, physical weakness, infertility and polycystic ovary syndrome, erectile dysfunction, and pregnancy complications. Currently, human obesity costs the US healthcare system $147 billion a year. Improving nutrition (particularly nutrient and energy intakes) and increasing energy expenditure through regular physical exercise are key to preventing and treating obesity.

Physiological Responses to Starvation or Fasting Regarding Energy Metabolism

Starvation is the involuntary act of having no or limited access to energy, food, or nutrients, whereas fasting is the voluntary act of abstaining from the consumption of food or nutrients (and thus no intake of energy or DM) beyond the postabsorptive period. The effects of fasting and complete starvation on energy metabolism are the same, namely reductions in whole-body metabolic rates.

Animals have strategies (such as the mobilization of body fat and protein to provide energy) to adapt to starvation or fasting so that they can survive for various periods, depending on the species. In mammals, birds, and fish, starvation is characterized by three phases: (a) the mobilization of glycogen, fat, and protein reserves as the major sources of energy and glucose; (b) the mobilization of fat as the primary source of energy and the conservation of body protein and glucose, with fat reserves reaching a critical threshold; and (c) a shift to the mobilization of the protein reserve as the main sources of energy and glucose. Changes in energy metabolism during starvation or fasting differ among animals with different capacities for hepatic ketogenesis. Ketone bodies provide the bulk of energy for all extrahepatic tissues and cells under such nutritional conditions in animals that are capable of ketogenesis (Chapter 6).

In humans, the initial phase (the first 7 days) of starvation is associated with gradual increases in: (a) the degradation of glycogen to provide glucose; (b) the hydrolysis of TAGs in the WAT to release fatty acids and glycerol, (c) the oxidation of fatty acids in the liver to produce ketone bodies (acetoacetate and β-hydroxybutyrate), (d) protein degradation in the whole body (primarily skeletal muscle) to generate amino acids, (e) the use of ketone bodies as major metabolic fuels in extrahepatic tissues (primarily the brain, kidneys, and the small intestine), and (f) the conversion of glucogenic amino acids, glycerol, lactate, and odd-carbon number fatty acids into glucose in the liver and kidneys (Palmer and Clegg 2021; Owen 2005; Wu and Marliss 1992). Between Days 0 and 7 of human starvation, the plasma concentrations of glucose decrease gradually from ~5 mM to the nadir value (~4 mM), whereas those of acetoacetate and β-hydroxybutyrate gradually increase from ~0.05 to ~1 mM and from ~0.1 mM to ~4.2 mM, respectively. During the mid- and late phases of human starvation, protein degradation in tissues is reduced gradually due to its inhibition by elevated ketone bodies to conserve protein in the whole body. The loss of 50% protein from the body is incompatible with survival. When sufficient drinking H_2O is available, nonobese humans generally do not survive a 30-day starvation, but obese individuals can survive at least 37 days of starvation (Owen 2005).

Some species of animals naturally experience starvation for days or months. For example, most birds can survive several days without food (McCue 2010), and hibernating bears can survive during the cold winter without eating food or drinking H_2O (Carey et al. 2003). Likewise, in their natural habitat or aquaculture, many species of fish (e.g., rainbow trout and sturgeon) can survive months of starvation, particularly during times of climate changes, seasonal variations, competition for food, breeding, and migration (Furne and Sanz 2017). Of particular note, the European eel (*Anguilla anguilla*) exhibits extraordinary resilience to 1594 days (53 months) of starvation (Boëtius and Boëtius 1985). It is largely unknown why different species of animals have different resilience to prolonged starvation.

SUMMARY

Energy is an important concept in nutrition and may be defined as the capacity to do work. Energy itself is not a nutrient but is contained within nutrients, primarily carbohydrates, lipids, protein, and amino acids. According to the first law of thermodynamics, energy is conserved in any system. Namely, energy is neither created nor destroyed but is interconverted from one form into another. The chemical energy of feedstuffs is transformed into biological energy after they are digested, and the nutrients are metabolized in animals. As metabolic transformers, animals utilize different nutrients as energy sources through different pathways at different efficiencies. This provides a biochemical basis for assessing the energy values of feedstuffs for animals (e.g., mammals, poultry, fish, and crustaceans) as GE, DE, ME, and NE (NE_m and NE_g or NE_L). Although the apparent digestibility of nutrients (leading to fecal energy) has traditionally been used to estimate apparent DE, the true DE values can be calculated according to the true digestibility of dietary carbohydrates, fats, and protein. Dietary intake of fiber greatly affects fermentation and gaseous production by the GIT. Thus, the ME values of feedstuffs or habitual diets for animals differ widely. NE is the portion of the feed energy that is available to the animal for maintenance and production. The relationship between the NE and ME is relatively constant for nonruminant mammals and birds (i.e., NE = 0.80 or 0.82 × ME, depending on animal species), but can vary greatly for ruminants, depending on methane production by the rumen.

The rates of energy metabolism and, therefore, the requirements of animals for energy are influenced by a plethora of factors, including species, breeds, gender, physiological state, health status, body size, body composition (e.g., fat and lean tissue mass), physical activity, and environmental temperature. The measurement of heat production by direct calorimetry, indirect calorimetry (based on RQ values), or the comparative slaughter technique is necessary to determine the NE of any feedstuffs for animals. Total heat production by the animal includes the heat generated from basal metabolism and HI and is the single largest loss of the ingested energy. In all animals, the efficiency of utilization of dietary ME for maintenance is greater than that for production, and the efficiencies of the dietary ME from fat or carbohydrates for both maintenance and production are greater than those from protein. These differences result from the facts that (a) the use of energy for productive purposes (e.g., tissue growth, egg laying, lactation, pregnancy, and wool growth) is associated with heat production; (b) the energetic efficiency of biosynthetic processes (e.g., fat accretion and protein deposition) is always less than 100%; and (c) the energetic

efficiency of oxidation of protein for ATP production is always lower than that of fat and glucose in mammals and birds due to the need for energy to dispose of ammonia as urea or uric acid. Because extensive fermentation in the rumen produces a large amount of methane, the efficiencies in the utilization of dietary ME for maintenance and production are generally lower in ruminants than in nonruminants. In contrast, compared with mammals and birds, fish have a greater NE value because of low HI by releasing NH_3 plus NH_4^+ directly through their gills. With the increasing inclusion of nonconventional feedstuffs in animal diets, the use of NE will better predict the efficiency of dietary energy for both maintenance and production and, therefore, precise formulation of diets. Improving the efficiency of dietary energy utilization is essential to reducing the costs of animal production and resource use, thereby sustaining the global livestock, poultry, and aquaculture industries.

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9 Nutrition and Metabolism of Vitamins

Vitamins are organic compounds required in small amounts for the normal metabolism, growth, and production of animals. Hopkins (1912) demonstrated that a synthetic diet containing purified casein, lard, sucrose, starch, and inorganic salts was inadequate for the weight gain of young rats and that adding a small quantity of milk (4.7 calories/g diet) to this synthetic diet helped the animals grow normally. The unknown but essential growth-promoting compounds were called "accessory factors". In the same year, Casimir Funk thought that these substances contained amino nitrogen (N) and named them "vitamines" (meaning vital amines). It is now known that only a few vitamins contain amino-N (Berdanier 1998). The seminal finding of Hopkins challenged the dogma held in the early 1910s that only five classes of nutrients were essential for animals: water, proteins, carbohydrates, fats, and minerals.

Associations between the deficiencies of some vitamins (despite a lack of knowledge about their chemical structures or physiological functions) and certain diseases were recognized much earlier than the beginning of the 20th century (Semba 2012). In 1753, James Lind (a British naval physician) reported that scurvy in sailors could be cured by the inclusion, in diets, of salads and fruits, which are rich in a substance that was first isolated in 1928 as ascorbic acid. In addition, Alexander Wynter Blyth (a British chemist) observed in 1872 a pigment with yellow-green fluorescence in cow's milk that was known as vitamin B_2 (Chick and Roscoe 1928). Furthermore, between 1889 and 1898, Christiaan Eijkman [a Dutch physician working in the Dutch East Indies (now Indonesia)] found that the disease beriberi in humans occurred in chickens fed polished rice without husk and could be cured by giving the patients brown rice grain rather than polished rice. In 1934, the rice husk was known to contain thiamin. Only after the discovery of vitamins, was it recognized that their specific deficiencies result in characteristic diseases such as scurvy, beriberi, rickets, pellagra, and xerophthalmia (Ross and Gardner 1994; Zempleni et al. 2013).

Historically, vitamins were named alphabetically in order of discovery. This nomenclature was convenient and was generally accepted before the discovery of their chemical nature. However, chemical names are increasingly used to describe vitamins of the B complex (B_1, B_2, B_3, B_5, B_6, B_{12}, biotin, and folic acid). Vitamins are classified as water- or lipid-soluble based on their solubility in water or fat, respectively (Table 9.1). The absorption and transport of dietary lipid-soluble vitamins are closely linked to the metabolism of lipids, low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), and high-density lipoprotein (HDL). Although some substances [e.g., *para*-aminobenzoic acid, carnitine, choline, flavonoids, lipoic acid, *myo*-inositol, pyrroloquinoline quinone (PQQ), and ubiquinones] have been called vitamins at various times, such classifications have not generally been accepted. These factors can be classified as quasi-vitamins, namely vitamin-like compounds (Combs and McClung 2017). Some vitamins are single substances (e.g., riboflavin and pantothenic acid), and others are members of the families of chemically related compounds – vitamers (e.g., nicotinic acid and nicotinamide for niacin; retinol, retinal, and retinoic acid for vitamin A). In animal nutrition, requirements for vitamins are expressed as milligrams or international units (UIs) per kilogram of body weight (BW) or percentage of a complete diet (McDonald et al. 2021). The main objective of this chapter is to highlight the sources, metabolism, and functions of vitamins.

TABLE 9.1
Vitamins Important in Animal Nutrition

Water-Soluble Vitamins		Lipid-Soluble Vitamins	
Conventional Name	**Chemical Name**	**Conventional Name**	**Chemical Name**
Vitamin B_1	Thiamin	Vitamin A	Retinol
Vitamin B_2	Riboflavin	Vitamin D_2 (plant)	Ergocalciferol
Vitamin B_3	Niacin (Nicotinamide)	Vitamin D_3 (animal)	Cholecalciferol
Vitamin B_5	Pantothenic acid	Vitamin E	Tocopherol
Vitamin B_6	Pyridoxine	Vitamin K_1 (plant)	Phylloquinone
Vitamin B_{12}	Cyanocobalamin	Vitamin K_2 (bacteria)	Menaquinone
Biotin	Biotin	Vitamin K_3 (synthetic)	Menadione
Folate (B_9)	Pteroylglutamic acid		
Vitamin C	Ascorbic acid		

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CHEMICAL AND BIOCHEMICAL CHARACTERISTICS OF VITAMINS

General Characteristics of Vitamins

Vitamins have various structures but share some common biochemical characteristics (Combs and McClung 2017). These features are as follows: (a) vitamins are organic nutrients that are required in small amounts for biochemical reactions; (b) many vitamins (especially lipid-soluble vitamins, as well as vitamins B_1, B_3, B_6 and B_{12}) are destroyed by oxidation, heat, light, or certain metals and, therefore, conditions of storage and processing of feeds can influence their biological values; (c) most vitamins, except a few (niacin, vitamin C, and vitamin D), cannot be synthesized by animal cells, making almost all vitamins dietary essential nutrients for nonruminant animals; (d) vitamins are neither degraded to provide energy nor used for cell structural purposes; (e) many vitamins or their derivatives (including B-complex vitamins, vitamin C, and vitamin K) serve as coenzymes for enzymes, while lipid-soluble vitamins fulfill specific functions in the body, such as the maintenance of vision (vitamin A), the utilization of dietary calcium (Ca) and phosphorus (vitamin D), blood clotting (vitamin K), and regulation of gene expression; (f) some vitamins (e.g., vitamins C and E) are potent antioxidants; (g) most vitamins are not utilized in the form in which they are absorbed from the intestine, and are converted or modified to their active forms; (h) some vitamins are covalently attached to enzymes or tightly associated with enzymes via a noncovalent linkage; (i) small-intestinal absorption of water-soluble vitamins occurs via specific carrier proteins, whose expression is regulated at transcriptional and/or post-transcriptional levels; and (j) the absence or relative deficiency of vitamins in the diet results in characteristic deficiency states and diseases. Like any other nutrients, excess vitamins are toxic to animals (DiPalma and Ritchie 1977). The chemical properties of vitamins are summarized in Table 9.2.

TABLE 9.2
Discovery and Chemical Properties [Including Molecular Weights (MWs) and Melting Points] of Vitamins

Vitamin	Year of Discovery	Vitamers	MW (Da)	Absorption Maximum (nm)[a]	Melting Point (°C)	Color at 25°C	Form at 25°C
				Lipid-soluble vitamins			
Vitamin A[a]	1915	Retinol	286.4	325	62–64	Yellow (YL)	Crystal
		Retinal	284.4	373	61–64	Orange	Crystal
		Retinoic acid	300.4	351	180–182	Yellow	Crystal
Vitamin D	1919	Vitamin D_2	396.6	265	115–118	White	Crystal
		Vitamin D_3	384.6	265	84–85	White	Crystal
Vitamin E	1922	α-Tocopherol	430.7	294	2.5	Yellow	Oil
		γ-Tocopherol	416.7	298	–2.4	Yellow	Oil
Vitamin K	1929	Vitamin K_1	450.7	242, 248, 260, 269, 325	–20	Yellow	Oil
		Vitamin K_2	649.2	243, 248, 261, 270, 325–328	54	Yellow	Crystal
		Vitamin K_3	172.2	246, 262, 333	105–107	Yellow	Crystal
				Water-soluble vitamins			
Vitamin C	1907	Free acid	176.1	245	190–192	White	Crystal
		Sodium salt	198.1	245	218	White	Crystal
Thiamin	1906	Free form	265.4	243 (pH 2.3), 235, 265	164	White	Crystal
		Disulfide form	562.7	---	177	Yellow	Crystal
		Hydrochloride	337.3	---	164	White	Crystal
		Mononitrate	327.4	---	196–200	White	Crystal
Riboflavin	1933	Free form	376.4	220–225, 266, 371, 444, 475	278	Orange to YL	Crystal
Niacin	1926	Nicotinic acid	123.1	263	237	White	Crystal
		Nicotinamide	122.1	263	128–131	White	Crystal
Vitamin B_6	1934	Pyridoxal	167.2	293	165	White	Crystal
		Pyridoxine-HCl	205.6	255, 326	160	White	Crystal
Biotin	1926	Free form	244.3	very weak absorption at 204	167	White	Crystal
Pantothenic acid	1931	Free form	219.2	very weak absorption at 212	184	Yellow	Oil
		Calcium salt	476.5	very weak absorption at 212	195	White	Crystal
Folate	1931	Monoglutamate	441.1	256, 283, 368	250	Orange to YL	Crystal
Vitamin B_{12}	1926	Cyanobalamin	1355.4	278, 361, 550	>300	Red	Crystal

Source: Combs and McClung 2017; and Open Chemistry Database, https://pubchem.ncbi.nlm.nih.gov.

[a] Measured at neutral pH unless indicated otherwise.

General Sources of Vitamins for Animals

Many plants can synthesize and directly provide vitamins except for vitamins A, B_{12}, and D and can form precursors for vitamins A and D_2 (Berdanier 1998). Thus, most feedstuffs supply vitamins, but their content varies widely. For example, concentrate feeds contain B vitamins, whereas forages are good sources of carotenes. In contrast, dried feedstuffs from plant sources provide vitamin D_2, but it is virtually absent from typical corn- and soybean meal (SBM)-based diets for poultry and swine. Furthermore, animal products are rich in most vitamins (including lipid-soluble vitamins) but are deficient in vitamin C and pantothenic acid, whereas plants are good sources of many water-soluble vitamins, vitamin E, and vitamin K (Table 9.3). Finally, microbial synthesis of the B complex vitamins and vitamin K occurs in the rumen of ruminants and in the large intestine of animals (Combs and McClung 2017). Except for vitamin B_{12}, vitamin K, and biotin, intestinal bacteria do not contribute a nutritionally significant quantity of vitamins to the animal hosts. The jejunal portion of the small intestine is the major site for the absorption of all dietary vitamins but vitamin B_{12}. The latter is absorbed primarily by the ileum. Although the epithelial cells of the large intestine express vitamin transporters, the rates of vitamin transport by colonocytes of the large intestine are much lower than those for enterocytes of the small intestine (Said 2011). Intestinal transport of water- and lipid-soluble vitamins is summarized in Table 9.4.

Provitamins are compounds that are not vitamins themselves but function as vitamins after a chemical change (Zempleni et al. 2013). The best examples are the carotenes (precursors of vitamin A), subcutaneous provitamin D (the precursor of vitamin D_3), and tryptophan (a precursor of niacin). The efficiency of conversion of provitamins into vitamins varies with the substrates, as well as the species and developmental stage of animals (Combs and McClung 2017).

WATER-SOLUBLE VITAMINS

Apart from their solubility properties, water-soluble vitamins have little in common from the chemical point of view. They are present in plant- and animal-sourced foods at various concentrations (Table 9.5). As noted previously, most of these nutrients are not synthesized in animal cells and must be supplied in nonruminant diets (Pond et al. 1995). Their synthesis in the rumen may not be sufficient for maximal growth and production performance of ruminants (McDonald et al. 2021). Water-soluble vitamins are absorbed in the small intestine (primarily in the jejunum for all vitamins but vitamin B_{12}) via specific transporters into the portal vein (Halsted 2003). Most of the water-soluble vitamins are transported in the blood in their free forms, but some are carried in their protein-bound forms. Except for vitamin B_{12}, these nutrients are excreted from the body primarily in the urine (Zempleni et al. 2013). Water-soluble vitamins are stored in tissues in limited amounts and rarely accumulate at toxic concentrations (DiPalma and Ritchie 1977).

Thiamin (Thiamine, Vitamin B_1)

Structure: Thiamin is the first member of the family of water-soluble vitamins to be discovered. In 1911, Casimir Funk first isolated thiamin from rice husk. In 1936, Robert Williams identified the chemical structure of thiamin,

TABLE 9.3
Vitamins from Major Plant- and Animal-Sourced Foods

Micronutrient	Plant-Sourced Food	Animal-Sourced Food
Thiamin (vitamin B_1)	Peas and other legumes, nuts whole grains, and wheat germ	Meat, liver, milk, eggs and other animal products
Riboflavin (vitamin B_2)	Wheat germ, whole grains, nuts legumes, and green vegetables	Meat, liver, milk, eggs and other animal products
Niacin (vitamin B_3)	Whole grains, wheat germ, nuts legumes, and vegetables	Meat, liver, milk, eggs and other animal products
Vitamin B_6	Nuts, legumes, whole grains vegetables, and bananas	Meat, liver, milk, eggs and other animal products
Pantothenic acid	Whole grains, legumes, nuts and vegetables	Meat, liver, milk, eggs and other animal products
Biotin	Whole grains, legumes, nuts and vegetables	Meat, liver, milk, eggs and other animal products
Folic acid (Vitamin B_9)	Peas and other legumes, nuts, juice whole grains, and leafy vegetables	Liver, milk, eggs, meat and other animal products
Vitamin B_{12}	Absent from plants	Meat, liver, milk, eggs and other animal products
Vitamin C	Abundant in fresh vegetables, juice tomatoes, green tea, and potatoes, but virtually absent from whole grains	Liver, milk, eggs, meat and other animal products
Vitamin A	Absent from plants; however, dark, green, orange or yellow vegetables are good sources of provitamin A	Liver, milk, eggs, meat and butter
Vitamin D	Absent from plants; however, sun-dried vegetables contain vitamin D_2	Milk, eggs, and liver are good sources of vitamin D_3
Vitamin E	Vegetable oils and wheat germ oil	Meat, liver, milk, eggs, fat and other animal products
Vitamin K	Alfalfa, pepper, whole grains, Vegetables, and bananas	Meat, liver, milk, eggs and other animal products

Source: Wu et al. (2014).

TABLE 9.4
Transport of Vitamins and Choline from the Intestinal Lumen across Enterocytes and Colonocytes in Animals

	Apical Membrane Transporter (Intestinal Lumen into Enterocyte)		Basolateral Membrane Transport (Enterocyte into the Lamina Propria)
Vitamin	**Conventional Name**	**Gene Name**	
Thiamin	Thiamin transporter 1 (ThTr1; Na^+-independent)	SLC19A2	Transmembrane protein
	Thiamin transporter 2 (ThTr2; Na^+-independent)	SLC19A3	
	Thiamin pyrophosphate transporter in colonocytes (energy-dependent but Na^+-independent)	SLC44A4	
Riboflavin	Riboflavin transporter-1 (RF-1; Na^+-independent)	SLC52A1	Transmembrane protein
	Riboflavin transporter-2 (RF-2; Na^+-independent)	SLC52A3	
Niacin	MCT1 (H^+-dependent, Na^+-independent)	SLC16A1	Transmembrane protein
	Na^+-coupled monocarboxylate transporter	SLC5A8	
	Organic acid transporter-2	SLC22A7	
Pantothenate	Multivitamin transporter (Na^+-dependent)	SLC19A6	Na^+-independent transporter
		SLC5A6	
Vitamin B_6	TPN1 (H^+-dependent, Na^+-independent)	SLC19A2	Transmembrane protein
		SLC19A3	
Biotin	Multivitamin transporter (Na^+-dependent)	SLC19A6	Na^+-independent transporter
		SLC5A6	
Vitamin B_{12}[a]	Receptor-mediated endocytosis	ABCC1	Multidrug resistance protein-1
Folate	Reduced-folate carrier-1 (RFC-1, Na^+-independent)	SLC19A1	Multidrug resistance proteins
	Reduced-folate carrier-2 (RFC-2, Na^+-independent)	Splice variant*	Multidrug resistance proteins
	Proton (H^+)-coupled folate transporter	SLC46A1	
	Folate receptors (negligible in small and large intestines)	FOLR1, 2, 3	
	Mitochondrial folate transporter	SLC25A32	N/A
Vitamin C	Vitamin C transporter-1 (Na^+-dependent)	SLC23A1	Transmembrane protein
	Vitamin C transporter-2 (Na^+-dependent)	SLC23A2	
DHAA	GLUT2 (facilitative, Na^+-independent)	SLC2A2	Transmembrane protein
	GLUT8 (facilitative, Na^+-independent)	SLC2A8	
Vitamins A, D, E. K	Passive diffusion via lipid micelles	N/A	Passive diffusion via chylomicrons and VLDL
Choline	Organic cation transporter (OCT, Na^+-dependent)	SLC5A7	Transmembrane protein

[a] Cubilin for the intrinsic factor-vitamin B_{12} complex, and the asialoglycoprotein receptor for the haptocorrin-vitamin B_{12} complex.

Note: DHAA, dehydroascorbic acid; MCT1, monocarboxylate transporter 1; N/A, not applied; TPN1, vitamin B_6 transporter (transport of pyridoxine protein 1); VLDL, very low-density lipoprotein; * a splice variant of SLC19A1.

which consists of a substituted pyrimidine joined by a methylene bridge to a substituted thiazole (Figure 9.1). This vitamin has maximum absorption wavelengths of 235 and 265 nm, which correspond to its pyrimidine and thiazole moieties, respectively. The active form of vitamin B_1 is thiamin diphosphate (pyrophosphate), which is synthesized from thiamin by ATP-dependent thiamine diphosphotransferase (Manzetti et al. 2014). Thiamin is unstable, particularly under alkaline conditions because of its quaternary N, cleaved to the thiol form in water, and easily oxidized to thiamin disulfide and other derivatives (e.g., thiochrome).

Sources: Thiamin is present in a variety of foods of plant (particularly cereal grains) and animal origin, as well as in yeast. However, most manufactured foods contain only low concentrations of this vitamin (Combs and McClung 2017). Thiamin is concentrated in the outer layers of seeds, the germ, and in the growing areas of roots, leaves, and shoots. Yeasts (e.g., dried brewer's and baker's yeasts), fermentation products, unrefined cereal grains, and animal products (e.g., egg yolk, liver, kidney, and pork muscle) are good sources of thiamin. The synthetic vitamin is sold in the form of thiamin-HCl because it is more stable.

Absorption and transport: Thiamin pyrophosphate is released from dietary proteins during hydrolysis in the gastrointestinal tract and then hydrolyzed to thiamin. Thiamin is absorbed into enterocytes mainly through thiamine

TABLE 9.5
Content of Water-Soluble Vitamins and Choline in Feedstuffs[a]

Feedstuffs	Biotin	Choline	Folate	Niacin	Pantothenic Acid	Riboflavin	Thiamin	Vitamin B_6	Vitamin B_{12}
Alfalfa, 17% CP	0.54	1.40	4.36	38	29	13.6	3.4	6.5	0.00
Barley grain	0.14	1.03	0.31	55	8.0	1.8	4.5	5.0	0.00
Blood meal[b]	0.03	852	0.10	31	2.0	2.4	0.4	4.4	44
Blood meal[c]	0.28	485	0.40	23	3.7	3.2	0.3	4.4	---
Canola meal	0.98	6700	0.83	160	9.5	5.8	5.2	7.2	0.00
Casein, dried	0.04	205	0.51	1	2.7	1.5	0.4	0.4	---
Corn grain	0.06	620	0.15	24	6.0	1.2	3.5	5.0	0.00
CSM[d]	0.30	2933	1.65	40	12	5.9	7.0	5.1	0.00
Feather meal	0.13	891	0.20	21	10	2.1	0.1	3.0	78
Fish meal, Menhaden	0.13	3056	0.37	55	9.0	4.9	0.5	4.0	143
Flax meal	0.41	1512	1.30	33	14.7	2.9	7.5	6.0	0.00
MBM[e]	0.08	1996	0.41	49	4.1	4.7	0.4	4.6	90
Milk powder[f]	0.25	1393	0.47	12	36.4	19.1	3.7	4.1	36
Oat grain	0.24	946	0.30	19	13	1.7	6.0	2.0	0.00
Peanut meal, sol extr	0.39	1854	0.50	170	53	7.0	5.7	6.0	0.00
PBM[g]	0.09	6029	0.50	47	11.1	10.5	0.2	4.4	---
Rice bran	0.35	1135	2.20	293	23	2.5	22.5	26	0.00
Rice grain[h]	0.08	1003	0.20	25	3.3	0.4	1.4	28	0.00
Safflower meal, sol extr	1.03	820	0.50	11	33.9	2.3	4.6	12	0.00
Sorghum grain	0.26	668	0.17	41	12.4	1.3	3.0	5.2	0.00
SBM[i]	0.27	2794	1.37	34	16	2.9	4.5	6.0	0.00
Sunflower meal, sol extr	1.40	3791	1.14	264	29.9	3.0	3.0	11.1	0.00
Wheat bran	0.36	1232	0.63	186	31	4.6	8.0	12	0.00
Wheat grain[j]	0.11	778	0.22	48	9.9	1.4	4.5	3.4	0.00
Yeast (Brewers' dried)	0.63	3984	9.90	448	109	37.0	91.8	42.8	1

Source: National Research Council (NRC) 1998.

[a] Values are mg/kg (on an as-fed basis), except for vitamin B_{12} (μg/kg; on an as-fed basis).

[b] Conventional blood meal.

[c] Blood meal, spray or ring dried (93% dry matter).

[d] Cottonseed meal (solvent extract, 41% CP).

[e] Meat & bone meal (93% dry matter).

[f] Bovine milk (skim, dried; 96% dry matter).

[g] Poultry byproduct meal (93% dry matter).

[h] Polished, broken.

[i] Soybean meal (solvent extracted, 89% dry matter).

[j] hard red, winter.

Note: CP, crude protein; sol extr, solvent extraction.

transporters 1 and 2 (ThTr1 and ThTr 2), which are pH-dependent and Na^+-independent carriers (Said 2011). The rate of thiamin transport is greatest in the jejunum and ileum. There are reports that thiamin pyrophosphate itself can be directly transported by colonocytes via an energy-dependent, Na^+-independent transporter (SLC44A4) (Nabokina et al. 2014). Thiamin exits the intestinal epithelial cell through its basolateral membrane via a specific transporter. After entering the portal vein, thiamin is transported in the protein-bound form into various tissues (e.g., the liver), where it is converted back into thiamin pyrophosphate by the action of thiaminokinase (Brown 2014). A specific thiamin-binding protein has been identified in rat serum and liver, as well as hens' eggs (Combs and McClung 2017). A small amount of the body's thiamin also exists as thiamin monophosphate and thiamin triphosphate.

Functions: Thiamin diphosphate is a coenzyme in enzymatic reactions involving the transfer of an aldehyde unit: (a) oxidative decarboxylation of α-ketoacids for ATP production, and (b) transketolase reactions in the pentose phosphate pathway and for valine synthesis in microbes, plants, and yeasts (Bettendorff et al. 2014). In these reactions, thiamin diphosphate provides a reactive carbon on the thiazole that forms a carbanion, which is then free to add to the carbonyl group of α-keto acids. Examples of the oxidative decarboxylation of α-keto acids are catalyzed

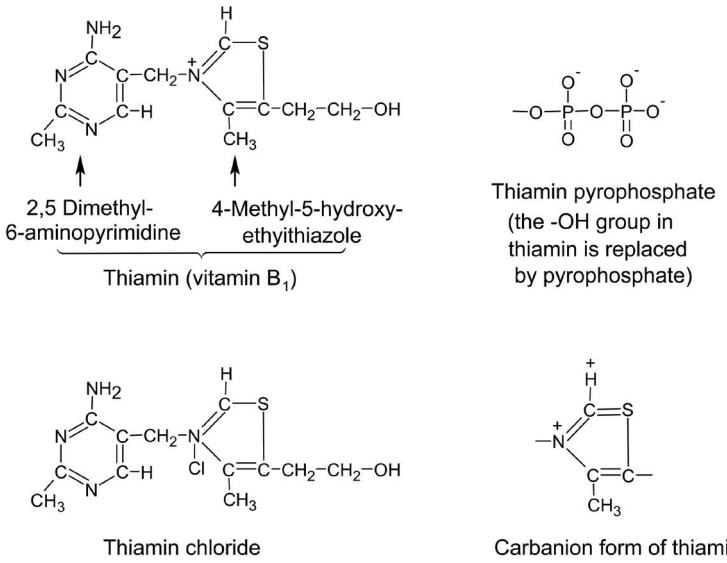


FIGURE 9.1 The chemical structure of thiamin (vitamin B_1). This vitamin consists of a pyrimidine ring and a thiazole ring that are linked by a methylene bridge. The pyrimidine and thiazole rings contain an amino group and a sulfur atom, respectively. Thiamin is chemically unstable in aqueous solutions.

by pyruvate, α-ketoglutarate (α-KG), or branched-chain α-ketoacid dehydrogenase (BCKAD) complex. Their reactions are outlined as follows:

$$\text{Pyruvate} \rightarrow \text{Acetyl-CoA} + CO_2\,(\text{pyruvate dehydrogenase complex})$$

$$\alpha\text{-Ketoglutarate} \rightarrow \text{Succinyl-CoA} + CO_2\,(\alpha\text{-ketoglutarate dehydrogenase complex})$$

$$\alpha\text{-Ketoacids of branched-chain amino acids (BCAAs)} \rightarrow \text{R-CO-CoA} + CO_2\,(\text{BCKAD})$$

Deficiency and diseases: Thiamin deficiency produces poor appetite, anorexia, weight loss, edema, heart problems, muscular and nerve degeneration, and progressive dysfunction of the neurological system. These deficiency symptoms are collectively called the disease of beriberi (Jones and Hunt 1983), a term originally used by the natives of Indonesia for "leg disorders, including weakness, edema, pain and paralysis". In humans, the populations most at risk of developing thiamin deficiency are chronic alcoholics, those overly dependent on polished rice (which lacks the outer germ layer) as a staple, and those consuming large amounts of improperly cleaned raw seafood due to the activity of thiaminase in bacteria (Vedder et al. 2015). Thiaminase activity is destroyed by high temperatures or cooking, and bacteria can be killed by alcohol. The ataxia and ocular symptoms associated with thiamin deficiency in alcoholics are known as Wernicke's disease, which is caused by decreased intake of nutrients, reduced absorption, and impaired ability to utilize the absorbed vitamin.

In addition to the general symptoms of beriberi, some characteristics of thiamin deficiency occur in farm animals. For example, pigs have inflammatory lesions of the gastrointestinal tract, diarrhea, occasional vomiting, dermatitis, hair loss, and respiratory dysfunction (Roche 1991). In chicks fed a thiamin-deficient diet for 10 days, they develop polyneuritis, which is characterized by head retraction (known as "stargazing") and paralysis. In ruminants, microbial synthesis of the vitamin in the digestive tract plus exogenous sources in the diet normally provides adequate amounts of thiamin (McDonald et al. 2021). However, when there is high activity of bacterial thiaminase in the rumen (e.g., lactic acidosis caused by feeding rapidly fermentable feeds), the deficiency condition known as cerebrocortical necrosis occurs, particularly in young animals (Jones and Hunt 1983). This disease is characterized by circling movements, head pressing, stargazing (a sign of neurological disorders), blindness, and muscular tremors. Thiamine-deficient cattle may display polioencephalomalacia (cerebrocortical necrosis). In horses and ruminants that consume thiaminase-containing bracken fern or horsetail fern, thiamin deficiency symptoms appear. In all animals, beriberi can be treated through the consumption of meat and vegetables, as well as dietary supplementation with thiamin (Pond et al. 1995).

Riboflavin (Vitamin B_2)

Structure: Riboflavin consists of a heterocyclic isoalloxazine ring attached to ribitol (Figure 9.2). It is a yellow and fluorescent pigment. This vitamin is relatively heat-stable in either acid or neutral solutions but is destroyed under alkaline conditions (Thakur et al. 2017). Riboflavin decomposes to lumiflavin and lumichrome in the presence of visible light.

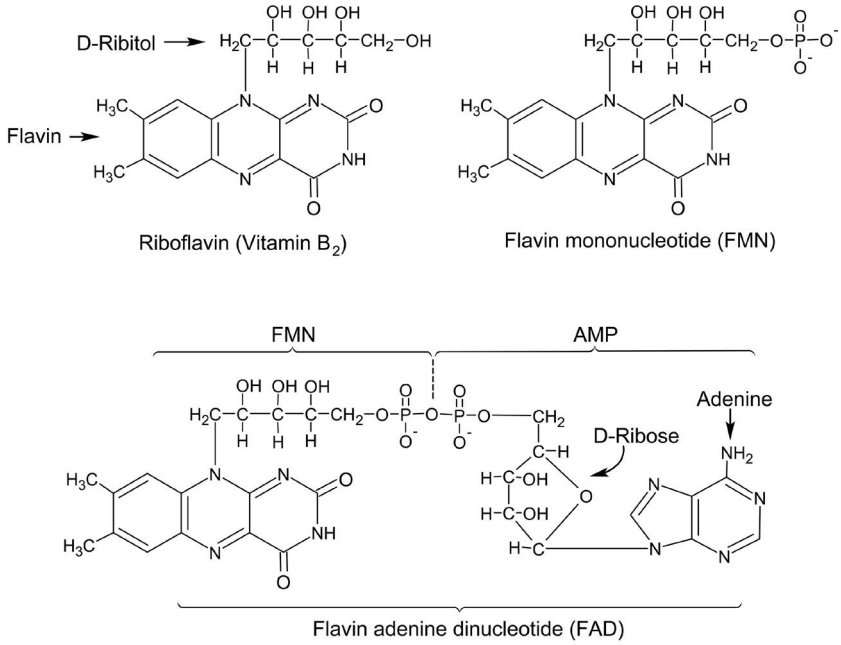


FIGURE 9.2 The chemical structure of riboflavin (vitamin B_2). This vitamin consists of a heterocyclic isoalloxazine ring attached to D-ribitol. The flavin moiety contains four nitrogen atoms. Riboflavin is a component of FMN and FAD. This vitamin is destroyed under alkaline conditions or by visible light.

Sources: Riboflavin is synthesized by plants and microbes but not by animal cells (García-Angulo 2017). Animals obtain riboflavin by consuming foods of plant and animal origins. The liver is an excellent source of riboflavin, and considerable amounts are present in kidneys, meat, and dairy products. Dark green leafy vegetables are good sources of riboflavin, but cereal grains are poor sources of riboflavin. Thus, riboflavin is widely present in foods (Thakur et al. 2017). In plant- and animal-sourced feedstuffs, this vitamin is almost exclusively bound to proteins in the form of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD).

Absorption and transport: Dietary FMN and FAD are hydrolyzed by intestinal phosphatases [alkaline phosphatase, FAD pyrophosphatase (which converts FAD into FMN), and FMN phosphatase] to generate free riboflavin prior to absorption by enterocytes. The major site of digestion is the jejunum. The digestibility of riboflavin from animal products is greater than that from plant products (Bates 1997). The apical membrane of the enterocytes contains riboflavin transporters (RF-1 and RF-2), which are Na^+-independent but moderately sensitive to pH (Said 2011). These cells can convert most of the absorbed riboflavin into FMN by ATP-dependent flavokinase, with FMN being further metabolized into FAD. The remaining free riboflavin exits the enterocyte across its basolateral membrane into the lamina propria through a specific transporter, enters the venous blood circulation, and is transported in plasma in both free and protein-bound forms (~50% in either form). About 80% of FMN in plasma (which is derived primarily from the lysis of cells) is bound to proteins through relatively weak hydrogen bonding. Both riboflavin and FMN in plasma bind weakly to globulins and fibrinogen, with albumin being the predominant protein.

Functions: Riboflavin is a major component of FMN and FAD. FMN is formed by ATP-dependent phosphorylation of riboflavin. FAD is synthesized from FMN with ATP by FAD synthase; in this reaction, the AMP moiety of ATP is transferred to FMN. FMN and FAD are co-factors of oxidoreductase enzymes known as flavoproteins. FMN and FAD are usually bound to their apoproteins via noncovalent linkage. Many flavoprotein enzymes contain one or more metals as essential cofactors and are known as metalloflavoproteins (Powers 2003). About 10% of FAD in the cell is covalently bound to enzymes such as succinate dehydrogenase and monoamine oxidase. In reactions catalyzed by flavoprotein enzymes, $FMNH_2$ and $FADH_2$ are produced from FMN and FAD, respectively. The flavoprotein enzymes are widespread in animals and participate in many biochemical reactions, including the synthesis of cholesterol, steroids, and vitamin D (Pinto and Cooper 2014). Examples of flavoproteins are given in Table 9.6. Murgia et al. (2023) recently reported that the human flavoproteome contains over 90 proteins that regulate a plethora of biochemical pathways involving nutrient metabolism and antioxidant signaling.

Deficiency and diseases: The signs of riboflavin deficiency in animals include: low appetite, reduced growth, reduced feed efficiency, lesions of the mouth known as cheilosis (cracks and fissures at the corners of the mouth) and angular stomatitis (inflammation of the oral mucosa), and reduced glutathione reductase activity in erythrocytes. Other symptoms include dermatitis, a rash on the

TABLE 9.6
Flavoprotein Enzymes in Animals

Enzyme	Cofactor	Function
L-Amino acid oxidase	FMN	L-Amino acids → α-Ketoacids + NH_3
NADH dehydrogenase[a]	FMN	Mitochondrial electron transport
Pyridoxine phosphate oxidase	FMN	Vitamin B_6 metabolism
D-Amino acid oxidase	FAD	D-Amino acids → α-Ketoacids + NH_3
Choline dehydrogenase	FAD	Choline degradation
Dihydrolipoyl dehydrogenase	FAD	Oxidative decarboxylation of α-ketoacids
Dimethylglycine dehydrogenase	FAD	Choline degradation
Fatty acyl-CoA dehydrogenase	FAD	Fatty acid oxidation
Glutathione reductase	FAD	Reduction of GSSG (oxidized glutathione) to 2 GSH
Methylene-H_4folate reductase	FAD	Production of 5-methyl-H_4folate
Mitochondrial glycerol-3-P dehydrogenase	FAD	Transfer of cytosolic reducing equivalents into mitochondria
Monoamine oxidase	FAD	Metabolism of neurotransmitters
Monomethylglycine dehydrogenase	FAD	Choline degradation
Nitric oxide synthase	FAD/FMN	L-Arginine + O_2 → L-Citrulline + NO
Sphinganine oxidase	FAD	Sphingosine synthesis
Succinate dehydrogenase[b]	FAD	Krebs cycle
Xanthine oxidase	FAD	Purine degradation

[a] Also known as NADH-ubiquinone reductase: NADH + H^+ + UQ ⇌ NAD^+ + UQH_2 (ubiquinol)
[b] Also known as succinate-ubiquinone reductase: Succinate + UQ ⇌ Fumarate + UQH_2

scrotum or vulva, photophobia, degenerative changes in the nervous system, endocrine dysfunction, and anemia (Jones and Hunt 1983). A severe deficiency, which can be induced experimentally in animals, results in failure to grow and reproduce, dermatitis, and nerve degeneration. In humans, symptoms of riboflavin deficiency (e.g., poor appetite, reduced growth, muscular weakness, gastrointestinal ulcers, anemia, edema, hepatic steatosis, paralysis, and photophobia) occur only after 3–4 months of deprivation of the vitamin (Combs and McClung 2017). In view of the many metabolic functions of riboflavin, it is surprising that a short-term deficiency of the vitamin is not lethal. This is perhaps because riboflavin, FMN, and FAD are tightly bound to intracellular proteins (including enzymes) in animals. The turnover rate of riboflavin may be very slow, and thus it may take a very long time to deplete this vitamin in cells. Nonetheless, a severe, long-term deficiency of riboflavin can cause death in animals.

In addition to these general symptoms, some characteristics of riboflavin deficiency occur in farm animals (Pond et al. 1995). For example, in pigs, deficiency symptoms include poor appetite, growth restriction, vomiting, skin eruptions, and eye abnormalities. For sows, dietary riboflavin is essential to maintain normal estrus activity and prevent premature birth. In chicks, riboflavin-deficient chicks grow poorly and develop "curled toe paralysis," in which chicks walk on their hocks with the toes curled inward (Baker et al. 1999). This is a specific symptom caused by peripheral nerve degeneration. In breeding hens, a deficiency of riboflavin causes decreased hatchability. Embryonic abnormalities occur, including the characteristic "clubbed down" symptom in which the down feather continues to grow inside the follicle, forming a coiled feather (Wyatt et al. 1973). In ruminants, riboflavin is synthesized by ruminal microbes. Thus, deficiencies of riboflavin are rare in ruminants with a functional rumen. However, riboflavin deficiencies have been reported for young calves and lambs, and symptoms include loss of appetite, diarrhea, and cheilosis (lesions in the corners of the mouth).

Niacin (Vitamin B_3)

Structure: Niacin is the generic name for nicotinic acid and nicotinamide (Figure 9.3). They are colorless and have an optical absorption maximum at 262 nm (Kamanna et al. 2013). Nicotinic acid is a monocarboxylic acid derivative of pyridine. Nicotinamide is a relatively stable vitamin and is not easily destroyed by heat, acids, alkalis, or oxidation.

Sources: Niacin is found widely as nicotinamide in animal tissues and as protein-bound nicotinic acid in plants (Table 9.3). In cereal grains, much of the niacin is present in a bound form that is not readily available to humans, pigs, and poultry. In corn, niacin is in a bound, unavailable form, niacytin, from which niacin can be released by pretreatment with hot alkali (calcium oxide). In Central America, soaking corn in alkaline water before the preparation of tortillas can effectively protect against pellagra (Carpenter 1983). Rich sources of niacin are liver, meats, yeasts (brewer's and baker's yeasts), groundnut, and sunflower meals.

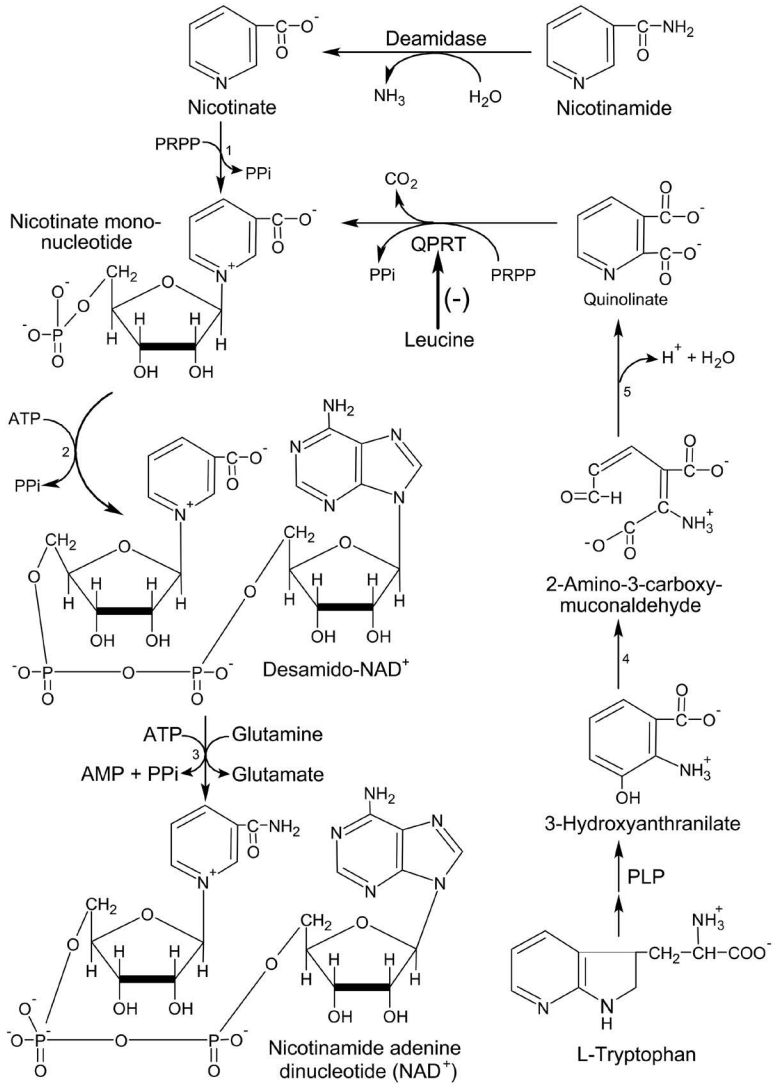


FIGURE 9.3 The chemical structure of niacin (vitamin B_3) and its synthesis from tryptophan in animals. Niacin is the generic name for nicotinic acid (a monocarboxylic acid derivative of pyridine) and its derivative, nicotinamide. Nicotinic acid is synthesized from tryptophan via a series of reactions in animals. PLP, pyridoxal phosphate; PRPP, 5-phosphoribosyl-1-pyrophosphate; QPRT, quinolinate phosphoribosyl transferase.

L-Tryptophan can be converted to nicotinate mononucleotide in most animal species, including humans, dogs, pigs, rats, and ruminants (Figure 9.3), but the efficiency of the conversion is low, especially in fish and poultry (e.g., 45:1 on a weight basis in chicks; Yao et al. 2011). Unlike dogs, cats cannot synthesize niacin from tryptophan due to (a) a high activity of picolinate carboxylase (also known as 2-amino-3-carboxymuconic acid-6-semialdehyde decarboxylase, ACMSD) to divert 2-amino-3-carboxymuconate semialdehyde (an intermediate for the formation of nicotinate mononucleotide) into α-ketoadipate, and (b) a low activity of quinolinate phosphoribosyltransferase for converting 2-amino-3-carboxymuconate semialdehyde into nicotinate mononucleotide (Badawy 2017). For comparison, the hepatic activity of picolinate carboxylase (μmol/g wet tissue/h) in animals was: cat, 50.5; lizard, 29.6; frog, 13.7; cattle, 8.3; pig, 7.1; pigeon, 7.0; rabbit, 4.3; mouse, 4.2; guinea pig, 4.0; chicken, 3.9; man, 3.2; hamster, 3.1; toad, 3.1; and rat, 1.6 (Ikeda et al. 1965). These data indicate a species difference in niacin synthesis among animal species.

Absorption and transport: Hydrolysis of the niacin-containing pyridine nucleotides, such as the oxidized and reduced forms of nicotinamide adenine dinucleotide [(NAD(H)] and the oxidized and reduced forms of nicotinamide adenine dinucleotide phosphate [NADP(H)], by NAD(P)-glycohydrolase (an intestinal mucosal enzyme) generates nicotinamide and ADP-ribose. Nicotinamide and nicotinic acid are absorbed by enterocytes through a specific high-affinity (apparent K_m of 0.53 μM), acidic-pH-dependent, and Na^+-independent carrier (Nabokina et al. 2005). The Na^+-coupled monocarboxylate transporter

SLC5A8 (apparent K_m of 0.23–0.3 mM) may play a role in the absorption of high pharmacological doses of niacin. Niacin exits the enterocyte through its basolateral membrane into the lamina propria through a specific transporter. This vitamin is transported in plasma as nicotinic acid and nicotinamide in the unbound form and is taken up by tissues via the anion transport system (e.g., SLC5A8 in erythrocytes), Na^+-dependent transport system (e.g., renal tubules), and energy-dependent system (e.g., brain; Kamanna et al. 2013). Inside the cells, nicotinic acid (nicotinate) is converted to NAD and NADP by enzymes present in the cytosol of most cells (Figure 9.4).

Functions: Niacin is a component of NAD and NADP (Bogan and Brenner 2008). Nicotinic acid (nicotinate) is the form of niacin required for the synthesis of NAD and NADP by enzymes present in the cytosol of most cells. NAD and NADP are coenzymes for many oxidoreductase enzymes involved in the metabolism of carbohydrates, fatty acids, ketone bodies, amino acids, and alcohol (MacKay et al. 2012). NAD(P)-dependent reactions involve the transfer of two electrons and two protons, and NAD(P) accepts two of the electrons and one of the protons, with the second proton remaining in solution. Because of its roles in nutrient metabolism, niacin (an oral dose of 1–4 g/day) has been used since 1955 to treat dyslipidemia in adult humans, with beneficial effects including decreases in plasma concentrations of triacylglycerols (by 20–50%) and low-density lipoprotein (LDL) cholesterol (by 10–25%), and increases in plasma concentrations of HDL (by 10–30%; Meyers et al. 2003). However, high doses of nicotinic acid result in cutaneous symptoms with flushing episodes and itching, as well as hepatotoxicity, insulin resistance, hyperglycemia, and hyperuricemia. The flushing symptoms can be alleviated by taking a niacin supplement immediately after meals.

In addition to its usual role as an oxidant or reductant, NAD^+ serves as a substrate for poly(ADP-ribose) polymerase, which catalyzes the attachment of ADP-ribose to various chromosomal proteins. Thus, NAD^+ is used in the post-translational modification of a variety of proteins. In addition, NADPH is a cofactor for the reduction of disulfide bonds in redox proteins such as glutaredoxin and thioredoxin.

$$NAD(P)^+ + AH_2 \rightleftharpoons NAD(P)H + H^+ + A$$

Deficiency and diseases: Severe niacin deficiency results in a disease known as pellagra, first noted in the 1760s in Spain and Italy, where corn (not treated with calcium oxide) was popular. Affected people consumed little or no meat. Given the roles of ATP and redox signaling in cellular metabolism, it is not surprising that pellagra is characterized by severe dermatitis and fissured scabs, diarrhea, and mental depression. The disease is also associated with the four D's: dermatitis, diarrhea, dementia, and death (Carpenter 1983). Dependence on sorghum is also pellagragenic, not because of low tryptophan but because of sorghum's high leucine content (Gopalan and Jaya Rao 1975). Excess dietary leucine can bring about niacin deficiency by inhibiting quinolinate phosphoribosyl transferase (QPRT), a key enzyme in the conversion of tryptophan to NAD. Because vitamin B_6 is involved as a cofactor in the synthesis of niacin from tryptophan, vitamin B_6 deficiency can potentiate a deficiency in niacin. Other conditions leading to symptoms of pellagra include administration of some drugs such as the antituberculosis drug isoniazid (which

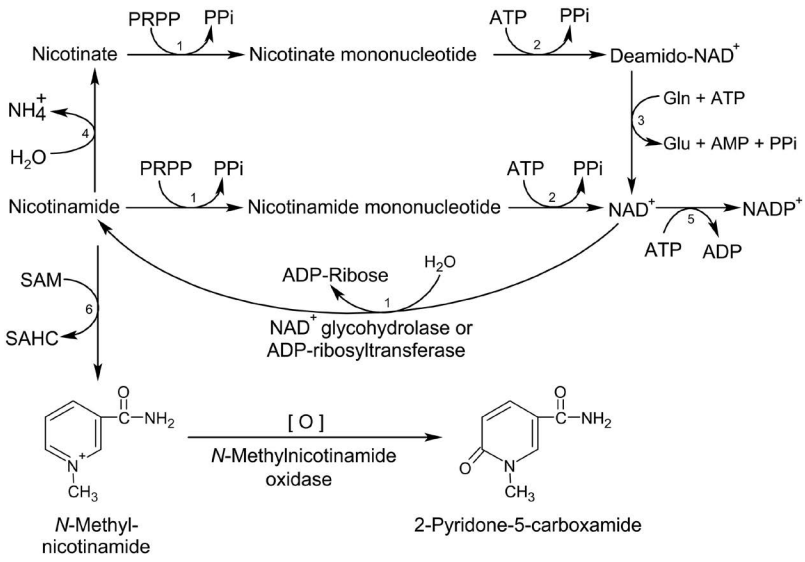


FIGURE 9.4 Nicotinate metabolism in animals. Nicotinate is converted into NAD^+ and $NADP^+$ via ATP-dependent reactions. Nicotinic acid and nicotinamide are interconvertible in animals. PRPP, 5-phosphoribosyl-1-pyrophosphate; SAHC, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine.

directs tryptophan metabolism to serotonin formation). Hartnup disease is a disorder in which tryptophan absorption by the small intestine is impaired (Bogan and Brenner 2008). In pigs, deficiency symptoms include poor growth, anorexia, enteritis, vomiting, and dermatitis (Roche 1991). In poultry, niacin deficiency causes bone disorders, feathering abnormalities, and inflammation of the mouth and upper part of the esophagus (Jones and Hunt 1983). When pigs and poultry are fed diets with a high maize content, niacin deficiency may occur, but niacin deficiency rarely occurs in ruminants with a functional rumen (Niehoff et al. 2009). Pellagra in nonruminants can be cured by dietary supplementation with niacin.

Pantothenic Acid (Pantothenate)

Structure: Pantothenic acid (vitamin B_5) is an amide of pantoic acid and β-alanine (Figure 9.5).

The anion form of this vitamin is called pantothenate, and its more stable form (commercially available) is D-calcium pantothenate. Thus, vitamin B_5 can be considered as a peptide consisting of β-alanine and a butyrate derivative. Pantothenic acid is a yellow, viscous oil, but its salts (with Ca and other minerals) are white crystalline substances. In the dry form, the salts of pantothenic acid are stable at 25°C but are hygroscopic.

Sources: Pantothenic acid is widely distributed in nature, and thus its name was derived from the Greek *pantothen*, meaning "present everywhere". This vitamin is synthesized by plants and microbes (Figure 9.6) but not by animal cells (Coxon et al. 2005). Pantothenic acid is present in foods primarily as coenzyme A (CoA) and, to a lesser extent, protein-bound 4'-phosphopantetheine. In the latter, a 4-phosphopantetheine moiety is linked via its phosphate group to the hydroxyl group of serine in protein. Pantothenic acid is particularly abundant in animal products (e.g., liver, egg yolk), yeast, groundnuts, whole grain cereals, alfalfa, and legumes. Long-term dietary supplementation with high doses of pantothenic acid (6 g/day for 16 weeks) in adult humans did not affect intramuscular CoA-SH concentration or muscle strength (Whitfield et al. 2021).

Absorption and transport: CoA and protein-bound 4'-phosphopantetheine in diets do not readily cross cell membranes (including those of the intestine) and therefore must be hydrolyzed to pantothenic acid prior to its intestinal absorption. Specifically, in the lumen of the small intestine, dietary CoA is hydrolyzed to dephospho-CoA, 4'-phosphopantetheine, and pantetheine (Shibata et al. 1983). The responsible two phosphatases are (a) pyrophosphatase, which acts on diphosphate bonds; and (b) orthophosphatase (also known as orthophosphoric monoester phosphohydrolase), which acts on orthophosphoric monoesters. The protein-bound 4'-phosphopantetheine in the diet is hydrolyzed by orthophosphatase to release 4'-phosphopantetheine. The 4'-phosphopantetheine is then dephosphorylated by pyrophosphatase or phosphatase to yield pantetheine, which is hydrolyzed by the intestinal pantetheinase into pantothenic acid and cysteine. Ultimately, pantothenic acid is readily absorbed into the enterocytes through the Na^+-dependent multivitamin transporter (Vadlapudi et al. 2012). The exit of this vitamin across the basolateral membrane of the enterocyte into the lamina propria is mediated by a Na^+-independent carrier. In plasma, pantothenic acid is transported in the free form. Interestingly, erythrocytes carry most of this vitamin in the blood.

Functions: In animal cells, pantothenic acid is converted to 4'-phosphopantetheine, which is the prosthetic group of CoA, acyl carrier protein (ACP) of

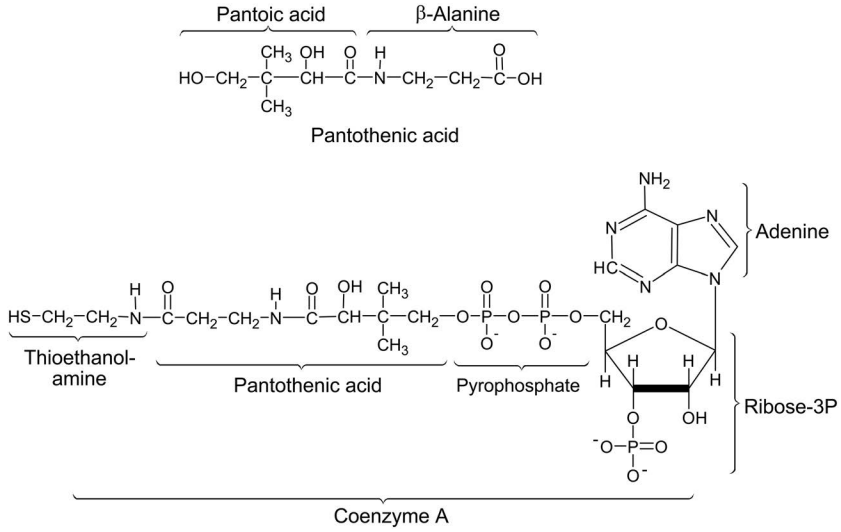


FIGURE 9.5 The chemical structure of pantothenic acid (vitamin B_5). This vitamin (dihydroxy-β,β-dimethylbutyryl-β-alanine) is an amide of pantoic acid and ß-alanine. Pantothenic acid is stable at neutral pH but is readily destroyed by acid, alkali, and heat.

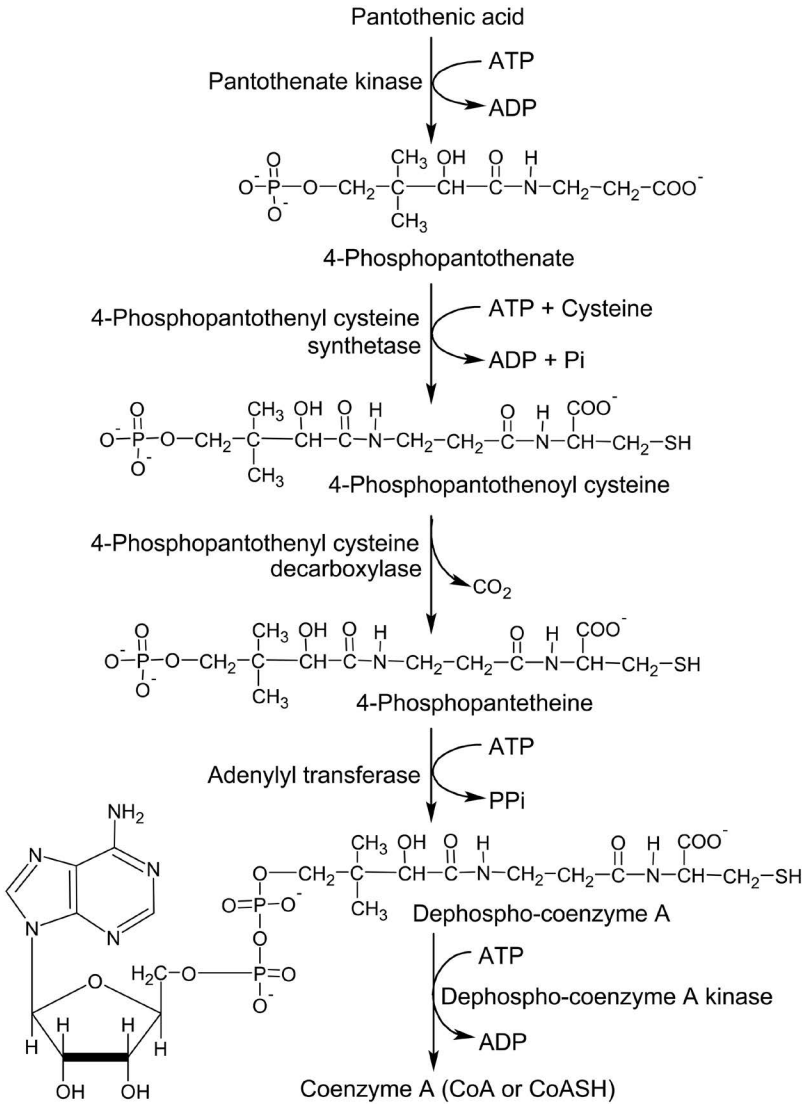


FIGURE 9.6 Conversion of pantothenic acid into coenzyme A (CoA) in animal cells. Four of the five reactions require the hydrolysis of ATP to provide energy. Phosphopantothenylcysteine decarboxylase is a carboxy-lyase that cleaves a carbon-carbon bond and does not require vitamin B_6 for its catalytic activity.

the fatty acid synthase complex (Figure 9.7), and N^{10}-formyltetrahydrofolate dehydrogenase. The latter catalyzes the conversion of N^{10}-formyltetrahydrofolate to tetrahydrofolate, an essential cofactor in the metabolism of nucleic acids and amino acids (Smith and Song 1996). CoA is involved in many reactions: the Krebs cycle; the metabolism (synthesis and oxidation) of fatty acids, amino acids, and ketone bodies; syntheses of cholesterol, acetylcholine, and heme; and the conjugation of bile acids with glycine or taurine (Zempleni et al. 2013). ACP plays

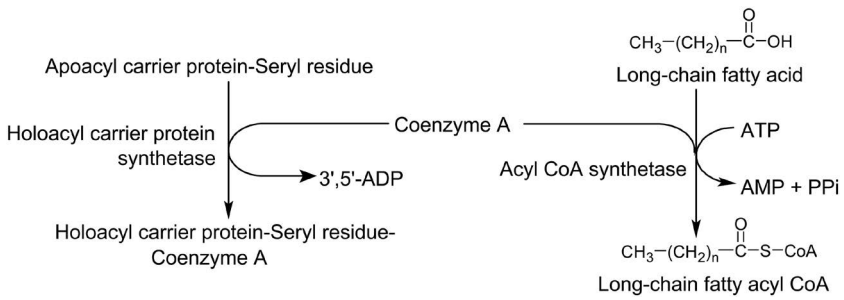


FIGURE 9.7 Role of coenzyme A (CoA) as a coenzyme for holoacyl carrier protein synthetase and acyl-CoA synthetases in animal cells. As a major carrier of activated acyl groups, CoA forms thioester bonds with carbon chains. This facilitates enzyme recognition and biotransformation reactions.

an important role in fatty acid synthesis. Dietary pantothenic acid is essential for all mammalian species studied (including humans, pigs, dogs, rodents, and cats) as well as for poultry and fish (Combs and McClung 2017).

Deficiencies and diseases: Deficiency of pantothenic acid is rare because the substance is widely distributed in foods. Deficiency of pantothenic acid can be induced experimentally, which results in a loss of appetite, slow growth, skin lesions, loss of hair, depression, fatigue, ulceration of the intestine, weakness, and eventually death (Zempleni et al. 2013). Interestingly, the "burning foot syndrome" has been ascribed to pantothenic acid deficiency in prisoners of war (Berdanier 1998). In pigs, a deficiency of pantothenic acid results in poor growth, diarrhea, loss of hair, scaliness of the skin, and a characteristic "goose-stepping" gait. In severe cases, pigs cannot stand (Roche 1991). Deficiency of this vitamin has been reported for commercial herds of pigs. In poultry, deficiency of this vitamin results in growth restriction, dermatitis, and reduced hatchability (Jones and Hunt 1983). Deficiency of pantothenic acid is rare in ruminants because ruminal microbes can synthesize all B-complex vitamins (Ragaller et al. 2011).

Pyridoxal, Pyridoxine, and Pyridoxamine (Vitamin B_6)

Structure: The name pyridoxine given to vitamin B_6 reflects its structure as a pyridine derivative. Three biologically active forms of vitamin B_6 are now recognized: pyridoxal, pyridoxine, and pyridoxamine (Figure 9.8). These three forms are interconvertible in animals, with the parent substance of vitamin B_6 being pyridoxine (Hayashi 1995). Active vitamin B_6 is mainly pyridoxal phosphate. Pyridoxine, pyridoxal phosphate, and pyridoxamine phosphate are the main representatives of vitamin B_6 in the diet, and all three have equal vitamin activity because they can be interconverted in the body (Figure 9.9). The amine and aldehyde derivatives of pyridoxine are less stable than pyridoxine. Vitamin B_6 is destroyed by heat.

Sources: The vitamin is present in plants mainly as pyridoxine glycoside, whereas animal products contain pyridoxine, pyridoxal, and pyridoxamine (Ueland et al. 2015). Poultry, fish, liver, eggs, meat, milk, yeast, bananas, vegetables, cereal grains, and nuts are good sources of vitamin B_6. A large proportion of vitamin B_6 in foods is bound to protein via the ε-amino groups of lysine residues and the sulfhydryl groups of cysteine residues. In plants, this vitamin is also present in glycosylated forms [e.g., 5′-O-(β-D-glucopyranosyl) pyridoxine]. Pyridoxine is the major vitamin B_6 in plant-sourced foods, whereas pyridoxal and pyridoxamine are the primary forms of the vitamin in animal-sourced foods. Pyridoxine-HCl is stable at 25°C and used for dietary supplementation.

Absorption and transport: Much of the vitamin B_6 in foods is not biologically available due to low digestibility. This vitamin in glycosides and proteins must first be released through hydrolysis before its intestinal absorption. The enterocyte membrane-bound alkaline phosphatase hydrolyzes pyridoxal phosphate and pyridoxamine phosphate into pyridoxal and pyridoxamine, respectively. All forms of vitamin B_6 (i.e., pyridoxal, pyridoxine, and pyridoxamine) are absorbed into the enterocytes (mainly of the jejunum and ileum) through a H^+-dependent but Na^+-independent carrier (Said 2011). At present, little is known about the molecular identity of the transport protein on the basolateral membrane of the enterocyte. In the small-intestinal mucosa, pyridoxal is phosphorylated by pyridoxal kinase to pyridoxal phosphate, whereas pyridoxine and pyridoxamine are metabolized to pyridoxal phosphate. Pyridoxal phosphate is the major form of the vitamin transported in the blood and is tightly bound to proteins (primarily albumin in plasma, hemoglobin in erythrocytes, and surface proteins in platelets) via the Schiff-base linkage. A smaller amount of pyridoxal, pyridoxine, and pyridoxamine is also transported in plasma in the protein-bound forms. Phosphorylation of the absorbed pyridoxine, pyridoxal, and pyridoxamine and their binding to proteins, which occur in both the small-intestinal mucosa and blood, play important roles in the intestinal absorption of vitamin B_6.

Extraintestinal cells take up vitamin B_6 through a specific membrane transporter. The rate of uptake of pyridoxal by animal cells is greater than that of pyridoxal phosphate (Combs and McClung 2017). Most tissues contain pyridoxal kinase, which catalyzes the phosphorylation by ATP of the unphosphorylated forms of the vitamin to their respective phosphate esters. Pyridoxic acid is the end product of vitamin B_6 degradation and is excreted in urine.

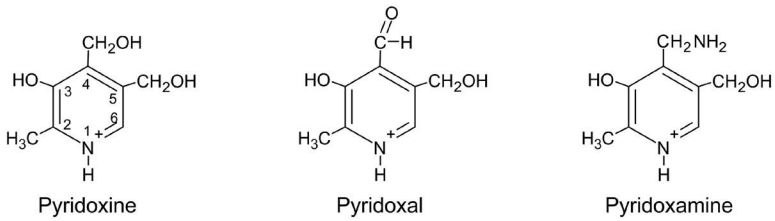


FIGURE 9.8 The chemical structure of vitamin B_6 as a pyridine derivative. The three biologically active forms of vitamin B_6 are pyridoxal, pyridoxine, and pyridoxamine (an amine). Pyridoxal, pyridoxine, and pyridoxamine contain an aldehyde group (–CHO), a methylhydroxyl group ($–CH_3OH$), and an aminomethyl group ($–CH_3NH_2$), respectively.

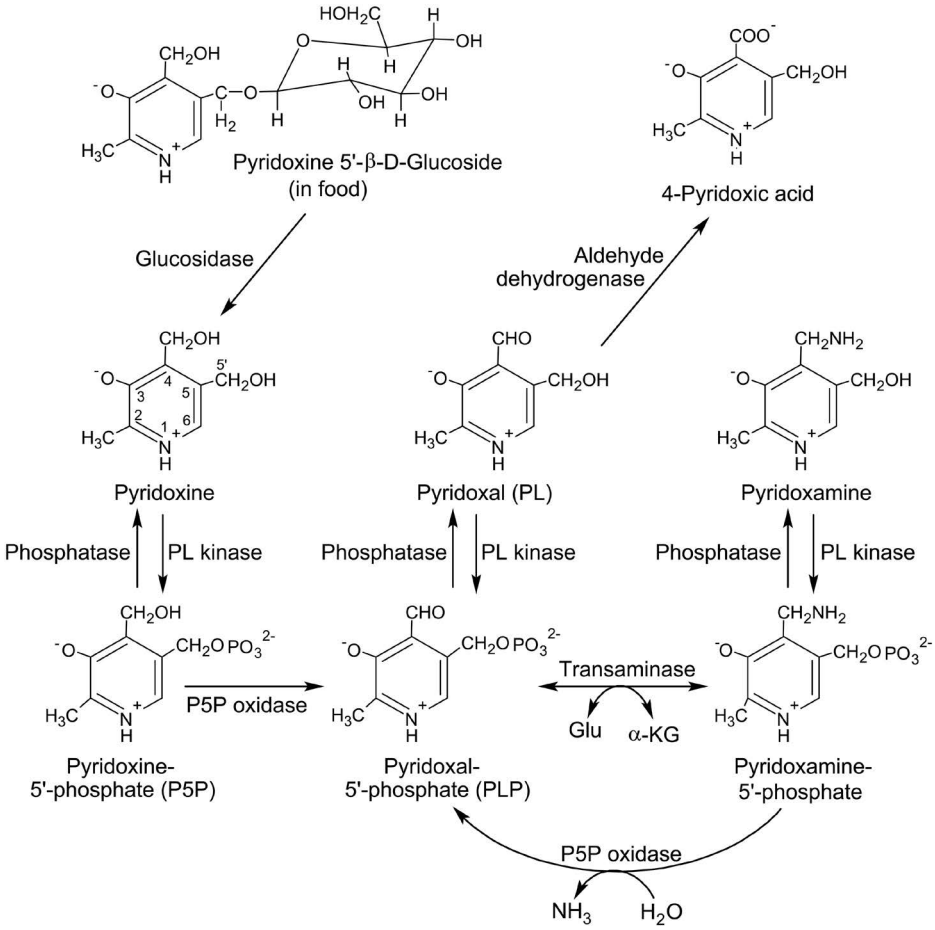


FIGURE 9.9 Interconversion of pyridoxal, pyridoxine, and pyridoxamine in animal cells. The required reactions are catalyzed by a series of enzymes, including transaminases, kinases, oxidases, and phosphatases. Pyridoxal is oxidized by aldehyde dehydrogenase to 4-pyridoxic acid. Free pyridoxal and 4-pyridoxic acid can exit cells.

Functions: Pyridoxal phosphate is the coenzyme of several enzymes involved in amino acid metabolism (Table 9.7). By entering into a Schiff base combination between its aldehyde group and the amino group of an α-amino acid, pyridoxal phosphate can facilitate changes in the three remaining bonds of the α-amino carbon to allow (a) transamination, (b) decarboxylation, or (c) threonine aldolase activity (Figure 9.10). The mechanism whereby vitamin B_6 participates in amino acid transamination is outlined in Figure 9.11. In addition, pyridoxal phosphate is covalently bound to glycogen phosphorylase via an initial Schiff base with the ε-amino group of a lysine residue of the enzyme and plays an important role in stabilizing the conformational structure of the enzyme (Helmreich 1992). Muscle glycogen phosphorylase accounts for 70–80% of total vitamin B_6 in the body. Furthermore, through participation in one-carbon metabolism that regulates DNA methylation, dietary vitamin B_6 may increase gene expression and protein synthesis in tissues. Such an effect was demonstrated for the liver of animals, including Atlantic salmon (Saito et al. 2024).

Deficiencies and diseases: Amino acid metabolism is impaired in animals with vitamin B_6 deficiency. The symptoms of vitamin B_6 deficiency are generally similar among animal species: hyperammonemia,

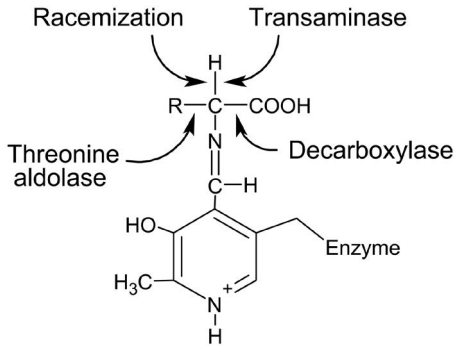


FIGURE 9.10 Roles of vitamin B_6 in amino acid transamination, certain decarboxylation, and threonine degradation, as well as some deamination and racemization reactions. Pyridoxal phosphate is the active form of vitamin B_6 for these reactions.

TABLE 9.7
Pyridoxal Phosphate-Dependent Enzymes in Animals

Enzyme	Function
Amino acid racemase	L-Amino acid ⇌ D-Amino acid
Amino acid (AA) transaminase	AA_1 + α-$Ketoacid_1$ ⇌ AA_2 + α-$Ketoacid_2$
Aminolevulinic acid (AL) synthase	Heme synthesis; Succinyl-CoA + Glycine → AL + CO_2
Arginine decarboxylase	Arginine → Agmatine (Argamine) + CO_2
Aspartate decarboxylase	Aspartate → ß-Alanine + CO_2
Cystathionase	Cystathionine → Cysteine + α-Ketobutyrate
Cysteine sulfinate decarboxylase	Cysteine sulfinate → Taurine
Cystathionine synthase	Homocysteine + Serine → Cystathionine
DOPA decarboxylase	Dihydroxyphenylalanine (DOPA) → Dopamine + CO_2
Glutamate (Glu) decarboxylase	Glutamate → γ-Aminobutyrate + CO_2
Glycine cleavage system	Glycine + H_4-folate → CO_2 + NH_3 + N^5N^{10}-CH_2-H_4 folate
Glycogen phosphorylase	Glycogen → Glucose-1-P
Histidine decarboxylase	Histidine → Histamine + CO_2
Ketosphinganine synthase[a]	Palmitoyl-CoA + Serine → Ketosphinganine + CO_2
Kynureninase	3-Hydroxykynurenine + H_2O → 3-Hydroxyanthranilate + Ala
Kynurenine transaminase	Kynurenine + α-Ketoglutarate → Kynurenate + Glutamate
Methionine (Met) synthase	Homocysteine + 5-Methyl-H_4folate → Met + H_4folate
Ornithine aminotransferase	Ornithine + α-Ketoglutarate ⇌ Pyrroline-5-carboxylate + Glu
Ornithine decarboxylase	Ornithine → Putrescine + CO_2
Phosphatidylserine decarboxylase	Synthesis of phosphatidylethanolamine
Serine hydroxymethyltransferase	Serine + H_4-folate → Glycine + N^5,N^{10}-Methylene-H_4-folate
Threonine aldolase	Threonine → Glycine + Acetaldehyde

[a] Also known as 3-dehydrosphinganine-L-serine synthetase.
Note: Ala, alanine; DOPA, L-3,4-dihydroxyphenylethyl amine; H_4folate, tetrahydrofolate.

hyperhomocysteinemia, oxidative stress, cardiovascular dysfunction, alterations in the skin and nervous tissue, anemia, growth restriction, depression, confusion, seizure (a neurological problem), skin lesions, and sometimes convulsion (Ahmad et al. 2013). Vitamin B_6 deficiency is often found in chronic alcoholics because of (a) a low intake of the vitamin, (b) alcohol-induced impairments in the metabolism of the vitamin, and (c) reduced hydrolysis of the phosphate bond of pyridoxal phosphate (Halsted and Medici 2011). Vitamin B_6 deficiency can occur in nursing infants, whose mothers are depleted of the vitamin due to its inadequate intake. A widely used antituberculosis drug, isoniazid, can induce vitamin B_6 deficiency by forming a hydrazone with pyridoxal. In pigs, deficiency of vitamin B_6 results in poor growth, convulsions, impaired immune function, and anemia with high levels of iron in the spleen (Roche 1991).

Biotin

Structure: Biotin is an imidazole derivative widely distributed in foods of animal and plant origin. It is a sulfur (S)-containing vitamin (Figure 9.12). In the crystalline form, this vitamin is stable at 25°C. In an aqueous solution, biotin is sensitive to degradation under oxidative, strongly acidic, or strongly alkaline conditions (Waldrop et al. 2012). In plant and animal tissues, biotin is usually bound to its enzymes through an amide bond between the C-2 of its thiophane nucleus and the ε-amino group of a protein-bound lysine residue (Berdanier 1998).

Sources: Biotin is synthesized by plants, bacteria, and fungi but not by animal cells (Fugate and Jarrett 2012). This vitamin is widely distributed in natural foods, in both free and enzyme-bound forms, but mostly in very low concentrations (McMahon 2002). Liver, milk, egg yolk, dried yeast, oilseeds, alfalfa, and vegetables are rich sources of biotin. In plant-sourced foods (e.g., barley and wheat), much of the protein-bound vitamin is not released during digestion and therefore is biologically unavailable. A large portion (about 50%) of the requirement for biotin may be met by synthesis from intestinal bacteria. The bioavailability of biotin in feedstuffs for intestinal absorption is usually <50% (Pond et al. 1995).

Absorption and transport: In the lumen of the small intestine, protein-bound biotin is digested by gastrointestinal proteases and peptidases to form biotinyl-L-lysine (biocytin). This lysyl–biotin adduct is further cleaved into lysine and biotin by biotinidase, an enzyme present on the apical membrane of the enterocytes. Milk and blood also contain biotinidase to release biotin. Free biotin is

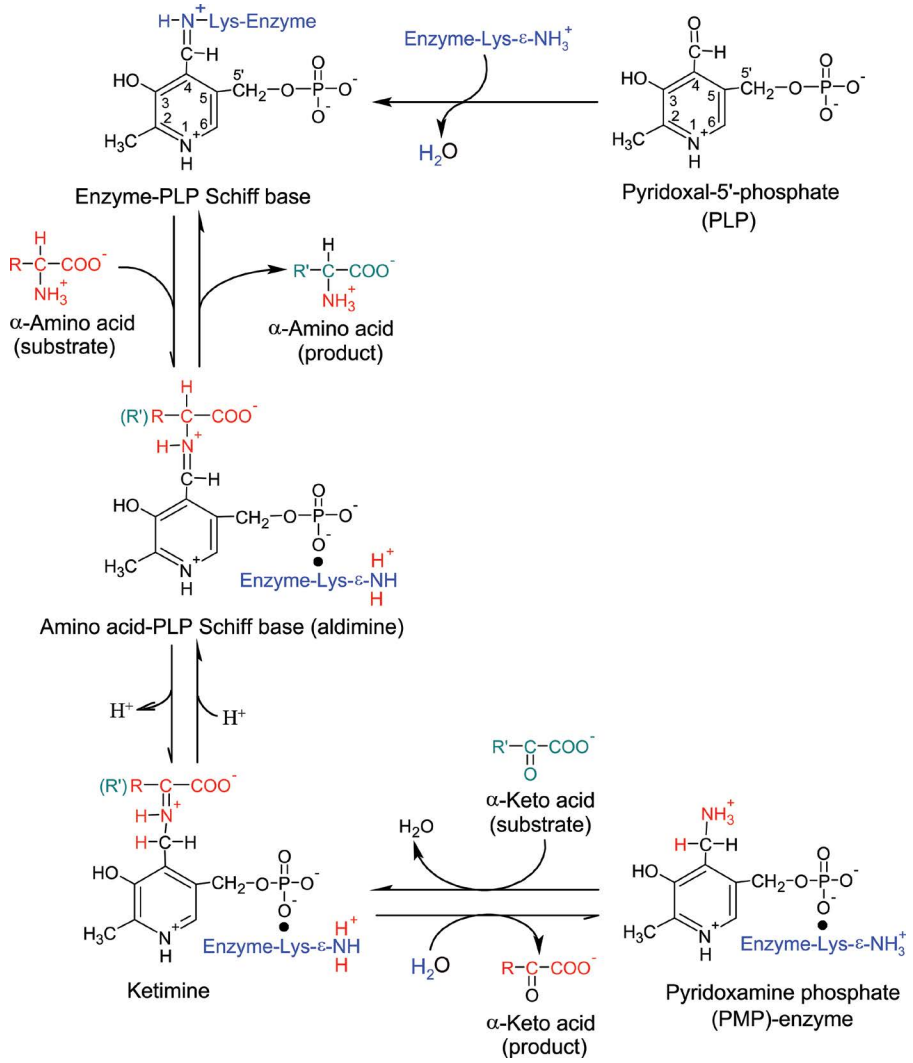


FIGURE 9.11 The mechanism whereby vitamin B_6 participates in amino acid transamination. In this reaction, pyridoxal phosphate (PLP) forms an enzyme–PLP Schiff base, which then accepts an amino acid to form an amino acid–PLP Schiff base intermediate. The latter is rearranged to a ketimine, which is then hydrolyzed to an α-keto acid and a pyridoxamine phosphate-enzyme complex. A series of reverse reactions transfer the amino group of pyridoxamine phosphate to a new α-ketoacid to form a new amino acid.

absorbed into the enterocytes through the Na^+-dependent multivitamin transporter (Quick and Shi 2015). This transporter is also expressed on the apical membrane of the epithelial cells of the large intestine. Biotin exits the enterocytes and colonocytes through their basolateral membranes into the lamina propria via a specialized Na^+-independent transporter. Intestinal biotin uptake is upregulated by a dietary deficiency of biotin, but down-regulated by a dietary excess of biotin (Said 2011). This adaptive regulation may be mediated via changes in the

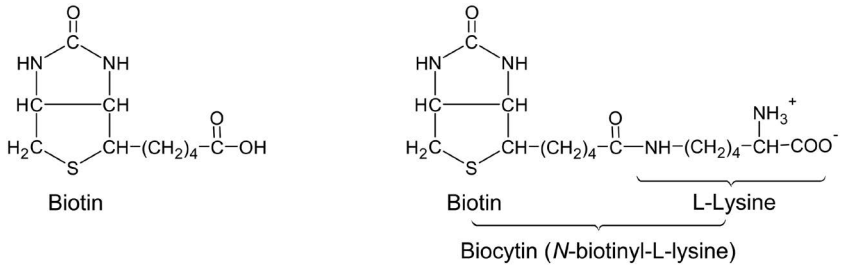


FIGURE 9.12 The chemical structure of biotin. It is an organic heterobicyclic compound, with a sulfur-containing tetrahydrothiophene ring fused to a ureido group, and with a valeric acid side chain attached to the thiophene ring. This vitamin is an imidazole derivative.

TABLE 9.8
Biotin-Dependent Enzymes in Animal and Microbes

Enzyme	Function
Both animals and microbes	
Pyruvate carboxylase	Pyruvate + CO_2 → Oxaloacetate (gluconeogenesis; and provision of OAA for the Krebs cycle)
Acetyl-CoA carboxylase	Acetyl-CoA + CO_2 → Malonyl-CoA (fatty acid synthesis, Regulation of CPT I activity in the outer mitochondria membrane, Fuel sensing mechanism)
Propionyl-CoA carboxylase	Propionyl-CoA + CO_2 → D-Methylmalonyl-CoA (gluconeogenesis from propionate)
ß-Methylcrotonyl-CoA carboxylase	ß-Methylcrotonyl-CoA + CO_2 → ß-Methylglutaconyl-CoA (catabolism of leucine and certain isoprenoid compounds)
Microbes	
Urea amidolyase (urea carboxylase)	ATP + Urea + HCO_3^- → ADP + Phosphate + Urea-1-carboxylate (Providing a source of nitrogen in microbial metabolism)
Geranyl-CoA carboxylase	ATP + Geranoyl-CoA + HCO_3^- → ADP + Phosphate + 3-(4-Methylpent-3-en-1-yl) pent-2-enedioyl-CoA

number (and/or activity) of the biotin transporters, but not in their affinity.

Biotin is transported in plasma in its free form. About 12% of the total biotin in plasma is covalently bound, and another 7% is reversibly bound to proteins (Zempleni et al. 2013). Extraintestinal tissues take up biotin through specific transporters, including the Na^+-dependent multivitamin transporter (SLC5A6) (Uchida et al. 2015). Inside the cells, biotin is attached to its apoenzymes through the formation of an amide linkage with the ε-amino group of a specific lysine residue. This biochemical reaction, which is called protein biotinylation, is catalyzed by biotin protein ligase.

Functions: In mammals, birds, fish, and microbes, biotin is a coenzyme of four ATP-dependent carboxylases: pyruvate carboxylase, acetyl-CoA carboxylase, propionyl-CoA carboxylase, and β-methylcrotonyl-CoA carboxylase (Table 9.8). Biotin is covalently bound to the enzymes via the terminal amino group of a lysine residue (Figure 9.13). Two additional biotin-dependent carboxylases are present in microbes but not in animal cells: urea amidolyase (which enables microbes to hydrolyze urea to ammonia and CO_2) and geranyl-CoA carboxylase (which participates in isoprenoid catabolism in microbes; Waldrop et al. 2012). Furthermore, dietary biotin plays an important role in increasing the expression of acetyl-CoA carboxylase 2 in the hypothalamus, leading to reduced feed intake (Sone et al. 2016). Thus, this vitamin may be beneficial for the prevention and treatment of obesity in animals.

Deficiencies and diseases: Biotin deficiency is rare but can occur in humans consuming raw eggs (Zempleni et al. 2013). This is because egg white contains a heat-labile protein (avidin), which binds very tightly with biotin via a noncovalent linkage, thereby preventing the absorption of biotin by the intestine. Avidin is destroyed during cooking. Absence of carboxylate synthase, which attaches biotin to the lysine residue of the biotin carrier protein, results in multiple carboxylase deficiencies and causes biotin deficiency symptoms (Tong 2013). A rare genetic deficiency of biotinidase also causes biotin deficiency in 1/40,000 infants. The symptoms of biotin deficiency include growth restriction, depression, muscle pain, dermatitis, neurological disorders, anorexia, and extreme tiredness. In pigs, biotin deficiency results in poor growth, foot lesions, hair loss, dry scaly skin, and impaired reproductive function (Roche 1991). In poultry, biotin deficiency causes reduced growth, dermatitis, leg bone abnormalities, cracked feet, poor feathering, and fatty liver and kidney syndrome (Jones and Hunt 1983). The fatty liver and kidney syndrome, which occurs mainly

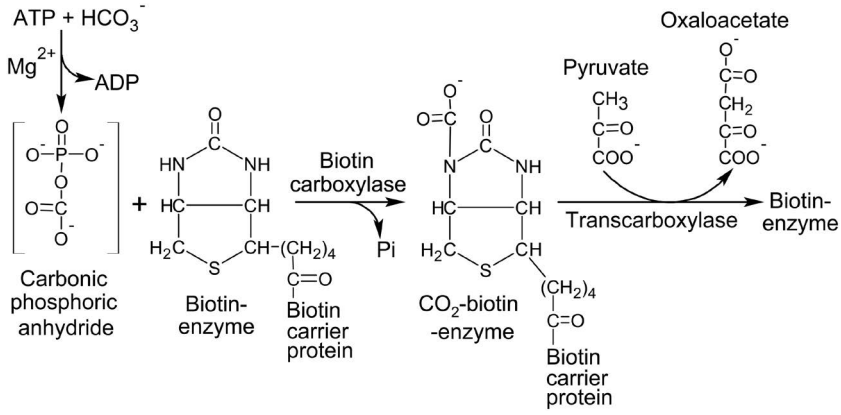


FIGURE 9.13 The mechanism whereby biotin participates in the carboxylation of monocarboxylic acids. Biotin (a coenzyme) acts as a "swinging arm" to shuttle intermediates between two active sites when it is covalently attached to its protein enzyme.

in 2- to 5-week-old chicks, is a fatal condition in which the liver and kidneys are pale and swollen due to abnormal fat accumulation. In rats, a deficiency of biotin suppresses the expression and activity of ornithine carbamoyltransferase (a urea-cycle enzyme), leading to hyperammonemia (Maeda et al. 1996).

Vitamin B_{12} (Cobalamin)

Structure: Vitamin B_{12} (cobalamin) is an octahedral cobalt complex consisting of a porphyrin-like ring and a cobalt ion at its center (Figure 9.14). Cobalt occurs in three oxidation states: Co^{+}, Co^{2+}, and Co^{3+} (Stadtman 2002). For example, in the liver, vitamin B_{12} exists as methylcobalamin (Co^{+}), 5-deoxy-adenosylcobalamin (Co^{2+}), and hydroxocobalamin. Methylcobalamin and 5-deoxy-adenosylcobalamin are the coenzymatically active forms of vitamin B_{12}. The commercial preparation of vitamin B_{12} is cyanocobalamin (Co^{3+}) and is the most stable form of the vitamin. Hydroxocobalamin (also known as aquacobalamin) and cyanocobalamin can be converted into methylcobalamin and 5-deoxy-adenosylcobalamin in cells. Vitamin B_{12} has the most complex structure of all the vitamins. The free form of this vitamin is sensitive to acidic conditions and is stabilized after binding to specific proteins.

Sources: Vitamin B_{12} is synthesized exclusively by microbes but not animal cells (Raux et al. 2000). Thus, this vitamin is not needed in ruminant diets that provide sufficient cobalt, such as inorganic cobalt salts (Georgievskii 1982). Vitamin B_{12} is absent from plants and plant-derived cereals unless they are contaminated by microbes. All animal tissues contain vitamin B_{12}, which is derived from their diets and intestinal bacteria synthesis. Good sources of vitamin B_{12} are the liver, meats (particularly beef), poultry, milk (e.g., 0.4 μg/L in human breast milk and 4.0 μg/L in cow's milk), and fish (Gille and Schmid 2015). Meat contains hydroxocobalamin and 5-deoxy-adenosylcobalamin, whereas dairy products contain hydroxocobalamin and methylcobalamin. All vitamin B_{12} found in animals and our environment originated from B_{12} synthesis in microbes. The naturally occurring vitamin B_{12} in foods is bound to its enzyme proteins in the coenzyme form (Gruber et al. 2011). Preformed vitamin B_{12} must be supplied to preruminants and nonruminants.

Absorption and Transport

Vitamin B_{12}-binding Proteins in the GIT

In the lumen of the stomach, there are two glycoproteins for binding vitamin B_{12} in a species-specific manner (Figure 9.15). They are the intrinsic factor (IF) and haptocorrin (HC, also known as the R-protein, transcobalamin 1, or cobalophilin). The IF binds one of the four forms of cobalamin (methylcobalamin, adenosylcobalamin, cyanocobalamin, and hydroxocobalamin) with equal affinity. IF is resistant, but HC is sensitive, to degradation by proteases (trypsin > chymotrypsin > elastase) in the lumen of

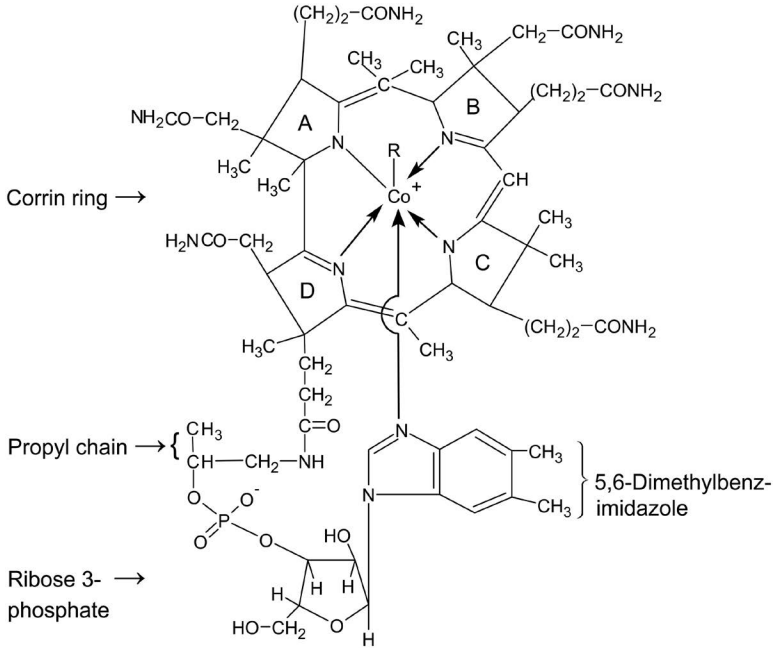


FIGURE 9.14 The chemical structure of cobalamin (vitamin B_{12}). This vitamin is an octahedral cobalt complex consisting of a porphyrin-like corrin ring, a cobalt ion (coordinated with the four pyrrole nitrogen atoms) at its center, a dimethylbenzimidazolyl nucleotide, a propyl chain, and an R group. Naturally occurring cobalamins are adenosylcobalamin, hydroxycobalamin (also known as hydroxocobalamin), aquacobalamin (the conjugated acid of hydroxocobalamin), and methylcobalamin, in which the R group is 5'-deoxyadenosyl, –OH, H_2O, and CH_3, respectively. Chemically synthetic forms of cobalamin are cyanocobalamin (with the R group being CN) and hydroxocobalamin. Cobalamin is the only known example of a cobalt-carbon bond.

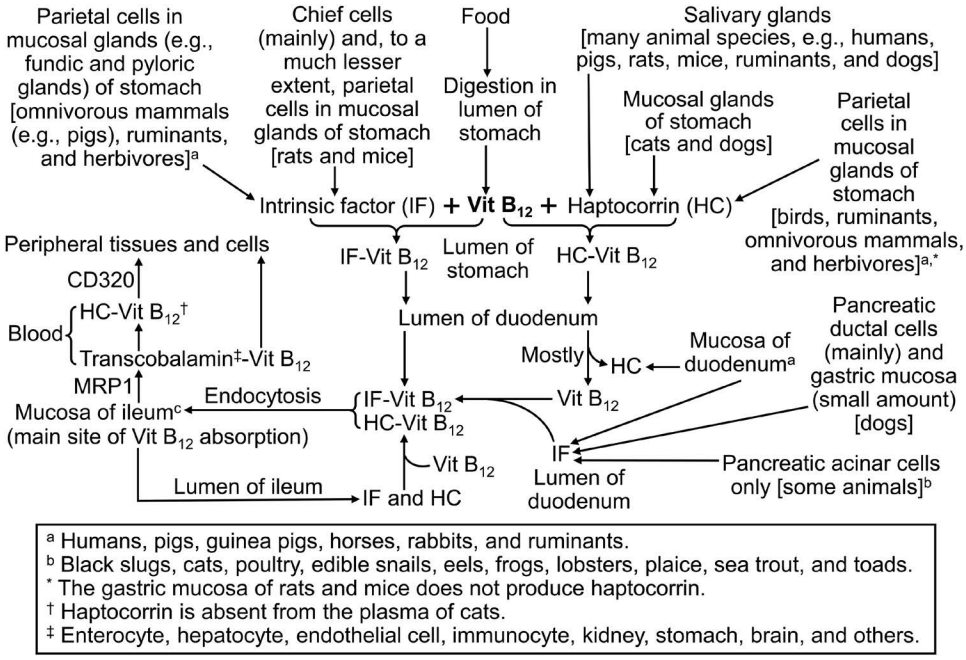


FIGURE 9.15 Digestion, absorption, and transport of vitamin B_{12} (Vit B_{12}) in animals. In the stomach, gastric digestive enzymes release Vit B_{12} from the food matrix. This vitamin binds to the intrinsic factor (IF) and haptocorrin (HC) in the lumen of the stomach and the small intestine, with the major sources (e.g., the gastric mucosa, intestinal mucosa, and exocrine pancreas) of the binding proteins varying among animal species (see the inserted box). In the lumen of the duodenum, the IF-Vit B_{12} complex is stable, but the HC-Vit B_{12} complex is unstable, and its Vit B_{12} moiety is transferred to IF. The ileum is the primary site for the absorption of Vit B_{12} via receptor-mediated endocytosis (cubam for the IF-Vit B_{12} complex and the asialoglycoprotein receptor for the HC-Vit B_{12} complex) in enterocytes. After entering the enterocyte, the protein components of the IF-vitamin B_{12} and the HC-vitamin B_{12} complexes are hydrolyzed by lysosomal proteases to release vitamin B_{12} into the cytosol, where vitamin B_{12} is ultimately attached to transcobalamin. The transcobalamin-vitamin B_{12} complex exits the enterocyte across its basolateral membrane into the lamina propria through the multidrug resistance protein 1 (MDRP1). Immediately after entering the venous blood circulation, most (e.g., 75% in humans) of vitamin B_{12} binds to transcobalamin in plasma. After the absorbed vitamin B_{12} reaches equilibrium with the total body vitamin B_{12}, ~25% and 75% of the plasma vitamin B_{12} are carried by transcobalamin and HC, respectively. Transcobalamin-vitamin B_{12} and HC-vitamin B_{12} complexes in plasma are taken up by extraintestinal cells through an endocytic receptor called the transmembrane CD320 protein.

the small intestine. The third vitamin B_{12}-binding protein is called transcobalamin (a nonglycoprotein secretory protein and also known as transcobalamin II). All the known cobalamin-bonding proteins share the same overall structural scaffold and each of them carries a single vitamin B_{12} molecule (Nielsen et al. 2012).

The sources of IF in the GIT vary with animal species. Salivary glands of many animal species (including humans, pigs, rats, mice, ruminants, and dogs) synthesize and secrete IF (Alpers and Russell-Jones 2013). In addition, IF is synthesized and secreted predominantly by parietal cells in mucosal glands (e.g., fundic and pyloric glands) of the stomach (or abomasum) and the mucosa of the small intestine (e.g., duodenum and ileum) in humans, pigs, guinea pigs, horses, rabbits, and ruminants such as cattle and sheep (Marcoullis et al. 1977, 1978; McKay and McLeay 1981). In contrast, IF is synthesized and secreted predominantly by chief cells in mucosal glands (e.g., fundic and pyloric glands) of the stomach in rats and mice. Interestingly, IF is synthesized and released by pancreatic ductal cells and, to a small extent, the parietal cells of the gastric mucosa in dogs (Simpson et al. 1993) but exclusively by pancreatic acinar cells in cats (Fyfe 1993). The feline stomach does not produce IF. Of interest, stomach mucosal preparations from black slugs, chickens, edible snails, eels, frogs, lobsters, plaice, sea trout, and toads do not produce or contain IF (Hippe and Schwartz 1971); the major source of the IF in the lumen of their small intestine may be the pancreatic acinar cells (Kather et al. 2020; Siani et al. 2023; Vaillant et al. 1990). Thus, gastric glands are the major source of IF in birds, ruminants, omnivorous mammals (including pigs and rodents), and herbivores, whereas the exocrine pancreas is almost the exclusive origin of IF in terrestrial omnivorous mollusk, omnivorous birds, and carnivores; aquatic carnivorous fish and crustaceans; and carnivorous amphibians.

HC is synthesized and released by mucosa glands of the stomach (or abomasum) in noncarnivorous vertebrates (e.g., humans, pigs, guinea pigs, horses, rabbits, poultry, and ruminants) and the salivary glands of many animal species, including humans, pigs, rats, mice, and ruminants, but only from mucosal glands of the stomach in dogs and cats. HC is not synthesized by the gastric mucosa of rats

and mice. In addition, the milk of HC-producing mammals (e.g., humans, pigs, rats, mice, ruminants, and carnivores) is also a source of HC. Interestingly, this vitamin B_{12}-binding protein is present in the plasma of humans, pigs, ruminants, dogs, and chickens but is absent from the plasma of cats (Siani et al. 2023). Thus, the proportions of IF to HC vary greatly with different animal species.

Transcobalamin is synthesized and released by many types of cells and tissue, including enterocytes, hepatocytes, endothelial cells, astrocytes, immunocytes (e.g., macrophages and lymphocytes), kidneys, and gastric mucosa. This protein occurs in all vertebrates and invertebrates studied to date. Transcobalamin binds vitamin B_{12} within the enterocytes of the small intestine and in plasma.

Uptake of Dietary Vitamin B_{12} by the Intestine

Within the lumen of the stomach, vitamin B_{12} is released from the food matrix through gastric acidification and proteolysis (particularly the action of pepsin) and then binds to IF and/or HC in a species-dependent manner. The IF-vitamin B_{12} complex exits the stomach into the duodenum, where pancreatic enzymes (trypsin and chymotrypsin) digest most HC of the gastric HC-vitamin B_{12} complex, releasing vitamin B_{12} that subsequently binds available IF (e.g., the pancreas-derived IF). The IF-vitamin B_{12} complex is taken up into enterocytes (mainly in the ileum) across their apical membrane via endocytosis after the complex binds to its specific receptor known as cubam (Figure 9.15). The latter consists of two subunits: cubilin (for binding to vitamin B_{12}) and amnionless (for endocytosis). The asialoglycoprotein receptor mediates the endocytosis, into enterocytes, of the HC-vitamin B_{12} complex after its terminal sialic acid is cleaved by sialidase (also known as neuraminidase). Both cubam and the asialoglycoprotein receptor are most abundant in the ileum, among the intestinal segments. Accordingly, in all animals studied, the intestinal absorption of vitamin B_{12} occurs primarily in the ileum and, to a lesser extent, the jejunum and large intestine (Alpers and Russell-Jones 2013).

Upon entering the enterocyte, the IF-vitamin B_{12} and the HC-vitamin B_{12} complexes are transported into lysosomes, where proteases hydrolyze the IF and HC in these complexes to release vitamin B_{12}. A lysosomal membrane protein, called cobalamin F [now identified as ATP-binding cassette subfamily D member 4 (ABCD4)], transports vitamin B_{12} from the lysosome into the cytosol (Kitai et al. 2021), where the free vitamin B_{12} binds to a cytosolic protein called cobalamin C [now identified as metabolism of cobalamin associated C (MMACHC)], which is involved in the decyanation of cyanocobalamin and dealkylation of alkylcobalamins (Froese et al. 2015). Subsequently, vitamin B_{12} is passed onto another cytosolic protein called cobalamin D [now identified as methylmalonic aciduria and homocystinuria type D protein (MMADHC)] (Yamada et al. 2015) and then attached to transcobalamin. The transcobalamin-vitamin B_{12} complex exits the enterocyte across its basolateral membrane into the lamina propria through the multidrug resistance protein 1 (MRP1) (Beedholm-Ebsen et al. 2010). MRP1 is a member of the ATP-binding cassette (ABC) transporter family of proteins that couple the hydrolysis of ATP to the transport of substances against a concentration gradient.

Immediately after entering the venous blood circulation, most (e.g., ~75% in humans) of vitamin B_{12} binds to transcobalamin in plasma (Hall 1977). Thus, transcobalamin is the chief transporter of vitamin B_{12} in blood immediately following its absorption by the small intestine. After the absorbed vitamin B_{12} reaches equilibrium with the total body vitamin B_{12}, ~25% and 75% of the plasma vitamin B_{12} are carried by transcobalamin and HC, respectively. Transcobalamin-vitamin B_{12} and HC-vitamin B_{12} complexes in plasma are taken up by extraintestinal cells through an endocytic receptor called the transmembrane CD320 protein (Nielsen et al. 2012). The latter is heavily glycosylated and belongs to the LDL receptor family. Upon endocytosis, transcobalamin is degraded in the lysosomes to release vitamin B_{12} (hydroxocobalamin), which is either converted to methylcobalamin in the cytosol or enters the mitochondria for conversion into 5'-deoxyadenosylcobalamin.

Unlike other water-soluble vitamins, vitamin B_{12} is stored primarily in the liver and kidneys, bound to its binding proteins (primarily transcobalamin). This vitamin also occurs in other tissues (e.g., skeletal muscle and kidneys). Interestingly, cats have a much lower capacity to store vitamin B_{12} than dogs and humans. Thus, the half-lives of vitamin B_{12} are 11–14 days, 42–112 days, and ~ 1 year in adult cats, dogs, and humans, respectively (Siani et al. 2023).

There are developmental changes in the role of IF and HC in the intestinal absorption of vitamin B_{12}. In neonatal mammals, as demonstrated for 1- to 28-day-old pigs (Ford et al. 1975; Trugo et al. 1985), when IF production is low, HC plays an important role in the uptake of dietary vitamin B_{12} by the small and large intestines. By contrast, in adulthood, vitamin B_{12} absorption is dependent primarily on its binding to the IF.

Excretion of Vitamin B_{12}

Unlike other water-soluble vitamins, vitamin B_{12} is excreted from animals mainly via biliary secretion into the lumen of the duodenum and then the feces and, to a much lesser extent, the urine. In nonruminants (particularly patients with pernicious anemia), urinary excretion of the vitamin after its oral administration is often used to determine whether the animal can absorb vitamin B_{12} properly; this is called the Schilling test. In ruminants, vitamin B_{12} exits the rumen via microbes or is bound to salivary HC and released during digestion in the abomasum (Smith and Marston 1970). Lactating cows fed normal diets excrete approximately 87% of all excreted cobalt in the feces, 1.0% in urine, and 12% in milk, whereas nonlactating cows excrete approximately 98% of all excreted cobalt in the feces and 2.0% in urine (Georgievskii 1982). Because of the high efficiency of the enterohepatic circulation in reabsorbing vitamin B_{12} in the ileum via the IF-mediated transport, most

of the bile-derived vitamin B_{12} is retained in the body. The total daily excretion of this vitamin via the feces and urine represents <1% of the total body reserves in animals.

Functions: The active vitamin B_{12} coenzymes are methylcobalamin and 5'-deoxy- adenosylcobalamin (Gruber et al. 2011). Methylcobalamin is the coenzyme required for the synthesis of (a) methionine from homocysteine and (b) tetrahydrofolate from N^5-methyltetrahydrofolate by methionine synthase (Figure 9.16). The metabolic significance of this reaction is to detoxify homocysteine and maintain the intracellular concentration of methionine, while regenerating H_4folate from N^5-methyltetrahydrofolate for the syntheses of purine, pyrimidine, and nucleic acids. Through its role in one-carbon metabolism that regulates DNA methylation, dietary vitamin B_{12} may contribute to fetal programming of postnatal growth, development, and health in offspring (Rush et al. 2014). In addition, 5'-Deoxyadenosylcobalamin is the coenzyme required for the synthesis of succinyl-CoA from L-methylmalonyl-CoA by methylmalonyl-CoA mutase (Nielsen et al. 2012). This is a key reaction in the conversion pathway of propionate to an intermediate of the Krebs cycle and therefore to glucose. The synthesis of glucose from propionate is particularly important for ruminants because propionate is a major product of microbial fermentation in the rumen and because little glucose is absorbed from the small intestine of ruminants.

Deficiencies and diseases: Without supplementation, a deficiency of vitamin B_{12} is common among nonruminants fed plant-sourced ingredients, ruminants with inadequate intake of cobalt, and farm-raised fish (Pond et al. 1995). In humans and most nonprimate species, gastric bypass surgery and gastric atrophy (typically caused by persistent infection with *Helicobacter pylori*) reduce IF production, resulting in vitamin B_{12} deficiency (Jones and Hunt 1983). In humans, vegans are at risk of actual dietary deficiency of vitamin B_{12} because the vitamin is found only in foods of animal origin or in microbes, but not in plants (Stover 2004). In addition, the lack of bioavailable IF due to its binding with self-antibodies results in a deficiency of vitamin B_{12} (pernicious anemia, an autoimmune disease). Furthermore, an inherited defect in the synthesis of methylcobalamin or 5'-deoxyadenosylcobalamin causes vitamin B_{12} deficiency (Gruber et al. 2011). Likewise, vitamin B_{12} deficiency may occur as a result of a parasitic infection by tapeworms, which can arise

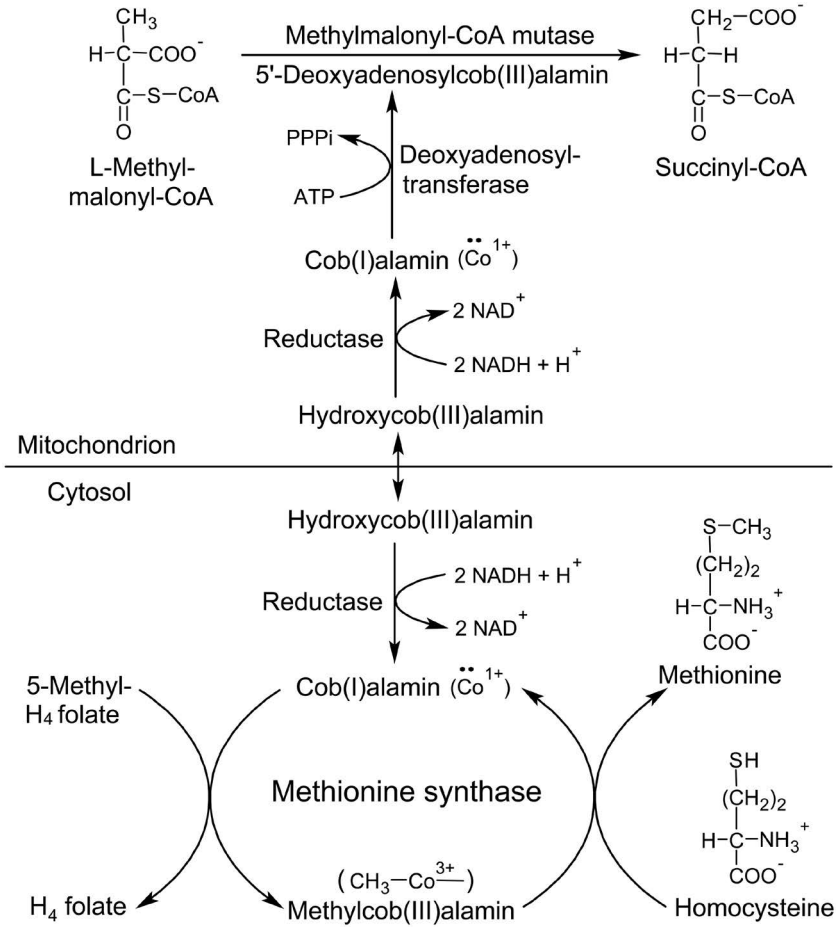


FIGURE 9.16 Roles of vitamin B_{12} as a coenzyme for methylmalonyl-CoA mutase and methionine synthase. Adenosylcobalamin, hydroxycobalamin, and methylcobalamin participate in the indicated reactions that occur in hepatocytes. These forms of cobalamin can be generated from cyanocobalamin in animals.

from the consumption of infected raw fish and be passed to the consumer's gut, thereby limiting the availability of vitamin B_{12} to the animal host.

Numerous metabolic disorders are caused by inadequate intake of dietary vitamin B_{12}. For example, a deficiency of this vitamin results in decreased activity of methionine synthase and methylmalonyl-CoA mutase, diminished DNA (purine and thymidylate) synthesis, reduced cell division, and impaired formation of the nucleus of new erythrocytes with consequent accumulation in the bone marrow of megaloblasts. Homocystinuria (the accumulation of homocysteine) and methylmalonic aciduria (the accumulation of methylmalonic acid) also occur in animals and humans with vitamin B_{12} deficiency. Neurological disorders associated with vitamin B_{12} deficiency may be secondary to a relative deficiency of methionine.

Folate

Structure: Folate (vitamin B_9) refers to folic acid (also known as pteroylglutamic acid) and its derivatives, which act as coenzymes for one-carbon metabolism (Shane 2008). Folic acid consists of a pteridine base, *p*-aminobenzoic acid, and glutamic acid (Figure 9.17), and is the parent structure of the large family of pteridine derivatives. Folates may contain one or more glutamate residues as a γ-linked polypeptide chain. For example, in plants, folate exists as a polyglutamate conjugate consisting of seven glutamate residues (Butterworth and Bendich 1996). In animal tissues, the major form of folate is a pentaglutamyl conjugate containing five glutamate residues (Zhao and Goldman 2013). Essentially, all cellular folates occur in the polyglutamyl form. Folates that carry one-carbon units at varying oxidation states and bound to one or two N atoms (e.g., N^5,N^{10}-methylene tetrahydrofolate) are named according to the specific carbon moiety bound. The reduced folates include dihydrofolate and tetrahydrofolate. Most folates in foods can be easily oxidized under aerobic conditions of processing and storage (Combs and McClung 2017).

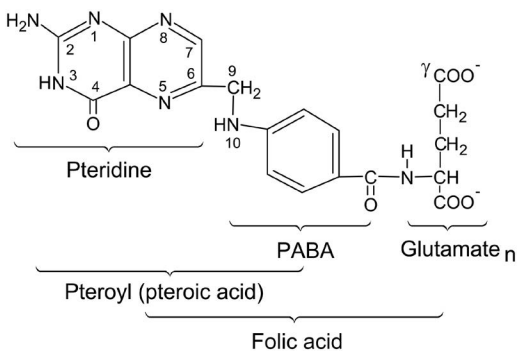


FIGURE 9.17 The chemical structure of folate (vitamin B_9). This vitamin refers to folic acid (also known as pteroylglutamic acid) and its derivatives. The number (n) of glutamate residues range from 1 to 15. In folate, the pteridine ring is the core structure linked to a *p*-aminobenzoic acid (PABA) group. The latter is linked to either one glutamate residue (in chemically synthetic folate) or a poly-glutamate peptide (in plants and animals).

Sources: Folic acid is synthesized by plants and microbes (Sahr et al. 2005). This vitamin is widely distributed in foods of plant and animal origin. Green leafy materials, cereals, extracted oilseed meals, and orange juice are good sources of folic acid. The liver, egg yolk, and milk are also major sources of the vitamin. Note that animal cells cannot synthesize *p*-aminobenzoic acid or attach glutamate to pteroic acid and therefore require folate from the diet for metabolism. Folate is readily degraded by moisture, particularly at high temperatures, and is destroyed by ultraviolet light (Lucock 2000). The bioavailability of dietary folate is 30–80% depending on the sources of food, with the folate in animal products being better digested and absorbed than the folate in plant products.

Absorption and transport: As noted previously, dietary folate exists in the form of mono- and polyglutamates, which are called folymonoglutamates and folypolyglutamates, respectively. Folymonoglutamates, but not folypolyglutamates, are absorbed by enterocytes and colonocytes. Thus, folypolyglutamates must be hydrolyzed into folymonoglutamates prior to their absorption. This digestion process occurs mainly in the proximal part of the small intestine and involves the enzyme folylpoly-γ-glutamate carboxypeptidase (also known as γ-glutamyl hydrolase or conjugase) that is localized on the apical membrane of the enterocyte. The intestinal activity of folylpoly-γ-glutamate carboxypeptidase is increased by folate deficiency but decreased by folate excess (Said et al. 2000). Both the proton-coupled folate transporter (PCFT, SLC46A1) and two reduced-folate carriers [RFC-1 (SLC19A1) and RFC-2], which are expressed in the apical membrane of intestinal epithelial cells, are responsible for intestinal absorption of folymonoglutamate (Said 2011). RFCs, which are H^+-dependent but Na^+-independent, are the major transporters for folates in mammalian cells. Upon entering the enterocytes, most folymonoglutamate is reduced to tetrahydrofolate (H_4folate) by folate reductase, which uses NADPH as a donor of reducing equivalents (Figure 9.18). N^5-methyl-H_4folate is also formed in these cells, which is the major form of folate in plasma. Little is known about the molecular identity of the folate transport system on the basolateral membrane of intestinal epithelial cells. However, members of the multidrug resistance proteins may play an important role in transporting intracellular folate across the basolateral membrane of the enterocyte into the lamina propria (Zhao and Goldman 2013).

In most animal species, free folate is transported in plasma as folymonoglutamate derivatives (mainly N^5-methyl-H_4folate) (Zempleni et al. 2013). They are taken up by extraintestinal cells through PCFT, RFCs, and high-affinity folate receptors (FRs). Except for RFCs, which are ubiquitously expressed in animal tissues, the distribution of PCFT and the high-affinity FRs is tissue-specific. In particular, PCFT is highly expressed in the small intestine, as noted previously, and also in the kidneys, liver, placenta,

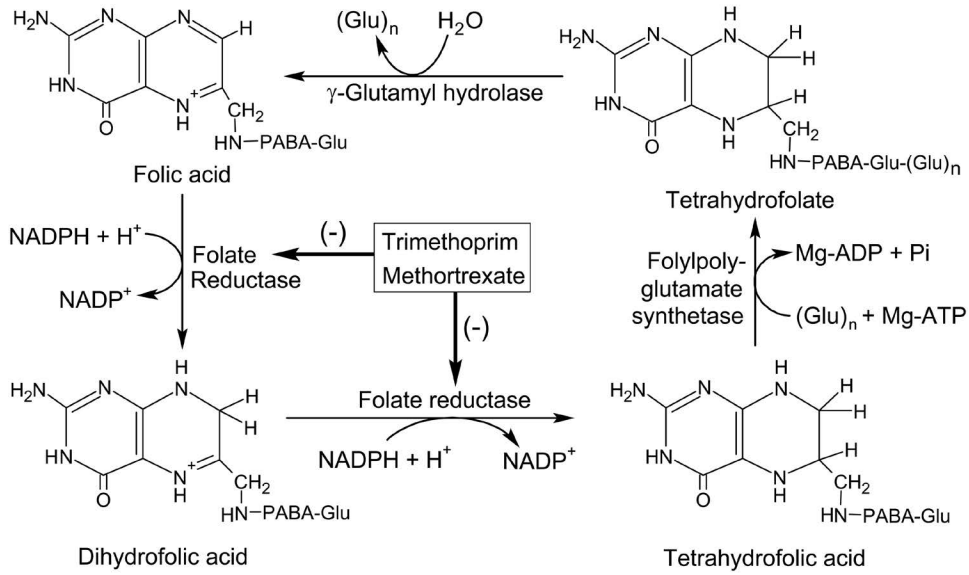


FIGURE 9.18 Conversion of folate into tetrahydrofolate in animal cells. Folic acid is converted by folate (or dihydrofolate) reductase in virtually all cell types into tetrahydrofolic acid. The latter is subsequently linked to a poly-glutamate peptide. The number (n) of glutamate residues is up to 15.

and spleen, but to a lesser extent in the brain, testes, and lung. Much lower levels of PCFT are expressed in the terminal ileum and colon. Likewise, three FRs are expressed at the cell membrane in a tissue-specific manner (Zhao et al. 2011). For example, FRα is expressed on the membrane of epithelial tissues (e.g., the placenta, the apical brush-border membrane of proximal renal tubular cells, retinal pigment epithelium, and the choroid plexus). FRβ is present in hematopoietic tissues (e.g., spleen, thymus, and $CD34^+$ monocytes) and macrophages. FRδ is highly abundant in natural and TGFβ-induced regulatory T cells, whereas FRγ is a secreted protein. The kidneys play an important role in folate homeostasis. In particular, folates are filtered via the glomerulus and reabsorbed via the apical FRα-mediated endocytosis from the lumen of the renal tubule into the tubular epithelial cell. The reabsorbed folate will then be transported across the tubular epithelial cell into the bloodstream by its basolateral folate transporters.

Functions: Active folate in cell metabolism is tetrahydrofolate (H_4folate) in the form of tetrahydrofolate polyglutamates (Stover 2004). H_4folates are the carriers of activated one-carbon units and are used as cofactors in reactions that comprise one-carbon metabolism. The one-carbon units carried by H_4folates represent methyl (CH_3-), methylene (-CH_2-), methenyl (-CH=), formyl (O=CH-), and formimino (HN=CH-), which are all metabolically interconvertible (Figure 9.19). N^{10}-formyl-H_4folate contributes to the C-2 and C-8 of the purine ring structure. N^5-N^{10}-methylene-H_4folate provides the methyl group for the synthesis of thymidylate, an event that is essential to DNA synthesis (Figure 9.20). Thus, folate is required for the following processes: (a) interconversion of serine and glycine, (b) synthesis of purines, (c) histidine degradation, and (d) provision of methyl groups essential for methylation reactions such as choline and methionine synthesis (Ducker and Rabinowitz 2017). Note that serine is the major source of one-carbon units carried by folate, whereas formiminoglutamate (a metabolite of L-histidine) can also transfer its formimino group to H_4folate to form N^5-formimino-H_4folate (Wang et al. 2012). Finally, folate is required for the microbial production of methane from formate or CO_2 plus H_2 (Chapter 5).

Deficiencies and diseases: Risk factors in folate deficiency include (a) the inadequate intake of folate, (b) the chronic consumption of excess alcohol, (c) the heating of foods and dilution in cooking water, (d) increased requirement due to pregnancy and lactation in mammals or egg laying in birds, (e) treatment with methotrexate (an inhibitor of folate reductase), and (f) vitamin B_{12} deficiency. Inadequate intake of vitamin B_{12} induces folate deficiency because vitamin B_{12} is required for the conversion of folate from a form (N^5-methyl-H_4folate) with limited use into a form (H_4folate) that participates in a variety of reactions. Severe metabolic disorders occur in folate-deficient animals, particularly during embryonic, fetal, and neonatal development (Ducker and Rabinowitz 2017). For example, folate deficiency causes megaloblastic anemia because one-carbon metabolism is necessary for the formation of erythrocytes. Folate deficiency during pregnancy can cause neural tube defects such as spina bifida in infants (Fleming and Copp 1998). The complexities of the interaction between vitamin B_{12} and folate are a consequence of their common participation in the methionine synthase reaction. Folate deficiency can occur in chickens and turkeys that are

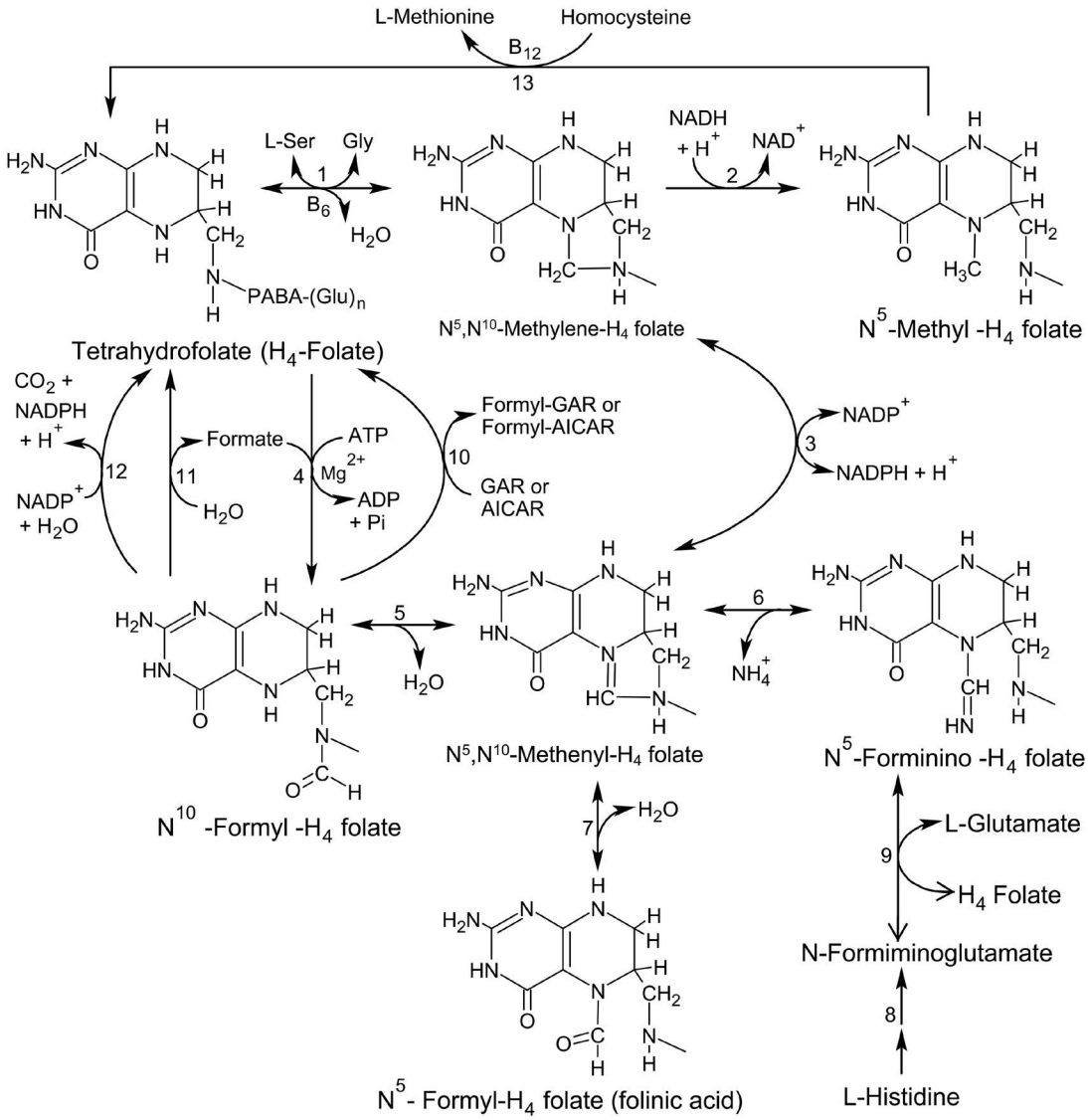


FIGURE 9.19 One-carbon metabolism in animal cells. The one-carbon units carried by H_4folates represent methyl (CH_3-), methylene (-CH_2-), methenyl (-CH=), formyl (O=CH-), and formimino (HN=CH-), which are all metabolically interconverted in animal cells. Enzymes that catalyze the indicated reactions are (1) serine hydroxymethyl transferase; (2) N^5-N^{10}-methylene H_4-folate reductase; (3) N^5-N^{10}-methylene H_4-folate dehydrogenase; (4) N^{10}-formyl H_4-folate synthetase; (5) N^5-N^{10}-methenyl H_4-folate cyclohydrolase activities; (6) formimidoyl H_4-folate cyclodeaminase; (7) N^5-N^{10}-methenyl H_4-folate cyclohydrolase; (8) a sequential series of enzymes: histidase, urocanase, and imidazolone propionate hydrolase; (9) glutamate formiminotransferase; (10) glycinamide ribonucleotide transformylase; (11) N^{10}-formyl H_4-folate deformylase; (12) N^{10}-formyl H_4-folate dehydrogenase; and (13) methionine synthase. Enzymes 3, 4, and 5 are also collectively called N^5-N^{10}-methylene H_4-folate dehydrogenase, which is a trifunctional enzyme (possessing N^{10}-formyl H_4-folate synthetase, N^5-N^{10}-methylene H_4-folate dehydrogenase, and N^5-N^{10}-methenyl H_4-folate cyclohydrolase activities) in the cytosol of mammalian cells, but is a bifunctional enzyme (possessing N^5-N^{10}-methylene H_4-folate dehydrogenase and N^5-N^{10}-methenyl H_4-folate cyclohydrolase activities) in the mitochondria of mammalian cells. Enzymes 6 and 9 are also collectively called formiminotransferase-cyclodeaminase, which is a bifunctional enzyme possessing glutamate formiminotransferase and formimidoyl H_4-folate cyclodeaminase activities in mammals. AICAR, 5-amino-4-imidazolecarboxamide riboside; GAR, glycinamide ribonucleotide.

fed diets containing practical ingredients, with symptoms including poor growth, reduced feed efficiency, anemia, poor bone development, and poor egg hatchability (Pesti et al. 1991; Sherwood et al. 1993). This may be due to the low ability of poultry to synthesize folate in their intestinal bacteria and a low ability of folate absorption by their hindguts. In addition, corn- and SBM-based diets without folate supplementation do not provide sufficient folate for the optimal growth, reproduction, or lactation of swine (Matte et al. 1992). In aquatic animals (e.g., fish and crustaceans), folic acid deficiency causes reductions in feed intake, feed efficiency, and growth, as well as anemia, splenic infarction, dark skin pigmentation, and impaired immunity (NRC 2011).

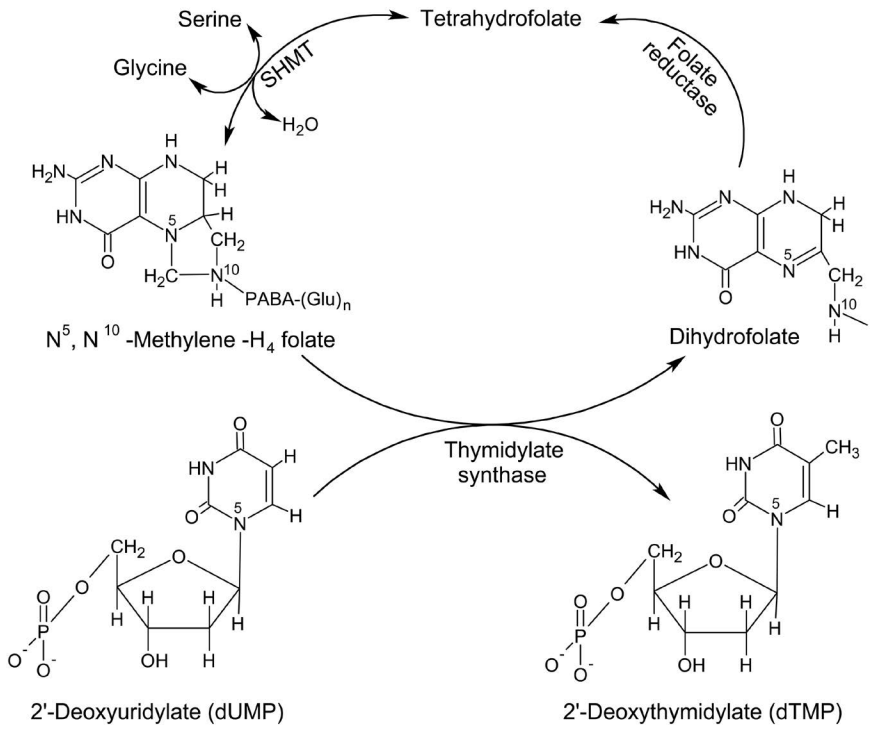


FIGURE 9.20 Roles of tetrahydrofolate in the formation of 2'-deoxythymidylate (dTMP), a precursor of DNA. Serine hydroxymethyltransferase (SHMT) catalyzes the conversion of serine and tetrahydrofolate into glycine and N^5,N^{10}-methylene-H_4 folate. The latter is used for the methylation of 2'-deoxyuridylate (dUMP) thymidylate synthase to dTMP. A coproduct (dihydrofolate) of the reaction is reduced by NADPH-dependent folate reductase to tetrahydrofolate.

Folate deficiency rarely occurs in ruminants because of the presence of the vitamin in commonly used ingredients and the synthesis of the vitamin by intestinal bacteria. However, under conditions (e.g., gestation and lactation) when the requirements of folic acid are increased, dietary supplementation of folic acid is necessary. Thus, adding high doses of folic acid (>0.5 mg/kg of BW) to diets for lactating cows increased serum folate concentrations, milk yield, and milk fat content (Ragaller et al. 2009).

Folinic acid (5-formyl tetrahydrofolic acid) is a metabolically active analog of tetrahydrofolate and can be used to treat folate deficiency in animals. For example, folinic acid is often administered to methotrexate-treated cancer patients at the appropriate time to alleviate damage to their bone marrow and gastrointestinal mucosa cells (Zempleni et al. 2013).

Ascorbic Acid (Vitamin C)

Structure: Vitamin C (also known as ascorbic acid) is a glucose derivative with the chemical name of 2,3-didehydro-L-threo-hexano-1,4-lactone. It is a white, crystalline, and water-soluble substance. The oxidized form of this vitamin is dehydroascorbic acid (also called L-dehydroascorbic acid; Wells and Xu 1994). Vitamin C has weak acidic and strong reducing properties and can stabilize unpaired electrons. Therefore, it is used to scavenge free radicals, protect biomolecules (e.g., tetrahydrobiopterin) from oxidation in an aqueous solution, and maintain metal ions (e.g., iron and copper) in their reduced forms (Englard and Seifter 1986). This vitamin is heat-stable in an acidic solution and readily decomposed under alkaline conditions (Figure 9.21). The destruction of vitamin C at high temperatures is enhanced by exposure to light. Dehydroascorbic acid is not ionized in a weakly acidic solution or a neutral solution and is not stable in an aqueous solution because it is degraded through the hydrolytic ring opening to form 2,3-dioxo-L-gulonic acid (Combs and McClung 2017).

Sources: Vitamin C is essentially absent from grains (McDonald et al. 2021). It is synthesized from glucose via the uronic acid pathway in the liver of most mammals (e.g., pigs, dogs, cats, and ruminants) and in the liver and kidneys of poultry, and in GIT microbes (Mahan et al. 2004; Maurice et al. 2004; Ranjan et al. 2012). However, in primates (e.g., humans and monkeys) and guinea pigs, as well as some species of insects, birds, teleost fish, and invertebrates, vitamin C cannot be synthesized from glucose due to the lack of L-gulonolactone oxidase (Zempleni et al. 2013). In contrast to other water-soluble vitamins, gastrointestinal bacteria in nonruminants do not have a net synthesis of vitamin C (Wrong et al. 1981).

Good sources of vitamin C are fresh vegetables, including green leafy plants, bell peppers, broccoli, citrus fruits, spinach, tomatoes, and potatoes. Animal products (e.g., milk and eggs) contain some vitamin C. In plant- and

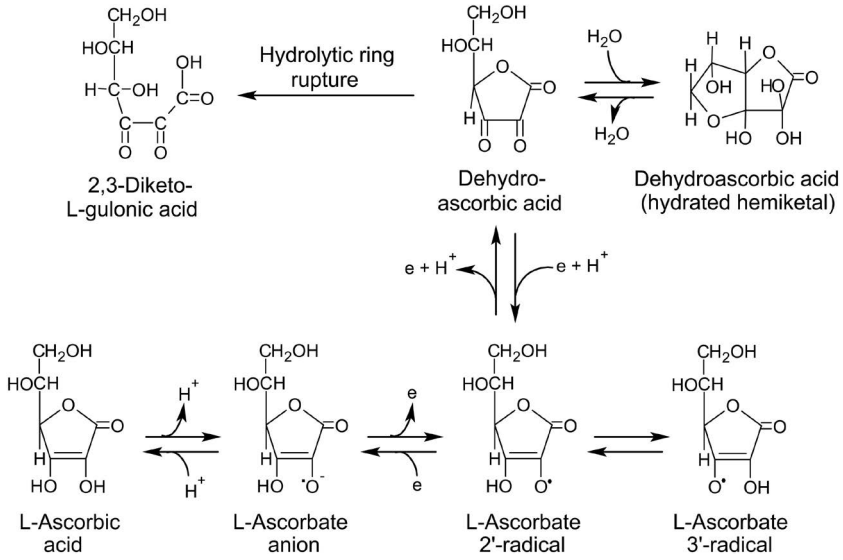


FIGURE 9.21 The chemical structure of vitamin C and its oxidation to L-ascorbate 2'-radical and dehydroascorbic acid in animal cells. Vitamin C (also known as ascorbic acid) is synthesized from D-glucose in most nonprimate species and is stable in water. Ascorbic acid is reversibly oxidized into dehydroascorbic acid (DHA) upon exposure to light, heat, transition metal ions, and oxygenated alkaline solutions. The oxidation of vitamin C can generate radical species.

animal-sourced foods, vitamin C exists in the reduced and oxidized forms. The oxidized form of the vitamin is converted into ascorbic acid in intestinal epithelial cells by glutathione-dependent dehydroascorbic acid reductase, which is the intrinsic activity of thioltransferases (glutaredoxins; Wells et al. 1990). In ruminants, dietary vitamin C is totally degraded in the rumen, and there is virtually no net synthesis of this vitamin by the microbes (Ranjan et al. 2012). Thus, it is difficult to increase milk's vitamin C content through dietary supplementation of the unprotected vitamin.

Absorption and transport: Vitamin C is absorbed by the small intestine into the blood circulation. The apical membrane of the enterocyte contains Na^+-dependent vitamin C transporters (SVCT) 1 and 2 (the products of the SLC23A1 and SLC23A2 genes, respectively) to take up dietary vitamin C (Bürzle et al. 2013). SVCT1 is expressed in the intestine, liver, and kidney, but SVCT2 is widely distributed in animal tissues. In contrast, dehydroascorbic acid is absorbed into the enterocyte via facilitative (Na^+-independent) glucose transporters GLUT2 and GLUT8 that are competitively inhibited by hexoses (e.g., glucose and fructose) and flavonoids (e.g., phloretin and quercetin; Corpe et al. 2013). Vitamin C exits the enterocyte across its basolateral membrane into the lamina propria via a specific protein carrier. Thus, dietary sugars and flavonoids (e.g., derived from fruits and vegetables) may modulate vitamin C bioavailability in animals. In addition, intestinal absorption of vitamin C is upregulated by its dietary deficiency but down-regulated by its dietary excess (Said 2011).

In animals, vitamin C is transported in plasma in the free form, which usually has no detectable dehydroascorbic acid. Vitamin C is taken up by extraintestinal tissues via SVCT2 and participates in many biochemical reactions (see the section below). In cells, this vitamin may be oxidized to an ascorbyl free radical and then to dehydroascorbic acid by losing one electron in each step (Figure 9.21). Dehydroascorbic acid undergoes irreversible hydrolysis to 2,3-diketo-L-gulonic acid, which is oxidized to C_5 fragments (xylose, xylonic acid, and lyxonic acid), C_4 fragments (L-erythrulose and L-threonate), oxalate, H_2O, and CO_2 (Figure 9.22). The proportions of these products

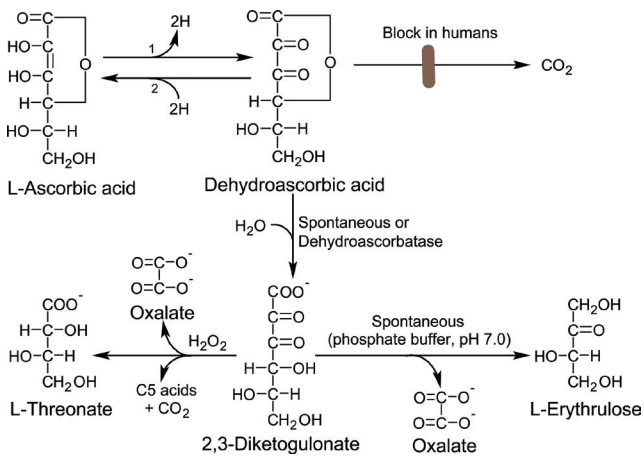


FIGURE 9.22 Vitamin C catabolism in animals. This vitamin is oxidized to dehydroascorbic acid and then 2,3-diketogulonate, either spontaneously or via specific enzymes. The end degradation products of vitamin C include CO_2, L-threonate, oxalate, and L-erythrulose in a species-dependent manner and are excreted in urine.

vary with animal species (e.g., almost completely as H_2O and CO_2 in rats and guinea pigs, but primarily as oxalate in humans; Combs and McClung 2017). Vitamin C can be regenerated from (a) ascorbyl free radicals by NADPH-dependent semidehydroascorbyl reductase and (b) dehydroascorbic acid through its reduction by the reduced form of glutathione (GSH) or lipoic acid nonenzymatically or by GSH-dependent dehydroascorbic acid reductase. All these reactions help to conserve vitamin C.

Functions: Vitamin C is a donor of reducing equivalents and can reduce such compounds as O_2, nitrate, and cytochromes a and c (Englard and Seifter 1986; Padayatty and Levine 2016). Vitamin C can help to maintain a metal cofactor of some enzymes in the reduced state (e.g., Cu^+ in monooxygenase and Fe^{2+} in dioxygenases, including α-KG- and O_2-dependent histone demethylases). The following reactions depend on vitamin C and, in some cases, one or more additional cofactors.

1. Synthesis of hydroxyproline by prolyl hydroxylase (Fe^{2+}) in collagen (post-translational reaction) that requires vitamin C and α-KG.

$$\text{-Pro-}\left(\text{peptide}\right) + {}^{18}O_2 + \alpha\text{-KG} \rightarrow \text{-}{}^{18}\text{OH-Pro-} + \left[{}^{18}O\right]\text{Succinate} + CO_2$$

2. Synthesis of hydroxylysine by lysyl hydroxylase (Fe^{2+}) in collagen (post-translational reaction).

$$\text{-Lys-}\left(\text{peptide}\right) + {}^{18}O_2 + \alpha\text{-KG} \rightarrow \text{-}{}^{18}\text{OH-Lys-} + \left[{}^{18}O\right]\text{Succinate} + CO_2$$

3. Oxidation of tyrosine at the steps catalyzed by p-hydroxyphenylpyruvate hydroxylase (Fe^{2+}) and homogentisate oxidase (Fe^{2+})

$$\text{p-Hydroxyphenylpyruvate} + O_2 \rightarrow \text{Homogentisate} + CO_2$$
$$\text{Homogentisate} + O_2 \rightarrow \text{Maleylacetoacetate}$$

4. Synthesis of catecholamines from tyrosine by dopamine β-hydroxylase.

$$\text{Dopamine} + O_2 \rightarrow \text{Norepinephrine}$$

5. Synthesis of bile acid (vitamin C is required at the initial 7α-hydroxylase step).

$$\text{Cholesterol} + O_2 + \text{NADPH} + H^+ \rightarrow 7\alpha\text{-Hydroxycholesterol} + NADP^+$$

6. Synthesis of carnitine (vitamin C is required for two Fe^{2+}-containing hydroxylases).

$$\varepsilon\text{-N-Trimethyllysine} + \alpha\text{-KG} + O_2 + \text{Vitamin C} \rightarrow \beta\text{-Hydroxy-trimethyllysine} + \text{Succinate} + CO_2 + \text{Dehydroascorbate}$$
$$\gamma\text{-Butyrobetaine} + \alpha\text{-KG} + O_2 + \text{Vitamin C} \rightarrow \text{Carnitine} + \text{Succinate} + CO_2 + \text{Dehydroascorbate}$$

7. Steroidogenesis in the adrenal cortex requires vitamin C.
8. The absorption of iron is significantly enhanced by the presence of vitamin C.
9. Vitamin C inhibits the formation of nitrosamines during digestion.
10. Vitamin C is a water-soluble antioxidant. This vitamin increases intracellular concentrations of tetrahydrobiopterin (BH4) in endothelial cells by protecting BH4 from oxidation. Therefore, vitamin C promotes endothelial NO synthesis, which is essential for the regulation of cardiovascular function (Wu and Meininger 2002).

Deficiencies and diseases: The classic syndrome of vitamin C deficiency is scurvy, which can be cured by the consumption of fruits and fresh vegetables (Padayatty and Levine 2016). The disease is related to defective collagen synthesis in the connective tissue (e.g., blood vessels). In addition to connective tissue abnormalities [including subcutaneous and other hemorrhages (bleeding), soft swollen gums, loose teeth, and capillary fragility], impaired wound healing, muscle weakness, fatigue, depression, poor growth, reduced feed intake, and increased risk for infectious diseases can also occur in animals with vitamin C deficiency (Jones and Hunt 1983). Oral administration of vitamin C is effective in treating these symptoms. Indeed, as early as 1617, John Woodall (a British physician) recommended the use of lemon juice (abundant in vitamin C) to prevent and treat scurvy in humans. Because farm animals can synthesize vitamin C, its severe deficiency normally does not occur. However, under certain conditions (e.g., heat stress), the demand for vitamin C may be greater than its endogenous synthesis, and therefore a dietary supplement may be beneficial for improving animal health and productivity (Ranjan et al. 2012; Roche 1991).

LIPID-SOLUBLE VITAMINS

Lipid-soluble vitamins are all isoprene derivatives. Concentrations of vitamin E and β-carotene in plant- and animal-sourced foods are shown in Table 9.9. Except for vitamin D_3, they are not synthesized in animal cells and must be supplied in ruminant diets (Pond et al. 1995). In ruminants, ruminal microbes can synthesize vitamin K_2, but not either vitamins A, D, and E or β-carotene. Lipid-soluble vitamins are absorbed efficiently by the small intestine (primarily in the jejunum) when diets contain lipids (Meydani and Martin 2001). The absorbed vitamins are assembled into chylomicrons (vitamins A, D, E, and K) and VLDL (vitamin E) in the enterocyte for export across its basolateral membrane into either the intestinal lymphatics (mammals and fish) or the portal vein (birds and reptiles) (Chapter 6). Like any other hydrophobic lipid, lipid-soluble vitamins are transported in plasma in their protein-bound forms and are excreted from the body primarily in the feces via the bile

TABLE 9.9
Content of Lipid-Soluble Vitamins and β-Carotene in Feedstuffs[a]

Feedstuff	Vitamin E	β-Carotene	Feedstuff	Vitamin E	β-Carotene
Alfalfa	49.8	94.6	Poultry byproduct meal	Trace	0.00
Barley grain	7.4	4.1	Peanut meal	2.7	0.00
Blood meal[b]	1.0	0.00	Pea seeds	0.2	1.0
Blood mealc	1.0	0.00	Rice bran	9.7	0.00
Canola meal	13.4	0.00	Rice grain[h]	2.0	0.00
Corn grain	8.3	0.8	Safflower meal	16.0	0.00
Cottonseed meal[d]	14.0	0.2	Sorghum grain	5.0	0.00
Feather meal	7.3	0.00	SBM[i]	2.3	0.2
Fish meal	5.0	0.00	Sunflower meal	9.1	0.00
Flax meal	2.0	0.2	Wheat bran	16.5	1.0
Meat and bone meal[e]	1.6	0.00	Wheat grain[j]	11.6	0.4
Milk[f]	4.1	0.00	Whey (dried)	23	0.00
Oat grain	7.8	3.7	Yeast[k]	10.0	0.00

Source: National Research Council (NRC) 1998.

[a] Values are mg/kg (on the as-fed basis).
[b] Conventional blood meal.
[c] Blood meal, spray or ring dried.
[d] Solvent extract; containing 41% crude protein.
[e] Meat and bone meal (93% dry matter).
[f] Bovine milk (skim, dried; 96% dry matter).
[g] Poultry byproduct meal (93% dry matter).
[h] Polished, broken.
[i] Soybean meal (solvent extracted, 89% dry matter).
[j] hard red, winter.
[k] Yeast, brewer's dried.

(Zempleni et al. 2013). Because of the body's ability to store surplus lipid-soluble vitamins, toxicity can result from their excessive intake, with the risk order: vitamin A > vitamin D > vitamin K > vitamin E (Zempleni et al. 2013).

Vitamin A

Structure: Vitamin A is chemically known as all-*trans*-retinol (the alcohol form). Its 13-cis isomer has a lower biological activity (e.g., 50% of all-*trans*-retinol in chicks). The term "retinoids" has been used to describe both the natural forms and the synthetic analogs of retinol. Vitamin A is a polyisoprenoid compound containing a cyclohexenyl ring (Figure 9.23). It is present in animal tissues and stored in the liver, mainly as retinol esters. Retinol derivatives are retinal (the aldehyde form) and retinoic acid (the acid form), which are called retinoids (Cascella et al. 2013). Most of the preformed vitamin A in animal-sourced foods is in the form of retinyl esters. Vitamin A is a pale yellow crystalline solid, insoluble in water but soluble in fat and various

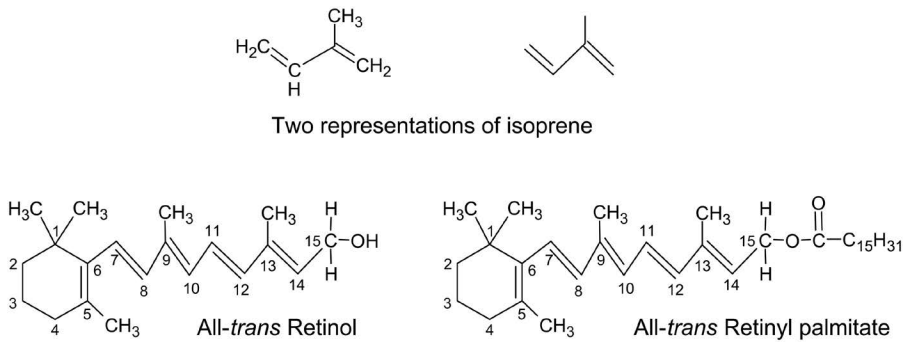


FIGURE 9.23 The chemical structure of vitamin A (also known as all-*trans*-retinol). This vitamin is an isoprene derivative containing a six-membered cyclohexenyl ring with methyl substitutions, as well as four double bonds in the side chain. The term "retinoids" has been used to describe both the natural forms and the synthetic analogs (e.g., retinyl palmitate) of retinol.

fat solvents. It is readily destroyed by oxidation on exposure to air and light. A related compound with the formula $C_{20}H_{27}OH$, originally found in fish, has been designated dehydroretinol or vitamin A_2.

Retinol and retinal are interconverted by NAD(P)H -dependent retinaldehyde reductase. However, once formed from retinal, retinoic acid cannot be converted back to retinal or to retinol (Blomhoff et al. 1992). Dietary β-carotene is converted to all-*trans*-retinol (vitamin A) by β-carotene dioxygenase and retinaldehyde reductase mainly in enterocytes (Figure 9.24), but this reaction is absent from the cat. Because ß-carotene is not efficiently metabolized to vitamin A, the biological activities of β-carotene and vitamin A are not equivalent on a per-weight basis. In humans, 6.0 mg of β-carotene is equivalent to 1.0 mg of retinol (Combs and McClung 2017). In animals expressing intestinal β-carotene dioxygenase, all-*trans*-retinaldehyde is also oxidized to all-*trans*-retinoic acid (another bioactive lipid metabolite of ß-carotene).

Sources: Foods of animal origin (e.g., liver, eggs, milk, and butter) contain vitamin A largely in the form of retinyl esters [i.e., retinol esterified with a molecule of long-chain fatty acid (LCFA) palmitic acid]. The liver is a good source of vitamin A. For example, concentrations of vitamin A in the livers of various animal species (μg/g liver) are as follows: pig, 30; cow, 45; rat, 75; man, 90; sheep, 180; horse, 180; hen, 270; codfish, 600; halibut, 3000; polar bear, 6000; and soup-fin-shark, 15000 (McDonald et al. 2021). Plants do not contain vitamin A. However, some plants are rich sources of provitamin A, which takes the form of a family of compounds called carotenoids, including α-carotene, β-carotene, γ-carotene, and β-cryptoxanthin (the major carotenoid in corn). Among these carotenoids, β-carotene has the greatest vitamin A-equivalent activity in ruminants and nonruminants (Gross and Budowski 1966; McDonald et al. 2021; Yeum and Russell 2002). Vegetables that are dark green, orange, and yellow are good sources of carotenoids. The efficiencies (%) of conversion of β-carotene to vitamin A in animals are as follows: chickens, 100; cattle, 24; dogs, 67; horse, 33; man, 33; pig, 30; rats, 100; and sheep, 30 (Berdanier 1998). Unlike dogs, cats cannot convert dietary β-carotene into vitamin A due to the absence of β-carotene 15,15-dioxygenase, although β-carotene is absorbed by the enterocytes of the feline small intestine (Schweigert et al. 2002).

An IU is used to standardize the biological activity of vitamin A and provitamin A carotenoids in various forms

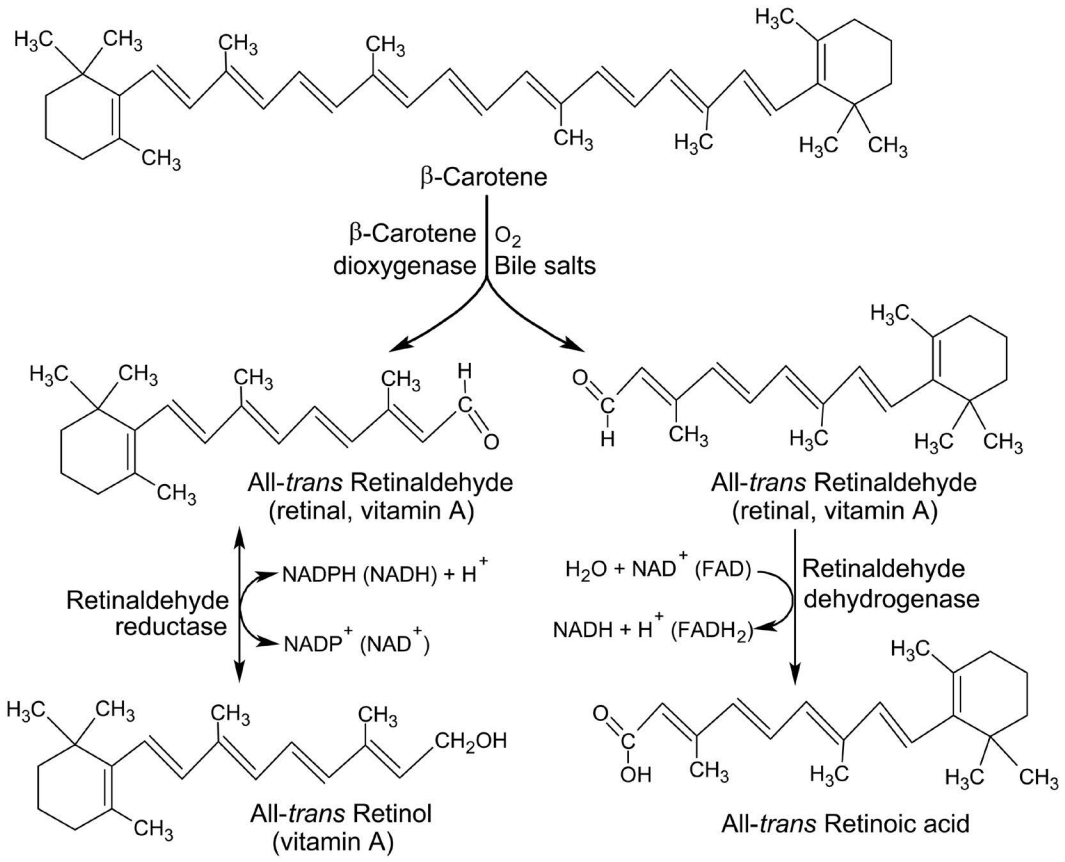


FIGURE 9.24 Conversion of β-carotene into vitamin A. This NADPH-dependent reaction occurs in the enterocytes of many mammals, birds, and fish. β-Carotene dioxygenase cleaves β-carotene to two molecules of all-*trans*-retinaldehyde (also known as retinal, an aldehyde form of vitamin A). The latter is either reduced by NADPH-dependent retinaldehyde reductase to all-*trans*-retinol or oxidized by retinaldehyde dehydrogenase to all-*trans*-retinoic acid. Cats lack β-carotene dioxygenase and thus cannot form vitamin A from β-carotene.

(Combs and McClung 2017). The equivalencies of vitamin A precursors to vitamin A in rats and chickens are as follows:

$$\begin{aligned}1\text{ IU of vitamin A} &= 0.30\ \mu g \text{ of crystalline all-}\textit{trans}\text{-retinol}\\ &= 0.344\ \mu g \text{ of retinyl acetate}\\ &= 0.55\ \mu g \text{ of retinyl palmitate}\\ &= 0.6\ \mu g \text{ of all-}\textit{trans}\text{-}\beta\text{-carotene}\\ &\quad (\text{synthetic, supplemental form})\\ &= 1.2\ \mu g \text{ other provitamin A carotenoids}\end{aligned}$$

In the United States, retinol activity equivalent (RAE) is currently the unit for the amount of vitamin A in food and the recommended dietary allowance (RDA) of vitamin A for humans. For calculation, 1 μg of RAE is equivalent to 1 μg of all-*trans*-retinol, 2 μg of synthetic (supplemental) all-*trans*-β-carotene, 12 μg of all-*trans*-β-carotene in plant foods (e.g., carrots and fruits), 24 μg of α-carotene, or 24 μg of β-cryptoxanthin (IOM 2001). There is no difference in biological function between naturally occurring and chemically synthetic β-carotene (Grune et al. 2010). However, the rate of intestinal absorption of synthetic β-carotene is generally greater than that for β-carotene in plant foods.

Retinol and retinal in the retina of the eyes and many other tissues are interconvertible through either NAD^+-dependent retinol dehydrogenase or a bifunctional retinoid oxidoreductase complex (ROC; Belyaeva et al. 2017). ROC is composed of at least two subunits of an NAD^+-dependent retinol dehydrogenase (which catalyzes the oxidation of retinol to retinal) and two subunits of an NADPH-dependent retinal reductase (which catalyzes the reduction of retinal back to retinol; Belyaeva et al. 2017). In animal cells, including photoreceptors and the retinal pigment epithelium, all-*trans*-retinal is oxidized by NAD^+-dependent retinal dehydrogenase to all-*trans*-retinoic acid.

$$\text{all-}\textit{trans}\text{-Retinol} + NAD^+ \rightleftharpoons \text{all-}\textit{trans}\text{-Retinal} + NADH + H^+ \quad (\text{Retinol dehydrogenase})$$

$$\text{all-}\textit{trans}\text{-Retinal} + NAD^+ + H_2O \rightleftharpoons \text{all-}\textit{trans}\text{-Retinoic acid} + NADH + H^+ \quad (\text{Retinal dehydrogenase})$$

The action of ROC is as follows:

$$\text{all-}\textit{trans}\text{-Retinol} + NAD^+ \rightleftharpoons \text{all-}\textit{trans}\text{-Retinal} + NADH + H^+ \quad (\text{Retinol dehydrogenase})$$

$$\text{all-}\textit{trans}\text{-Retinal} + NADPH + H^+ \rightleftharpoons \text{all-}\textit{trans}\text{-Retinol} + NADP^+ \quad (\text{Retinal reductase})$$

Absorption and transport: In the lumen of the small intestine, retinol esters are dispersed in bile droplets and then hydrolyzed by pancreatic enzymes (triglyceride lipase and lipase-related protein 2, as well as intestinal phospholipase B) to yield retinol (Reboul et al. 2006). Both retinol and β-carotene are solubilized with lipids and bile salts to form micelles, from which retinol is taken up by the enterocyte (primarily in the jejunum) through its apical membrane via passive diffusion. In contrast to retinol, carotenoids (including β-carotene) are taken up by enterocytes through their binding to a scavenger receptor class B, type I (SCARB1), which is also a receptor for HDL and other lipophilic compounds including tocopherols (Van Bennekum et al. 2005). Esterification of vitamin A in the enterocyte is essential to generate a concentration gradient across its apical membrane for the absorption of the vitamin and carotenoids. This is accomplished through (a) the conversion of carotenoids into vitamin A; (b) the binding of retinol to specific cellular retinoid-binding proteins (CRBPs), including the abundant CRBP2, which transports vitamin A from the cytosol to the endoplasmic reticulum; and (c) enzymatic esterification with saturated LCFAs by the action of lecithin:retinol acyltransferase and diacylglycerol *O*-acyltransferase 1 in the endoplasmic reticulum (O'Byrne et al. 2005; Wongsiriroj et al. 2008).

In rats and poultry, most of the dietary β-carotene absorbed into the intestinal mucosa is cleaved to retinol, and only a small amount of the absorbed β-carotene enters the lymph or blood circulation. In humans, cattle, horses, pigs, and sheep, some carotenoids escape conversion in the small intestine and enter the circulation. The absorbed carotenoids are transported in plasma, carried to tissues (see the paragraphs below), and contribute to the pigmentation of meat, eggs, and milk, although peripheral tissues can convert β-carotene into retinol. Being water-soluble, dietary retinoic acid is absorbed by the small intestine into the portal vein (Arnhold et al. 2002).

Inside enterocytes, retinol esters are incorporated into chylomicrons (O'Byrne et al. 2005). The chylomicrons exit the enterocyte across its basolateral membrane (via passive diffusion) into the intestinal lymphatics and then the bloodstream in mammals and fish, or into the portal circulation in birds and reptiles (Chapter 6). Following the hydrolysis of chylomicrons, their remnants (containing retinyl esters) are taken up by hepatocytes via an LDL receptor-mediated process (Chelstowska et al. 2016). The internalized REs then undergo enzymatic hydrolysis by carboxyl ester lipases, carboxylesterases, and hepatic lipases to release retinol. In the liver, vitamin A is stored as an ester in lipocytes (perisinusoidal stellate cells, located between the capillaries and the hepatocytes) as a lipoglycoprotein complex. This complex consists of about 13% protein [retinol-binding protein (RBP)], 42% retinyl esters, 28% triglycerides, 13% cholesterol, and 4% phospholipids (Vogel et al. 1999). The RBP is secreted by the liver (Blaner 1989) and, in gestating mammals, also by their conceptuses before the onset of their elongation and throughout the period of their periimplantation (Harney et al. 1990). For the transport of vitamin A from the liver to other tissues, the vitamin A ester of its lipoglycoprotein complex is hydrolyzed intracellularly to retinol, which is then bound to RBP. The resulting retinol-RBP is processed in the Golgi apparatus and secreted into plasma. The processed retinol-RBP complex exits the liver into the blood and is taken up into extrahepatic cells via plasma-membrane receptors. Thus, after its mobilization

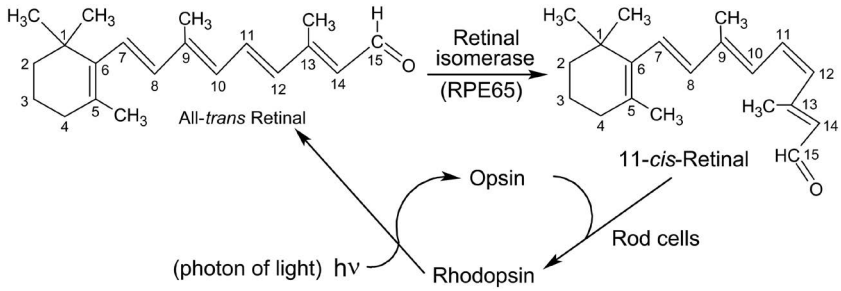


FIGURE 9.25 The mechanism whereby vitamin A is essential for vision. In the photoreceptor cells of the retina, all-*trans*-retinol is converted into all-*trans*-retinal. The latter is isomerized into 11-*cis*-retinal in the retinal pigment epithelium (RPE) by retinal isomerase [RPE65 (a 65-kDa protein)]. In rod cells, 11-*cis*-retinal is bound to a protein called opsin to generate rhodopsin. Upon exposure to light, rhodopsin dissociates to form all-*trans*-retinal and opsin; this reaction is coupled with the opening of a calcium ion channel in the membrane of rod cells. The rapid flux of calcium ion triggers a nerve impulse, allowing light to be perceived by the brain.

from the liver, vitamin A is transported in plasma in the RBP-bound form. In extrahepatic cells, retinol is bound to a specific cellular RBP (Kono and Arai 2015). The lactating mammary glands secrete most vitamin A as retinyl esters. Interestingly, in the eggs of reptiles, retinal is the major retinoid, and retinol is present at much lower levels, whereas significant amounts of both retinol and retinal are stored in the eggs of birds (Irie et al. 2010).

Concentrations of retinoic acid in plasma and tissues are much lower than those of retinol. For example, in mice, the concentrations of all-*trans*-retinoic acid in plasma (6 pmol/ml) and tissues (e.g., 15 and 20 pmol/g in the liver and kidney, respectively) are 2- to 3-orders of magnitudes less than total retinol (Vogel et al. 1999). Unlike vitamin A, retinoic acid is transported in plasma primarily in an albumin-bound form. Carotenoids that are not converted into vitamin A in enterocytes are incorporated into chylomicrons, which exit the cells into lymphatic circulation. Through lipoprotein metabolism in the blood, carotenoids retained in the chylomicron remnants are internalized by the liver. This organ assembles vitamin A into VLDL for secretion into the blood circulation.

Functions: Vitamin A has multiple functions in animals (Figures 9.24 and 9.25). Except for vision, most physiological actions of vitamin A are mediated by its bioactive metabolite retinoic acid (Cascella et al. 2013). For proper vision, retinol is converted into retinal, which is a component of the visual pigment known as rhodopsin (Figure 9.25). Rhodopsin occurs in the rod cells of the retina, cells that are responsible for vision under low-light conditions. When rhodopsin is exposed to light, it dissociates to form all-trans-retinal and opsin; this reaction is coupled with the opening of a Ca^{2+} channel in the membrane of the rod cell (Blaner 2020). The rapid flux of Ca^{2+} triggers a nerve impulse, allowing light to be perceived by the brain.

β-Carotene has an antioxidative property by forming a resonance-stabilized carbon-centered radical complex with a peroxyl radical (ROO•; Figure 9.26). Both retinol

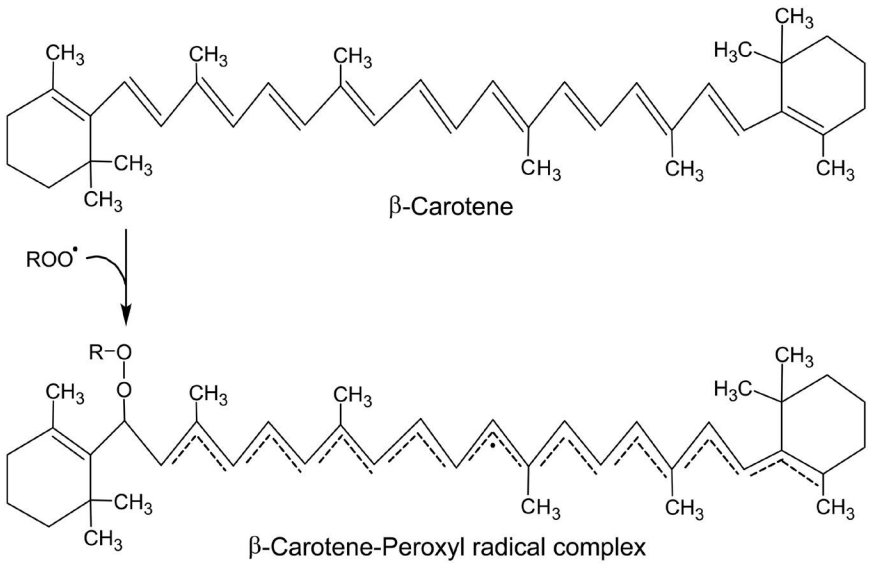


FIGURE 9.26 The formation of a resonance-stabilized carbon-centered radical complex from ß-carotene and a peroxyl radical (ROO•). This reaction occurs spontaneously. Carotene-like substances (shown in Figure 9.27) also have antioxidative functions.

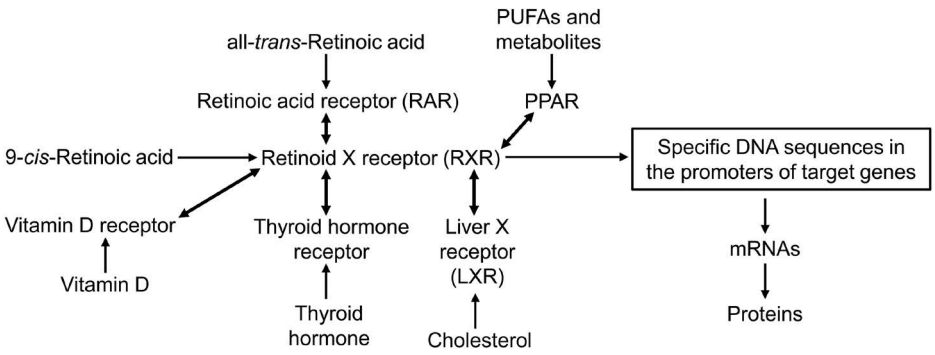


FIGURE 9.27 Retinoic acid, vitamin D, thyroid hormone, PUFAs, and cholesterol regulate gene expression in animal cells. In the nucleus, each of these molecules (ligands) binds to its respective receptor (nuclear transcription factor), which binds RXR to form a functional heterodimer. The latter binds to specific DNA sequences in the promoters of target genes to regulate the transcription of downstream target genes into mRNAs and proteins. PPAR, peroxisome proliferator-activated receptor; PUFAs, polyunsaturated fatty acids.

and retinoic acid play an important role in regulating gene expression by binding to the nuclear retinoid X receptor (RXR; Figure 9.27), as steroid hormones do. Retinoic acid can also alter the activities of transcription factors to promote the transcription of downstream target genes into mRNAs and eventually proteins (Chelstowska et al. 2016). There are reports that retinol and 9-*cis* retinoic acid upregulate the expression of ornithine aminotransferase in intestinal epithelial cells (Dekaney et al. 2008) and inducible NO synthase (Zou et al. 2007). In addition, retinoic acid participates in the synthesis of glycoproteins. This may account for, in part, the effect of retinoic acid in promoting cell growth and differentiation. Compelling evidence shows that retinol or retinoic acid is required for (a) maintenance of the integrity of normal epithelial tissues (e.g., the skin, small intestine, kidneys, blood vessels, uterus, placenta, and male reproductive tract); (b) spermatogenesis, embryonic survival, and fetal growth and development; (c) synthesis of glycosaminoglycans and growth of osteoclasts; (d) hematopoiesis; and (e) lymphoid organ development, antibody production by B-lymphocytes, and immune responses to pathogens (Ross 2012). Finally, β-carotene can stabilize organic peroxide free radicals within its conjugated alkyl structure and is an antioxidant at low oxygen concentration. A group of carotene-like compounds with an antioxidative activity are shown in Figure 9.28, but lycopene, lutein, and zeaxanthin have no vitamin A activity (Yeum and Russell 2002). Collectively, vitamin A is essential for vision, growth and development of cells (particularly epithelial cells), embryonic survival, and immunocompetence.

Deficiencies and diseases: Because vitamin A is stored in the liver, well-nourished animals (including humans) do not manifest signs or symptoms of its deficiency within weeks or months of consuming vitamin A-insufficient diets. Vitamin A deficiency occurs in mammals, birds, and fish consuming poor diets such as those with low contents of vitamin and provitamin A. Vitamin A deficiency restricts animal growth, impairs the function of many tissues and cells, increases risk for infectious diseases, induces testicular damage, and causes embryonic death/fetal resorption (Clagett-Dame and DeLuca 2002; Stephensen 2001). The initial symptom of vitamin A deficiency is defective night vision (night blindness, a defect in the retina). This disorder occurs when liver stores of the vitamin are nearly exhausted (Collins and Mao 1999). Further depletion of vitamin A leads to keratinization of epithelial tissues of the eyes, lungs, gastrointestinal and genitourinary tracts, and decreased mucous secretion. Finally, the deterioration of the eye's tissues (e.g., cornea), which is known as xerophthalmia (the extreme dryness of the conjunctiva) and keratomalacia (dryness with ulceration and perforation of the cornea), leads to blindness.

Excesses: Vitamin A toxicity occurs when the capacity of RBP for binding vitamin A has been exceeded, such that the cells are exposed to unbound retinol or retinoic acids. Thus, the chronic consumption of high levels of vitamin A is dangerous (Soprano and Soprano 1995). Assessing vitamin A status in animals with subclinical toxicity is complicated by the fact that this range of retinol concentrations in plasma are not sensitive indicators of hepatic vitamin A reserves. However, the symptoms of overt hypervitaminosis A include severe liver fibrosis, bone and eye abnormalities, pain in the bones, hair loss, dry or rough skin, neurological disorders, headache, dizziness, irritability, nausea, vomiting, and teratogenicity such as birth defects (Penniston and Tanumihardjo 2006; Soprano and Soprano 1995). Excess vitamin A as a teratogen has been demonstrated in many animal species, including chicks, dogs, guinea pigs, hamsters, mice, monkeys, rabbits, rats, and pigs (Collins and Mao 1999). Of note, there are species differences in sensitivity to hypervitaminosis A due to differences in vitamin A metabolism, as indicated by the toxicity of 13-*cis*-retinoic acid (isotretinoin, a derivative of vitamin A) that is mediated by its isomerization to the all-*trans*-retinoic acid (Nau 2001). Specifically, the sensitive species (primates and rabbits) metabolize 13-*cis*-retinoic acid to the active 13-*cis*-4-keto-retinoic acid, and their placental transfer of 13-*cis*-retinoic acid is extensive, whereas the insensitive species (rats and mice) eliminate 13-*cis*-retinoic acid rapidly through the detoxification to the β-glucuronide, and their placental transfer of 13-*cis*-retinoic acid is limited. In

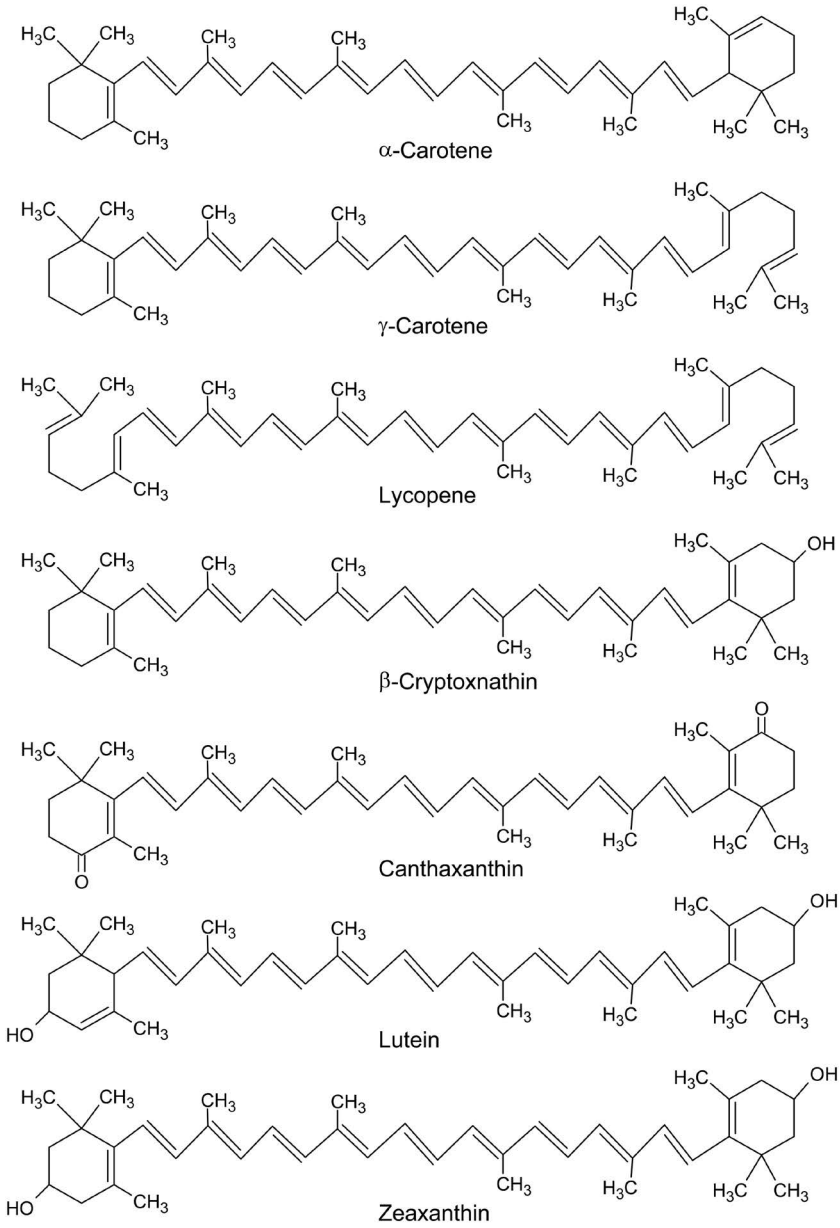


FIGURE 9.28 Carotene-like substances with antioxidative capacities in animal cells. α-Carotene, γ-carotene, β-cryptoxanthin, and canthaxanthin can be converted into vitamin A in the small-intestinal enterocytes and gastrointestinal bacteria of most animals, including ruminants, swine, dogs, poultry, and fish, but the efficiency of conversion is very low as compared to β-carotene. Cats cannot synthesize vitamin A from any carotenoids. Lycopene, lutein, and zeaxanthin are not precursors of vitamin A in mammals, birds, or fish.

addition, excess vitamin A not only stimulates the production of bone-resorbing osteoclasts (and thus bone resorption) but also inhibits bone formation, leading to bone loss and osteoporosis development (Lerner 2024). Results of research involving gene-knockout mice indicate that excess vitamin A causes acute and chronic toxicity by hyperactivation of the nuclear retinoic acid receptor (RAR) and RXR (Collins and Mao 1999). Carotenemia, which is manifested by a yellow-orange coloring of the skin, results from the ingestion of excessive amounts of vitamin A precursors in food (e.g., carrots). This may be desirable for the production of certain breeds of poultry in some regions of the world.

Vitamin D

Structure: Vitamin D is a steroid prohormone (Figure 9.29). The two most important forms are ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3). In animals, vitamin D is metabolized to 1,25-dihydroxyvitamin D (Jones et al. 1998). Vitamin D_3 is a hormone known as calcitriol, which plays a central role in Ca and phosphorus metabolism. The term "vitamin D_1" was originally suggested by earlier workers for an active component, but it was later found to consist mainly of vitamin D_2 and some impurities. Thus, the name vitamin D_1 has been abolished. Vitamin D is not water-soluble, but is

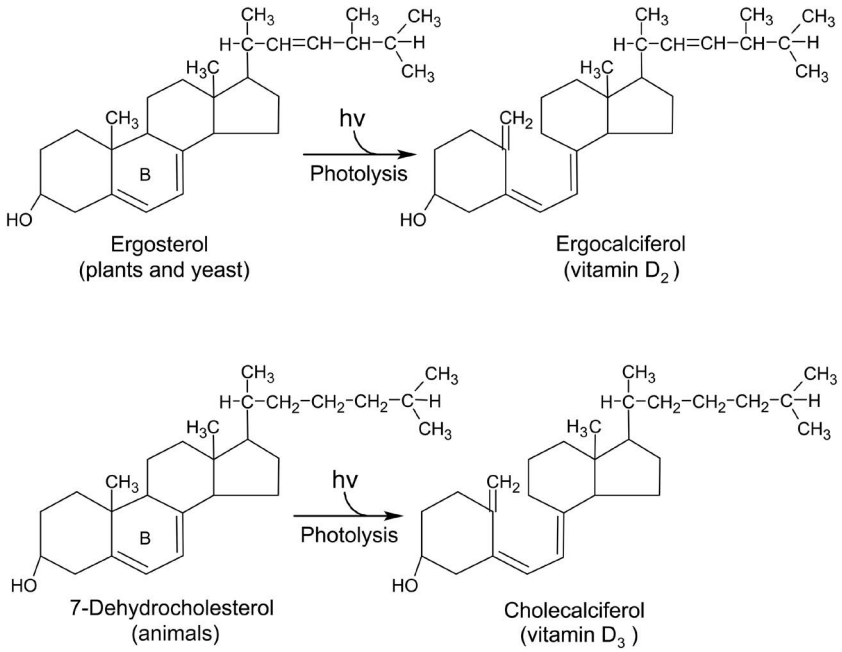


FIGURE 9.29 The chemical structures of vitamin D_2 and D_3, as well as their formation. In response to high-energy (hv) ultraviolet (UV) light from the sun, vitamin D_2 is formed from ergosterol in plants and yeast, and vitamin D_3 is synthesized from 7-dehydrocholesterol (a subcutaneous component of the skin) in most animals (including humans, pigs, poultry, and ruminants).

soluble in fat and organic solvents. Although a water-soluble sulfate derivative of vitamin D was reported to be present in cow's milk, this finding could not be verified for human breast milk or cow's milk (Okano et al. 1986). Both vitamin D_2 and vitamin D_3 are unstable upon over-irradiation and oxidized during storage. Vitamin D_3 is more stable than vitamin D_2. Vitamin D concentrates, like vitamin A, are usually protected by the addition of antioxidants.

Sources: Vitamin D is limited in feedstuffs for animals. This vitamin is rarely present in plants, except in sun-dried roughages (Peterlik 2012). An unfortified, typical corn- and SBM-based diet contains no vitamin D (Pond et al. 1995). In animals, this vitamin is present in small amounts in certain tissues and is abundant only in some fish. Halibut liver and cod liver oils are rich sources of vitamin D_3 (Egaas and Lambertsen 1979). The content of this vitamin in muscle meat is generally much lower than that in the liver, and the content of vitamin D in egg yolks ranges between the values for meat and offal. Unfortified cow's milk and dairy products are generally low in vitamin D, but butter has a high content of this vitamin (Schmid and Walther 2013).

Most animals (e.g., cattle, poultry, pigs, sheep, and rats) can synthesize vitamin D_3. Specifically, when the skin is exposed to sunlight (ultraviolet irradiation), the B-ring of 7-dehydrocholesterol is cleaved to yield cholecalciferol (vitamin D_3; DeLuca 2016). This conversion is most active with the light of wavelengths between 280–310 nm. Therefore, vitamin D is called the "sunshine vitamin". The rates of vitamin D_3 synthesis in animals vary with their living environments because (a) the amount of ultraviolet radiation that reaches the earth's surface depends on latitude and atmospheric conditions, (b) ultraviolet radiation is greater in the tropics than in the temperate regions, and (c) the presence of clouds, smoke and dust will reduce ultraviolet radiation (Christakos et al. 2016). Thus, air pollution impairs vitamin D_3 synthesis in grazing animals, including lactating cows (Weir et al. 2017). Because ultraviolet radiation cannot pass through ordinary window glass, animals housed indoors lack a subcutaneous synthesis of vitamin D_3. Ultraviolet radiation is more effective in animals with light-colored skins than those with dark skins. Interestingly, vitamin D-deficient rainbow trout can synthesize vitamin D3 in the skin when exposed to blue light (that can result from the photochemical action of visible light in water) at wavelengths between 380 and 480 nm (Pierens and Fraser 2015).

There are reports that cats and dogs, when exposed to appropriate ultraviolet irradiation, have a very limited ability to synthesize vitamin D_3 from 7-dehydrocholesterol in their skins (How et al. 1994). This is because of a low concentration of subcutaneous 7-dehydrocholesterol and a high activity of 7-dehydrocholesterol Δ^7 reductase that rapidly converts 7-dehydrocholesterol into cholesterol in both cats and dogs. For example, the concentration of 7-dehydrocholesterol in the skin of dogs is only about 10% of that in the skin of rats (How et al. 1994). Interestingly, unlike dogs, cats cannot use dietary vitamin D_2 (the vitamin D in sun-dried plants). Thus, cholecalciferol is generally added to commercial diets for cats. It is possible that the synthesis of vitamin D is also negligible in other carnivores.

In plants, the ultraviolet radiation of sunlight also cleaves the B-ring of ergosterol to generate ergocalciferol (vitamin D_2). The latter differs from vitamin D_3 only by possessing

a double bond between carbons 22 and 23, as well as an additional methyl group at carbon-24. There are reports that 1 kg of hay contains between 800 and 1700 IU of vitamin D_2 (McDonald et al. 2021). Like plants, fungi (e.g., yeast and mushrooms) are also rich in ergosterol, and its radiation results in the formation of vitamin D_2. The zooplankton (plants in rivers and ponds) exposed to UV light at the water surface can form vitamin D_2, which is a source of vitamin D for fish and crustaceans (Pierens and Fraser 2015).

Vitamin D_2 is almost as effective as vitamin D_3 in stimulating intestinal absorption of Ca and phosphate for most mammals (e.g., cattle, sheep, pigs, and humans; Christakos et al. 2016). However, vitamin D_2 has a very low biological activity in chickens and other birds (only ~10% of the potency of vitamin D_3; Proszkowiec-Weglarz and Angel 2013). Thus, poultry feeds must be fortified with vitamin D_3. For a few of the mammalian species (e.g., New World monkeys), vitamin D_3 is much more potent than vitamin D_2 on a weight basis (DeLuca 2016). In practice, with exceptions for poultry and certain monkeys, safe levels of vitamin D_2 can prevent abnormal bone development, as can vitamin D_3. Thus, the equivalency of vitamin D_3 to vitamin D_2 varies due to the factors mentioned previously for humans.

$$1 \text{ IU of vitamin D} = 0.025 \text{ μg of crystalline vitamin } D_3.$$

Absorption and transport: In the lumen of the small intestine, dietary vitamin D_3 and vitamin D_2 are solubilized with lipids and bile salts and then absorbed from the micelles by enterocytes via passive diffusion (Reboul 2015). Following absorption (primarily in the jejunum), vitamin D_3 and D_2 are assembled into chylomicrons in enterocytes. The vitamin D-containing chylomicrons exit the enterocyte across its basolateral membrane (via passive diffusion) into the intestinal lymphatics and then the bloodstream in mammals and fish, or into the portal circulation in birds and reptiles (Chapter 6). Through the metabolism of lipoproteins in the blood, the vitamin D-containing chylomicrons are converted into chylomicron remnants, which are subsequently taken up by the liver through receptor-mediated mechanisms (Chapter 6). Unlike other lipid-soluble vitamins, vitamin D is not stored in the liver but is distributed almost evenly among various tissues (Combs and McClung 2017). Transport of vitamin D_3 from the sites of its cutaneous synthesis into the liver via the blood is mediated by a specific protein, called vitamin D binding protein (also known as transcalciferin, an α-globulin). This binding protein has a higher affinity toward 1,25-dihydroxyvitamin D_3 than the vitamin D_2 derivative. In the liver, vitamin D is hydroxylated into 25-hydroxyvitamin D_3 or 25-hydroxyvitamin D_2, which is subsequently exported into the blood. The hepatocyte-derived 25-hydroxy vitamin D is transported in plasma bound to the vitamin D-binding protein. Vitamin D, if used externally, can also be absorbed effectively across the lipid bilayers of the skin.

Functions: Interorgan metabolism of vitamin D (Figure 9.30) is required for exerting its potent biological activity (DeLuca 2016). Specifically, in the liver of

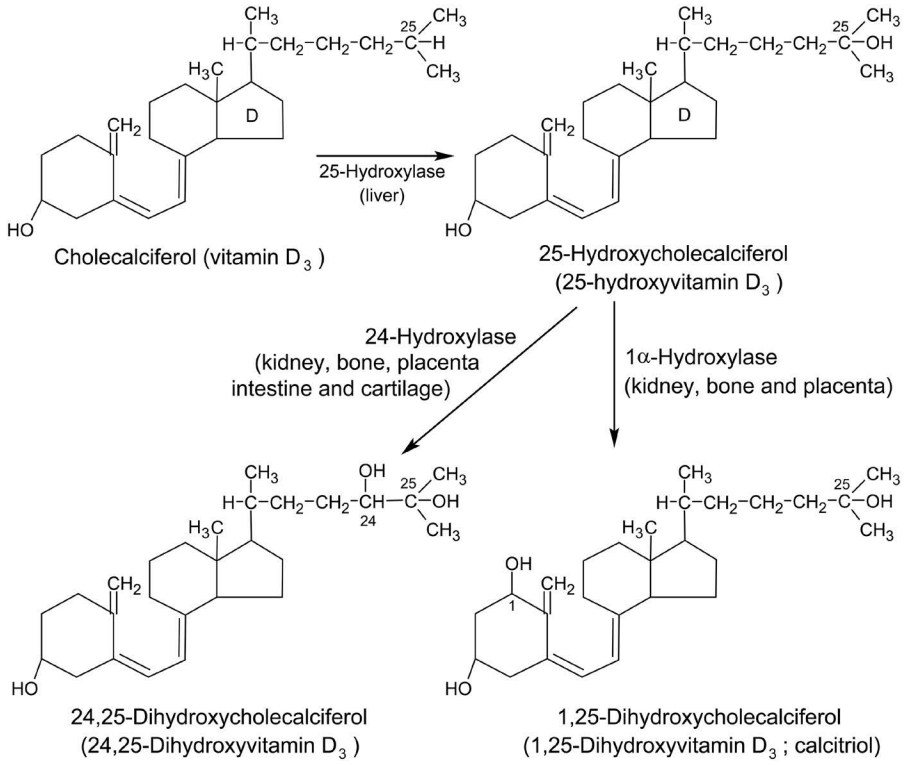


FIGURE 9.30 Conversion of vitamin D_3 into 1,25-dihydroxyvitamin D_3 (calcitriol) through interorgan cooperation involving the liver, kidneys, and bone, and, in the case of pregnant mammals, also placentae. Calcitriol is a hormone in animals.

mammals as well as both the liver and the kidneys of birds, vitamin D_3 is converted to 25-hydroxyvitamin D_3 by vitamin D_3-25-hydroxylase. This enzymatic activity involves cytochrome P-450-dependent mixed-function oxygenase. 25-hydroxyvitamin D_3 is the major form of vitamin D_3 in the blood circulation and the major storage form in the liver. In renal tubules (kidneys), bone, and placenta, the 25-hydroxyvitamin D_3 is further converted to 1,25-dihydroxyvitamin D_3 (calcitriol) by 25-hydroxyvitamin D_3-1-hydroxylase. As a compensatory response, renal vitamin D_3-25-hydroxylase activity is increased in heat-stressed laying hens that have low concentrations of Ca and P in plasma (Bailey and Creger 1982). Hypocalcemia, hypophosphatemia, and parathyroid hormone increase the activity of 1-hydroxylase, whereas 1,25-dihydroxyvitamin D_3 decreases the enzyme's activity. Vitamin D_3-25-hydroxylase and 25-hydroxyvitamin D_3-1-hydroxylase are mitochondrial enzymes. Vitamin D_2 undergoes the same transformations as vitamin D_3 in animals. The 1,25-dihydroxyvitamin D_3 is transported in plasma bound to the vitamin D binding protein.

The vitamin D hormone (1,25-dihydroxyvitamin D_3 or 1,25-dihydroxyvitamin D_2) binds the calcitriol receptor in the nucleus, thereby stimulating gene transcription and the formation of specific mRNAs that code for Ca- and phosphate-binding proteins (Christakos et al. 2016). 1,25-Dihydroxyvitamin D_3 has three important physiological effects: (a) activation of the vitamin D-dependent Ca and phosphate transport systems of the enterocyte; (b) stimulation of the osteoclasts for the release of Ca and phosphate from bone; and (c) enhancement of Ca and phosphate resorption by the kidney (DeLuca 2016). Approximately 65% and 80% of the filtered Ca and phosphate are reabsorbed, respectively, primarily within the proximal tubule under normal dietary intakes of the minerals (Christakos et al. 2016). Thus, vitamin D is essential for the regulation of Ca and phosphorus metabolism and consequently for the calcification and growth of bones.

Let us use "milk fever" to further highlight the function of vitamin D. Milk fever is a disease in cows whose plasma Ca levels drop below 5 mg/100 mL (DeGaris and Lean 2008). The disease occurs when a cow has been fed an alfalfa diet high in Ca before giving birth to a calf (calving) and lactation. When calving, the cow has a low appetite, and the intake of Ca from the diet becomes inadequate. The body requires a period of adjustment to activate the Ca-mobilizing mechanisms. Because the prior consumption of high-Ca diets leaves these mechanisms in the nonactivated state, the sudden decrease in feed intake by the lactating cow does not allow the animal to adjust and mobilize bone Ca at an adequate rate. The consequence is a rapid drop in plasma Ca concentration, which can lead to coma and sometimes death. Milk fever can be treated by intravenous administration of Ca or 1,25-dihydroxyvitamin D_3 into the sick lactating cow.

Deficiencies and diseases: There is no need for a dietary source of vitamin D if animals (except for cats and dogs) are exposed to sunlight for at least a short period of time during the day. However, vitamin D (preferably vitamin D_3) must be supplemented to plant-based diets of the livestock and poultry housed entirely indoors. Also, because the content of vitamin D_2 in dried hay varies considerably, vitamin D supplementation is desirable, especially for young ruminants and pregnant animals on winter diets. When animals and humans are not exposed to sunlight or do not receive adequate amounts of vitamin D_3 in their diets, a deficiency of vitamin D occurs. Animals with vitamin D deficiency exhibit abnormal growth and development (DeLuca 2016). Specifically, vitamin D deficiency causes rickets in young animals and children, and this disease is characterized by the low deposition of Ca and phosphate in bones, low mineral density of bones, and low breaking strength of bones (Leyva-Jimenez et al. 2019). Vitamin D deficiency also results in osteomalacia in adults, in which there is reabsorption of deposited bone tissue. Rickets and osteomalacia can also be caused by a dietary deficiency of Ca or P or by an imbalance between these two minerals. In young cattle, vitamin D deficiency symptoms include swollen knees. In pigs, deficiency symptoms are usually enlarged joints, broken bones, joint stiffness, and occasional paralysis (Roche 1991). In poultry, a deficiency of vitamin D causes weak legs as in all other animals, as well as reduced egg production and poor eggshell quality (Proszkowiec-Weglarz and Angel 2013). In young animals, vitamin D deficiency restricts their skeletal growth and development, leading to short stature. Dietary supplementation with vitamin D can effectively treat its deficiency symptoms in all vertebrates and crustaceans (Biasucci et al. 2024).

Excesses: Excess vitamin D or 25-hydroxyvitamin D is toxic to animals (DeLuca 2016). The symptoms of vitamin D intoxication are thirst, itchiness, diarrhea, malaise, weight loss, polyuria, diminished appetite, neurological deterioration, hypertension, irritability, nausea, vomiting, and headache. In addition, severe hypercalcemia, hyperphosphatemia, and hypermineralization occur in many tissues to cause excessive calcification, particularly the kidneys, aorta, heart, lung, and subcutaneous tissue. Bone pain is common in hypervitaminosis D. Different animals may have different sensitivity to excess vitamin D in diets. There are reports that 1,25-dihydroxyvitamin D is not responsible for toxicity caused by vitamin D or 25-hydroxyvitamin D (DeLuca et al. 2011). Of note, the elevated plasma concentration of Ca in hypervitaminosis D can be reduced by treatment with adrenal cortical steroids (Dipalma and Ritchie 1977).

Vitamin E

Structure: Vitamin E was discovered nearly 100 years ago in vegetable oils as a factor required for reproduction in rats. This nutrient was required to prevent fetal resorption in gestating rats fed lard-containing diets that were easily oxidizable (Niki and Traber 2012). It was given the name tocopherol (*tokos* = childbirth, *phero* = bear, *ol* = an alcohol

or phenol). Vitamin E refers to a group of tocol and tocotrienol derivatives that exhibit the biological activity of D-α-tocopherol (Combs and McClung 2017). However, based on essential roles in reproduction, only D-α-tocopherol is considered the active form of vitamin E in human nutrition (IOM 2000).

Tocopherols and tocotrienols are called chromanols, which protect cells from lipid peroxidation. Tocopherols are all isoprenoid-substituted 6-hydroxychromanes (tocols), namely side-chain derivatives of a methylated 6-chromanol nucleus. The most active form of tocopherol is D-α-tocopherol (Figure 9.31), and other tocopherols with much weaker biological activity include D-β-tocopherol, D-γ-tocopherol, and D-δ-tocopherol (Traber 2007). These four naturally occurring tocol compounds differ in the number of methyl groups (3, 2, or 1 $-CH_3$ groups) present in their aromatic ring but have the same three chiral centers (2R, 4'R, and 8'R) in the phytol (side) chain. Thus, tocopherol has 8 possible stereoisomers. D-α-Tocopherol has the widest natural distribution and the greatest biological activity of the vitamin E group (Table 9.10). The β, γ, and δ forms of D-tocopherol have only about 8.1%, 3.4%, and 0.4% of the activity of D-α-tocopherol (Combs and McClung 2017). Like tocopherol, tocotrienol also has α, β, γ, and δ isomers but has only one chiral center (2R in naturally occurring tocotrienols or 2S in synthetic tocotrienols). Tocotrienols differ from tocopherols because their phytol (side) chain has three double bonds, which are absent from tocopherols (Figure 9.31).

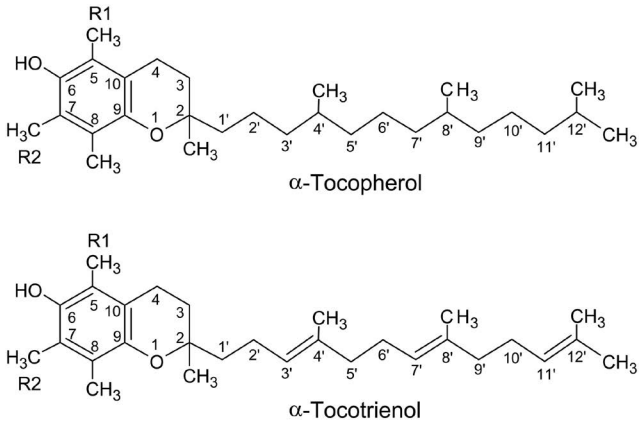


FIGURE 9.31 The chemical structures of tocopherols and tocotrienols. They are called chromanols. Tocopherols are all isoprenoid-substituted 6-hydroxychromanes (tocols). α-, β-, γ-, and δ-Tocopherols differ in R1 and R2 groups. Tocotrienols differ from tocopherols because they have three double bonds in the phytol (side) chain, and these bonds are absent from tocopherols. α-, β-, γ-, and δ-Tocotrienols differ in R1 and R2 groups.

Tocopherol	R1	R2	Tocotrienol	R1	R2
α-	CH_3	CH_3	α-	CH_3	CH_3
β-	CH_3	H	β-	CH_3	H
γ-	H	CH_3	γ-	H	CH_3
δ-	H	H	δ-	H	H

TABLE 9.10
Synthetic and Naturally Occurring Compounds with Vitamin E Activity[a]

Compound	Systematic Name	Biopotency (IU/mg of material)
	Synthetic tocopherol	
All-*rac*-α-Tocopheryl acetate[b]	2RS,4'RS,8'RS-5,7,8-Trimethyltocylacetate	1.0
All-*rac*-α-Tocopherol[b]	2RS,4'RS,8'RS-5,7,8-Trimethyltocol	1.1
	Naturally occurring tocopherol	
R,R,R-α-Tocopherol[b]	2R,4'R,8'R-5,7,8-Trimethyltocol	1.49
R,R,R-ß-Tocopherol	2R,4'R,8'R-5,8-Dimethyltocol	0.12
R,R,R-γ-Tocopherol	2R,4'R,8'R-5,7-Dimethyltocol	0.05
R,R,R-δ-Tocopherol	2R,4'R,8'R-8-Monomethyltocol	0.006
	Derivative of tocopherol	
R,R,R-α-Tocopheryl acetate	2R,4'R,8'R-5,7,8-Trimethyltocyl acetate	1.36
	Naturally occurring tocotrienol	
R-α-Tocotrienol	*trans*-2R-5,7,8-trimethyltocotrienol	0.32
R-β-Tocotrienol	*trans*-2R-5,8-dimethyltocotrienol	0.05
R-γ-Tocotrienol	*trans*-2R-5,7-dimethyltocotrienol	0.00
R- δ-Tocotrienol	*trans*-2R-8-momomethyltocotrienol	0.00

Source: Combs and McClung 2017.

[a] The four naturally occurring tocopherols differ in the number of methyl groups present in their aromatic ring, but have the same three chiral centers (2R, 4'R, and 8'R) in the phytol (side) chain. In contrast, tocotrienol has only one chiral center (2R in naturally occurring tocotrienols). Both tocopherols and tocotrienols have α, β, γ, and δ isomers, and they are all antioxidants.

[b] Recognized as having vitamin E activity.

Note: *Rac,* racemo. This term refers to a racemic mixture or racemate that has equal amounts of left- and right-handed enantiomers of a chiral molecule.

DL-α-tocopherol was chemically synthesized in the early 1970s, and its acetate ester was then adopted as the international standard for assessing the biological activities of all other forms of the vitamin. To date, the synthetic preparations of vitamin E (the acetate ester form) for animal feeding include all eight possible stereoisomers and are designated with the prefix all-*rac*-.

Sources: Vitamin E is widely distributed in feedstuffs. Vegetable oils (e.g., corn, soya bean, sunflower seed, safflower seed, and peanut oil) are good sources of this vitamin. Animal fats (e.g., butter and lard) and products (e.g., fish, eggs, and beef) also provide some vitamin E. However, corn and wheat grains are poor sources of this vitamin. For example, concentrations of total tocopherols in feedstuffs [mg/kg of dry matter (DM)] are as follows: corn grain, 4–10; fish meal, 21; lucerne meal, 190–250; safflower oil,

500; soybean oil, 1000; SBM, 3–6; various green forages, 200–400; wheat germ oil, 1700–5000; and wheat grain, 30–35 (McDonald et al. 2021). Accordingly, the concentrations of α-tocopherol in feedstuffs (mg/kg of DM) are as follows: corn grain, 0.5–3; fish meal, 21; lucerne meal, 180–240; safflower oil, 350; soybean oil, 100; SBM, 1; various green forages, 200–400; wheat germ oil, 800–1200; and wheat grain, 15–18 (McDonald et al. 2021). Thus, α-tocopherol accounts for nearly 100% of total tocopherols in fish meal and lucerne meal, but only 10% of total tocopherols in corn grain and SBM. Vitamin E is not very stable in storage, and its stability can be greatly improved by esterifying its hydroxyl group with acetic acid to form α-tocopheryl acetate (Byers and Perry 1992). Although fish liver oils are good sources of vitamin A and D, they have insignificant amounts of vitamin E. Vitamin E is destroyed by cooking and food processing including deep-freezing.

$$\begin{aligned}1\ \text{IU of vitamin E} &= 0.67\ \text{mg of D-}\alpha\text{-tocopherol (natural)}\\ &= 0.90\ \text{mg of DL-}\alpha\text{-tocopherol (synthetic)}\end{aligned}$$

Absorption and transport: In the lumen of the small intestine, dietary vitamin E (in the acetate ester or free alcohol form) is solubilized with lipids and bile salts. The esterified forms of the vitamin are hydrolyzed by pancreatic and duodenal-mucosal esterases to release its free alcohol form. The vitamin E-containing micelle is absorbed by enterocytes (primarily in the jejunum) via passive diffusion (Niki and Traber 2012). The efficiency of absorption of the acetate ester form of vitamin E is similar to that of its free alcohol form. Active fat absorption promotes the absorption of vitamin E by the small intestine. Impaired fat absorption leads to vitamin E deficiency because tocopherol is found dissolved in the fat of the diet and is liberated and absorbed during fat digestion (Zingg 2015).

Inside the enterocyte, vitamin E is assembled into chylomicrons and VLDL. These vitamin E-containing lipoproteins exit the enterocyte across its basolateral membrane (via passive diffusion) into the intestinal lymphatics and then the bloodstream in mammals and fish, or into the portal circulation in birds and reptiles (Chapter 6). Through the metabolism of lipoproteins in the blood, vitamin E-rich chylomicrons and VLDL are converted into chylomicron remnants and LDL, respectively. Unlike vitamins A and D, there is no specific carrier protein for vitamin E in plasma.

The vitamin E-containing lipoproteins in plasma are taken up by the liver through receptor-mediated mechanisms (Chapter 5). Inside hepatocytes, a protein (called α-tocopherol transfer protein) stimulates the movement of vitamin E between membrane vesicles and carries this vitamin to the nascent VLDL (Ulatowski and Manor 2013). The vitamin E-containing VLDL is then released from the hepatocytes into the blood. In the circulation, some VLDL is converted into LDL by lipoprotein lipases, and some vitamin E is transferred to HDL. The presence of vitamin E in lipoproteins helps to protect both proteins and polyunsaturated fatty acids in plasma. In the blood, vitamin E exchanges rapidly between lipoproteins and erythrocytes, which have a high rate of vitamin E turnover (25%/h), to protect these cells. Thus, vitamin E is transported in plasma via lipoproteins. The vitamin E-containing LDL is taken up into cells through the LDL receptor, and the intracellular degradation of the endocytosed LDL (Chapter 5) releases vitamin E. The intracellular transport of vitamin E requires specific tocopherol-binding proteins (Kono and Arai 2015). In white adipose tissue, vitamin E is stored mainly in the triacylglycerol fraction. In contrast, in most nonadipose cells, this vitamin is present almost exclusively in plasma and organelle membranes. In animals, vitamin E is stored primarily in white adipose tissue and liver. Note that vitamin E is released from white adipose tissue in response to exercise but not starvation (Landrier et al. 2012).

Functions: Vitamin E is an antioxidant against the peroxidation of polyunsaturated fatty acids in cellular and subcellular membrane phospholipids (Fang et al. 2002). Tocopherols act as antioxidants by breaking free-radical chain reactions because of their ability to transfer a phenolic hydrogen to a peroxyl free radical of a peroxidized polyunsaturated fatty acid (Figure 9.32).

$$\begin{aligned}&\text{ROO}^{\bullet} + \text{Tocopherol-OH} \rightarrow \text{ROOH} + \text{Tocopherol-O}^{\bullet}\\ &\text{ROO}^{\bullet} + \text{Tocopherol-O}^{\bullet} \rightarrow \text{ROOH}\\ &\quad + \text{Non-free radical product}\\ &\text{Tocopherol-O}^{\bullet} + \text{Vitamin C (reduced)} + 2\ \text{GSH} \rightarrow\\ &\quad \text{Tocopherol-OH} + \text{Vitamin C (oxidized)} + \text{GSSG}\end{aligned}$$

The non-free radical oxidation product is conjugated with glucuronic acid via the 2-hydroxy group and excreted in bile. In this chemical reaction, tocopherol is not recycled after carrying out its functions and must be replenished from the diet. The antioxidant action of tocopherol is effective in solutions with high oxygen concentrations and in tissues exposed to high O_2 partial pressures, for example, the erythrocyte membrane, the membranes of the respiratory tree, the retina, and nerve tissues (Traber 2007). By keeping the cell membrane and nucleus intact, vitamin E α-tocopherol is necessary for the structure of lipid bilayers, cell adhesion, nutrient transport, and tissue-specific gene expression. Furthermore, by affecting protein-membrane and lipid-membrane interactions, vitamin E can alter the trafficking of intracellular proteins and lipids, as well as cell signaling (Zingg 2015). Because excessive amounts of reactive oxygen species are harmful to macromolecules and cell membranes, antioxidant nutrients (including vitamin E) may prevent inflammation, infertility, muscular and neurological degeneration, cardiac dysfunction, skin lesions, hemolytic anemia, and aging (Kim and Han 2019). Finally, vitamin E can enhance immunity in animals, such that neonatal immunity is improved in chicks hatched from hens fed vitamin E-supplemented diets (Haq et al. 1996).

Because synthetic vitamin E is relatively expensive as a food supplement, there is an increasing interest in using

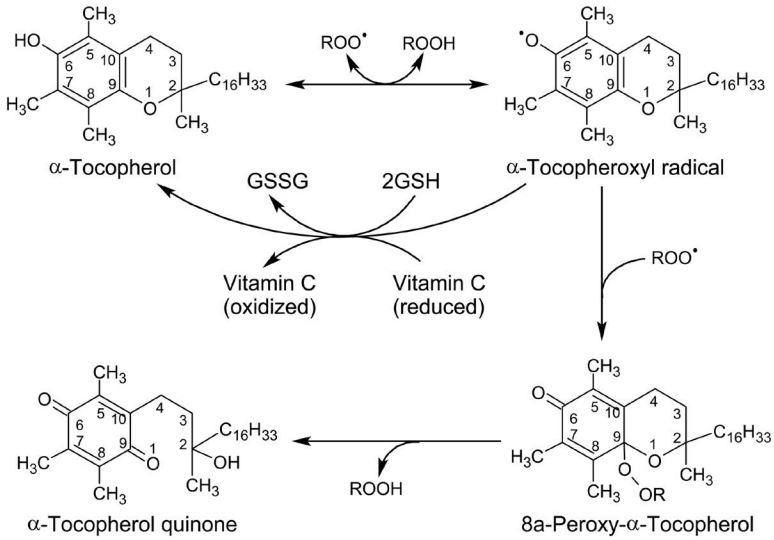


FIGURE 9.32 Role of vitamin E (α-tocopherol) in protecting cells against oxidants. Vitamin E donates a hydrogen from the hydroxyl group on its chromane ring to reactive oxygen species (e.g., lipid peroxyl radical, ROO•). In this process, the reactive oxygen species is converted to a relatively stable and inactive substance (e.g., lipid hydroperoxide, ROOH), and vitamin E is oxidized to α-tocopheroxyl radical. The latter is converted back into α-tocopherol via glutathione- and vitamin C-dependent enzymatic reactions. Thus, vitamin E can prevent against lipid peroxidation and scavenge free radicals in animals.

dietary polyphenols as its alternatives in animal feeding (Bešlo et al. 2023). Polyphenols (e.g., flavonoids, quercetin, caffeic acid, gallic acid, and ferulic acid in plants) are a group of water-soluble compounds that contain multiple phenol units with hydroxyl (-OH) groups and possess diverse and complex chemical structures. The name of polyphenol is derived from the Greek word *polus*, meaning "many or much," and has been used at least since 1894. As antioxidants that can form hydrogen bonds, metal ion-chelate complexes, and nonreactive adducts, polyphenols act by (a) protecting vitamin E, vitamin C, unsaturated fatty acids, and other organic nutrients from oxidation (including peroxidation); (b) regenerating oxidized vitamin E and oxidized vitamin C into biologically active forms; and (c) beneficially modulating the composition and activity of the intestinal microbiota. Thus, dietary supplementation with polyphenols can preserve vitamin E and increase its concentration in the blood, leading to a partial replacement of vitamin E in rations (Gessner et al. 2017). However, polyphenols and vitamin E are quantitatively and qualitatively not equivalent in either biology or nutrition. Because polyphenols and vitamin E differ in chemical, biochemical, and physiological properties, polyphenols cannot substitute for vitamin E in animal reproduction, immune responses, the integrity and functions of cells (including erythrocytes), or the regulation of cell-specific gene expression.

Deficiencies and diseases: Deficiency of vitamin E may cause anemia in newborns and adults due to the decreased production of hemoglobin and a shortened erythrocyte life span (Niki and Traber 2012). In all females and males, vitamin E deficiency results in impaired reproduction (e.g., a decrease in sperm production and dead fetuses, spontaneous abortion, and fetal resorption), muscle weakness and muscular dystrophy, skin and ocular lesions, and edema (Jones and Hunt 1983). Hepatic damage and muscular degeneration (myopathy) are the most frequent manifestations of vitamin E deficiency in laboratory and farm animals and are useful signs for diagnosis. These diseases include liver necrosis in rats and pigs, exudative diathesis in chicks due to increased capillary permeability, and white muscle disease in lambs and calves. Diets for animals (e.g., gestating and lactating sows, cows in lactating and dry periods, growing broilers, and growing fish) must contain vitamin E to maintain optimal health, growth, and feed efficiency (Politis 2012; Pond et al. 1995). Of particular note, D-α-tocopherol, but not its isomers or tocotrienols, can prevent neural damage or fetal resorption in vitamin E-deficient animals (Zempleni et al. 2013).

Excesses: When tocopherol is oxidized, it becomes a free radical species (Fang et al. 2002). Thus, high levels of dietary vitamin E can be toxic to animals. Symptoms of hypervitaminosis E include hepatic injuries and excess white-fat accumulation in rats, testicular atrophy in hamsters, slow development of secondary sex characteristics in roosters, and a teratogenic effect in mice (Dipalma and Ritchie 1977). Chicks with vitamin E toxicity exhibit reductions in growth rate, thyroid function, mitochondrial respiration, bone calcification, and hematocrit, but increases in reticulocytosis (March et al. 1968, 1973) and lethality of chick embryos (Bencze et al. 1974). Finally, dietary supplementation with excess vitamin E enhances the risk of developing heart failure after myocardial infarction (Marchioli et al. 2006) and all-cause mortality (Miller et al. 2005) in human subjects. Thus, excess vitamin E should be avoided in animal diets.

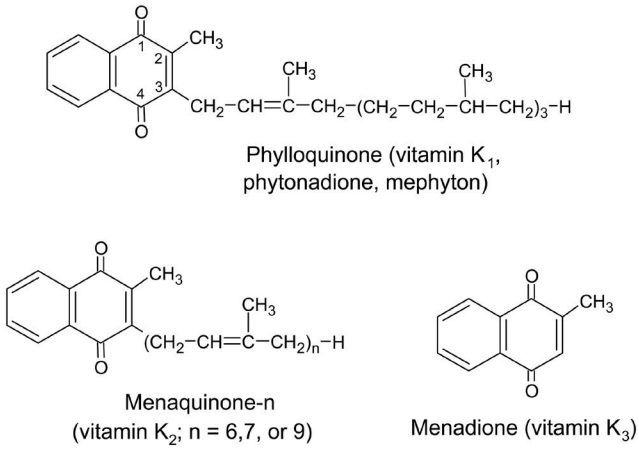


FIGURE 9.33 The chemical structure of vitamin K. Vitamins of the K group are polyisoprenoid-substituted napthoquinones. Phylloquinone (K_1) is the major form of vitamin K found in plants, whereas menaquinone (vitamin K_2) is synthesized by intestinal bacteria. Vitamin K_3 (menadione) is a synthetic, water-soluble vitamin K.

Vitamin K

Structure: Vitamin K is the generic name for 2-methyl-1,4-naphthoquinone and all of its derivatives with the biological activity of phylloquinone. Vitamins of the K group are polyisoprenoid-substituted napthoquinones (Figure 9.33). Phylloquinone (K_1) is the major form of vitamin K found in plants. Menaquinone (vitamin K_2) is synthesized by intestinal bacteria and found in animal tissues. The letter "K" originates from the German word "koagulation" (coagulation, blood clotting). Vitamin K_3 (menadione) is a synthetic vitamin K and is not found naturally. The catabolism of vitamin K_1 in the intestine can generate vitamin K_3, whereas the metabolism of vitamin K_3 in animals can lead to vitamin K_2 (menaquinone; Hirota et al. 2013). Dihydrovitamin K is the active form of vitamin K. Vitamins K_1 and K_2 are lipid-soluble, whereas vitamin K_3 is water-soluble.

The discovery of vitamin K had three independent beginnings with toxicological, nutritional, and biochemical studies (Ferland 2012). In toxicology, a hemorrhagic (bleeding) disease arose in cattle that had been eating spoiled sweet clover. Clover contains a bitter chemical called coumarin. During spoilage, microbes convert coumarin into dicumarol (4-hydroxydicoumarin), which inhibits 2,3-epoxide reductase and thus inhibits the recycling of vitamin K (Figure 9.34). The dicoumarol in the spoiled clover caused the death of many animals, especially after dehorning and castration procedures. Similarly, administration of Warfarin, which is structurally similar to dicoumarol (Figure 9.35), is now commonly used as a drug to (a) induce experimental anticoagulation in animals, (b) treat blood clotting (e.g., deep vein thrombosis and pulmonary embolism), and (c) prevent stroke in certain patients with

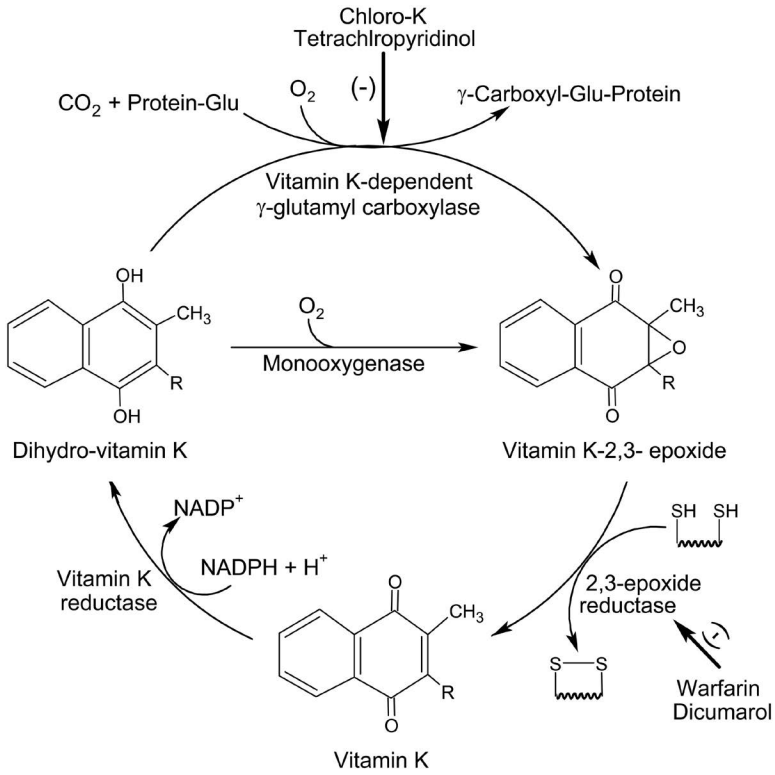


FIGURE 9.34 Vitamin K recycling (also known as the vitamin K-epoxide cycle) in animal cells. Vitamin K is reduced enzymatically to dihydro-vitamin K, which is subsequently oxidized to Vitamin K-2,3-epoxide. The latter is converted back into vitamin K by a thiol cofactor (e.g., reduced thioredoxin)-dependent 2,3-epoxide reductase. This enzyme is inhibited by Warfarin and dicumarol.

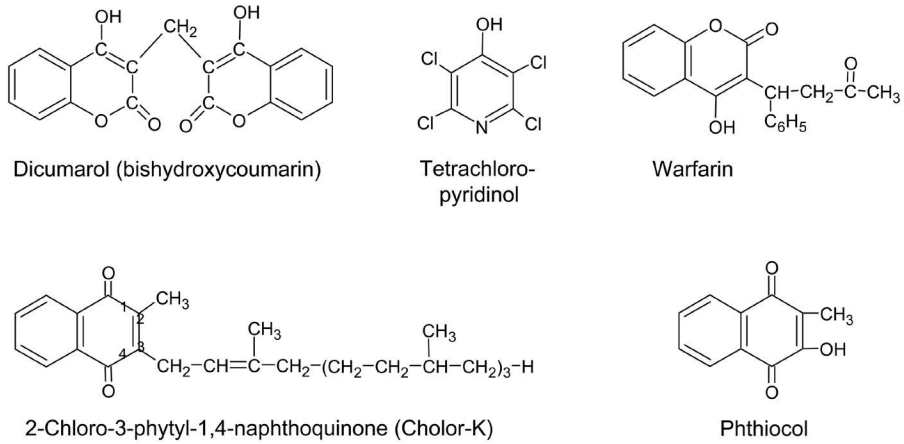


FIGURE 9.35 The chemical structures of vitamin K antagonists. Dicumarol (also known as dicoumarol) is a natural compound found in sweet clover, whereas tetrachloropyridinol, Warfarin, 2-chloro-3-phytyl-1,4-naphthoquinone, and phthiocol are chemically synthetic substances. They are highly effective anticoagulants that inhibit vitamin K-dependent coagulation factors.

blood-thickening conditions (Clark et al. 2015). In nutrition, rats fed a lipid-deficient diet suffered from a bleeding disease. However, this disease could be cured by treatment with (a) an extract of alfalfa that contained vitamin K_1 and (b) spoiled fishmeal that contained vitamin K_2. The finding that phthiocol was effective in treating the bleeding disease provided an early clue to the structure of vitamin K. In biochemistry, as noted previously, vitamin K was found to be required for the biological activities of blood clotting factors and vitamin K-dependent proteins. This work helped to elucidate the mechanisms whereby vitamin K is an essential nutrient in all animals.

Sources: Plants and green algae can synthesize K_1 from chorismate (Basset et al. 2017), whereas bacteria can synthesize vitamin K_2 from chorismate and isoprene (glucose metabolites; Bentley and Meganathan 1982). In contrast, there is no de novo synthesis of any vitamin K in animal cells. Vitamin K is widely distributed in plants, with bananas and green leafy vegetables (e.g., spinach, lettuce, broccoli, brussels sprouts, and cabbage) being good sources of vitamin K (Shearer et al. 1996). Certain plant (e.g., alfalfa)- and animal (e.g., meat)-sourced feedstuffs are good sources of this vitamin in animal tissues. Milk also provides some vitamin K for mammalian neonates. Fruits and grains contain little vitamin K. In ruminants, ruminal bacteria supply a nutritionally significant quantity of vitamin K_2 to the host. In all animals, the production of vitamin K_2 by intestinal bacteria can also contribute vitamin K for metabolic utilization by the hosts. However, bacterial synthesis of vitamin K_2 is insufficient to meet the needs of the animals for this vitamin. Preterm neonates often receive administration of vitamin K to prevent bleeding because they have only a limited number and species of bacteria in their intestine (Clarke 2010).

As noted previously, the vitamin K cycle in the endoplasmic reticulum allows reduced vitamin K to be regenerated (Booth and Suttie 1998). In this cycle, the 2,3-epoxide product of the carboxylation reaction is converted to the quinone form of vitamin K by 2,3-epoxide reductase, using a dithiol reductant (Figure 9.34). NADPH is required for the reduction of the quinone form of vitamin K to the dihydroxyquinone form of vitamin K. Note that the vitamin K cycle helps to conserve vitamin K but does not result in a net synthesis of this vitamin.

Absorption and transport: In the lumen of the small intestine, dietary vitamin K_1 and K_2 (naturally occurring vitamin K) are solubilized with lipids and bile salts and absorbed from the micelles by enterocytes via passive diffusion. Absorption of naturally occurring vitamins K_1 and K_2 by the intestine requires normal fat absorption and occurs only in the presence of bile salts, like other lipid-soluble vitamins. Inside the enterocyte, vitamins K_1 and K_2 are assembled into chylomicrons, which exit the enterocyte across its basolateral membrane (via passive diffusion) into the lymphatics and then the bloodstream in mammals and fish, or into the portal circulation in birds and reptiles (Chapter 6). The water-soluble vitamin K_3 and its naturally occurring structural analog [5-hydroxy-menadione (plumbagin)] are absorbed into enterocytes and colonocytes via their apical membrane multidrug resistance–linked ABC drug transporter-2 (ABCG2; Shukla et al. 2007). Both vitamin K_3 and plumbagin exit the intestinal epithelial cells across their basolateral membranes via MRP1 [also known as ABC drug transporter-1 (ABCC1)]. Absorbed vitamin K_3 and plumbagin enter the portal circulation. In contrast to vitamins K_1 and K_2, intestinal absorption of vitamin K_3 and plumbagin does not directly depend on bile salts.

Through the metabolism of lipoproteins in the blood, vitamin K-rich chylomicrons are converted into chylomicron remnants, which are taken up by the liver through receptor-mediated mechanisms (Chapter 6). In hepatocytes, the catabolism of the chylomicron remnants releases vitamin K, which is subsequently transferred into VLDL and LDL for export into the blood. As noted previously, dietary vitamin K_1 and K_3 are metabolized to yield vitamin K_2 in certain tissues (e.g., liver, kidneys, and brain) via the conversion of vitamin K_1 into vitamin K_3, followed by its prenylation

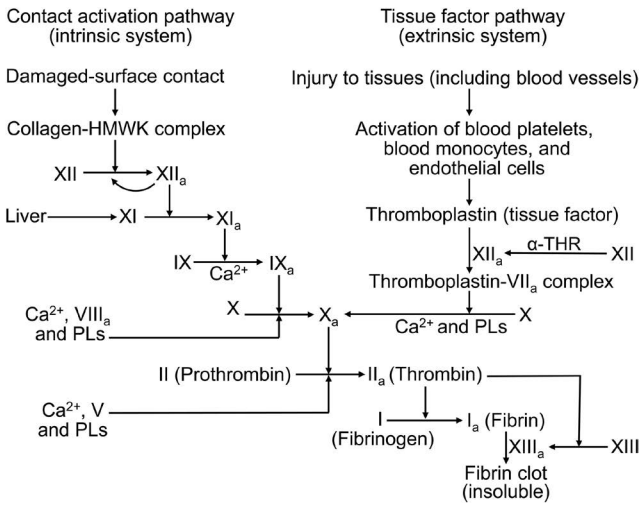


FIGURE 9.36 Contact activation (intrinsic) and tissue factor (extrinsic) pathways for blood clotting. Coagulation requires many proteins, which are synthesized by the liver and circulate as zymogens (inactive forms) in the blood. Most of these proteins are serine proteases, but factors VIII and V are glycoproteins, and factor XIII is a transglutaminase. Procoagulants (factors II, VII, IX, and X) depend on vitamin K for activity. An active form of coagulants is indicated by a subscript letter "a". In the intrinsic pathway, all factors are present in plasma, and the formation of a collagen/high-molecular-weight kininogen complex due to contact with the damaged surface of tissues triggers a cascade of reactions to form an insoluble fibrin clot. In the extrinsic pathway, some cell types (e.g., blood platelets and monocytes, as well as endothelial cells) of the injured tissues release thromboplastin (tissue factor plus phospholipids), which triggers a cascade of reactions to form an insoluble fibrin clot. The extrinsic pathway is the primary pathway for coagulation in animals. PLs, phospholipids; α-THR, α-thrombin.

through the action of UbiA prenyltransferase in the endoplasmic reticulum (Nakagawa et al. 2010). Unlike vitamins A and D, there is no specific carrier protein for vitamin K in plasma. Through the metabolism of lipoproteins, the VLDL is converted into LDL, which is taken up by the extrahepatic tissues via receptor-mediated mechanisms (Chapter 6).

Functions: In animals, a long-chain vitamin K_2 (menaquinone-4) is the major constituent of the hepatic vitamin K pool, with vitamin K_1 being a minor one (Combs and McClung 2017). Menaquinone-4 is ubiquitously present in extrahepatic tissues, with high concentrations in the brain, kidneys, and pancreas. Vitamin K is a coenzyme of procoagulation factors (II, VII, IX, and X) via contact-activation (intrinsic) and tissue-factor (extrinsic) pathways (Figure 9.36). Specifically, procoagulation factors (including II, VII, IX, and X) are synthesized by the liver and circulate in the blood as zymogens (inactive forms) in the blood. Many of them are serine proteases. Activation of these zymogens requires a cascade of vitamin K-dependent reactions, which include the carboxylation of some glutamate residues in the zymogens to form γ-carboxyglutamates (Figure 9.37). The γ-carboxyglutamates of zymogens chelate Ca^{2+} to form biologically active enzymes (proteins). For example, 10 of the glutamate residues in prothrombin (factor II) are carboxylated by factor X_a in the presence of Ca^{2+}, factor V, and phospholipids, resulting in the generation of thrombin (factor II_a). The latter converts the soluble fibrinogen into the insoluble fibrin clot in the presence of factor $XIII_a$ (Figure 9.36) to stop bleeding (Basset et al. 2017).

Vitamin K also plays a key role in the control of hemostasis by serving as a cofactor of anticoagulation proteins, namely proteins S, C, and Z (Esmon et al. 1987; Yin et al. 2000). Protein S (named for Seattle, Washington, where it was discovered in 1979) is a cofactor to protein C, which inhibits factors V_a and $VIII_a$ (Castoldi and Hackeng 2008). Protein Z is a cofactor of a serpin (serine protease inhibitor) that inhibits factor X_a (Yin et al. 2000). Proteins S, C, and Z are important for normal blood flow.

Other vitamin K-dependent proteins include osteocalcin (a bone-specific protein), matrix Gla protein (an inhibitor of soft tissue calcification), and growth arrest specific gene 6 (Gas-6) protein (which plays a role in platelet aggregation; migration and proliferation of vascular smooth muscle cells; thromboembolism, inflammation, and immune response; Shearer et al. 1996; Shiraki et al. 2015). Thus, vitamin K also plays an important role in bone growth and health, as well as cardiovascular and immunological functions.

Deficiencies and diseases: Hemorrhagic disease can occur in newborn infants, who are particularly vulnerable to vitamin K deficiency (Rai et al. 2017). This is because (a) the placenta does not efficiently pass vitamin K to the fetus,

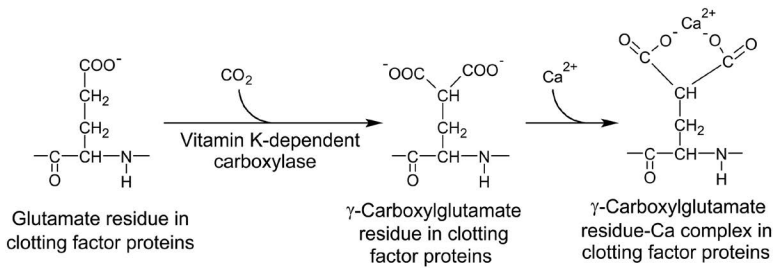


FIGURE 9.37 Vitamin K-dependent carboxylation of coagulating protein factors. This post-translational modification of glutamate residues in the clotting factors (proteins) to form γ-carboxyglutamate residue (Gla) is catalyzed by a vitamin K-dependent carboxylase. This allows for the chelation of Gla with Ca^{2+} that is necessary for the function of coagulating protein factors.

and (b) the gut is essentially sterile immediately after birth. If prothrombin (factor II) concentrations in plasma drop too low because of vitamin K deficiency, the hemorrhagic (bleeding) syndrome may appear. Both subcutaneous and internal hemorrhages manifest in the young and adults when vitamin K is grossly inadequate, leading to the loss of blood and anemia. In all animals, vitamin K deficiency can be caused by fat malabsorption, which may be associated with pancreatic dysfunction, biliary disease, atrophy of the intestinal mucosa, or any cause of steatorrhea (excess fat in fecal discharges; Ferland 2012; Jones and Hunt 1983). In addition, sterilization of the large intestine by antibiotics can result in vitamin K deficiency when its dietary intake is limited. Diseases of vitamin K deficiency can be cured through oral (nonruminants) or intravenous (ruminants) administration of this vitamin.

Excesses: Vitamin K_3 is an oxidant and also undergoes monovalent reduction to the semiquinone radical, which can be further oxidized by O_2 to the quinone with the formation of a superoxide anion (Basset et al. 2017). This form of vitamin K has been reported to oxidize hemoglobin into methemoglobin (Broberger et al. 1960). Thus, high concentrations of vitamin K_3 in the blood cause erythrocyte instability, hemolysis, and fatal anemia (Finkel 1961), as well as jaundice, hyperbilirubinemia, and kernicterus in infants (Owens 1971). In addition, the intramuscular administration of K_1 into the buttock may result in sciatic nerve paralysis (Dipalma and Ritchie 1977). Irritability, nausea, vomiting, and headache also occur in hypervitaminosis K. Furthermore, administration of high doses of vitamin K_3 can induce renal toxicosis in the horse, as indicated by renal colic, hematuria, azotemia, and electrolyte abnormalities (Rebhun et al. 1984). This is likely due to oxidative stress in the kidneys.

QUASI-VITAMINS

Quasi-vitamins include *p*-aminobenzoic acid, carnitine, choline, flavonoids, *myo*-inositol, lipoic acid, PQQ, and ubiquinones (Table 9.11). Most of them are soluble in water. They are

TABLE 9.11
Dietary and Metabolically Active Forms of Vitamins and Quasi-Vitamins

Vitamin	Major Dietary Form	Metabolically Active Form	Major Function
	Lipid-soluble vitamins		
Vitamin A	Retinyl palmitate and acetate; Provitamin A (β-carotene)	Retinol, retinal, retinoic acid	Vision, epithelial cell function, and gene expression
Vitamin D	Cholecalciferol (D_3), ergocalciferol (D_2)	1,25-dihydroxy-vitamin D	Skeletal growth and development
Vitamin E	All-*rac*-α-tocopherol acetate	α-,β-,γ-, and δ-Tocopherols	Anti-oxidative function
Vitamin K	Phylloquinones, menaquinones, and menadione sodium bisulfite	Phylloquinones, menaquinones	Blood clotting
	Water-soluble vitamins		
Vitamin C	L-Ascorbate	L-Ascorbate, dehydroascorbate	Hydroxylation of Pro and Lys residues in collagen; antioxidant
Thiamin	Thiamin, thiamin pyrophosphate	Thiamin pyrophosphate	Oxidative decarboxylation of KA
Riboflavin	FMN, FAD, flavoproteins, riboflavin	FMN, FAD	Co-factors of flavoproteins
Niacin	NAD(P), nicotinamide, nicotinic acid	NAD(P)	Co-enzymes of oxidoreductases
Vitamin B_6	Pyridoxal and pyridoxamine 5'-phosphate	Pyridoxal 5'-phosphate	Coenzyme for AA transaminase
Biotin	Biocytin, D-biotin	D-Biotin	ATP-dependent carboxylation
Pantothenate	Coenzyme A, acyl-CoA, Ca pentothenate	Coenzyme A (CoA)	A component of CoA and ACP
Folate	Mono-, poly-, and pteroyl-glutamates	THF, 5-MTF, 5-10-MTF	One-carbon metabolism
Vitamin B_{12}	Cyano-, methyl-, DA-cobalamins	Methyl- or DA-cobalamin	Removal of homocysteine
	Quasi-vitamins		
Choline	Phosphatidylcholine, choline chloride	Acetyl-choline	Neurotransmission
Carnitine	Carnitine (meat), acetylcarnitine	Carnitine	Oxidation of LCFAs
myo-Inositol	Phosphatidylinositol, phytate	Phosphatidylinositol	Cell signaling
PQQ	Pyrroloquinoline quinone (PQQ)	PQQ	A redox cofactor
Lipoic acid	Protein-bound lipoic acid, free lipoic acid	Protein-bound lipoic acid	Oxidative decarboxylation of KA
Ubiquinones	Ubiquinones	Coenzyme Q (in animals)	Mitochondrial electron transport
Bioflavonoids	Glycosides of bioflavonoids	Bioflavonoids	Antioxidants, pigments
PABA	*para*-Aminobenzoic acid (PABA)	PABA	Synthesis of folate in bacteria

Source: Combs and McClung 2017; and Open Chemistry Database, https://pubchem.ncbi.nlm.nih.gov.

Note: AA, amino acid; ACP, acyl carrier protein; DA, 5'-deoxyadenosyl; KA, α-ketoacids; LCFAs, long-chain fatty acids; 5,10-MTF, N^5-N^{10}-methylene-tetrahydrofolate; 5-MTF, N^5-methyl-tetrahydrofolate; THF, tetrahydrofolate.

absorbed by enterocytes via apical membrane carrier mediators and enter either the portal vein or lymphatics depending on their chemical structures (Figure 9.38). Except for *p*-aminobenzoic acid and flavonoids, these nutrients are known to be essential for metabolism and physiology in animals. Some of them act like vitamins but have not been generally recognized as vitamins because of (a) historical reasons, (b) much higher amounts of dietary requirements than most vitamins, and (c) the lack of evidence for the occurrence of diseases resulting from their dietary deficiencies in diets. Excess quasi-vitamins are rare but can occur after high doses of oral, subcutaneous, or intravenous administration to cause toxicity (e.g., reduced feed intake, growth depression, dizziness, nausea, vomiting, and diarrhea; Combs and McClung 2017).

Choline

Structure: Choline was first isolated by Adolph Strecker from pig and cattle bile in 1862 and first chemically synthesized by Oscar Liebreich in 1865. The chemical structure of choline is 2-hydroxy-*N*,*N*,*N*,-trimethylethanaminium [also known as (β-hydroxyethyl)trimethylammonium] (Chapter 4). As a quaternary saturated amine, choline is a strong organic base and is soluble in water. When heated in an alkaline solution, choline decomposes to trimethylamine $^{+}N\text{-}(CH_3)_3$ (a compound with a fishy odor) and glycol ($HO\text{-}CH_2\text{-}CH_2\text{-}OH$). Slow degradation of choline occurs during storage.

Sources: Choline is widely present in feedstuffs, mostly in the form of phosphatidylcholine. Less than 10% of choline occurs as the free base or sphingomyelin (Chapter 3). Animal liver, brain, and kidney, as well as egg yolk, wheat germ, and soybeans, are rich sources of choline. Corn contains a low content of both choline (only half the levels found in barley, oats, and wheat) and nondetectable betaine (<0.1% of the value found in whole wheat grain; Bruce et al. 2010). Because betaine has a choline-sparing effect (Combs and McClung 2017), dietary requirements for choline by animals fed a wheat-based diet are much lower than animals fed a corn-based diet.

Most animal species synthesize de novo choline from methionine, serine, and palmitate in the liver via phosphatidylserine (PS) as an immediate (Figure 9.39). The chemical structures of some metabolites are illustrated in Figure 9.40. In this metabolic pathway, SAM is used as the methyl donor to sequentially convert PS into phosphatidylethanolamine (PE) via PS *N*-methyltransferase and then into phosphatidyl methylethanolamine and phosphatidyl dimethylethanolamine via PE *N*-methyltransferase. Rat tissues possess a low activity of PS *N*-methyltransferase and, therefore, have a limited capacity to de novo synthesize choline (Zempleni et al. 2013). In chicken tissues, PS *N*-methyltransferase activity is absent until after 13 weeks of age (Combs and McClung 2017). In mammals, birds, and most fish, the synthesis of choline cannot meet its requirements, and, therefore, choline

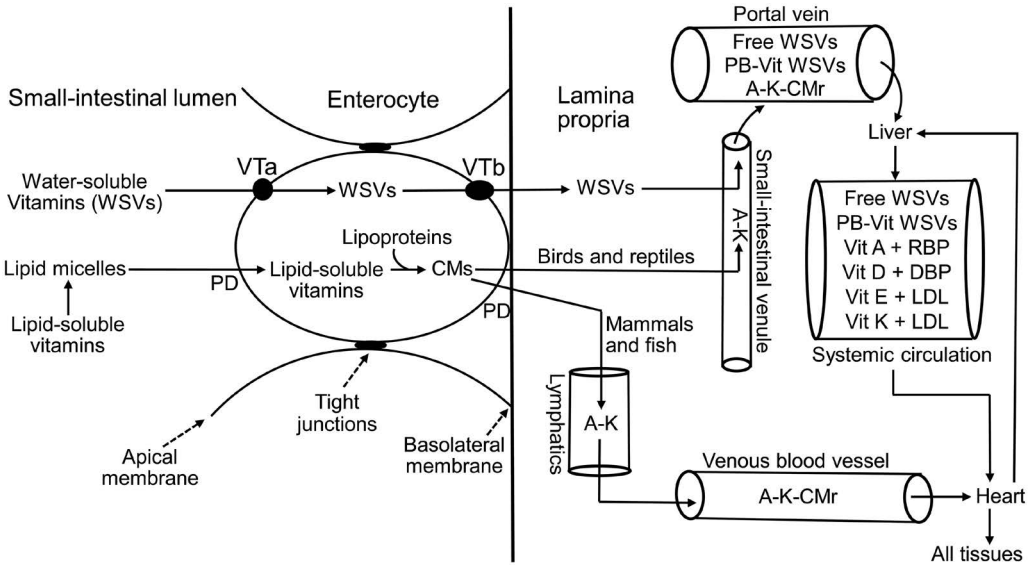


FIGURE 9.38 Absorption and transport of water-soluble and lipid-soluble vitamins in animals. Water- and lipid-soluble vitamins are absorbed by enterocytes into the lamia propria via specific transporters and passive diffusion, respectively. These vitamins enter the portal vein and lymphatics, respectively, in mammals and fish, but all the vitamins are absorbed into the portal vein in birds and reptiles. The vitamins are transported in the blood in their free or protein-bound forms and are ultimately taken up by the liver and other cells of the body via specific transporters (for water-soluble vitamins except for vitamin B_{12}) and receptor-mediated endocytosis (for vitamin B_{12} and all lipid-vitamin vitamins). A-K, chylomicrons (CM) containing vitamins A, D, E, and K; A-K-CMr, chylomicron remnants (CM) containing vitamins A, D, E, and K; BDP, vitamin D-binding protein; free WSVs, vitamins (niacin, pantothenic acid, biotin, folate, vitamin C, and 50% riboflavin) are transported in plasma in free (unbound) forms; PB-Vit-WSVs, vitamins (thiamin, vitamin B_6, vitamin B_{12}, and 50% riboflavin) are transported in plasma in their protein-bound forms; PD, passive diffusion; RBP, retinol-binding protein; VTa, apical membrane transporters for water-soluble vitamins; VTb, basolateral membrane transporters for water-soluble vitamins.

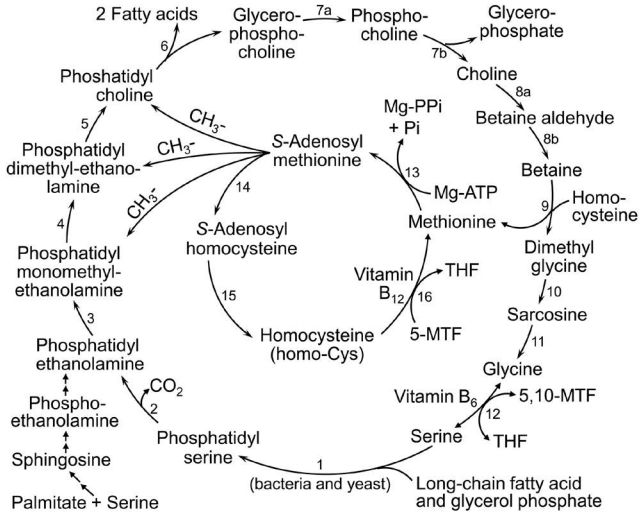


FIGURE 9.39 De novo synthesis of choline from methionine, serine, and palmitate in the liver of animals. Rats have a limited capacity for choline synthesis. In chickens, this pathway is not developed until after 13 weeks of age. Reaction 1 occurs only in bacteria, whereas all other reactions are present in animal cells and bacteria. The enzymes that catalyze the indicated reactions are (1) a series of enzymes for phosphatidylserine synthesis (Chapter 6), (2) phosphatidylserine decarboxylase, (3) phosphatidyl aminoethanol (phosphatidylethanolamine) *N*-methyltransferase, (4) phosphatidyl *N*-monomethylaminoethanol (phosphatidyl *N*-monomethylethanolamine) *N*-methyltransferase, (5) phosphatidyl *N*-dimethylaminoethanol (phosphatidyl *N*-dimethylethanolamine) *N*-methyltransferase, (6) phospholipases A_1 and A_2, (7a) glycerophosphocholine phosphodiesterase, (7b) phosphocholine phosphatase, (8a) choline dehydrogenase, (8b) betaine aldehyde dehydrogenase, (9) betaine:homocysteine methyltransferase, (10) dimethylglycine dehydrogenase (a flavoprotein) that catalyzes the oxidative demethylation of dimethylglycine to sarcosine, (11) sarcosine dehydrogenase (a flavoprotein) that catalyzes the oxidative demethylation of sarcosine to glycine, (12) serine hydroxymethyltransferase, (13) *S*-adenosylmethionine synthetase (methionine adenosyltransferase); sarcosine (*N*-methylglycine), (14) *S*-adenosylmethionine-dependent enzymes, (15) S-adenosylhomocysteine hydrolase, and (16) N^5-methylt etrahydrofoate:homocysteine methyltransferase. SAM, *S*-adenosylmethionine; 5,10-MTF, N^5-N^{10}-methylene-tetrahydrofolate; 5-MTF, N^5-methyl-tetrahydrofolate; THF, tetrahydrofolate.

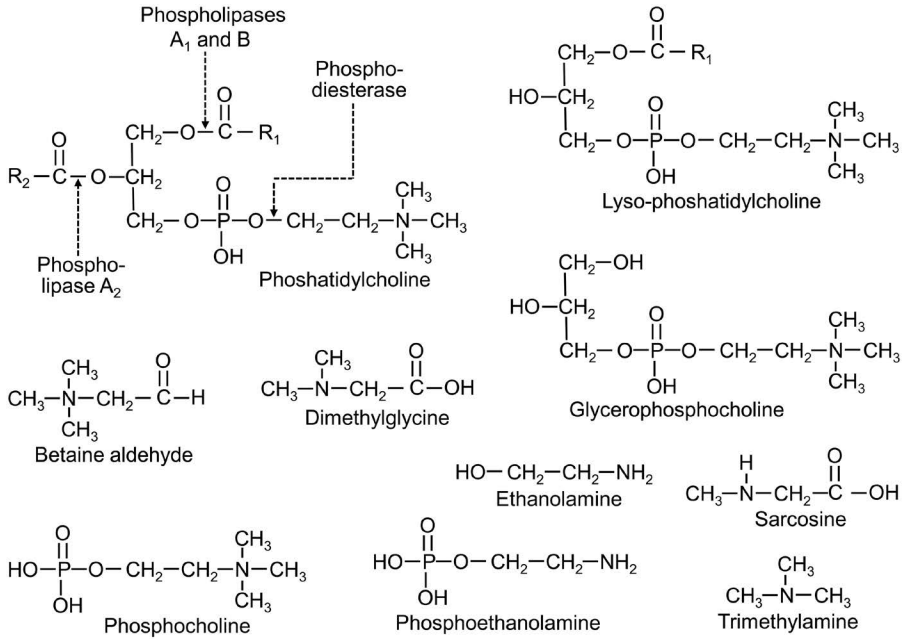


FIGURE 9.40 The chemical structures of some metabolites in the pathway of choline synthesis. They contain nitrogen atoms and occur in nature (e.g., animals, plants, and microbes). Note that trimethylamine is primarily responsible for the fishy odor associated with fouling fish, bacterial infections, and bad breath.

[as choline chloride, choline bitartrate, or phosphatidylcholine (PC)] must be supplied in diets.

Absorption and transport: Depending on the form of dietary choline, it is absorbed by the small intestine into the lymphatic or portal circulation. In the lumen of the small intestine, dietary PC is hydrolyzed by a high activity of phospholipase A_2 (from the pancreas; cleaving the β-ester bond) to generate lyso-PC and one molecule of fatty acid. Some PC is hydrolyzed by phospholipase A_2, as well as phospholipases A_1 and B (from the intestinal mucosa; cleaving the α-ester bond), to yield glycerolphosphorylcholine (GPC) and two molecules of fatty acids. GPC is further hydrolyzed by GPC diesterase (from the pancreas) to form choline and glycerol phosphate. Because the activity of phospholipase A_2 is much greater than that of phospholipases A_1 and B, lyso-PC is the major product of PC digestion in the small intestine. Lyso-PC is absorbed into enterocytes primarily via simple diffusion (Chapter 6). About one-third of free choline in the lumen of the small intestine is taken up into enterocytes via choline transporter-1 (an apical membrane Na^+-dependent, high affinity transporter, K_M <10 μM), as well as organic cation transporters (OCTs) 1 and 2 (Kato et al. 2006). Once inside the enterocytes, lyso-PC and free choline are re-esterified into PC. The PC is then packaged into chylomicrons and VLDL for export into the lymphatics (mammals) or the portal vein (birds, fish, and reptiles) and is transported in plasma as lipoproteins (Chapter 6). The choline that is not esterified in the enterocyte exits the cell through its basolateral membrane into the lamina propria via a choline/H^+ antiport (Zempleni et al. 2013). About two-thirds of free choline in the lumen of the small intestine is degraded by bacteria to form trimethylamine, which is readily absorbed into the portal vein.

In the lumen of the small intestine, some sphingomyelin is hydrolyzed by intestinal alkaline sphingomyelinase and neutral ceramidase to sphingosine, phosphoethanolamine and LCFAs (Nilsson and Duan 2006). Sphingosine is absorbed into enterocytes primarily via simple diffusion. In these cells, most sphingosine is acylated into sphingomyelin, while some sphingosine is converted to palmitate and ethanolamine via a series of enzyme-catalyzed reactions, and the resultant palmitate is esterified into triacylglycerols (Chapter 6). Sphingomyelin, along with triacylglycerols, is packaged into chylomicrons and VLDL for export into the lymphatics and is transported in the blood as lipoproteins (Chapter 6).

Functions: Choline has many physiological functions in animals (Ennis and Blakely 2016). Choline is converted by choline kinase into phosphocholine for PC synthesis in a variety of tissues. As a component of PC, choline is essential to (a) maintain the structure and function of biological membranes and (b) promote the interorgan transport of lipids and the efflux of lipids from the liver and small intestine. In addition, choline is the precursor of betaine (a direct methyl-group donor) that is required via the methylation of homocysteine to methionine by betaine homocysteine methyltransferase (Figure 9.41). Also, via the activation of

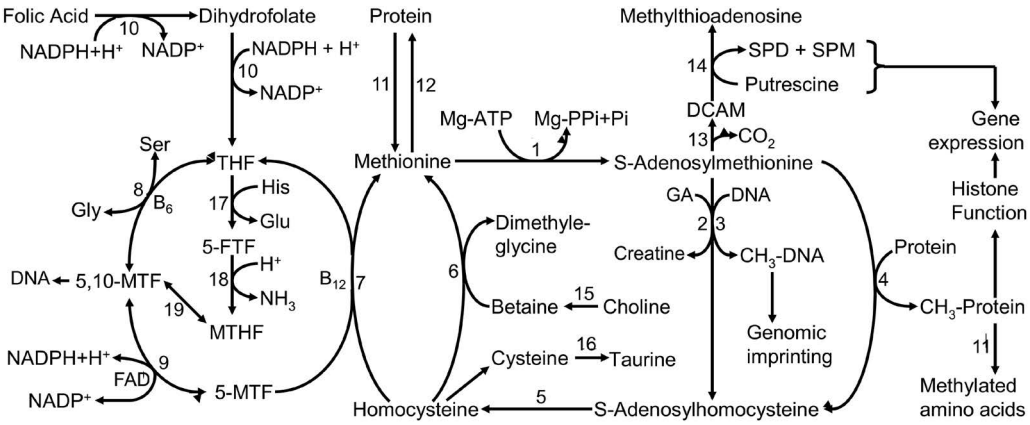


FIGURE 9.41 Roles of nutrients in the provision of methyl group donors, as well as DNA and protein methylation. Both amino acids and vitamins play a crucial role in the provision of S-adenosylmethionine for methylation of DNA and protein as well as the synthesis of creatine, cysteine, taurine, and polyamines. Enzymes catalyzing the indicated reactions are (1) methionine *S*-adenosyltransferase; (2) guanidinoacetate *N*-methyltransferase; (3) DNA methyltransferase; (4) protein methyltransferase; (5) *S*-adenosylhomocysteine hydrolase; (6) betaine:homocysteine methyltransferase; (7) N^5-methyltetrahydrofolate:homocysteine methyltransferase; (8) serine hydroxymethyltransferase; (9) N^5,N^{10}-methylenetetrahydrofolate reductase; (10) folate dehydrogenase; (11) enzymes of protein degradation (including calpains, ubiquitin-dependent proteases, proteasome, and lysosomal proteases); (12) enzymes for protein synthesis; (13) *S*-adenosylmethionine decarboxylase; (14) spermidine synthase and spermine synthase; (15) choline dehydrogenase; (16) enzymes of taurine synthesis (cysteine dioxygenase and cysteinesulfinate decarboxylase); (17) enzymes of N^5-formimino-tetrahydrofolate formation (histidase, urocanase, imidazolonepropionase, and glutamate formiminotransferase); (18) formiminotetrahydrofolate cyclodeaminase; and (19) methylene-tetrahydrofolate reductase. B_6, vitamin B_6; B_{12}, vitamin B_{12}; CH_3-DNA, methylated DNA; CH_3-protein, methylated protein; DCAM, decarboxylated *S*-adenosylmethionine; 5-FTF, N^5-formimino-tetrahydrofolate; GA, guanidinoacetate; Glu, glutamate; Gly, glycine; His, histidine; 5-MTF, N^5-methyl-tetrahydrofolate; 5,10-MTF, N^5,N^{10}-methylenetetrahydrofolate; MTHF, N^5,N^{10}-methenyltetrahydrofolate; Ser, serine; SPD, spermidine; SPM, spermine; THF, tetrahydrofolate. (Reproduced from Wu et al. 2006, with permission from Oxford University Press.)

methionine to S-adenosylmethionine (a major methyl-group donor) by methionine *S*-adenosyltransferase, choline can regulate the methylations of amino acids, DNAs, and proteins (including histones) that play important roles in cell function and gene expression via epigenetics (Figure 9.41). Furthermore, as a precursor of ceramide, choline plays an important role in transmembrane signaling. Likewise, as a component of platelet-activating factor (Chapter 3), choline participates in blood clotting, inflammation, conceptus implantation, and uterine contraction. Finally, as a substrate for the synthesis of acetylcholine (a neurotransmitter) by choline acetyltransferase, choline is required for neurological function in animals.

$$\text{Choline} + \text{ATP} \rightleftharpoons \text{Phosphocholine} + \text{ADP}$$
$$(\text{Choline kinase, } Mg^{2+})$$

Deficiencies and diseases: Choline deficiency occurs in: (a) young poultry fed diets containing a low content of choline, and (b) other animals fed diets containing a low content of choline or methionine (Combs and McClung 2017). Symptoms of choline deficiency in animals include hepatic steatosis, fatty liver, impaired growth, reduced feed efficiency, brain defects, and neurological dysfunction (Zeisel and da Costa 2009). In addition, choline deficiency increases the risk of carcinogenesis. Thus, choline is an essential nutrient for animals that cannot synthesize sufficient amounts to meet their metabolic requirements.

Carnitine

Structure: Carnitine was found to be a constituent of vertebrate skeletal muscle in 1905. It is now known to be present in nearly all animal tissues, especially the skeletal muscle, heart, and liver, where the rates of LCFA oxidation are high. Carnitine is a quaternary amine, and its chemical structure [β-hydroxy-γ-*N*-trimethylaminobutyrate (Chapter 4)] was established in 1927. Carnitine is a strong organic base and is soluble in water.

Sources: Carnitine is abundant in meats and dairy products (Combs and McClung 2017) but is low or absent in plants (Panter and Mudd 1969). The liver of animals can synthesize carnitine from peptide-bound lysine, methionine, α-KG, vitamin C, and iron via a series of enzyme-catalyzed reactions (Vaz and Wanders 2002). However, the synthesis of carnitine in insects is limited. De novo synthesis of carnitine has not been demonstrated in any bacterial species (Meadows and Wargo 2015). A dietary supplemental form of carnitine is acetylcarnitine, which is also a physiological metabolite of carnitine in animal tissues.

Absorption and transport: In the lumen of the small intestine, acylcarnitine is cleaved by pancreatic carboxyl ester lipase to form carnitine and LCFAs. Carnitine is absorbed into enterocytes primarily via (a) OCT2, which is a Na^+-dependent, high affinity transporter localized in the apical membrane; and (b) amino acid transporter $B^{0,+}$. In these cells, some carnitine is acetylated by carnitine acetyltransferase to form acetylcarnitine (Gross et al. 1986). Carnitine exits the enterocyte into the lamina propria via OCT3, which is a Na^+-independent transporter localized in the basolateral membrane (Durán et al. 2005). Absorbed carnitine enters the portal circulation and becomes available for utilization by extraintestinal tissues. Unabsorbed carnitine is degraded to trimethylamine, malic acid, and betaine by microbes in the small and large intestines (Meadows and Wargo 2015). These metabolites are absorbed into the portal circulation via transmembrane transporters. Carnitine is transported in the blood in both free and acetylated forms, and is taken up by extraintestinal tissues via OCT2 against concentration gradients. Like amino acids and glucose, carnitine is reabsorbed by the renal glomerulus into the blood circulation.

$$\text{Acetyl-CoA} + \text{Carnitine} \rightleftharpoons \text{CoA} + \text{Acetylcarnitine}$$
$$(\text{Carnitine acetyltransferase})$$

Dietary acetylcarnitine is absorbed into enterocytes via amino acid transporter $B^{0,+}$ (Wu 2022). Inside these cells, some acetylcarnitine is deacetylated into carnitine, which is transported as noted previously. The transporter $B^{0,+}$ and OCT3 mediate the efflux of acetylcarnitine from the enterocyte across its basolateral membrane into the lamina propria. The rate of intestinal acetylcarnitine transport is faster than that of carnitine (Gross et al. 1986). Absorbed acetylcarnitine enters the portal circulation and becomes available for utilization by extraintestinal tissues to form carnitine.

Functions: Carnitine is required for the transport of LCFAs from the cytosol into mitochondria for oxidation to CO_2 and H_2O and ATP production (Chapter 6). This is the major source of energy for the skeletal muscle, heart, and liver in (a) nonruminants and preruminants under fed or fasting conditions, and (b) ruminants when the ruminal production of short-chain fatty acids is low or under food deprivation conditions.

Deficiencies and diseases: Animals exhibit a deficiency of carnitine when its dietary intake, intestinal absorption, or de novo synthesis is inadequate. Symptoms include (a) skeletal myopathy, muscle necrosis, cardiomyopathy, and fatigue because of insufficient energy provision to muscle fibers and cardiomyocytes; (b) reduced protein synthesis, increased proteolysis, and increased amino acid oxidation to ammonia in skeletal muscle due to excess fat-induced insulin resistance; (c) fatty liver and hypoglycemia due to insufficient oxidation of LCFAs (resulting in their accumulation) and insufficient energy supply for gluconeogenesis in hepatocytes; (d) hyperammonemia due to impaired ammonia detoxification via the urea cycle that results from an inhibition of carbamoylphosphate synthase-I by LCFA-CoA (e.g., palmitoyl-CoA) in hepatocytes; (e) neurological dysfunction as a result of hypoglycemia

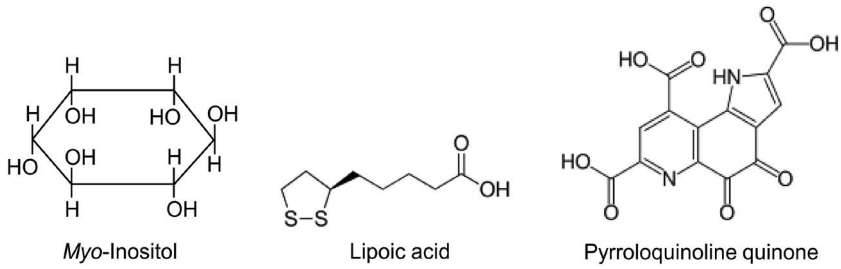


FIGURE 9.42 Chemical structures of *myo*-inositol, lipoic acid, and pyrroloquinoline quinone. These substances are present in mammals, birds, fish, and microbes. They are all soluble in water. Lipoic acid (a coenzyme) acts as a "swinging arm" to shuttle intermediates between two active sites when it is covalently attached to its protein enzyme.

and hyperammonemia; and (f) hyperlipidemia because of impaired oxidation of LCFAs to CO_2 and H_2O in the whole body (Zempleni et al. 2013).

Myo-Inositol

Structure: *myo*-Inositol is a water-soluble 6-carbon sugar related to D-glucose and occurs naturally in 9 possible isomeric forms. However, only *myo*-inositol (*cis*-1,2,3,5 *trans*-4,6-cyclohexane-hexanol) is biologically important as a nutrient (Figure 9.42).

Sources: *myo*-Inositol is widely distributed in plants and animal products, as part of phosphatidylinositol in cell membranes, as free inositol, and as a component of phytic acid. The latter is present in many grain products. Phytate and phytic acid can bind Ca, magnesium, iron, zinc, and other divalent ions, thereby reducing their absorption by the small intestine. Animal tissues (e.g., liver, kidney, brain, and testes) and bacteria can synthesize *myo*-inositol from D-glucose (Geiger and Jin 2006), and this metabolic pathway is outlined in Figure 9.43.

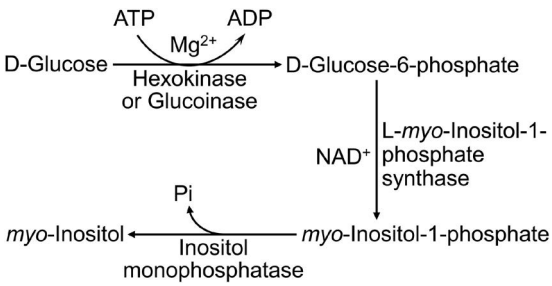


FIGURE 9.43 Synthesis of *myo*-inositol from D-glucose in animal cells and microbes. D-Glucose is converted into glucose-6-phosphate by hexokinase or glucokinase. The internal cyclization of glucose 6-phosphate by L-*myo*-inositol-l-phosphate synthase forms *myo*-inositol-1-phosphate, and this enzyme has the absolute requirement for NAD^+ with no net gain in NADH. Thus, the overall reaction for the generation of *myo*-inositol-1-phosphate is tightly coupled with oxidation and reduction. Finally, inositol monophosphatase catalyzes the dephosphorylation of *myo*-inositol-1-phosphate to yield *myo*-inositol.

Absorption and transport: Depending on the form of *myo*-inositol, it is absorbed from the small intestine into the lymphatics (phosphatidylinositol) or portal circulation (free inositol) with a high efficiency. In the rumen of ruminants and in the lumen of the small intestine of all animals, phytic acid can be converted into *myo*-inositol by microbial phytase with the removal of the phosphate groups (Chapter 10). The efficiency of this conversion in the digestive tract depends on the animal species. Ruminants and horses have a greater capacity for utilizing dietary phytate than swine and poultry. *myo*-Inositol is absorbed into the enterocyte via Na^+-coupled *myo*-inositol transporter-2 (SMIT2), which is a secondary active transporter (Aouameur et al. 2007). This sugar exits the enterocyte across its basolateral membrane via a diffusive (Na^+-independent) carrier-mediated mechanism (Reshkin et al. 1989). Glucose transport systems in the apical membrane of the enterocyte, such as SGLT1 or GLUT5, do not contribute to *myo*-inositol uptake. Likewise, GLUT2 does not mediate *myo*-inositol efflux from the enterocyte across its basolateral membrane. Depending on the form of *myo*-inositol, it is transported in the blood as lipoproteins (phosphatidylinositol) or a predominantly free form (free inositol). *myo*-Inositol in the blood is taken up by the liver via carrier-mediated diffusion, by the brain via SMIT1 and SMIT2, by the renal medulla via SMIT1, and by proximal tubule cells in the kidney cortex via SMIT2 (Aouameur et al. 2007). SMIT2 is responsible for the reabsorption of *myo*-inositol from the glomerular filtrate. A major difference between SMIT1 and SMIT2 is that D-chiro-inositol (an epimer of *myo*-inositol) is transported by SMIT2 with high affinity but is not transported by SMIT1, whereas L-fucose is transported by SMIT1 but not by SMIT2.

Functions: *myo*-Inositol is incorporated into phosphatidylinositol in animal cells. Phosphatidylinositol plays an important role in cell metabolism and signaling by serving as (a) a constituent of cell membranes and (b) a mediator of cellular responses to external stimuli. For example, inositol-1,4,5-triphosphate (IP_3) is an intracellular second messenger that stimulates the release of Ca^{2+} from the endoplasmic reticulum to the cytosol, which in turn activates a variety of Ca^{2+}-dependent protein kinases (including protein kinase C) and pathways (Gill et al. 1989).

Deficiencies and diseases: Most animal species (including fish species such as abalone and channel catfish) can synthesize sufficient *myo*-inositol, and therefore it is not considered to be a dietary essential nutrient (Burtle and Lovell 1989; Mai et al. 2001; Zempleni et al. 2013). However, a lack of *myo*-inositol in diets results in feed intake depression, impaired growth, reduced feed efficiency, and skin lesions in some aquatic animals, including common carp, red sea bream, Japanese eel, rainbow trout, chinook salmon, and shrimp (Mai et al. 2001). Of interest, male gerbils can sufficiently synthesize *myo*-inositol, but female gerbils cannot (Chu and Hegsted 1980). The absence of *myo*-inositol from the diet causes intestinal lipodystrophy and reduced survival in female gerbils. Under some conditions such as diabetes (with large amounts of *myo*-inositol being excreted in urine), infections, and heat stress (impaired *myo*-inositol synthesis), *myo*-inositol may be a conditionally essential nutrient in diets for animals (Combs and McClung 2017).

Lipoic Acid

Structure: Lipoic acid (a yellow solid compound) is also known as α-lipoic acid and thioctic acid (Figure 9.42). This water-soluble organosulfur compound consists of two S atoms (at C_6 and C_8) connected by a disulfide bond (the oxidized form). The C_6 atom is chiral, and the molecule exists as two enantiomers [(*R*)-(+)-lipoic acid and (*S*)-(-)-lipoic acid] and as a racemic mixture (*R*/*S*)-lipoic acid. The reduced form of lipoic acid is known as dihydrolipoic acid.

Sources: Lipoic acid is present in plant and animal products. Exogenous free lipoic acid from the diet is activated by an ATP-dependent lipoate-activating enzyme, and the product is then transferred by lipoyltransferase to lipoic acid-dependent enzymes (Figure 9.44). Gastrointestinal bacteria and the mitochondria of animal cells can synthesize a small amount of lipoic acid from octanoic acid (Cronan 2016). In the presence of *S*-adenosylmethionine, lipoyl synthase inserts two S atoms from the Fe-S centers of the enzyme to form lipoyl-ACP. The latter is transferred to lipoic acid-dependent enzymes (e.g., pyruvate dehydrogenase). Thus, either the exogenous or the endogenously synthesized lipoic acid (the R-isomer) can be bound to the ε-NH_2 group of a lysine residue in a lipoyl-specific protein. In animal cells, oxidized lipoic acid can be converted by an NADPH-dependent enzyme (e.g., glutathione reductase) into dihydrolipoate.

Absorption and transport: In the lumen of the small intestine, protein-bound lipoic acid is degraded by proteases to release lipoic acid. The latter is absorbed into enterocytes via the Na^+-dependent monocarboxylic acid transporter and the Na^+-dependent multivitamin transporter (Quick and Shi 2015). In the cells, some lipoic acid is reduced to dihydrolipoate. Both lipoic acid and dihydrolipoate exit enterocytes through their basolateral membranes into the lamina propria via a specialized Na^+-independent transporter. Absorbed lipoic acid and dihydrolipoate enter the portal circulation and are transported in the blood primarily as free acids.

Functions: Lipoic acid is a component of lipoamide (Chapter 5), which is a coenzyme for α-ketoacid dehydrogenase complexes, including pyruvate dehydrogenase, α-KG dehydrogenase, and BCKAD complexes, in animal cells and microbes. These enzymes play important roles in pyruvate decarboxylation to acetyl-CoA, the activity of the Krebs cycle, and BCAA catabolism, respectively. Therefore, lipoic acid is essential for ATP production from the oxidation of glucose, amino acids, and fatty acids in a tissue-specific manner. In addition, lipoic acid is a cofactor for the acetoin dehydrogenase complex (also known as acetyl-CoA:acetoin *O*-acetyltransferase), which degrades acetoin (a product of the decarboxylation of α-acetolactate in bacteria that gives butter its characteristic flavor; Cronan 2016). Like α-ketoacid dehydrogenase complexes, the acetoin dehydrogenase complex requires thiamine diphosphate as a cofactor. Furthermore, the H-protein [one of the four

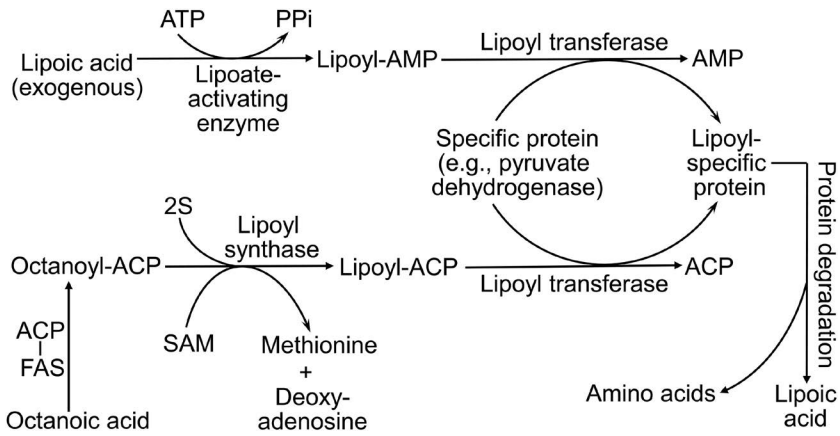
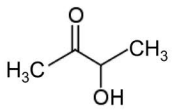


FIGURE 9.44 Synthesis of specific protein-bound lipoic acid from octanoic acid in the mitochondria of animal cells and in bacteria. This process involves the activation of octanoate, the insertion of sulfur atoms from an iron-sulfur cluster within the enzyme, and the transfer of the octanoyl moiety from the octanoyl-acyl carrier protein complex to a specific protein. ACP, acyl carrier protein; FAS, fatty acid synthase; SAM, *S*-adenosylmethionine.

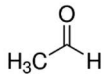
proteins in the glycine cleavage system (also known as the glycine decarboxylase complex)] contains lipoamide; therefore, lipoic acid is essential for glycine degradation in the liver and kidneys of animals and in microbes. Finally, high concentrations of dihydrolipoate can scavenge reactive oxygen and N species (Moura et al. 2015).

$$\alpha\text{-Ketoacid} + CoA + NAD^+ \rightarrow \text{Acyl-CoA} + NADH + H^+ \quad (\alpha\text{-ketoacid dehydrogenase complex in animal cells and bacteria})$$

$$\text{Acetoin} + CoA + NAD^+ \rightarrow \text{Acetaldehyde} + \text{Acetyl-CoA} + NADH + H^+ \text{ (acetoin dehydrogenase complex, bacteria)}$$



Acetoin
($C_4H_8O_2$, a bacterial metabolite)



Acetaldehyde
(C_2H_4O, a natural metabolite in bacteria and plants; a product of ethanol oxidation in the liver of animals)

Deficiencies and diseases: A deficiency of lipoic acid is rare in animals fed diets consisting of plant- or animal-sourced ingredients. However, subjects with inborn errors of enzymes responsible for endogenous lipoic acid synthesis exhibit impaired ATP production, neurological dysfunction, muscle weakness, and abnormal amino acid catabolism.

Pyrroloquinoline Quinone

Structure: PQQ (4,5-dihydro-4,5-dioxo-1H-pyrrolo-[2,3-f]quinoline-2,7,9-tricarboxylic acid) or methoxatin (Figure 9.41) was discovered in the late 1970s as a water-soluble cofactor of bacterial oxidoreductases. This aromatic tricarboxylic acid with a fused heterocyclic (*o*-quinone) ring system is now known to be present in yeast, plants, and animals. It is a stable substance in physiological solutions, although its C-5 carbonyl group is reactive toward nucleophiles (e.g., thiol groups). Enzymes containing PQQ are called quinoproteins. In bacteria, PQQ is a member of ortho-quinone cofactors that include tryptophan tryptophylquinone, trihydroxyphenylalanyl quinone, lysine tyrosylquinone, and the copper-complexed cysteinyltyrosyl radical (Stites et al. 2000).

Sources: PQQ is present in plant (e.g., vegetables, fruits, and legumes) and animal (e.g., milk, meats, and eggs) products (Stites et al. 2000). It can be synthesized from glutamate and tyrosine residues of a PqqA polypeptide (e.g., 24 amino acids in *Methylobacillus flagellatum*) by gastrointestinal bacteria as an endogenous source for their hosts (Puehringer et al. 2008). Animal cells do not contain enzymes for PQQ synthesis.

Absorption and transport: Little is known about the transporters of PQQ absorption by the small intestine. However, a study with mice showed that orally administered PQQ was readily absorbed (62%, range 19–89%) in the lower intestine into the portal circulation (Smidt et al. 1991). It is possible that ABCG2 mediates the uptake of PQQ into the enterocyte across its apical membrane and that MRP1 is responsible for the efflux of PQQ from the cell across its basolateral membrane. Absorbed PQQ enters the portal circulation. Greater than 95% of PQQ in the blood is associated with the cell fraction, with the remaining in plasma (Smidt et al. 1991). PQQ is taken up from plasma primarily by the kidneys and skin and, to a lesser extent, other tissues. Most (81%) of the absorbed PQQ is excreted by the kidneys within 24 h.

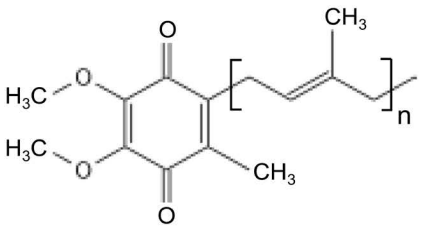
Functions: PQQ is a redox cofactor in cells. In certain (e.g., methane-generating) bacteria, it acts as a coenzyme for oxidoreductases, including methanol dehydrogenase, ethanol dehydrogenase, and membrane-bound glucose dehydrogenase. It is unknown whether PQQ is essential for enzymes in animal cells. However, there is evidence that PQQ catalyzes the nonspecific oxidation of (a) pyridoxamine phosphate to pyridoxal phosphate and (b) peptidyl lysine residues in elastin and collagen to aldehyde products (known as allysines). These reactive aldehydes undergo spontaneous chemical reactions with other aldehyde residues or unmodified lysine residues to form cross-linking, which is essential for the stabilization of collagen fibrils and for the elasticity of elastin in animals. Furthermore, the radical-scavenging activity of the reduced form of PQQ is 7.4-fold greater than that of vitamin C (Ouchi et al. 2009). As an antioxidant, PQQ has cardio- and neuro-protective effects, while improving mitochondrial biogenesis and function. Therefore, dietary supplementation with 0.2 ppm PQQ·Na_2 can enhance the anti-oxidative status, growth performance, and carcass yield of broiler chicks (Samuel et al. 2015; Wang et al. 2015).

Deficiencies and diseases: PQQ deficiency is rare in animals fed diets consisting of plant- and animal-sourced ingredients. There are reports that mice fed a PQQ-free diet exhibited suboptimal embryonic survival, neonatal growth, skin lesions, and impaired immunity (Killgore 1989; Steinberg et al. 1994). Of interest, maximal animal growth could be achieved when the basal diet of mice contained as little as 1 nmol or 300 ng of PQQ per gram of diet (Steinberg et al. 1994).

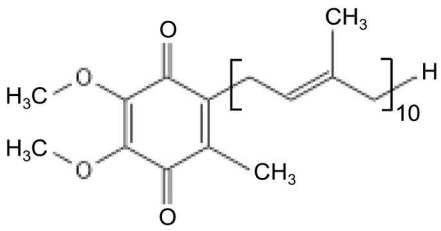
Ubiquinones

Structure: Ubiquinones are a group of lipid-soluble 1,4-benzoquinone derivatives with isoprenoid side chains of different lengths. A physiologically important

ubiquinone is ubiquinone Q_{10} [also known as ubiquinone Q or coenzyme Q (CoQ_{10})], where Q refers to the quinone group and the subscript "10" is the number of isoprenyl subunits. The structure of the 6-chromanol of coenzyme Q is similar to that of the oxidized form of vitamin E or vitamin K.



A ubiquinone



Coenzyme Q_{10} (coenzyme Q)

Sources: Ubiquinones occur in plant- and animal-sourced ingredients. Gastrointestinal bacteria and the hepatocytes of animals can synthesize coenzyme Q from (a) tyrosine (the precursor of the benzoquinone structure) and (b) acetyl-CoA (the substrate of the isoprene side chain), with mevalonate as an intermediate (Bentinger et al. 2010). In the hepatocytes of vertebrates (e.g., mammals, birds, and fish), coenzyme Q synthesis involves mitochondria, the endoplasmic reticulum, and peroxisomes (Figure 9.45).

Absorption and transport: In the lumen of the small intestine, dietary ubiquinones are solubilized with lipids and bile salts and absorbed from the micelles by enterocytes via passive diffusion. Upon entering the enterocyte, ubiquinones are assembled into chylomicrons, which exit the enterocyte across its basolateral membrane (via passive diffusion) into the lymphatics and then the bloodstream in mammals and fish, or into the portal circulation in birds and reptiles (Chapter 6). Ubiquinones in the blood are taken up by extraintestinal

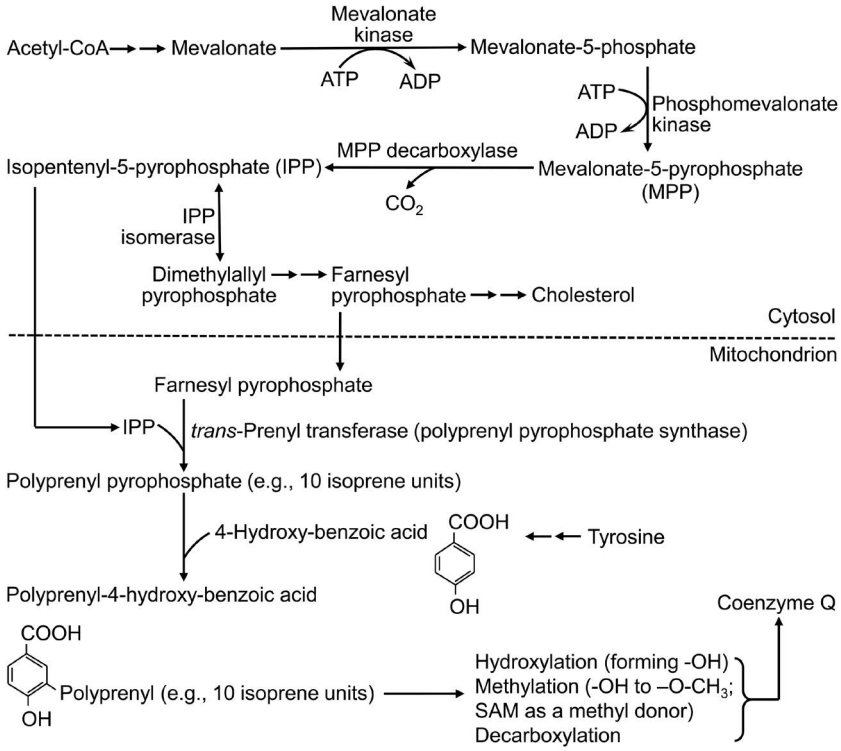


FIGURE 9.45 Synthesis of coenzyme Q in the hepatocytes of mammals, birds, and fish. Tyrosine is converted into the benzoquinone structure in mitochondria, and acetyl-CoA is the substrate for the formation of the isoprene side chain in the endoplasmic reticulum and peroxisomes. Hydroxymethylglutaryl-CoA (HMG-CoA) reductase, which is a rate-controlling enzyme in the generation of mevalonate from acetyl-CoA, is an integral protein of the endoplasmic reticulum membrane. The enzymes that convert mevalonate into farnesyl pyrophosphate are localized in peroxisomes. The last reactions involving the hydroxylation, methylation, and decarboxylation of polyprenyl-4-hydroxy-benzoic acid occur in mitochondria.

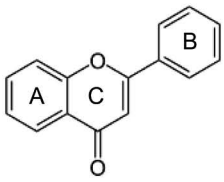
cells as a component of VLDL or LDL via specific receptors (Chapter 6). Dietary ubiquinones (e.g., CoQ_{10}) have a low bioavailability to an animal because of their poor solubility in gastrointestinal fluids and their large molecular weight (Acosta et al. 2016).

Functions: There are three redox states of CoQ_{10}: fully oxidized (ubiquinone), semiquinone (ubisemiquinone), and fully reduced (ubiquinol) (Chapter 1). The capacity of ubiquinone as a two-electron carrier (moving between the quinone and quinol forms) and as a one-electron carrier (moving between semiquinone and one of the other forms) is essential for its role in the mitochondrial electron transport chain (complexes I and II). This is because the Fe-S clusters of cytochromes can only accept one electron at a time. In addition, like vitamin E, ascorbic acid, and β-carotene, coenzyme Q has a potent antioxidant property and can scavenge free radical species. Thus, coenzyme Q can alleviate the disorders (except for reproduction) seen in vitamin E-deficient animals (e.g., mice and rats; Combs and McClung 2017). Oral administration of CoQ_{10} can (a) alleviate symptoms of cardiovascular disease and inflammation which are known to be associated with low circulating levels of CoQ_{10} and high oxidative stress, and (b) prevent or slow the progression of metabolic syndrome (including type 2 diabetes), aging, and neurodegeneration (Gasmi et al. 2024).

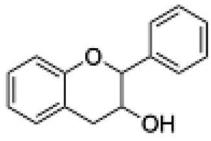
Deficiencies and diseases: There is little evidence for impaired metabolism or physiological function due to a lack of dietary coenzyme Q in animals, indicating a sufficient synthesis of this quasi-vitamin. However, because coenzyme Q shares a biosynthetic pathway with cholesterol, the interference with enzymes responsible for endogenous CoQ_{10} synthesis can cause a deficiency of coenzyme Q. For example, some β-blockers, blood pressure-lowering drugs, and statins (inhibitors of HMG-CoA synthase) inhibit the conversion of acetyl-CoA into mevalonate and may induce a deficiency of CoQ_{10} when its dietary intake is limited (Acosta et al. 2016).

Bioflavonoids

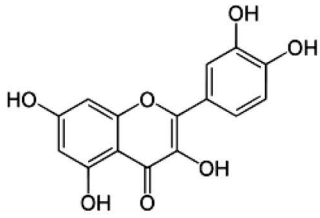
Structure: Bioflavonoids are a mixture of water-soluble polyphenolic derivatives of 2-phenyl-1,4-benzopyrone that consist of two phenyl rings (A and B) and one heterocyclic ring (C). They occur naturally as glycosides. Examples are flavan-3-ol, quercetin, and isoflavone (Schmitt and Dirsch 2009).



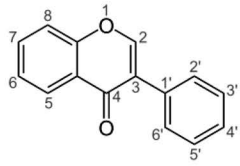
2-Phenyl-1,4-benzopyrone



Flavan-3-ol (flavanol)



Quercetin



Isoflavone [eg., genistein (5-OH, 7-OH, 4'-OH); and daidzein (7-OH, 4'-OH)]

Sources: Bioflavonoids are widely present in plants (e.g., fruits and vegetables) and, like carotenoids, contribute to their red, blue, and yellow pigments. Isoflavones (e.g., genistein and daidzein) occur in legumes, including soybeans, green beans, alfalfa sprouts, mung bean sprouts, cowpeas, kudzu roots, red clover blossoms, and red clover sprouts. Animals do not have enzymes for synthesizing bioflavonoids.

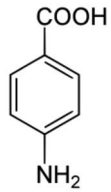
Absorption and transport: In the lumen of the small intestine, the glycosides of bioflavonoids are hydrolyzed by glycosidases of intestinal microbes before they are absorbed by enterocytes and colonocytes (Combs and McClung 2017). However, the molecular properties of bioflavonoid transporters are unknown. Absorbed bioflavonoids enter the portal circulation and are transported in the blood primarily as free compounds. In the liver, these compounds are conjugated as glucuronides or sulfates and can be degraded to various phenolic metabolites. The latter are excreted in the urine.

Functions: Bioflavonoids have a potent antioxidant property and can scavenge free radical species (Panche et al. 2016; Rahman and Hongsprabhas 2016). These phytochemicals can protect tissues from oxidative injury. Bioflavonoids can augment the action of vitamin C in the prevention of scurvy. Of note, genistein and daidzein possess estrogenic activity, which can enhance the synthesis of NO by endothelial cells to improve blood flow and nutrient transport (Schmitt and Dirsch 2009). In addition, soy isoflavones interact with estrogen receptors to improve endocrine function, thereby preventing bone resorption in elderly females, including postmenopausal women (Morabito et al. 2002) as well as possibly reducing risks for coronary heart disease and breast cancer (Chen and Chen 2021).

Deficiencies and diseases: Animals fed nutrient-balanced diets do not exhibit abnormal metabolism or physiology when they do not consume dietary bioflavonoids. There is no evidence for nutritional roles of these substances in mammals, birds, or fish. However, dietary supplementation with bioflavonoids is beneficial for animal health under conditions of oxidative stress.

Para-Aminobenzoic Acid

Structure: *para*-Aminobenzoic acid (also known as 4-aminobenzoic acid) consists of a benzene ring substituted with an amino group and a carboxyl group. It is a white solid that is soluble in water.



para-Aminobenzoic acid

Sources: *para*-Aminobenzoic acid occurs in plants, brewer's yeast, bacteria, and whole grains (Combs and McClung 2017). It is synthesized from chorismic acid (a product of phosphoenolpyruvate and erythrose-4-phosphate) by gastrointestinal bacteria but not animal cells. The contribution of these bacteria to folate in animal tissues is limited. Thus, mammals, birds, and fish must ingest folate or its rich sources (e.g., green leafy vegetables). Primarily as a component of diets, *para*-aminobenzoic acid is found in animal products such as the liver, kidneys, and skeletal muscle.

Absorption and transport: In the lumen of the small intestine, *para*-aminobenzoic acid is absorbed rapidly by enterocytes via a Na^+-independent transporter (passive diffusion; Imai et al. 2017). Upon entering the enterocyte, some of this substance is acetylated into *N*-acetyl-*para*-aminobenzoic acid by arylamine *N*-acetyltransferase. Absorbed *para*-aminobenzoic acid enters the portal circulation. In the liver, *para*-aminobenzoic acid is metabolized to *N*-acetyl-*para*-aminobenzoic acid. Both *para*-aminobenzoic acid and *N*-acetyl-*para*-aminobenzoic acid are excreted in the urine.

Functions: *para*-Aminobenzoic acid is a precursor for the synthesis of folate in bacteria (Combs and McClung 2017). Thus, this substance may play a role in the metabolism and growth of intestinal bacteria. *para*-Aminobenzoic acid antagonizes the bacteriostatic effect of sulfonamide drugs and can be used as a UV-screening agent due to its high absorbance in the UV range (Zempleni et al. 2013).

Deficiencies and diseases: Animals fed nutrient-balanced diets do not exhibit abnormal metabolism or physiology when they do not consume dietary *para*-aminobenzoic acid. There is no evidence for nutritional roles of this compound in mammals, birds, or fish. Synthesis of this compound by intestinal bacteria appears to meet their needs for folate, but such a microbial source of folate is inadequate for the host (Zempleni et al. 2013).

SUMMARY

Vitamins (organic substances needed in small amounts) are important for (a) nutrient metabolism by serving as coenzymes for a wide array of enzymes (e.g., pyruvate dehydrogenase complex, acetyl-CoA carboxylase, and ornithine decarboxylase); (b) anti-oxidative reactions (e.g., vitamins C and E); and (c) regulating gene expression and biological activities of proteins (e.g., nuclear RXR, intestinal and renal Ca^{2+} transporters, and coagulation factors). Based on their solubility in water, vitamins are classified as water-soluble (vitamins B_1, B_2, B_3, B_5, B_6, and B_{12}, as well as biotin and folate) or lipid-soluble (vitamins A, D, E, and K). All vitamins are sensitive to destruction by oxidation and high temperatures. Other than these chemical properties, vitamins have little in common in their structures. Terrestrial omnivores can synthesize vitamin A from β-carotene, niacin from tryptophan, and vitamin D_3 from subcutaneous 7-dehydrocholesterol. Although the requirements of animals for these nutrients are quantitatively low, almost all of them must be supplied in nonruminant diets, with the exception of vitamin D in terrestrial omnivores that are regularly exposed to sunlight. Compared with nonruminants, dietary requirements of ruminants for water-soluble vitamins and vitamin K are much lower because a functional rumen can synthesize all these nutrients when protein, carbohydrates, sulfur, and cobalt are adequate in diets. Some compounds with vitamin-like roles (e.g., carnitine, choline, *myo*-inositol, ubiquinones, PPQ, and bioflavonoids) are called quasi-vitamins. Most of them are soluble in water and nutritionally dispensable, but may provide health benefits to animals.

Vitamins are absorbed primarily by the small intestine and transported in the blood through different mechanisms (Figure 9.38). Specifically, water-soluble vitamins are absorbed into the enterocyte (primarily in the jejunum for most of them, but in the ileum for vitamin B_{12}) through its apical membrane via specific transporters. Some transporters are Na^+-dependent, whereas others are Na^+-independent. These vitamins exit the intestinal epithelial cell across its basolateral membrane into the lamina propria via specific transporters and then enter the portal circulation. Most vitamins (niacin, pantothenic acid, biotin, folate, vitamin C, and 50% riboflavin) are transported in plasma in their free (unbound) forms, but several vitamins (thiamin, vitamin B_6, vitamin B_{12}, and 50% riboflavin) are transported in plasma in their protein-bound forms. Water-soluble vitamins in the blood are taken up by all cells of the body via specific transporters and, in the case of vitamin B_{12}, via receptor-mediated endocytosis. In contrast, all dietary lipid-soluble vitamins are solubilized with lipids and bile salts as micelles in the lumen of the small intestine, and the micelles are subsequently taken up by enterocytes via passive diffusion. In the enterocytes, the absorbed lipid-soluble vitamins are assembled into chylomicrons and, in the case of vitamin E, also into VLDL. The intestine-derived lipoproteins exit the enterocyte across its basolateral membrane (via passive diffusion) into the intestinal lymphatics and then the bloodstream in mammals and fish, or into the portal circulation in birds and reptiles. Through the metabolism of lipoproteins in the blood, the lipid-soluble vitamins-containing chylomicrons and VLDL

TABLE 9.12
Vitamin-Related Metabolic Disorders (Including Metabolic Diseases) in Ruminants and Nonruminants

Metabolic Disorder	Cause	Major Symptoms
Beriberi[a]	Deficiency of thiamin	Tissue degeneration, brain dysfunction, leg disorders, and paralysis
Burning-foot syndrome	Deficiency of pantothenate	Skin lesions, hair loss, skin scaliness, and impaired reproduction
Capillary fragility	Deficiencies of Vit C + iron	Weak and leaky capillary blood vessels; cold and bluish skin
Hemorrhage	Deficiency of vitamin K	Hemorrhage, blood loss, anemia, abnormal bone growth, and death
Liver steatosis	Deficiency of choline	Hepatic steatosis, fatty liver, CNS dysfunction, and risk for cancer
Neural tube defects	Deficiency of folate	Anemia, homocysteinemia, embryonic/fetal death, and birth defects
Photophobia	Deficiency of riboflavin	Photophobia, itching eyes, dermatitis, and mouth lesions in mouth
Pellagra	Deficiency of niacin	Dermatitis, diarrhea, dementia, and death (four-D's)
Porcine stress syndrome[b]	Deficiencies of antioxidants	Oxidative stress in tissues; pale soft exudative (PSE) pork
Rickets	Deficiency of vitamin D	Abnormal bone structure, enlarged joints, and reduced egg production
Scurvy	Deficiency of ascorbate	Bleeding gums, abnormal connective tissue, and oxidative stress,
SKM myopathy	Deficiencies of antioxidants	Abnormal SKM structure and function; muscle weakness and pain
Tissue necrosis	Deficiency of vitamin E	Oxidative stress, muscular degeneration, hepatic necrosis, and anemia
Xerophthalmia	Deficiency of vitamin A	Defective night vision, abnormal epithelium, impaired reproduction

[a] Thiamin diphosphate participates in the oxidative decarboxylation of α-ketoacids for ATP production and in transketolase reactions for generation of NADPH.

[b] Also known as called malignant hyperthermia or transport myopathy. This syndrome is triggered by stress, excitement, or severe anesthetics (e.g., halothane).

CNS, central nervous system; SKM, skeletal muscle; Vit, vitamin

are converted into chylomicron remnants and LDL, respectively, which are subsequently taken up by the liver through receptor-mediated mechanisms. Lipid-soluble vitamins are transported in plasma in their lipoprotein-bound forms.

Most vitamins are not utilized in the form in which they are absorbed from the intestine but are converted or modified to their active forms (Table 9.11). Dietary requirements of mammals, birds, fish, and other animals for vitamins are affected by physiological states (e.g., pregnancy, lactation, physical activity, egg laying, and hatching), environment (e.g., low or high temperatures), and disease (e.g., bacterial, parasitic, and viral infections). Deficiencies of vitamins can greatly reduce animal growth, development, fertility, immunity, production, and feed efficiency, and can also be lethal in severe cases due to the impairments of ATP production, antioxidative responses, protein synthesis, and cellular signal transduction. Young animals are more sensitive to vitamin deficiencies than adults. The specific symptoms of deficiencies are usually characteristic of each vitamin (e.g., head retraction in thiamin-deficient chicks, connective tissue dysfunction in vitamin C-deficient animals, and xerophthalmia in vitamin A-deficient subjects). Major vitamin-related metabolic disorders in ruminants and nonruminants are summarized in Table 9.12. Most diseases of vitamin deficiencies, particularly at their early stages, can be cured by dietary supplementation or intravenous/intramuscular administration. Like any other nutrients, dietary intake or parenteral provision of excessive vitamins (particularly lipid-soluble vitamins) are toxic to animals, particularly during embryonic and fetal development. Therefore, the nutritional status of vitamins must be monitored regularly and their supply (including dietary supplementation) carefully controlled.

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10 Nutrition and Metabolism of Minerals

The word "mineral" is derived from the English "mine" or a substance in the earth's crust that can be obtained through "mining." There is a rich history of the discoveries and nomenclature of minerals (Miśkowiec 2023a,b). They are the second to the eighth most abundant elements in the earth's crust, only after oxygen (% by weight): oxygen, 46.6; silica (Si), 27.7; aluminum (Al), 8.1; iron (Fe), 5.0; calcium (Ca), 3.6; sodium (Na), 2.8; potassium (K), 2.6; magnesium (Mg), 2.1; and others, 1.5 (Lutgens and Tarbuck 2000). Minerals are inorganic elements that are present in both foods and animals. In contrast to organic compounds (e.g., carbohydrates, fatty acids, and amino acids), minerals are neither synthesized nor degraded by animals or microbes but may be incorporated into organic compounds to add functionality (Harris 2014). There are about 47 different minerals in organisms and feeds (Pond et al. 1995; Sampath et al. 2023; Smith 1982; Stanier 1983). Some minerals are present in the body at concentrations ≥400 mg/kg body weight (BW) and are called macrominerals: Na, K, chlorine (Cl), Ca, phosphorus (P), sulfur (S), and Mg (Table 10.1). P usually exists as phosphates in cells and physiological fluids (Takeda et al. 2012). Animals contain small amounts of about 40 minerals at concentrations <100 mg/kg BW, which are termed microminerals or trace minerals (Mertz 1987). To date, the following 16 microminerals have been shown to have important physiological functions in animals: Fe, copper (Cu), cobalt (Co), manganese (Mn), zinc (Zn), iodine (I), selenium (Se), molybdenum (Mo), chromium (Cr), fluorine (F), tin (Sn), vanadium (V), silicon (Si), nickel (Ni), boron (B), and bromine (Br) (Arthington and Ranches 2021; McCall et al. 2014; McDonald et al. 2021; Mertz 1974). These microminerals and the seven macrominerals noted previously are classified as nutritionally essential for animals. In animal feeding systems, dietary requirements for macrominerals are generally expressed as the percentage of the diet dry matter (DM), and microminerals are generally expressed as parts per million (ppm) or milligrams per kilogram of the diet DM. The content of minerals (particularly microminerals) in foods likely varies greatly with soil types worldwide. Deficiencies of minerals cause specific symptoms, such as reduced feed intake, growth restriction, impaired development, and even death (Mahan 1990; Spears 2019; Suttle 2010). Although some of the above minerals may become toxic to animals when fed at high levels, other minerals [e.g., cadmium (Cd), mercury (Hg), lead (Pb), beryllium (Be), arsenic (As), and Al] are toxic to animals at much lower levels and should be avoided at all times in their diets.

TABLE 10.1
Content of Macrominerals in the Whole Bodies of Newborn and Adult Animals

Mineral	Man	Pig	Dog	Cat	Rabbit	Guinea Pig	Rat	Mouse	Cattle	Chicken
	Amounts/kg of fat-free body weight									
Newborns										
Body weight, kg	3.56	1.25	0.328	0.118	0.054	0.080	0.0059	0.0016	55	0.04
Water, g	823	820	845	822	865	775	862	850	748	805
Calcium, g	9.6	10.0	4.9	6.6	4.6	12.3	3.1	3.4	12	4.0
Phosphorus, g	5.6	5.8	3.9	4.4	3.6	7.5	3.6	3.4	7	3.3
Magnesium, g	0.56	0.32	0.17	0.26	0.23	0.46	0.25	0.34	0.30	0.3
Sodium, mmol	82	93	81	92	78	71	84	72	80	83
Potassium, mmol	53	50	58	60	53	69	65	70	49	56
Chloride, mmol	55	52	60	66	56	60	67	61	52	60
Adults										
Body weight, kg	65	125	6.0	4.0	2.6	0.50	0.35	0.027	545	2.5
Water, g	720	750	740	740	730	646	720	780	705	740
Calcium, g	22.4	12.0	14.0	13.0	13.0	14.8	12.4	11.4	18.0	13.0
Phosphorus, g	12.0	7.0	6.8	8.0	7.0	9.43	7.5	7.4	10.0	7.1
Magnesium, g	0.47	0.45	0.40	0.45	0.50	0.94	0.40	0.43	0.41	0.50
Sodium, mmol	80	65	69	65	58	77	59	63	69	51
Potassium, mmol	69	74	65	77	72	107	81	80	49	69
Chloride, mmol	50	41	43	41	32	48	40	46	31	44

Source: Cheek and Holt (1963); Engle and Lemons (1986); Georgievskii et al. (1982); Kienzle et al. (1998); Pond et al. (1995); Sheng et al. (1981).

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The chemical properties of minerals affect their nutritional and physiological functions (Harris 2014). They can gain or lose electrons. Some minerals are metals (e.g., Na and Fe), which become cations by loss of electrons and have good electrical and thermal conductivity. Many of the metals are transition metals (e.g., Fe, Cu, and Zn), which are in Groups 3 through 12 of the periodic table (Figure 10.1) and have a strong tendency to form coordination compounds (Rayner-Canham and Overton 2006). However, many minerals are nonmetals (e.g., Cl, I, and P), which lack the characteristics of a metal and become anions by gaining electrons. Most minerals either form complexes with proteins for transport and biological functions, or interact with each other in the body. Metal-binding proteins are uniquely rich in cysteine, further underscoring the role of S-containing amino acids in mineral metabolism and function (Harris 2014). Thus, some minerals may affect the absorption and function of other minerals, and some can regulate gene expression in animal cells (Beckett et al. 2014; Cousins 1994; Spears and Weiss 2014). Furthermore, some minerals form coordination compounds with amino acids, such that the complexes are absorbed into enterocytes and extraintestinal cells at high rates. These kinds of interactions, which can be beneficial or detrimental, are an important aspect of mineral nutrition in animals. An imbalance of minerals, distinct from a simple deficiency, is a cause of certain nutritional disorders in livestock, poultry, fish, and crustaceans (Georgievskii et al. 1982; Lall and Kaushik 2021). Likewise, because minerals are not degraded, even those that are nutritionally essential can cause toxicity when their dietary intake exceeds requirements. Therefore, adequate knowledge of the chemistry and metabolism of minerals is essential to understanding animal nutrition and feeding practices. Formulating diets on the bioavailabilities of minerals (particularly Ca and P) can minimize their output in the manure and urine and can have both economic and ecological implications. The major objective of this chapter is to highlight the sources, absorption, transport, utilization, and functions of minerals in animals.

OVERVIEW OF MINERALS

Chemistry of Minerals

Minerals consist of elements, but not all elements are nutritionally essential. For example, hydrogen, which is the first element in the periodic table, is not considered a mineral in its elemental form. Like any nonhydrogen atom, an atom of a mineral is composed of a nucleus and the electrons surrounding the nucleus. The nucleus consists of protons that have positive charges and generally a similar number of neutrons that have no charge. An electron has one negative charge. More than 99.94% of an atom's mass is in the nucleus (Brown et al. 2003). The proton number is also known as the atomic number (e.g., 11 for Na and 17 for Cl). Atomic mass is the total mass of protons and neutrons in an atom, whereas atomic weight is the average mass of all the naturally occurring isotopes of an element, with the unit

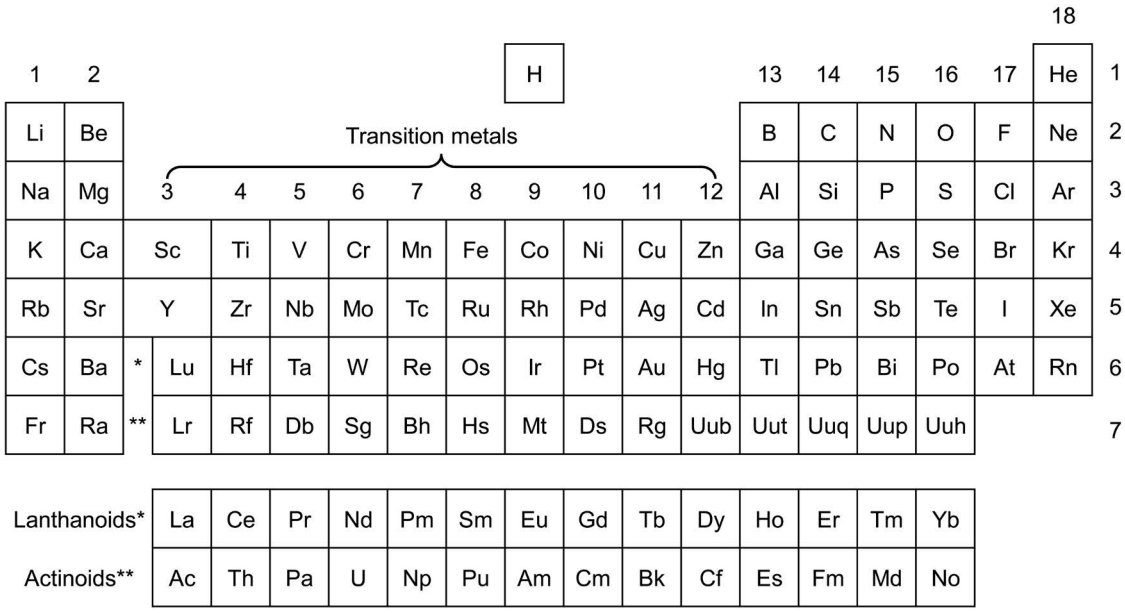


FIGURE 10.1 The periodic table of elements. This table is an arrangement of the chemical elements by their atomic number (number of protons), electron configurations, and chemical properties. Elements with similar behavior are ordered in the same column, whereas elements within a row (period) are metals on the left, transition metals (columns 3 to 12) in the middle, and nonmetals on the right. (Adapted from Rayner-Canham and Overton 2006.)

TABLE 10.2
Chemical Properties of Nutritionally Essential Minerals in Animals

Metals					Nonmetals				
Name	Symbol	Valence State[a]	Atomic Number	Melting Point (°C)	Name	Symbol	Valence State[a]	Atomic Number	Melting Point (°C)
Nutritionally essential macrominerals									
Calcium	Ca	+2	20	842	Chlorine	Cl	–1	17	–101.5
Magnesium	Mg	+2	12	650	Phosphorus	P	+3, +5, –3	15	44.2
Potassium	K	+1	19	63.5	Sulfur	S	+4, +6, –2	16	115.2
Sodium	Na	+1	11	97.8					
Nutritionally essential microminerals									
Chromium	Cr	+2, +3, +6	24	1907	Boron	B	+3	5	2076
Cobalt	Co	+1, +2, +3	27	1495	Bromine	Br	–1	35	–7.2
Copper	Cu	+1, +2, +4	29	1085	Fluorine	F	–1	9	–220
Iron	Fe	+2, +3	26	1538	Iodine	I	–1	53	113.7
Manganese	Mn	+2, +3	25	1246	Selenium	Se	+4, +6, –2	34	221
Molybdenum	Mo	+4, +5, +6	42	2623	Silicon	Si	+2, +4	14	1414
Nickel	Ni	+1, +2	28	1455					
Tin	Sn	+2, +4	50	232					
Vanadium	V	+2 to +5	23	1910					
Zinc	Zn	+2	30	420					

[a] Stable valence state.

being dalton (Da). If the number of protons and electrons is equal, an atom is electrically neutral and has no net electric charge. However, if an atom has more or fewer electrons than protons, it has one or more net negative or positive charges, respectively, and is called an ion. In other words, by gaining an electron, an atom becomes an anion, and by losing an electron, an atom becomes a cation. Two or more atoms form a compound (e.g., NaCl). After being dissolved in water, all binary ionic mineral compounds (e.g., NaCl) are ionized to yield their constituent ions (e.g., Na^+ and Cl^-). The stable valence states of some nutritionally essential minerals are shown in Table 10.2.

Niels Bohr's prototypical model of atomic structure based on quantum theory explains the structure of an atom. In 1913, Bohr proposed that electrons of an atom occupy circular orbits (shells) with different energy levels around its nucleus. Each of these shells is identified with an integer (e.g., 1, 2, 3, 4, …, n), which is called a principal quantum number. The maximum number of electrons in a shell is $2 \times n^2$ (i.e., 2, 8, 18, 32, … for shell 1, 2, 3, 4, …, respectively), where n is the principal quantum number (i.e., the number of shells). The higher a principal quantum number (or energy level), the farther the shell is from the nucleus. The atomic structures of Na, Cl, K, Mn, Cu, and Zn are shown in Figure 10.2.

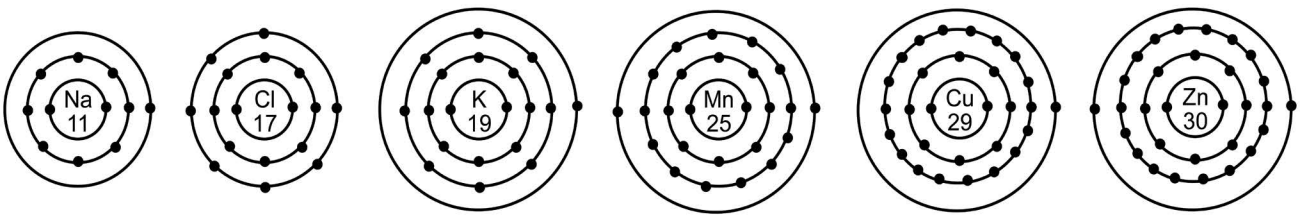


FIGURE 10.2 The atomic structures of sodium, chlorine, potassium, manganese, copper, and zinc. Electrons of an atom occupy circular orbits (shells) with different energy levels around its nucleus. The maximum number of electrons in a shell is $2 \times n^2$ (i.e., 2, 8, 18, 32, … for shell 1, 2, 3, 4, …, respectively), where n is the principal quantum number (i.e., the number of shells). The higher a principal quantum number (or energy level), the farther the shell is from the nucleus. In all atoms, electrons that are in the outermost shell are called valence electrons, which are involved in chemical bonding.

Within each shell, there are different spaces (called orbitals) with different shapes. The orbitals are denoted by a letter *s*, *p*, *d*, or *f*. The number of orbitals within a shell is equal to the shell number. For example, the first shell of an atom has 1 orbital (i.e., 1*s*); the second shell has 2 orbitals (i.e., 2*s* and 2*p*); the third shell has three orbitals (i.e., 3*s*, 3*p*, and 3*d*); and the fourth shell has four orbitals (i.e., 4*s*, 4*p*, 4*d*, and 4*f*). Orbitals *s*, *p*, *d*, and *f* have 1, 3, 5, or 7 suborbitals, respectively, with each suborbital holding up to two electrons (Rayner-Canham and Overton 2006). Thus, orbitals *s*, *p*, *d*, and *f* can have up to 2, 6, 10, and 14 electrons, respectively. The *s*-orbital primarily denotes elements in Groups 1 and 2 of the periodic table, the *d*-orbital denotes Groups 3–12 elements (transition metals), the *p*-orbital denotes Group 13, 14, 15, 16, 17, or 18 elements, and the *f*-orbital denotes the lanthanides and actinides group. Electrons generally fill the lowest energy-level orbital first and then a higher energy-level orbital, constituting an electron filling pattern of 1*s*, 2*s*, 2*p*, 3*s*, 3*p*, 4*s*, 3*d*, 4*p*, 5*s*, 4*d*, 5*p*, 6*s*, 4*f*, 5*d*, 6*p*, 7*s*, and 5*f*. Note the different energy levels of *d*, *p*, and *f* orbitals: $3d > 4s$, $4d > 5s$, $4f > 6s$, and $5f > 7s > 6p$. There are, however, exceptions for Cr and Cu, where the energy level of the 3*d* orbital is only slightly higher than that of the 4*s* orbital. An element is more stable when a *d* suborbital is either half-full (e.g., $4s^1$ and $3d^5$ for chromium) or completely full (e.g., $4s^1$ and $3d^{10}$ for Cu), compared to a partially filled *d* suborbital. In all atoms, electrons that are in the outermost shell are called valence electrons, which are involved in chemical bonding.

Electron configuration, which is the distribution of electrons of an atom in orbitals, is shown in Table 10.3 for nutritionally essential macro- and microminerals. Na, which has a single electron in its outer 3*s* orbital, can lose that electron to form a cation (Na^+), whereas chlorine, which has seven valence electrons, can gain one electron to form an anion (Cl^-). These two ions have low-energy states, are chemically stable, and exist as an ionic salt (NaCl) used in animal diets. Likewise, K cation (K^+), Ca dication (Ca^{2+}), and P in the form of phosphate (HPO_4^{2-}) are chemically stable. Of note, Na^+, Cl^-, K^+, Ca^{2+}, and PO_4^{3-} are not redox-active. In contrast, many transition metals, which can occur in more than one oxidation state (e.g., Fe^{2+} and Fe^{3+}), participate in redox reactions. As noted previously, all transition metals have incomplete 3*d* orbitals. Partially filling 3*d* orbitals allows a transition metal to accept or donate electrons, and these elements therefore play an important role in cellular oxidation-reduction reactions (e.g., the mitochondrial electron transport system). Thus, the theory of atomic structure aids in the understanding of the electronic states and chemical properties of minerals as related to animal nutrition and metabolism.

TABLE 10.3
Electron Configuration of Nutritionally Essential Minerals[a]

Element (Atomic Number)	Electron Configuration	Abbreviated Form
Boron (5)	$1s^2\ 2s^2\ 2p^1$	[He] $2s^2\ 2p^1$
Fluorine (9)	$1s^2\ 2s^2\ 2p^5$	[He] $2s^2\ 2p^5$
Sodium (11)	$1s^2\ 2s^2\ 2p^6\ 3s^1$	[Ne] $3s^1$
Magnesium (12)	$1s^2\ 2s^2\ 2p^6\ 3s^2$	[Ne] $3s^2$
Silicon (14)	$1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^2$	[Ne] $3s^2\ 3p^2$
Phosphorus (15)	$1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^3$	[Ne] $3s^2\ 3p^3$
Sulfur (16)	$1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^4$	[Ne] $3s^2\ 3p^4$
Chlorine (17)	$1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^5$	[Ne] $3s^2\ 3p^5$
Potassium (19)	$1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 4s^1$	[Ar] $4s^1$
Calcium (20)	$1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 4s^2$	[Ar] $4s^2$
Vanadium (23)	$1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 4s^2\ 3d^3$	[Ar] $4s^2\ 3d^3$
Chromium (24)	$1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 4s^1\ 3d^5$	[Ar] $4s^1\ 3d^5$
Manganese (25)	$1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 4s^2\ 3d^5$	[Ar] $4s^2\ 3d^5$
Iron (26)	$1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 4s^2\ 3d^6$	[Ar] $4s^2\ 3d^6$
Cobalt (27)	$1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 4s^2\ 3d^7$	[Ar] $4s^2\ 3d^7$
Nickel (28)	$1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 4s^2\ 3d^8$	[Ar] $4s^2\ 3d^8$
Copper (29)	$1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 4s^1\ 3d^{10}$	[Ar] $4s^1\ 3d^{10}$
Zinc (30)	$1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 4s^2\ 3d^{10}$	[Ar] $4s^2\ 3d^{10}$
Selenium (34)	$1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 4s^2\ 3d^{10}\ 4p^4$	[Ar] $4s^2\ 3d^{10}\ 4p^4$
Bromine (35)	$1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 4s^2\ 3d^{10}\ 4p^5$	[Ar] $4s^2\ 3d^{10}\ 4p^5$
Molybdenum (42)	$1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 4s^2\ 3d^{10}\ 4p^6\ 5s^1\ 4d^5$	[Kr] $5s^1\ 4d^5$
Tin (50)	$1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 4s^2\ 3d^{10}\ 4p^6\ 5s^2\ 4d^{10}\ 5p^2$	[Kr] $5s^2\ 4d^{10}\ 5p^2$
Iodine (53)	$1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 4s^2\ 3d^{10}\ 4p^6\ 5s^2\ 4d^{10}\ 5p^5$	[Kr] $5s^2\ 4d^{10}\ 5p^5$

[a] Electron configurations: Ar (argon), $1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6$; He (helium), ($1s^2$); Kr (krypton), $1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 4s^2\ 3d^{10}\ 4p^6$; Ne (neon), $1s^2\ 2s^2\ 2p^6$. These are all noble gases in Group 18 of the periodic table.

Concepts in the Digestion of Mineral Complexes in Diets and the Absorption of Minerals

The composition of minerals in common feedstuffs [including corn and soybean meal (SBM)] is summarized in Table 10.4. In addition, organic (e.g., amino acid and proteinate) and/or inorganic (e.g., limestone and $CaCl_2$) complexes of minerals are often supplemented to ruminant and nonruminant diets (Cromwell et al. 1998; Liao et al. 2019; Overton and Yasui 2014; Spears 1996, 2023). Furthermore, the sulfate form of Zn, Cu, Mn, or Fe is generally preferred over the oxide form because the former is much more bioavailable than the latter. During feeding, a large amount of water is secreted into the lumen of the small intestine to facilitate food digestion; almost all of this water, along with dietary nutrients, is subsequently reabsorbed by the small intestine (Goff 2018). Digestion of protein, lipids, and carbohydrates in the stomach and small intestine releases minerals from their food matrix. Phytase plays an important role in the release of minerals from plant-sourced ingredients because most minerals (particularly P) are bound to phytate and are entrapped within the complex fibers (Humer et al. 2015; Leyva-Jimenez et al. 2019). Additionally, phytate can

TABLE 10.4
Content of Minerals in Feedstuffs[a]

	Macrominerals							Microminerals				
Feedstuff	**Ca (%)**	**P (%)**	**Na (%)**	**Cl (%)**	**K (%)**	**Mg (%)**	**S (%)**	**Cu ppm**	**Fe ppm**	**Mn ppm**	**Se ppm**	**Zn ppm**
Alfalfa, 17% CP	1.53	0.26	0.09	0.47	2.3	0.23	0.29	10	333	32	0.34	24
Barley grain	0.06	0.35	0.04	0.12	0.45	0.14	0.15	7	78	18	0.19	25
Blood cells (spray-dried)	0.05	0.25	3.42	1.41	0.88	0.07	0.49	19	1770	6.5	8.5	68
Blood meal[b]	0.37	0.27	0.50	0.30	0.11	0.11	0.48	11	1922	6	0.58	38
Blood meal[c]	0.41	0.30	0.44	0.25	0.15	0.11	0.47	8	2919	6	0.60	30
BSFM	2.30	0.87	0.22	0.43	1.12	0.43	0.44[k]	9.5	191	191	1.2	95
Canola meal	0.63	1.01	0.07	0.11	1.22	0.51	0.85	6	142	49	1.10	69
Casein, dried	0.61	0.82	0.01	0.04	0.01	0.01	0.60	4	14	4	0.16	30
CBPM	4.46	2.41	0.44	0.54	0.53	0.18	0.52	10	442	9	0.88	94
Corn grain	0.03	0.28	0.02	0.05	0.33	0.12	0.13	3	29	7	0.07	18
CSM[d]	0.19	1.06	0.04	0.05	1.40	0.50	0.31	18	184	20	0.80	70
CVD	3.0	1.7	0.40	0.54	0.55	0.22	0.70[k]	14	440	11	0.75	120
Corn DDGS	0.12	0.73	0.22	0.26	0.90	0.29	0.66	7.65	126	18	0.20	65
Feather meal	0.33	0.50	0.34	0.26	0.19	0.20	1.39	10	76	10	0.69	111
Fish meal, Menhaden	5.21	3.04	0.40	0.55	0.70	0.16	0.71[k]	11	440	37	2.10	147
Fish meal, PA	4.10	2.46	0.65	0.89	1.16	0.24	0.85[k]	9	220	10	1.36	103
Fish meal, SE	5.33	3.41	0.88	1.30	1.52	0.25	0.87[k]	7	212	23	1.5	84
Flax meal	0.39	0.83	0.13	0.06	1.26	0.54	0.39	22	270	41	0.63	66
MBM[e]	9.99	4.98	0.63	0.69	0.65	0.41	0.38	11	606	17	0.31	96
Milk powder[f]	1.31	1.00	0.48	1.00	1.60	0.12	0.32	5	8	2	0.12	42
Oat grain	0.07	0.31	0.08	0.10	0.42	0.16	0.21	6	85	43	0.30	38
Peanut meal, sol extr	0.22	0.65	0.07	0.04	1.25	0.31	0.30	15	260	40	0.21	41
Plasma (spray-dried)	0.10	0.18	7.06	2.19	1.34	0.15	1.08	50	99.8	12	10	80
PBM[g]	4.46	2.41	0.49	0.49	0.53	0.18	0.52	10	442	9	0.88	94
Rice bran	0.07	1.61	0.03	0.07	1.56	0.90	0.18	9	190	228	0.40	30
Rice grain[h]	0.04	0.18	0.04	0.07	0.13	0.11	0.06	21	18	12	0.27	17
Safflower meal, sol extr	0.34	0.75	0.05	0.08	0.76	0.35	0.13	10	495	18	0.10	41
SDEP (PFG)	0.19	0.84	0.49	0.69	0.56	0.043	0.80[k]	2.7	78	1.5	1.2	57
SDPP (PFG)	0.22	1.07	4.10	4.70	0.13	0.038	1.19[k]	26	1782	3	1.7	42
Sorghum grain	0.03	0.29	0.01	0.09	0.35	0.15	0.08	5	45	15	0.20	15
Soybean meal[i], sol extr	0.32	0.65	0.01	0.05	1.96	0.27	0.43	20	202	29	0.32	50
Sunflower meal, sol extr	0.36	0.86	0.02	0.10	1.07	0.68	0.30	26	254	41	0.50	66
Wheat bran	0.16	1.20	0.04	0.07	1.26	0.52	0.22	14	170	113	0.51	100
Wheat grain[j]	0.06	0.37	0.01	0.06	0.49	0.13	0.15	6	39	34	0.33	40
Yeast (Brewers' dried)	0.16	1.44	0.10	0.12	1.80	0.23	0.40	33	215	8	1.00	49

Source: Jamroz et al. (2011); Li and Wu (2023); National Research Council (1988).

Note: BSFM, black soldier fly larvae meal; CBPM, chicken byproduct meal; DDGS, dried distillers grains with solubles; PA, Peruvian anchovy; PFG, pet-food grade; SDEP, spray-dried egg product; SDPP, spray-dried poultry plasma; SE, Southeast Asian miscellaneous marine fishes; sol extr, solvent extracted.

[a] Values are expressed on an as-fed basis.

[b] Conventional blood meal.

[c] Blood meal, spray or ring dried (93% dry matter).

[d] Cottonseed meal [solvent extract (sol extr), 41% CP].

[e] Meat & bone meal (93% dry matter).

[f] Bovine milk (skim, dried; 96% dry matter).

[g] Poultry byproduct meal (93% dry matter). Calcium content is 3.0% (on a dry matter basis) in some PBM products.

[h] Polished, broken.

[i] 44% crude protein on an as-fed basis.

[j] hard red, winter.

[k] Calculated on the basis of the content of cysteine, methionine and taurine in feedstuffs (Li and Wu 2020). By weight, the content of sulfur in cysteine, methionine, and taurine is 26.5%, 21.5%, and 25.6%, respectively.

form insoluble complexes with divalent cations (e.g., Ca and Zn) in the small intestine (Philippi et al. 2023). Moreover, phytate can react with tannins in feeds to further impair the digestion and absorption of dietary nutrients. In nonruminants, because the bacteria in the lumen of the small intestine cannot produce sufficient phytase or fiber-digesting enzymes, exogenous sources of these enzymes through dietary supplementation can improve the bioavailability of dietary minerals (Augspurger and Baker 2004; Kiarie and Nyachoti 2010).

In all animals, minerals are absorbed in the form of ions, but the major sites of absorption differ between ruminants and nonruminants for some minerals, as well as among minerals. In ruminants, the ruminal epithelium can absorb certain minerals (e.g., Na and Mg); however, most minerals, except for Mg, are absorbed primarily in the small intestine (Leonhard-Marek et al. 2005). There are reports that most of the dietary Mg is absorbed by the reticulorumen in sheep (Tomas and Potter 1976) and steers (Greene et al. 1983). In terrestrial nonruminants, except for a few (e.g., Co and Ca), hydrogen fluoride (HF), and the truly chelated mineral complexes, most macrominerals (Tables 10.5 and 10.6) are absorbed in the form of ions primarily by the enterocytes of the jejunum through specific transporters (either facilitated or active transporters) or receptor-mediated endocytosis. This is called transcellular transport or absorption (Goff 2018). The minerals that are truly chelated with organic compounds (e.g., heme, amino acids, dipeptides, and tripeptides) [with the chemical bonds being identified by the fast-Fourier transforming infrared (FT-IR) spectroscopy] are absorbed intact as part of their carriers via endocytosis or specific transporters (Sauer et al. 2017; Sun et al. 2020). In fish and crustaceans, the gills absorb minerals from the surrounding water. In a healthy gut with functional tight junctions between two enterocytes, passive diffusion of small molecules and ions through the tight junctions into the extracellular space, which is known as paracellular transport or absorption, is rather limited (Khanal and Nemere 2008).

Upon entering the enterocyte, macrominerals exist as free ions, whereas microminerals are carried by vesicles or protein transporters across its cytosol (Suttle 2010). All minerals exit the enterocyte through its basolateral membrane via exocytosis or specific transporters into the lamina propria (Tables 10.5 and 10.6) and ultimately enter the portal vein. These nutrients are transported in plasma as free or protein-bound ions, or in both forms, and are taken up by extraintestinal cells through exocytosis or specific transporters. In plasma or cells, most minerals (particularly microminerals) are present in a variety of complex forms, and only a few exist entirely as free elemental ions

TABLE 10.5
Absorption of Macrominerals by the Enterocyte, Their Transport in Plasma, and Their Uptake by Tissues

Element	Transporter in the Apical Membrane of Enterocyte	Transporter in the Cytosol of Enterocyte	Transporter in the Basolateral Membrane of Enterocyte	Transport in Plasma	Uptake by Extraintestinal Cells
Calcium	Ca transporter-1	Calbindin	Ca^{2+}-ATPase	Free and PB	Ca^{2+} channels
Chloride	Cl channels[a], NCT (HCO_3^-/Cl^- exchange)	No; Free anion	Cl channels (e.g., ClC-2) Na-K-2Cl CT (in)	Free anion	CACC (mainly)
Magnesium	TRPM6/7	No. Free cation	CNNM4	Free cation	TRPM6/7
Phosphate	NaPi2b, PiT1, PiT2	No; Free anion as PO_4^{2-}	Na^+-dependent transport	Free cation	NaPi2a, NaPi2c PiT1, PiT2
Potassium	K channels[a]	No; Free cation	K channels K-Cl cotransporter Na-K-2Cl CT (in) Na^+/K^+-ATPase (in)	Free cation	K channels[b] K-Cl cotransporter
Sodium	SGLT1, AMSC, NCT NHE2/3, AATs, NPT	No; Free cation	Na^+/K^+-ATPase, NHE1 Na-K-2Cl CT (in)	Free cation	Sodium channels SGLT2 (kidney)
Sulfur	AA transporters	No. As AAs	AA transporters	Free AAs	AA transporters

[a] Ligand- or voltage-gated chloride channels. CFTR (cystic fibrosis transmembrane conductance regulator) is the major Cl^- transporter in the apical membrane of the intestine.

[b] Potassium channels include K^+/H^+-ATPase, Na^+-Cl^--K^+ transporter, and K^+ conductance channels.

Note: AA, amino acid; AATs, sodium-dependent amino acid transporters; AMSC, amiloride-sensitive Na^+ channels; CACC, calcium-activated Cl channels; Cav, voltage-gated Ca^{2+} channels; CNNM4 (ancient conserved domain protein 4; (in), driving the influx of Na, K and Cl ions across the basolateral membrane of the enterocyte for secretion into the intestinal lumen; NHE, Na^+/H^+ exchanger; NPT, sodium-phosphate cotransporter; NCT, electroneutral Na^+-Cl^- absorption mediated by Na^+/H^+ (NHE2/3) and Cl^-/HCO_3^-; Na-K-2Cl CT (in), Na^+-K^+-$2Cl^-$ cotransporter; NaPi2b, sodium-phosphate cotransporter; PB, protein-bound; PiT1, phosphate transporter-1; PiT2, phosphate transporter-1; SLGT1, sodium-linked glucose transporter-1; TRPM6, transient receptor potential melastatin-related protein-6. The sign (in) indicates that minerals are transported from the lamina propria (ultimately the blood) into the intestinal epithelial cell.

TABLE 10.6
Absorption of Microminerals by the Enterocyte, Their Transport in Plasma, and Their Uptake by Tissues

Element	Transporter in the Apical Membrane of Enterocyte	Transporter in the Cytosol of of Enterocyte	Transporter in the Basolateral Membrane of Enterocyte	Transport in Plasma	Uptake by Extraintestinal Cells
Boron	NaBC1 and AQPs	Borate, $B(OH)_3$	NaBC1 and AQPs	Unbound $B(OH)_3$	NaBC1 and AQPs
Bromine	Cl^- and I^- transporters	Free anions	Anion exchangers	Free anions	Na^+-K^+-$2Cl^-$
Chromium	Passive diffusion	Chromodulin	Vesicle discharge	TF, Albumin	TF receptor*
Cobalt (free)	See Fe^{2+} transport	Cytosolic protein	See Fe^{2+} transport	Albumin	See Fe^{2+} transport
Cobalt (B_{12})	Receptor (endocytosis)	Cobalamins C/D	MRP1	TC-I, II, III	Receptor (CD320)
Copper	CTR1 (for Cu^{1+}) DCT1 (for Cu^{2+})	ATOX1, ATP7a COX-17, CCS Glutathione, MTs	Vesicle discharge powered by ATP7a (a Cu-ATPase)[a]	Albumin (10% Cu), Ceruloplasmin (90% Cu)	CTR1
Fluorine	F^-/H^+ cotransporter anion-exchange	Ca^{2+}, Mg^{2+}	Fluc protein (FEX)	Ca^{2+}, Mg^{2+}	F^-, Ca^{2+}, and Mg^{2+} channels
Iodine	Na^+/I^- symporter	No. Free anion	Fa passive diffusion	Albumin	Na^+/I^- symporter
Iron	DCT1 for Fe^{2+} HCP1 for heme Fe^{2+} Heme-R (for EDC)	Paraferritin complex	Ferroportin (only recognizing Fe^{2+})	Transferrin (TF)	Transferrin receptor
Manganese	DCT1	Vesicles, PMR1P ZIP8	Vesicle discharge PMR1P	Primarily TF (~50%)	Mn^{2+} transporter NRAMP1 in MΦ
Molybdenum	Active transporter	No. Free cation	SO_4^{2-} transporters	MoO_4^{2-}, RBCs	Active transporter
Nickel	DCT1	No. Free cation	Passive diffusion	Albumin	CME
Selenium[b]	NaS1/2, Fa diffusion	Selenoprotein P	Exocytosis	Selenoprotein P (Sepp1)	Sepp1 receptor (endocytosis)
Selenium[c]	AA transporters	No. Free AAs	AA transporters		
Silicon	NaPi2b; AQPs 3, 7, 9	No; as $Si(OH)_4$	Passive diffusion, AQP8	$Si(OH)_4$	NaPi2b; AQPs 3, 7, 9
Vanadium	Phosphate and other anion transporters	Ferritin (as vanadyl, V^{4+})	PO_4^{3-} transporter for VO_4^{3-}, nonheme iron pathways	Vanadyl-TF, -ferritin, HG	Anion transporters and the IF pathway
Zinc	ZIP4, DCT1, DMT-1	CRIP	Zn transporter-1 (ZnT1) and ZnT2	Albumin (60% Zn), MG (30% Zn), other factors (10% Zn)	Zip2/3 (liver) Zip4 (kidneys) Zips (other cells), Ca_v

[a] Found in all tissues except for the liver.

[b] Inorganic forms of selenium, with selenate and selenite being transported by NaS1/2 and facilitated (Fa) diffusion, respectively.

[c] Organic forms of selenium (seleno-methionine and seleno-cysteine).

Note: AA, amino acid; AQPs, aquaporins 3, 7, 8, 9, and 10, which can transport boric acid but not borate ions; B_{12}, vitamin B_{12}; CME, clathrin mediated endocytosis; CRIP, cysteine-rich intracellular protein; CTR1, copper transporter-1; DCT1, divalent cation transporter-1; EDC, endocytosis; Fa, facilitated; Fluc, fluoride carrier (also known as FEX, fluoride export protein); HCP1, heme carrier protein-1; HG, hemoglobin; heme-R, heme receptor; α_2-MG, α_2-macroglobulin; MΦ, macrophage and monocyte; MRP1, multidrug resistance protein 1; MTs, metallothioneins; NaBC1 (Na^+-driven boron channel-1); NaS 1/2, Na^+-sulfate cotransporters 1 and 2; PMR1P, manganese transporting ATPase; RBCs, red blood cells; TC, transcobalamins; * via endocytosis.

(i.e., Na^+, K^+, and Cl^-). Concentrations of macrominerals in the serum of animals are shown in Table 10.7. Absorbed minerals, except for Co, Cu, Mn, and mercury (which are excreted mainly via fecal excretion), are excreted from the body primarily through the urine (Georgievskii et al. 1982; Suttle 2010). Due to their low rates of intestinal absorption, many nonelectrolyte minerals (e.g., Ca, Mg, P, and Zn) in diets are generally excreted in greater amounts in feces than in urine. The efficiency of intestinal absorption of some trace minerals (e.g., Se and Cu) is much lower in ruminants than in nonruminants due to modifications [e.g., the reduction of dietary Se to insoluble forms (e.g., elemental Se or selenides)] in the ruminal environment (Spears 2003). The nutritional, metabolic, and physiological aspects of minerals in fish and crustaceans are generally similar to those in terrestrial animals except that the latter absorb minerals from both diets and the surrounding water (Lall and Kaushik 2021). Knowledge of the true rates of intestinal absorption of minerals is crucial for assessing the effects of dietary supplementation on acid-base balance in the body.

TABLE 10.7
Concentrations (mM) of Macrominerals in the Serum of Animals[a]

Mineral	Cat	Cattle	Chicken	Dog	Goat	Horse	Pig	Rabbit	Rat	Sheep
Sodium	149–158	132–152	150–158	145–150	142–155	134–142	139–145	125–150	148–150	140–149
Chloride	111–124	97–111	110–118	107–113	99–110	95–104	100–106	90–120	103–104	100–120
Calcium	2.2–2.8	2.4–3.1	2.0 –3.0	2.7–2.8	2.2–2.9	2.7–3.2	2.4–3.5	1.4–3.0	2.4–3.0	2.5–3.0
Phosphorus	0.8–2.7	1.8–2.1	1.3–2.3	1.2–1.8	1.4–2.9	2.2–3.5	2.3–3.3	1.3–1.9	1.9–2.4	1.3–1.9
Potassium	3.8–5.5	3.9–5.8	3.3–5.7	4.7–4.9	3.5–6.7	2.4–4.8	4.0–5.2	3.5–7.0	6.1–7.0	3.5–6.0
Magnesium	1.7–2.2	1.6–2.5	1.2–1.3	1.5–2.1	1.5–2.2	1.2–1.9	1.6–2.1	0.82–2.2	1.1–1.3	0.74–1.7

Source: eClinPath (2024); Fox et al. (2002); Georgievskii et al. (1982); Herzig et al. (2009); Laboklin (2024); Morgan and Chichester (1935); Rezaei et al. (2013).

[a] Concentrations (mM) of macrominerals in the serum of mice are: sodium, 157–166; chloride, 125–130; calcium, 2.2–2.6; phosphorus, 2.6–2.7; potassium, 7.8–8.0; and magnesium, 0.58–1.28.

Determination of Endogenous Losses of Minerals via the Digestive Tract

Minerals are excreted from animals via both urine and feces, as noted previously. The endogenous losses of minerals via the digestive tract (i.e., the amounts of endogenous minerals secreted into its lumen) underestimate the true digestibilities of dietary minerals and, therefore, must be measured. In all animals, endogenous losses of minerals (e.g., Ca, P, and Zn) into the intestinal tract can be determined using (a) diets either lacking test minerals or containing graded levels of test minerals (the regression method to identify the intercept); or (b) the intravenous administration of mineral tracers for analyzing their secretions into the gut (Alves et al. 2016; Anwar and Ravindran 2020; Anwar et al. 2016; David et al. 2019; Lee et al. 2021; Mutucumarana and Ravindran 2021; Mutucumarana et al. 2014; Nelson et al. 2022; Petersen and Stein 2006; Stein 2024; Walk et al. 2021; Zhang and Adeola 2017, 2018). The values for intestinal losses differ among different minerals and animal species (Table 10.8). There is generally a positive correlation between the endogenous fecal loss

TABLE 10.8
Intestinal Endogenous Losses of Minerals in Animals

Animal	Mineral	Intestinal Endogenous Loss
Growing pig[a]	Ca	349–659 mg/kg of DM intake when fed Ca-free corn-based diets without phytase; 186 mg/kg of DM intake when fed Ca-free corn-based diets with added phytase
	P	129–139 mg/kg of DM intake when fed P-free gelatin-based diets; 154 mg/kg of DM intake when fed a porcine plasma-based diet
	Zn	0.26 mg/kg BW/day; 9.2 mg/kg DM intake
Broiler chicken[b]	Ca	131, 253, 88–125, 77, and 46 mg/kg of DM intake, respectively, when fed Ca-free purified, Ca-free corn- based, Ca- and P-free, corn gluten meal-, and dried egg albumen-based diets
	P	25, 133, 110, 104, 438, 80, 609, and 418 mg/kg of DM intake, respectively, when fed P-free, Ca- and P-free, dried egg albumen-, gelatin-, casein-, wheat-, SBM-, and corn DDGS-based diets
Cattle[a]	Ca	15 mg/kg BW/day in 5- to 7-month-old calves; 16 mg/kg BW/day in adult steers
	P	9–10 mg/kg BW/day in calves weighing 140 kg; 11 mg/kg BW/day in adult cattle (pregnant or lactating)
	Zn	0.09 mg/kg BW/day in 5- to 7-month-old calves
Sheep[a]	Ca	4.93, 1.69, and 1.36 mg/kg BW/day, respectively, in growing, maintenance adult (nonpregnant and nonlactating), and production adult (pregnant or lactating) sheep
	P	8.5–31.5 mg/kg BW/day in adults
	Zn	0.11 mg/kg BW/day in adults
Goat[a]	Ca	7.4 mg/kg BW/day
	P	10 mg/kg BW/day
	Zn	0.17 mg/kg BW/day

Source: Alves et al. (2016); Anwar and Ravindran (2020); Anwar et al. (2016); Challa and Braithwaite (1988); Chu et al. (2009); David et al. (2019); Hattori et al. (2010); Lee et al. (2021); Mutucumarana and Ravindran (2021); Mutucumarana et al. (2014); Miller et al. (1991); Nelson et al. (2022); Payne and Sansom (1966); Suttle et al. (1982); Petersen and Stein (2006); Stein (2024); Walk et al. (2021); Zhang and Adeola (2017); Zhang and Adeola (2018).

[a] Total intestinal tract endogenous loss (measured in collected fecal samples).

[b] Ileal endogenous loss (measured in collected ileal digesta samples).

Note: BW, body weight; DDGS, dried distillers grains with solubles; DM, dry matter; SBM, soybean meal

of minerals and DM intake; for example, an increase of 0.64 mg Ca/kg BW/day for an increase of 1 g DM intake/kg BW/day in sheep (Braithwaite 1982). The endogenous fecal loss of minerals may not be altered by mineral intake, pregnancy, or lactation but may be slightly greater in young than in adult animals consuming the same amount of minerals per gram of BW (Braithwaite 1982).

Bioavailabilities of Minerals in Feedstuffs and Diets for Animals

Bioavailabilities of dietary minerals reflect their gastrointestinal digestion and absorption, as well as utilization by intestinal and extraintestinal tissues in animals (Baker 2001; Byrne and Murphy 2022). An absorbed mineral may not be usable for either metabolism or physiological function. Methods for estimating the bioavailabilities of minerals in feedstuffs and diets for animals include (a) slope ratio assays, (b) in vivo digestion trials, and (c) in vitro digestion experiments (Kiarie and Nyachoti. 2010). The use of these methods depends on animal species, individual minerals, and facilities. The values of bioavailabilities for macrominerals and microminerals in feed ingredients and diets are summarized in Tables 10.9 and 10.10, respectively. Because a nutritionally essential mineral participates in diverse physiological processes, its bioavailability

TABLE 10.9
Bioavailabilities of Macrominerals in Feedstuffs and Diets for Animals[a]

Animal	Mineral	Source	Bioavailability (%)
Human[b]	Ca	Mixed	Diets based on animal- and plant-sourced foods, 30
	K	Inorganic	Potassium chloride (KCl), 96–100
	P	Mixed	Diets based on animal- and plant-sourced foods, 70
	Na	Inorganic	Sodium chloride (NaCl), 96–100
Pig	Ca	Inorganic[b]	Calcium carbonate ($CaCO_3$), 70; dicalcium phosphate, 76
		Inorganic[c]	$CaCO_3$, 100; dicalcium phosphate ($CaHPO_4$), 100; a ragonite (a crystal form of $CaCO_3$), 99; calcium chloride ($CaCl_2$), poorly absorbed; calcium sulfate ($CaSO_4$), gypsum ($CaSO_4 \cdot 2H_2O$), and limestone (mainly $CaCO_3$): 99; dolomitic limestone (mainly magnesium calcium carbonate), 51–78; marble dust (mainly $CaCO_3$) and oyster shell (ground; 96% $CaCO_3$): 98
		Organic[c]	Calcium gluconate, calcium citrate, calcium lactate: 100
		Plant[b]	Canola meal, 47; corn, 50–55; soybean meal (SBM), 50–55
		Plant[c]	Dehydrated alfalfa meal, 21
		Animal[b]	Meat and bone meal, 77; poultry meal, 82
		Ration[b]	Corn- and SBM-based diets, 60–63
	P	Inorganic[b]	Monocalcium phosphate [also known as calcium dihydrogen phosphate; $Ca(H_2PO_4)_2$], 68
		Inorganic[d]	Calcium sodium phosphate ($CaNaO_4P$), 100; Curaçao Island phosphate, 81; DFP, 80–90; dicalcium phosphate, 97; monocalcium phosphate, 100; monosodium phosphate (NaH_2PO_4), 100; rock phosphate (soft), 53; tricalcium phosphate, 71
		Plant[b]	Canola meal (without added phytase), 34; canola meal (with added phytase), 61; field peas, 56; rice bran, 12; SBM (hulled), 47; SBM (low phytate), 63; SBM (fed to transgenic pigs expressing phytase), 93; sunflower meal, 20
		Plant[d]	Alfalfa meal, 95; Barley, 30; barley (dried grain), 25; canola meal, 15; corn DDGS, 77; corn gluten feed, 59; corn (dry), 18; corn (high moisture), 46; corn (hominy feed), 14; corn (low phytate), 62; cottonseed meal, 22; field peas, 28–56; oats, 29; oats (groats), 16; peanut meal, 12; rice bran, 25; sorghum (dry), 26; sorghum (high moisture), 43; soybean hulls, 78; SBM (low phytate), 63; SBM (dehulled), 23; SBM (hulled), 33; SBM (with added phytase), 69; sunflower meal, 20; triticale, 46; wheat, 50; wheat bran, 32; wheat middlings, 33
		Animal[d]	Blood cells (spray-dried), 95; blood meal, 92; bone meal, 85; dried whey, 94; feather meal, 31; fishmeal, 94; meat meal, 90; meat and bone meal, 73–90; milk products, 94; plasma (spray-dried), 95–97; and PBM, 90
		Yeast[b]	50–60
		Ration[b]	Corn- and SBM-based diets, 55
Poultry	Ca	Inorganic[b]	Biovalve shell, 27; calcium carbonate, 67; dicalcium phosphate, 67; limestone, 64; marble dust, 19; monocalcium phosphate, 65; monodicalcium phosphate, 60; and oyster shell, 60
		Inorganic[c]	Calcium sulfate, 95; dicalcium phosphate, eggshell, and tricalcium phosphate: 100; DFP, 92; limestone, 92; dolomitic limestone, 66; oyster shell, 100; rock phosphate (soft), 68
		Organic[c]	Calcium gluconate, calcium citrate, calcium lactate: 100
		Plant[b]	Canola meal, 53; corn, 50; SBM, 51
		Plant[c]	Canola meal, 45; dehydrated alfalfa meal, 21
		Animal[b]	Bone meal, 67; fishmeal, 56; meat and bone meal, 51–70

(*Continued*)

TABLE 10.9 (*Continued*)
Bioavailabilities of Macrominerals in Feedstuffs and Diets for Animals[a]

Animal	Mineral	Source	Bioavailability (%)
		Ration[b]	Corn- and SBM-based diets, 50–88 (depending on phytate content in diets)
	P	Inorganic[b]	Dicalcium phosphate, 69; monocalcium phosphate, 65; monodicalcium phosphate, 60
		Inorganic[d]	Curaçao Island phosphate, 81; DFP, 89; calcium sodium phosphate, 96; dicalcium phosphate, 91; monocalcium phosphate, 100; monosodium phosphate (NaH_2PO_4), 100; rock phosphate (raw), 75; rock phosphate (soft), 47; tricalcium phosphate, 62
		Plant[b]	Corn DDGS, 73; SBM (low phytate), 77–80; SBM (hulled), 60; sorghum, 33; wheat, 46.
		Plant[d]	Barley, 38; barley (brewers' dried grain), 32; canola meal, 45; corn DDGS, 77; corn (gluten feed), 95; corn (dried), 21; corn DDGS, 76; corn (hominy feed), 34; cottonseed meal, 42; field peas, 28; oats, 42; oats (groats), 21; pinto beans, 40; rice bran, 10; sorghum (dry), 26; sorghum (high moisture), 47; soybean hulls, 25; SBM (low phytate), 57; SBM (dehulled), 24; SBM (hulled), 16; SBM (with added phytase), 69; sunflower meal, 23; triticale, 31; wheat, 39; wheat bran, 30; wheat middlings, 41
		Animal[d]	Blood cells (spray-dried), 95; blood meal, 92; bone meal, 90; fishmeal, 103; meat meal, 90; meat and bone meal, 76; plasma (spray-dried), 100
		Ration[d]	Corn- and SBM-based diets, 25–68
Pig and poultry	Cl[f]	Inorganic	Sodium chloride (NaCl), potassium chloride (KCl), ammonium chloride (NH_4Cl), and $CaCl_2{\cdot}2H_2O$, 100
	K[h]	Inorganic	KCl, K_2CO_3, $KHCO_3$, and K_2HPO_4: 100
		Organic	Potassium acetate and potassium citrate, 100
		Plant	SBM, 100
	Mg	Inorganic[b]	$MgSO_4{\bullet}7H_2O$, 60
		Inorganic[g]	MgO, $MgCl_2$, $MgCO_3$, $MgSO_4$, $MgCO_3$, and magnesium phosphates: 100
		Plant[g]	Grains and concentrates, 50–60; SBM, 60
		Ration[b]	Corn- and SBM-based diets: 30–40
	Na[f]	Inorganic	NaCl, Na_2SO_4, $NaHCO_3$, sodium acetate, and sodium citrate: 100; DFP, 83
Ruminant	Ca	Inorganic[b]	Aragonite, 49; $CaCl_2$, 63; $CaCO_3$, 59; dicalcium phosphate, 73; monocalcium phosphate, 55; calcite flow, 49; DFP, 48; limestone, 50
		Plant[b]	Corn silage, 52; grass hays, 44; lucerne hay, 58
		Animal[b]	Dried skim milk, 56
		Rations[b]	Cattle fed concentrate diets, 50–70; goats fed concentrate diets, 23–39
	P	Inorganic[b]	Curaçao Island phosphate, 90; DFP, 54; dicalcium phosphate, 73; metaphosphates (Na and Ca), 61; monophosphates (Na and Ca), 76; phosphoric acid, 90; pyrophosphates (Na and Ca), 90; rock phosphate (raw), 48; rock phosphate (soft), 32; urea ammonium polyphosphate, 100.
		Plant[b]	Corn silage, 81; lucerne hay, 84; wheat bran, 26
		Ration[b]	Cattle fed concentrate diets, 58–75; goats fed concentrate diets, 70–75
	Others	Cattle[b]	Concentrate diets: K, 90–100; Mg, 17–29; Na, 90–91
		Sheep[b]	Concentrate diets: K, 90–100; Mg, 36–56; Na, 90

Source: Anwar et al. (2016); Baker (2001); Cromwell (2006); Kiarie and Nyachoti (2010); Liu et al. (2014); Meschy (2000); Mutucumarana et al. (2014); Philippi et al. (2023); Trairatapiwan et al. (2018); Wylie et al. (1985); Zhang and Adeola (2018).

[a] Values are expressed as either relative bioavailability value or true total tract digestibility (which includes absorption based on experimental methodologies), as specifically indicated for test animals, ingredients, and diets. All standard mineral sources are reagent grade.

[b] True total tract digestibility or absorption rate.

[c] Relative bioavailability value, with calcium carbonate as the standard source of Ca.

[d] Relative bioavailability value, with monocalcium phosphate as the standard source of P. The true total tract digestibility of P in monocalcium phosphate is 68%.

[e] Relative bioavailability value, with monopotassium phosphate (KH_2PO_4) as the standard source of P. The true total tract digestibility of P in monopotassium phosphate is estimated to be 70%.

[f] Relative bioavailability value, with NaCl as the standard source of Na or Cl. The true total tract digestibility of NaCl is 100%.

[g] Relative bioavailability value, with MgO as the standard source of Mg. The true total tract digestibility of Mg in MgO is 60%.

[k] Relative bioavailability value, with KCl as the standard source of K. The true total tract digestibility of K in KCl is 100%

Note: DFP, defluorinated phosphate [$Ca_4Na(PO_4)_3$]; DDGS, dried distillers grains with solubles; PBM, poultry by-product meal; SBM, soybean meal

TABLE 10.10
Bioavailabilities of Microminerals in Feedstuffs and Diets for Animals[a]

Animal	Mineral	Source	Bioavailability (%)
Human[b]	Cu	Mixed[b]	Diets based on animal- and plant-sourced foods: 30–40 (normal intake); 12–20 (high Cu intake), 36 (adequate Cu intake), and 50–56 (low Cu intake)
	Fe	Inorganic[b]	$FeSO_4$, 17
		Animal[b]	Organ meat, 25–30
		Mixed[b]	Diets based on animal- and plant-sourced foods, 14–18, 15; Vegetarian diets, 5–12
		Plants[b]	Dried legumes, 2; grain, 4; green leafy vegetables, 7–9
	Iodine	Inorganic[b]	Potassium iodide (KI) or sodium iodide (NaI), 96
	Zn	Plant[b]	5–10
		Animal[b]	30
		Mixed[b]	Diets based on animal- and plant-sourced foods: 20
Pig	Cu	Inorganic[b]	Cupric sulfate (copper sulfate) pentahydrate [$(CuSO_4)•5H_2O$], 36; 30–55 (depending on diets)
		Inorganic[c]	Cupric carbonate ($CuCO_3$), 100; cupric carbonate, basic [$CuCO_3•Cu(OH)_2$], 100; cupric oxide (CuO), 0; cuprous oxide (Cu_2O), 100; cupric sulfide (CuS), 10; $CuSO_4$, 100; $(CuSO_4)•5H_2O$ 100; feed-grade $(CuSO_4)•5H_2O$ and dicopper chloride trihydroxide [$Cu_2(OH)_3Cl$], 95
		Organic[c]	Cu-citrate, 84; Cu-lysine, 112; Cu-methionine, 110
		Plant[c]	Corn gluten meal, 50; cottonseed meal, 40; SBM (dehulled), 40; peanut, 45; soy mill run, 45
		Animal[c]	Beef liver, 80; chicken liver, 115; fecal material, 30; PBM, 90
		Ration[b]	Corn- and SBM-based diets, 36; 30–55
	Fe	Inorganic[b]	Ferrous sulfate monohydrate ($FeSO_4•H_2O$), 30
		Inorganic[e]	DFP, 35–85; $FeCl_2$, $FeSO_4•H_2O$, $FeSO_4•2H_2O$, $FeSO_4•7H_2O$: 100; ferric sulfate heptahydrate [$Fe_2(SO_4)_3$]•$7H_2O$, 37; Fe oxides, 0; Fe polyphosphate, 84–91
		Organic[e]	Fe-EDTA, 90–91; Fe-lysine, 100; Fe-methionine, 100; Fe-proteinate, 123; ferritin, 95–100
		Animal[e]	Blood meal (flash-dried), 28–50; PBM, 68
		Ration[b]	Corn- and SBM-based diets, 30–35
	Mn	Inorganic[b]	Manganese sulphate monohydrate ($MnSO_4•H_2O$), 29
		Inorganic[f]	Manganese chloride ($MnCl_2$), 100; manganese carbonate ($MnCO_3$), 55–95; manganese oxide (MnO), 75–96; manganese dioxide (MnO_2), 30
		Organic[f]	Mn-proteinate, 100; Mn-methionine, 100
		Plant[f]	Corn, SBM, and wheat bran, 0; rice bran, minimally available
		Ration[b]	Corn- and SBM-based diets, 30-35
	Zn	Inorganic[b]	Zinc sulfate heptahydrate ($ZnSO_4•5H_2O$), 36
		Inorganic[h]	Zinc carbonate ($ZnCO_3$), 98; $ZnSO_4•H_2O$, 100; $ZnSO_4•5H_2O$, 100; zinc oxide (ZnO), 87; Zn chloride, tetrabasic [$Zn_5(OH)_8Cl_2·H_2O$], 122–159
		Organic[h]	Zn-AA complex/chelate, 102; Zn-lysine, 78; Zn-methionine, 95
		Plant[h]	SBM, 34; soy protein concentrate, 18; soy protein isolate, 25
		Ration[b]	Corn- and SBM-based diets, 45–50
Poultry	Cu	Inorganic[b]	Cupric sulfate [$(CuSO_4)•5H_2O$], 30–55 (depending on diets)
		Inorganic[c]	Cupric chloride ($CuCl_2$), 106–110; $Cu_2(OH)_3Cl$, 70-134; $CuCO_3$: 64; $CuCO_3•Cu(OH)_2$, 100; cuprous chloride (CuCl), 81–145; cuprous iodide (CuI), 46–82; CuO, 0; cuprous oxide (Cu_2O), 92–98; CuS, 10; $CuSO_4$, 100; [$(CuSO_4)•5H_2O$], 100; feed-grade $(CuSO_4)•5H_2O$ and $Cu_2(OH)_3Cl$, 95
		Organic[c]	Cu-AA complex/chelate, 96–128; Cu-citrate, 84; Cu-lysine, 100; Cu-methionine, 91; Cu-proteinate, 95; HMTBa, 111–112
		Plant[c]	Corn gluten meal, 48; cottonseed meal, 41; SBM (dehulled), 38; peanut, 44; soy mill run, 47
		Animal[c]	Beef liver, 82; chicken liver, 97; fecal material, 34; PBM, 92; turkey liver, 83
		Ration[b]	Corn- and SBM-based diets, 30–50
	Fe	Inorganic[b]	$FeSO_4$, 33
		Inorganic[e]	DFP, 35–85; $FeSO_4•H_2O$, $FeSO_4•2H_2O$, $FeSO_4•7H_2O$, and $FeCl_2$: 100; [$Fe_2(SO_4)_3$]•$7H_2O$, 37; Fe-$ZnSO_4•H_2O$, 112–126; Fe oxides, 0; Fe-ammonium sulfate, 99–100; Fe orthophosphate, 4–36; Fe pyrophosphate, 45; Zn-$FeSO_4•H_2O$, 93–96
		Organic[b]	Fe-methionine, 38
		Organic[e]	Fe-ammonium citrate, 98–115; Fe-choline citrate, 102; Fe-citrate, 70–76; Fe-dextran, 100; Fe-fumarate, 100; and Fe-gluconate, 97; Fe-EDTA, 97–100; heme, 100; ferritin, 95–100; Fe-hemoglobin, 93; hemin, 9; Fe-methionine, 86–129; Fe-proteinate, 96–174; Fe glycerophosphate, 86–100

(*Continued*)

TABLE 10.10 (*Continued*)
Bioavailabilities of Microminerals in Feedstuffs and Diets for Animals[a]

Animal	Mineral	Source	Bioavailability (%)
		Plant[e]	Alfalfa meal, 65; corn (yellow), 20; cottonseed meal, 56; oilseeds, 40–50; rice bran, 65; SBM (dehulled), 45; sesame oil, 96
		Animal[e]	Blood meal, 22; feather meal, 39; fish meal, 32; meat and bone meal, 48; PBM, 68
		Ration[b]	Corn- and SBM-based diets, 30–35
	Mn	Inorganic[b]	Manganese sulphate monohydrate, 2–8
		Inorganic[f]	$MnCl_2$, 100; $MnCO_3$, 55; MnO, 75; MnO_2, 30; $MnSO_4 \cdot H_2O$, 100
		Organic[f]	Mn-AA complex/chelate, 84–148; Mn-HMTBa, 100; Mn-methionine, 100; Mn-propionate, 139; Mn-proteinate, 86–163
		Plant[f]	Corn, SBM, and wheat bran, 0; rice bran, minimally available
		Ration[b]	Corn- and SBM-based diets, 30
	Zn	Inorganic[b]	$ZnSO_4 \cdot 5H_2O$, 30
		Inorganic[h]	Fe-$ZnSO_4 \cdot H_2O$, 99; $ZnCl_2$, 88-107; $ZnCO_3$, 78–123; zinc chloride, basic, 108–119; zinc chloride, tetrabasic, 102–111; $ZnSO_4 \cdot H_2O$, 100; $ZnSO_4 \cdot 5H_2O$, 100; ZnO, 50–97; Zn dust, 99
		Organic[h]	Zn-AA complex/chelate, 76-164; Zn-citrate, 128; Zn-EDTA, 110–118; Zn-HMTBa, 161–441; Zn-lysine, 78–111; Zn-methionine, 117, 177, and 206 (for purified, semi-purified, and corn-SBM diets, respectively); Zn-picolinate, 31–104; Zn polysaccharide complex, 94; Zn-propionate, 116–119; Zn-proteinate, 70–200
		Plant[h]	SBM, 34; soy protein concentrate, 18; soy protein isolate, 25
		Ration[b]	Corn- and SBM-based diets, 23–30
Pig and poultry	Iodine	Inorganic[b]	KI or NaI, 96–100
		Inorganic[d]	Calcium iodate monohydrate [$Ca(IO_3)_2 \cdot H_2O$], cuprous iodide (CuI), potassium iodate (KIO_3), potassium iodide (KI), sodium iodide (NaI), and iodized salt: 100
		Organic[d]	Ethylenediamine dihydroiodide ($C_2H_8N_2 \cdot 2HI$), 100
	Se	Inorganic[b]	Na_2SeO_3 85; Na_2SeO_4, 85
		Organic[b]	Selenomethionine and selenocysteine, 100
		Plant[g]	Alfalha meal, 100; cereal grains, 60–80; SBM, 18–60
		Animal[g]	Fish meal, 10–40; PBM, 10–40
		Animal[g]	Yeast, 159
		Ration[b]	Inorganic Se in corn- and SBM-based diets, 65
Cattle	Cu	Inorganic[b]	$CuCl_2$, 12; $CuCO_3$, 6; Cu_2O, 10; $CuSO_4$, 16
		Inorganic[c]	$CuCl_2$, 98; $CuCO_3$, 86; CuO, poorly absorbed; CuS, 25; $CuSO_4$, 100; copper chloride, tribasic, [$Cu_2(OH)_3Cl$], 102–112
		Organic[c]	Cu-acetate, 100; Cu-citrate, 101; Cu-EDTA, 91–104; Cu-lysine, 90; Cu-proteinate, 82–112 (plasma Cu as the response criterion) or 147 (liver Cu as the response criterion)
		Ration[b]	Concentrate diets, 6; forage 1–5
	Fe	Inorganic[b]	$FeSO_4$, 25
		Inorganic[e]	$FeSO_4$, 100; ferrous carbonate ($FeCO_3$), 0–25
		Organic[b]	Ferrous ascorbate,
		Organic[e]	Fe-citrate, 107; Fe-EDTA, 93; Fe-phytate, 47
		Ration[b]	Liquid diets containing 40 and 100 mg Fe/kg for calves, 40 and 15, respectively; diets for growing cattle, 28; diets for adult cattle, 10
	Mn	Inorganic[b]	Manganese sulphate monohydrate, 2–8
		Inorganic[f]	MnO, 53–70; $MnSO_4 \cdot H_2O$, 100
	Se	Inorganic[b]	50
		Organic[b]	Se-methionine, 65; Se in yeast, 44
		Ration[b]	Concentrate diets: 40–50
	Zn	Inorganic[b]	$ZnSO_4$, 25 (steers and lactating dairy cows)
		Inorganic[h]	$ZnCO_3$, 58; $ZnCl_2$, 42; ZnO, 98–101; $ZnSO_4$, 100; zinc hydroxychloride [$Zn_5(OH)_8Cl \cdot H_2O$], 113; zinc sulfate (basic and tribasic), 100
		Organic[h]	Zn-lysine, 100; Zn-methionine, 98–133; Zn polysaccharide complex, 144
		Ration[b]	Preruminant calves, 50–85; ruminants: concentrate diets: 30–40, 35; forages, 15–18
Sheep	Cu	Inorganic[b]	$CuSO_4$, 10 (after weaning)
		Inorganic[c]	$CuCl_2$, 96; $CuCO_3$, 97; CuO, poorly absorbed; CuS, 25; $CuSO_4$, 100

(*Continued*)

TABLE 10.10 (*Continued*)
Bioavailabilities of Microminerals in Feedstuffs and Diets for Animals[a]

Animal	Mineral	Source	Bioavailability (%)
		Organic[c]	Cu-AA complex/chelate, 100; Cu-acetate, 98–110; Cu-citrate, 101; Cu-glycine, 96; Cu-lysine, 68–97; Cu-methionine, 150–152; Cu-proteinate, 103; Cu-EDTA, 96
		Ration[b]	Concentrate diets: 6; forages, 1–5
	Fe	Inorganic[b]	$FeSO_4$, 25
		Inorganic[e]	$FeSO_4$, 100; ferrous carbonate, 0–29
		Ration[b]	Concentrate diets: 10 (growing lambs), 21 (gestating ewes)
	Mn	Inorganic[b]	Manganese sulphate monohydrate, 2–8
		Inorganic[f]	$MnCO_3$, 20–93; MnO, 31–91; MnO_2, 25–67; $MnSO_4•H_2O$, 100
		Organic[f]	Mn-methionine, 93–164
	Se	Inorganic[b]	34–50
		Organic[b]	Se-methionine, 65; Se in yeast, 44
		Ration[b]	Concentrate diets: 40–50
	Zn	Inorganic[b]	$ZnSO_4$, 25 (lambs and ewes)
		Inorganic[h]	$ZnCO_3$, 105–106; ZnO, 74–106; zinc sulfate (basic and tribasic), 83–99
		Organic[h]	Zn-AA complex/chelate, 102–110; Zn-glycine, 82–335; Zn-lysine, 114; Zn-methionine, 95–134; Zn-proteinate, 56–254
		Ration[b]	Concentrate diets: 30–40, 35; forages, 15–18

Source: ARC (1980); Baker (2001); Cromwell (2006); Byrne and Murphy (2022); Chapman and Bell (1963); EFSA (2014); Espinosa and Stein (2021); Hattori et al. (2010); Jenkins and Hidiroglou (1991); Kiarie and Nyachoti (2010); Liu et al. (2014); NRC (2001); Philippi et al. (2023); Piskin et al. (2022); Shaeffer et al. (2017); Wapnir (1998); Wedekind et al. (1992); Wedekind et al. (1994); Zhang and Adeola (2018).

[a] Values are expressed as either relative bioavailability value or true total tract digestibility (which includes absorption based on experimental methodologies), as specifically indicated for test animals, ingredients, and diets. All sources of standard minerals are reagent grade.

[b] True total tract digestibility or absorption rate.

[c] Relative bioavailability value, with cupric sulfate as the standard source of Cu.

[d] Relative bioavailability value, with KI as the standard source of I.

[e] Relative bioavailability value, with $FeSO_4•H_2O$ as the standard source of Fe.

[f] Relative bioavailability value, with $MnSO_4•H_2O$ as the standard source of Mn.

[g] Relative bioavailability value, with Na_2SeO_3 as the standard source of Se.

[h] Relative bioavailability value, with $ZnSO_4•7H_2O$ as the standard source of Zn.

Note: AA, amino acid; DFP, defluorinated phosphate; HMTBa = 2-hydroxy-4-(methylthio)butanoate; PBM, poultry by-product meal; SBM, soybean meal.

may vary with measured response criteria. Organic minerals in feeds, except for phytate-complexed minerals and macrominerals, are absorbed by the gastrointestinal tract (GIT) at greater rates and may also have longer biological half-lives than inorganic minerals. Therefore, organic microminerals in feeds generally have greater bioavailability values and greater rates of retention in animal bodies than inorganic microminerals (Byrne and Murphy 2022). In ruminant diets, hydroxy trace minerals (e.g., Zn hydroxychloride) are relatively insoluble in water or at weakly acidic pH (e.g., in ruminal fluid) and, therefore, have minimal interactions with the ruminal environment for metabolism, as compared to soluble mineral compounds (e.g., Zn sulfate; Shaeffer et al. 2017). Due to their chemical properties, hydroxy trace minerals are readily solubilized at low pH (e.g., in the stomach of nonruminants or the abomasum of ruminants), yielding constituent ions that are available for absorption by the GIT. Thus, hydroxy trace minerals may have greater bioavailability values than water-soluble mineral compounds.

Methods for Determination of Bioavailabilities of Minerals in Feedstuffs

Slope Ratio Assays

This method involves the inclusion, in a diet, of graded levels of a mineral (e.g., Ca, P, or Zn) from either a test ingredient (e.g., SBM or limestone) or a known standard ingredient that is either high or 100% bioavailable (Kiarie and Nyachoti 2010). For example, $CaCO_3$ is often used as the standard source of Ca in slope ratio assays to assess the bioavailabilities of Ca in inorganic, organic, and feed-sources. Likewise, monosodium, monocalcium or dicalcium phosphate is employed as the standard source of P for the estimation of P bioavailability in feedstuffs. Caution should be taken in the choice and test amount of the standard source. For example, because Cu from CuO is poorly available to poultry, pigs, and ruminants, CuO should not be selected in studies as a reference or standard source for assessing the bioavailabilities of Cu in feed ingredients. Dietary levels of a test mineral range from a value below

the requirement to an optimum value, but the experimental diet must provide adequate amounts of all other nutrients. A response criterion (e.g., growth performance or bone density) is plotted against the intake of the test minerals by animals. The slope ratio is calculated by dividing the slope of the regression equation for the test ingredient by the slope of the regression equation for the standard ingredient. Thus, slope-ratio assays provide the relative bioavailabilities of minerals. This method reflects both the digestion and the postabsorptive utilization of a mineral in its ingredient matrix. Note that a high value of relative bioavailability for a dietary mineral (particularly a micromineral) does not necessarily mean a high efficiency in its use by animals if its digestibility and absorption rate are low.

In Vivo Digestion Trials

Basal and total endogenous intestinal flows of minerals can be determined to estimate their standardized and true total tract digestibilities, respectively. These methods provide more reliable values of mineral digestion than the fecal method that generates data on apparent total tract digestibilities. Because minerals are not formed in any animal and can be absorbed actively by both small and large intestines (Goff 2018), the true total tract digestibilities of minerals (calculated after correction for intestinal endogenous loss) are nutritionally more meaningful than their true ileal tract digestibilities. González-Vega et al. (2013) reported that the apparent and true total tract digestibilities of Ca in canola meal fed to growing pigs are 33.7–43.0% and 46.6%, respectively. Clearly, the use of apparent total tract digestibilities will overestimate dietary requirements of animals for minerals. A dietary mineral (classified as an essential nutrient) is generally absorbed by the small intestine in a form that can be used by animals for normal metabolism and physiological functions. Thus, the true total tract digestibility of this mineral (which includes its absorption based on experimental methodologies) is a good estimate of its bioavailability.

In Vitro Digestion Experiments

Animal experiments are time-consuming and expensive and, therefore, are not practical for the routine evaluation and screening of feedstuffs or diets. Thus, in vitro techniques may be useful alternatives to assess the bioavailabilities of minerals in feedstuffs and diets for animals. Using an in vitro procedure that simulates the digestive system of growing swine, Liu et al. (1998) reported that the in vitro digestibilities of P in plant-sourced ingredients (alfalfa meal, barley, canola meal, corn, grain sorghum, oats, rice bran, SBM, wheat, and wheat bran) were similar to published in vivo P availabilities but that in vitro digestibilities of P in animal-sourced ingredients (menhaden fish meal, meat and bone meal, spray-dried blood meal, and dry whey) were poorly correlated with published in vivo P availabilities. Fine grinding of feed ingredients and the addition of acid phosphatase to the digestion system increased the accuracy of this in vitro procedure. In ruminant nutrition, in vitro digestion methods include digestion with ruminal microbes or enzymes, and the in situ the nylon bag technique (i.e., placing feedstuffs in fabric bags in the rumen for hours of incubation; López 2005).

Factors Affecting the Bioavailabilities of Dietary Minerals in Animals

Bioavailabilities of dietary minerals can be affected by a plethora of factors, including animal species, the physiological state and nutritional status of animals, disease, interactions between and among nutrients, the chemical composition and physical properties of food ingredients, the chemical form and solubility of minerals, response criteria, the choice of standard source of minerals, and the environment (e.g., temperature and humidity). For example, because it is easier to release minerals from animal-sourced than plant-sourced ingredients, the bioavailabilities of various minerals (<1% for V in food to nearly 100% for F in water) in the former are generally higher than those in the latter (Georgievskii et al. 1982; Pond et al. 1995). Second, the bioavailabilities of minerals in diets for swine, poultry, and ruminants are generally greater for nonphytate organic than inorganic sources. Note that minerals (e.g., P) covalently bound to phytate (a carbohydrate) in plants are complexed with an organic substance. Third, Se bioavailability is reduced by high dietary S and the presence of cyanogenetic glycosides in certain legumes (Spears 2003). Fourth, organic sources of Zn and Cu are used differently than inorganic sources in ruminants. Specifically, although Zn from ZnO and zinc methionine was absorbed by lambs at the same rate, more Zn was retained from zinc methionine than inorganic Zn due to the reduced urinary excretion of organic Zn (Spears 1989). Similar results were reported by Kerley and Ledoux (1992) for zinc proteinate and ZnO fed to lambs. Likewise, Cu from copper proteinate resulted in lesser hepatic Cu deposition but greater ceruloplasmin activity than Cu from copper sulfate in ewes (Eckert et al. 1999). It is possible that the biological half-lives of organic minerals (absorbed as amino acid complexes) are longer than those for inorganic minerals in ruminants. In contrast, Liu et al. (2014) reported that the ratio of retention/absorption did not differ between organic and inorganic sources of Zn, Cu, Mn, and Fe in growing pigs, indicating that the organic forms of the absorbed microminerals are used at the same efficiency as their inorganic forms. Reasons for species differences in the metabolism of microminerals are unknown but may include diversities in their recirculation routes, interorgan transport, renal reabsorption, absorption by the large intestine, and excretion via the urine and feces.

General Functions of Minerals

Minerals have many physiological and metabolic functions (Engle and Lemons 1986; Fang et al. 2002; Suttle 2010). These nutrients are: (a) the major structural components of bones and teeth, with the content of minerals in the ash (%)

of adult animals being as follows: Ca, 28.5; P, 16.6; K, 4.8; S, 3.6; Cl, 3.5; Na, 3.7; Mg, 1.1; and Fe, 0.15 (Georgievskii et al. 1982); (b) contributors to the mechanical stability of the body (e.g., Ca, P, Mg, F, and Si); (c) electrolytes in the body (Na, K, and Cl) essential for the electrical charges of cells, as well as the regulation of acid-base balance and osmotic pressure; (d) modulators of transport activity, permeability, and excitability of cell membranes (e.g., Na, K, Cl, and P); (e) facilitators of the digestion of foods in the GIT; (f) regulators of food intake (e.g., Cl, Na, P, and Zn); (g) cofactors or components of enzymes (e.g., Se, Ca, Mg, Mn, Zn, and Cu) and other organic molecules (e.g., Fe in hemoglobin, Co in vitamin B_{12}, and I in thyroid hormones); and (h) participants in redox reactions (e.g., S, Cu, and Fe). Although V, Si, B, Br, Ni, and Sn have biochemical roles in cell metabolism, these microminerals are widely present in feedstuffs and, therefore, are not of particular importance to the formulation of animal diets.

About 40% of all proteins crystallized to date have a metal bound either loosely or tightly (Waldron et al. 2009). Enzymes that use metal ions as cofactors are classified as metal-activated enzymes or metalloenzymes. Metal-activated enzymes exhibit a greater catalytic activity in the presence of a mono- or divalent metal ion, compared with its absence; examples include alkaline phosphatase (Mg^{2+}), ATPase (Mg^{2+}), choline kinase (Mg^{2+}), DNAse (Mg^{2+}), hexokinase (Mg^{2+}), inosine monophosphate dehydrogenase (K^{+}), leucine aminopeptidase (Mn^{2+}), phosphoglucomutase (Mg^{2+}), pyruvate kinase (K^{+}), RNAse (Mg^{2+}), thrombin (Na^{+}), and β-xylosidase (Ca^{2+}). Metalloenzymes are enzyme proteins containing metal ions that are directly bound to the proteins; examples include alcohol dehydrogenase (Zn^{2+}), aminopeptidase (Zn^{2+}), α-amylase (Ca^{2+}), arginase (Mn^{2+}), carbonic anhydrase (Zn^{2+}), catalase (Fe^{2+}), cytochrome c oxidase (Fe), DNA polymerase (Zn^{2+}), lysyl oxidase (Cu^{2+}), RNA polymerase (Zn^{2+}), protein phosphatase-1 (Mn^{2+}), pyruvate carboxylase (Mn^{2+}), superoxide dismutase (Cu^{2+}, Zn^{2+}), and tyrosinase (Cu^{2+}). About one-third of all enzymes known so far are metalloenzymes (Valdez et al. 2014). There are differences between metal-activated enzymes and metalloenzymes. Metals have only a weak association with metal-activated enzymes but are tightly bound to metalloenzymes and retained by the enzymes on purification (Holm et al. 1996).

Overview of the Storage of Minerals in Animals

The biological half-lives of minerals in an animal differ substantially because some are stored in the body's skeleton, metalloproteins, and hormones but some are not (McDonald et al. 2021; Suttle 2010). For example, although Ca, P, and Mg have specific functions in the bone matrix, they can be mobilized from the bone during short-term dietary deficiencies to meet the needs of soft tissues for essential events such as energy metabolism, glycolysis, cellular signal transduction, nerve transmission, and muscle contractions. The minerals that are stored in metalloproteins (e.g., Fe, Zn, and Cu), hormones (e.g., I in thyroid hormones and Zn in insulin), and DNA (e.g., Zn in the Zn-finger) are released upon proteolysis or the degradation of the complexes to enter the cytosol of cells and the blood stream. Because most minerals have quasi- or true storage for a prolonged period (e.g., weeks), short-term (e.g., days) dietary deficiencies may not be problematic. However, the minerals (e.g., the electrolytes Na, K, and Cl) that do not have quasi- or true storage must be included daily in diets to prevent deficiencies. During fetal and postnatal growth, minerals are deposited in tissues, particularly bones (Pond et al. 1995; Wu et al. 2012). In contrast, under some physiological (e.g., lactation) and pathological conditions (e.g., cancers), minerals are mobilized from the body due to inadequate dietary intakes (Heurtault et al. 2024; Hu et al. 2010).

MACROMINERALS

Calcium (Ca)

Ca was discovered by Humphry Davy in 1808. However, Ca-containing compounds (e.g., gypsum, limestone, and marble) had been known for thousands of years prior to its isolation. The name of "calcium" originates from the Latin word *calx*, meaning "lime". It is a divalent cation. In 1840, Jean Boussigault (a French chemist) and Justus von Liebig (a German chemist) recognized that bone contains Ca and P. It is now known that Ca is a major component of the skeleton (bone) and teeth as a phosphate salt. A 70-kg animal contains about 1.3 kg Ca, with about 99% present in the skeleton. Depending on animal species, concentrations of total Ca^{2+} in plasma are 4.5–5.5 mM (approximately half of it binds to albumin), with total Ca^{2+} concentrations in serum being about 2.2–3.1 mM. Concentrations of free Ca^{2+} in plasma are 1.1–1.5 mM, depending on animal species.

Concentrations of Ca in plasma or serum are tightly regulated by not only dietary Ca intake but also intestinal Ca absorption and the mobilization of Ca from tissues (mainly bone) through the actions of hormones [including parathyroid hormone (PTH), calcitriol, and calcitonin]. When the dietary intake of Ca by animals is low, its circulating levels may remain within normal ranges due to these mechanisms of homeostatic control. Therefore, concentrations of free or total Ca in plasma or serum are generally not a good measure of Ca nutritional status in animals. Under conditions of severe Ca deficiency, concentrations of free or total Ca in plasma or serum may be substantially reduced, as shown for lactating cows with "milk fever" (see below).

Sources: Milk, legumes, and animal products (e.g., fish meal, meat and bone meal, and poultry byproduct) containing bones are good sources of Ca. Vegetables, cereals, and roots are poor sources of Ca (Baker 2001; Jamroz et al. 2011). For example, corn grain and SBM (44% crude protein) contain 0.03 and 0.34% Ca, respectively. Thus, plant-based diets must be supplemented with animal-sourced and/or inorganic feedstuffs (Kiarie and Nyachoti 2010). Ground limestone (mainly Ca carbonate; 36–38% Ca),

dicalcium phosphate ($CaHPO_4$; 29.46% Ca), Ca carbonate ($CaCO_3$; 40.04% Ca), Ca-citrate [$Ca_3(C_6H_5O_7)_2$; 8.04% Ca)], Ca-lactate ($C_6H_{10}CaO_6$; 18.37% Ca), and Ca-lactose ($C_{12}H_{22}CaO_{11}$; 10.46% Ca) are often used as Ca supplements for farm animals. Calcium chloride ($CaCl_2$), which is chemically a neutral substance, may slightly reduce blood pH because Ca^{2+} (alkaline) from this compound is absorbed by the small intestine at a lower rate than its Cl^-, resulting in the unmatched appearance of Ca^{2+} and Cl^- in the portal vein (Baker 2001). In addition, Cl^- can stimulate the excretion of HCO_3^- by animals via Cl^-/HCO_3^- exchanges (Chapter 1). However, Mun et al. (2022) recently reported that, compared to 0.178% or 0.374% limestone, adding 0.388% or 0.526% $CaCl_2$ to the corn- and SBM-based diets (containing 0.70 dicalcium phosphate as the major source of both Ca and P) of postweaning pigs for 28 days did not affect the blood pH but increased the digestibilities of dietary CP, Ca, and P by 2–3%, as well as BW gain and feed efficiency by 8–10%, without influencing feed intake. Thus, an appropriate dose of $CaCl_2$, which is highly soluble in water, is a good alternative to $CaCO_3$ for animals. Note that (a) Ca-oxalate is relatively insoluble in water and poorly absorbed by the intestine, and (b) any Ca salt is more soluble in acid than at neutral pH.

Absorption and transport: Ca^{2+} in the intestinal lumen is absorbed by the small intestine via Ca^{2+} transporter-1 (CaT1, a Ca^{2+} channel protein). The duodenum, jejunum, and ileum are responsible for the absorption of 5%, 15%, and 80% of Ca^{2+}, respectively (Khanal and Nemere 2008). Upon entering the enterocyte, Ca^{2+} is transported to intracellular organelles by a cytosolic protein called calbindin. Ca^{2+} exits the enterocyte across its basolateral membrane into the lamina propria via Ca^{2+}-ATPase. This basolateral membrane transporter is an antiporter, which transports Ca^{2+} out of the enterocyte in exchange for the entry of Mg^{2+} or Na^+ from the lamina propria (ultimately the blood). Thus, the hydrolysis of ATP drives intestinal Ca^{2+} absorption. Ca^{2+} enters the portal vein from the lamina propria, is transported in the blood in both free and protein-bound forms, and is taken up by extraintestinal cells through specific Ca^{2+} channels. Note that the relatively high content of fat or oxalic acid in animal diets results in the formation of Ca-fatty acid soaps or Ca-oxalate, which reduces the intestinal absorption of Ca^{2+}.

Calcitriol (1,25-dihydroxy-vitamin D_3) and PTH play an important role in regulating Ca^{2+} homeostasis in animals (Figure 10.3). Specifically, calcitriol, which is formed from vitamin D_3 or vitamin D_2, plays an important role in Ca^{2+} homeostasis in animals. Specifically, physiological concentrations of calcitriol enhance intestinal Ca^{2+} absorption by stimulating the expression of CaT1 and calbindin in enterocytes, the renal reabsorption of Ca^{2+}, and, in the case of low plasma Ca^{2+} concentration, the mobilization of Ca^{2+} from the bone matrix (Blaine et al. 2015). PTH, which is secreted by the parathyroid gland in response to low plasma Ca^{2+} concentration, increases the concentration of Ca^{2+} in the plasma by (a) upregulating the expression of 25-hydroxy-vitamin D hydroxylase (which converts 25-dihydroxy-vitamin D into 1,25-dihydroxy-vitamin D) and (b) activating osteoclasts to promote bone resorption [the mobilization of bone $Ca_3(PO_4)_2$ to Ca^{2+} for entry into the plasma] (Blaine et al. 2015). PTH cell signaling involves the activation of protein kinases A and C (Swarthout et al. 2002). Possibly through an increase in PTH concentration or activity, intestinal Ca absorption is enhanced during pregnancy, lactation, and the phase of rapid postnatal growth (Baker 2001).

In the small intestine, Ca and Mg influence each other's absorption. For example, a high Ca intake may decrease Mg absorption, and a high Mg intake may decrease Ca absorption. In addition to the factors noted previously, the consumption of Ca without food, the presence of phytate in diets, excessive intakes of dietary P, and fat malabsorption decrease the efficiency of Ca absorption (Baker 2001). Interestingly, a low intake of Mg^{2+} reduces Ca^{2+} concentrations in plasma and cells as well as ATP production and PTH activity, whereas concentrations of Mg and Na in plasma also regulate Ca^{2+} homeostasis in the body (Agus 1999). Decreases in ATP and PTH availabilities cause reductions in (a) energy-dependent intestinal absorption

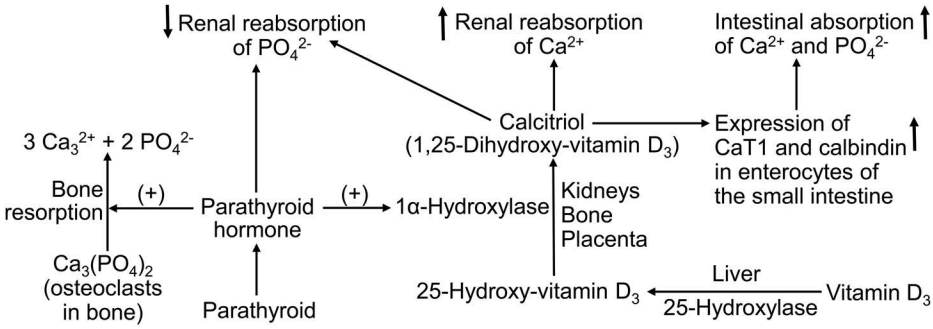


FIGURE 10.3 Regulation of intestinal absorption of calcium and phosphate, as well as renal reabsorption in the kidneys by vitamin D (a sterol) and parathyroid hormone. Vitamin D_3 is converted into calcitriol through interorgan metabolism involving mainly the liver and kidneys. Calcitriol and parathyroid hormone have anabolic effects on bone growth by enhancing intestinal absorption of calcium and phosphate, as well as renal reabsorption of calcium. ↑, increase; (+), activation; (–), inhibition.

of Ca^{2+} and renal reabsorption of Ca^{2+} and (b) concentrations of Ca^{2+} in plasma. Hypermagnesemia in plasma also results in hypocalcemia, and the underlying mechanisms include (a) inhibiting the secretion of PTH from the parathyroid gland and (b) promoting the excretion of Ca^{2+} by the kidneys due to low circulating levels of PTH. Similarly, hypernatremia (elevated Na^+ concentration in plasma) reduces the concentration of Ca^{2+} in plasma (Alexander et al. 2013). In the lumen of the renal tubule, Na^+ competes with Ca^{2+} for reabsorption into the blood. Thus, a high concentration of luminal Na^+ resulting from a high circulating level of Na^+ reduces the reabsorption of Ca^{2+}, leading to enhanced excretion of Ca^{2+} by the kidneys. Thus, high-salt diets increase the risk for kidney stones and osteoporosis in animals.

There is a paucity of information about the true digestibility or bioavailability of Ca in plant-sourced ingredients for animals. Inorganic and animal-sourced ingredients generally have high relative bioavailability values of ≥80% for Ca in poultry and swine diets and ≥50% for ruminant diets (Table 10.9). The lower values for ruminants than nonruminants result from complex interactions between Ca and the ruminal environment, as well as possibly lower rates of intestinal absorption in ruminants than in nonruminants. In animals (e.g., swine, poultry, ruminants, fish, and crustaceans), 65–95% of absorbed Ca is retained by their bodies, depending on dietary, physiological, and environmental factors (Lall and Kaushik 2021; Liu et al. 2014).

Functions: The phosphates of Ca^{2+} are the major minerals in bones, which protect internal organs and provide structural integrity to the body (Pond et al. 1995). The rigidity provided by the bones permits locomotion and the support of loads against gravity. In addition, the formation of Ca carbonate from Ca^{2+} and carbonate in the process of biomineralization is essential for eggshell production in laying birds. Furthermore, many enzymes and proteins require Ca^{2+} for their biological activities (Table 10.11). For example, in some blood-clotting proteins, Ca^{2+} is bound to residues of γ-carboxyglutamic acid, where this metal is required for their functions. Furthermore, as a cofactor of protein kinase C in different cell types, Ca^{2+} participates in signal transduction pathways (serving as a second messenger) in response to extracellular signals (e.g., hormones and neurotransmitters). Finally, Ca^{2+} is essential for skeletal muscle contractions, heartbeats, nerve transmission, and maximum milk production by lactating mammary glands. Thus, Ca^{2+} plays a vital role in nutrition and physiology.

Deficiency symptoms: In animals, symptoms of Ca deficiency are similar to those of vitamin D deficiency, including abnormal skeletal growth and development, as well as rickets (Chapter 9). Additionally, milk fever (parturient paresis) may occur in lactating cows shortly after calving (Chapter 9). This metabolic disease is characterized by low serum Ca^{2+} levels (causing hyper-excitability of the nervous system and weakened muscle contractions), muscular spasms, and, in severe cases, paralysis and unconsciousness. Intravenous administration of Ca-gluconate or vitamin D along with dietary cation-anion balance helps to restore serum Ca concentrations and treat this disease. When Ca is supplemented to animal diets, it is important to consider their Ca/P ratios. For most nonruminant farm animals, except laying hens, dietary ratios of bioavailable Ca to bioavailable P are usually in the range of 1:1 to 2:1

TABLE 10.11
Calcium-Activated Enzymes and Calcium-Binding Proteins

Enzyme or Protein	Function
Annexins	Cell adhesion
Blood clotting proteins	Ca binds to γ-carboxyglutamic acid residues of proteins
Ca-ATPase	Release Ca from the sarcoplasmic reticulum in skeletal muscle
Ca-activated proteases (calpain)	Protein degradation in skeletal and cardiac muscles
Cadherins	Epithelial cell adhesion
Calbindin	Cytosolic calcium transport
Calcitonin	Regulator of blood calcium and phosphate concentrations
Calmodulin	Calmodulin-dependent protein kinases and in Ca-dependent cell signaling. Calmodulin-dependent enzymes (e.g., NO synthase)
Caspases	Protease activation (widely present in cells)
Ca^{2+} transporter-1	Membrane calcium transport
Cell adhesion molecules	Binds with other cells or with the extracellular matrix
Lipase	Triacylglycerols → Fatty acids + Monoacylglycerol (the lumen of the small intestine)
NO synthase	Arginine + O_2 → NO + Citrulline
Pancreatic α-amylase	Hydrolysis of dietary starches and glycogen
Pancreatic phospholipase A_2	Hydrolysis of dietary phospholipids
Phospholipase A_2	Release of fatty acids (e.g. arachidonic acid) from membrane-bound lipids
Phospholipase C	Hydrolysis of the inositol phosphate group of phosphatidyl-4,5-diphosphate
Protein kinase C	Phosphorylate enzymes in cell signaling
Phosphorylase kinase	Glycogen breakdown and synthesis
Thermolysin (*Bacillus* species)[a]	Microbial hydrolysis of peptide bonds containing hydrophobic amino acids
Troponin C (TpC)	Muscle contraction
Trypsin	Protein → Amino acids + Small peptides (the lumen of the small intestine)
Trypsinogen	Trypsinogen (inactive) → Trypsin (active protease)

[a] This protease requires one zinc ion for enzymatic activity and four calcium ions for structural stability.

(e.g., 1.5:1 for growing mammals and fish). The proportion of Ca in the diet for laying hens (Ca:P ratio = 13:1) is much higher than that for 3- to 6-week-old growing broilers (Ca:P = 2.6:1), because of eggshell formation in the former (NRC 1994). Ruminants are not as sensitive to dietary Ca:P ratio as nonruminants. This is because some dietary Ca may be absorbed from the rumen and a large amount of plasma P is secreted back into the rumen, making the Ca/P ratio at the site of absorption in the small intestine much different than the dietary Ca/P ratio (Greene 2016). Excess P in diets reduces intestinal absorption of Ca^{2+} in all animals, and dietary P at levels higher than dietary Ca should be avoided.

Excess: As noted previously, the concentration of Ca^{2+} in plasma is regulated by dietary Ca intake, bone resorption and remodeling, intestinal absorption, and renal tubule resorption. Hypercalcemia most commonly results from overproduction of PTH by overactive parathyroid glands (hyperparathyroidism), but also from malignant diseases (e.g., cancer), as well as excessive use of Ca^{2+} and vitamin D supplements. In animals, symptoms of hypercalcemia include (a) constipation, nausea, and vomiting; (b) reduced feed intake and impaired growth; (c) excessive deposition of Ca^{2+} in blood vessels to form plaques that result in the dysfunction of the heart and brain; and (d) muscle pain, abdominal pain, and kidney stones (Žofková 2016). Severe hypercalcemia can be life-threatening. Of interest, concentrations of Ca^{2+} (as $CaCl_2$) in the ruminal fluid ≥450 μg/ml are toxic to ruminal microbes as assessed by the rate of cellulose digestion in ruminants (Georgievskii et al. 1982). For all animals, dietary P at levels higher than dietary Ca should be avoided. Furthermore, the inclusion of physically rough forms of particulate Ca in diets results in oral lesions.

Chlorine (Cl)

Cl was discovered by Carl W. Schelle in 1774 through heating HCl with pyrolusite (MnO_2) to generate Cl_2.

In 1810, Humphry Davy confirmed that Scheele's gas was actually the molecule of a distinct element, Cl. In 1834, William Prout discovered that HCl is produced by the stomach. Chloride (Cl^-), which is the anion of Cl, is the principal anion in the extracellular fluid. The concentrations of Cl^- are about 100–110 mM in plasma, and about 4–5 mM in the intracellular fluid. Cl^- is one of the halides (the other halides are F, Br, and I). The nonionized elemental forms of halides are called halogens. (The term "halogen" comes from a Greek word meaning "salt-producing"). The movement of Cl^- across a membrane is often seen as a passive movement that serves to balance a charge difference caused by the movement of Na^+ or K^+. However, active transport mechanisms exist for Cl^-.

$$MnO_2 + 4HCl \rightarrow MnCl_2 + Cl_2 \uparrow + 2H_2O$$

Sources: With the exception of fish and meat meals, most foods have a low Cl^- content. In contrast, animal products are rich sources of this mineral. For example, corn grain, SBM, rice bran, and wheat middlings contain only 0.05, 0.05, 0.07, and 0.04% Cl^-, respectively, whereas fishmeal (menhaden) provides 0.55% Cl^-. The major source of Cl^- for animal diets is NaCl, as noted previously.

Absorption and transport: In the lumen of the small intestine, the Cl^--containing salts (e.g., NaCl, KCl, $CaCl_2$, and $MgCl_2$) are readily ionized to give rise to Cl^-. In mammals (e.g., pigs and ruminants), birds, fish, and crustaceans, Cl^- enters the enterocyte from the intestinal lumen via (a) Cl^- channels [primarily cystic fibrosis transmembrane conductance regulator (CFTR)] and (b) electroneutral Na^+-Cl^- absorption mediated by Na^+/H^+ (NHE2/3) and Cl^-/HCO_3^- exchangers (Gawenis et al. 2002; Kato and Romero 2011). In the small intestine, the Na^+/H^+ exchanger takes up Na^+ and releases H^+, whereas the Cl^-/HCO_3^- exchanger releases HCO_3^- and takes up Cl^-. Thus, a major part of Cl^- absorption is due to Na^+ absorption, and normally, there is net water absorption together with HCO_3^- secretion from the enterocyte into the lumen in exchange for the entry of luminal Cl^- into the cell. This helps to neutralize the acidic digesta that moves from the stomach into the duodenum. Cl^- is present in the enterocyte as a free anion and is transported out of the cell by the basolateral Cl^- channels (e.g., Cl^- channel-2; Pena-Munzenmayer et al. 2005). Cl^- is absorbed into the portal vein and carried in plasma as a free cation for uptake by extraintestinal cells through Cl^- channels, primarily Ca^{2+}-activated Cl^- channels (Huang et al. 2012).

Functions: The biochemical functions of Cl^- are outlined as follows.

Acid-Base Balance

Cl^- is associated with Na^+ and K^+ in cells and tissues. These ions, along with bicarbonate ($HCO3^-$), are used to calculate the anion gap [i.e., $(Na^+ + K^+) - (Cl^- + HCO3^-)$] and contribute to the regulation of the acid-base balance in animals. Normal anion gap values in the serum of animals, as calculated above, are 10–20 mmol/L and are useful to assess the quantities of unmeasured cations (Ca and Mg) and anions (proteins, sulfates, phosphates, and certain organic acids). A high anion-gap value may be an indicator of metabolic acidosis (e.g., ketoacidosis or lactic acidosis). In contrast, a low anion-gap value may result from hypoalbuminemia, hypernatremia, or severe hypercalcemia.

Production of Gastric Hydrochloric Acid (HCl)

The parietal (oxyntic) cells of the stomach are the sources of gastric HCl. Gastric juice is a clear, yellow fluid of 0.2–0.5% HCl, with a pH of about 1.0. HCl denatures proteins and peptides, which is important for their hydrolysis by proteases and peptidases. HCl also kills bacteria, which helps to prevent infection.

Intestinal Secretion

In a postabsorptive state, the intestine secretes Cl^- into the lumen to maintain osmolarity and acid-base balance in the

lumen. The transporters involved in the secretion include (a) the basolateral Na^+-K^+-$2Cl^-$ cotransporter, which transports one Na^+, one K^+, and $2Cl^-$ ions from the lamina propria (ultimately the blood) into the intestinal epithelial cell; and (b) CFTR on the apical membrane of the intestinal epithelial cell. As noted previously, the Na^+ gradient, established by the basolateral Na^+/K^+-ATPase, provides the energy for the transport of ions into the cell. For a cotransporter, the presence of one substrate enhances the transport of the other. In cases of infection or intake of a noxious compound into the intestine, fluid (water and Cl^-) secretion into the lumen is enhanced due to the cAMP-mediated activation of Cl^- channel within intestinal epithelial cells.

Maintaining a Hydrated State of the Mucus

The transport of Cl^- ions across epithelial cells is associated with the movement of Na^+ and water, which helps to maintain a hydrated state of the mucus. This role is epitomized by the disease called "cystic fibrosis," one of the most common genetic disorders among Caucasians (Whites) (5% of the population carries the defective gene). Swine models of cystic fibrosis have been developed for research purposes (Rogers et al. 2008). The disease results from a genetic mutation of the Cl^- transport channel in the apical membrane of airway epithelial cells such that the channel is not activated normally by cyclic adenosine monophosphate (cAMP) and protein kinase (Cutting 2015). The failure to transport Cl^- ions from the lung epithelium into the air passage causes the failure of Na^+ and water to move passively into the airway. The impaired transport of water into the airway results in the development of thick and dehydrating mucus in the lungs. This mucus impairs breathing and invites recurrent infections that eventually destroy the lungs.

Deficiency symptoms: In all animals, a deficiency of Cl^- (also known as hypochloremia) reduces extracellular osmolarity and impairs acid-base balance (de Morais and Biondo 2006). Dietary Cl deficiency may cause an abnormal increase in the alkali reserve of the blood (alkalosis) due to an excess of bicarbonate. This is because inadequate levels of Cl^- in the body are partly compensated for by an increase in bicarbonate concentration. Animals with hypochloremia also exhibit increases in the urinary excretions of Ca and Mg. Furthermore, reduced food intake, impaired protein digestion, growth restriction, muscle weakness, and dehydration occur in Cl^--deficient animals (Jones and Hunt 1983). Intravenous or oral administration of NaCl is effective in treating Cl deficiency.

Chloride excess: A concentration of Cl^- in plasma greater than 110 mM is defined as hyperchloremia, which can result from excessive intake of dietary salts (e.g., NaCl, KCl, and NH_4Cl), high concentrations of Na^+ in plasma, and kidney failure. In animals, symptoms of hyperchloremia include nausea, vomiting, and diarrhea; reduced feed intake and impaired growth; sweating, dehydration, and high body temperature; fatigue and muscular weakness; high concentrations of Na^+ in plasma, hypertonicity, metabolic acidosis, and thirst; kidney disorders and failure; impaired blood flow; brain injury; and impaired oxygen transport by red blood cells (Cambier et al. 1998; Scarratt et al. 1985).

Magnesium (Mg)

Mg derives its name from Magnesia (a district in Thessaly, Greece), where the mineral magnesia alba [now known as magnesium carbonate ($MgCO_3$)] was naturally abundant. In 1722, Friedrich Hofman distinguished magnesia alba from other minerals. Joseph Black first recognized Mg as an element in 1755 when he found that magnesium oxide (MgO) differed from Ca oxide (CaO). Mg was first isolated from MgO by Humphry Davy in 1808. However, the discovery of Mg dates back to 1618, when a farmer in Epsom, England found that water from his well contained a bitter compound that could heal scratches and rashes. Interestingly, cows refused to drink this water, and the unknown substance was later identified as $MgSO_4{\cdot}7H_2O$. Mg is a divalent cation and the sixth most abundant macromineral in living organisms (the animal body: Ca > P > K > Na > S > Mg). The concentration of Mg^{2+} is about 0.8–2.2 mM in plasma, and about 1–3 mM in the intracellular fluid. About 90% of the intracellular Mg^{2+} is bound to ribosomes or polynucleotides (Wolf and Cittadini 2003).

Sources: Mg is widely present in plant- and animal-sourced feedstuffs. Wheat bran, dried yeast, and most vegetable protein concentrates (e.g., cottonseed cake and linseed cake) are good sources of Mg. Clovers usually contain more Mg than grasses. MgO, which has the highest content of Mg (60.3%) among its inorganic sources, is usually the form used for dietary supplementation to farm animals [including cattle, swine, and poultry (Davenport et al. 1990; Pinotti et al. 2021)] at relatively high bioavailability. In the chemical industry, MgO is prepared from the decomposition of $MgCO_3$ through heating at 350°C (i.e., $MgCO_3 \rightarrow MgO + CO_2$).

Absorption and transport: As noted previously, the reticulorumen is the major site for the absorption of dietary Mg in ruminants (Tomas and Potter 1976). For example, in growing lambs, 89% and 21% of dietary Mg are absorbed before the duodenum and in the whole small intestine, respectively (Wylie et al. 1985). In these species, there is little or no absorption of luminal Mg^{2+} by their omasum or abomasum. In all animals, Mg^{2+} in the intestinal lumen is absorbed by the enterocyte via the apical transient receptor potential melastatin-related protein-6 (TRPM6), which is a cation channel protein (Voets et al. 2004). TRPM7 is required for the activity of TRPM6. Both TRPM6 and TRPM7 are unusual bifunctional proteins that consist of an ion channel and a cAMP-dependent protein kinase (Ryazanova et al. 2010). In the enterocyte, Mg^{2+} is present both in its free form and bound to proteins and nucleic acids. This mineral exits the enterocyte across its basolateral membrane into the lamina propria via CNNM4 (an

ancient conserved domain protein 4), which is an electroneutral Na^+/Mg^{2+} exchanger. This basolateral CNNM4 transports Mg^{2+} out of the enterocyte in exchange for the entry of Na^+ without affecting transmembrane currents. Absorbed Mg^{2+} enters the portal vein from the lamina propria. This mineral is transported in plasma in both free and protein (albumin)-bound forms and is taken up by extraintestinal cells (including renal tubular epithelial cells) through Mg^{2+} transporters, including TRPM6/7 (Ryazanova et al. 2010). Renal reabsorption of Mg^{2+} is stimulated by PTH via cAMP-dependent cell signaling but is reduced in hypercalcemia due to the inhibition of PTH synthesis and release. Thus, Mg^{2+} homeostasis is regulated by gastrointestinal absorption, utilization (e.g., milk production), and excretion via the kidneys. Although about 80% of plasma Mg is filtered at the glomerulus during a single pass, only 3% of the filtered Mg is excreted in the urine, and most of the Mg within the lumen of the renal tubule is reabsorbed into the blood via the apical TRPM6/7 (Musso 2009).

Functions: Mg^{2+} interacts with ATP, DNA, and RNA to stabilize these polyphosphate compounds. This divalent ion is needed to alleviate electrostatic repulsion between phosphates, thereby stabilizing ATP structure, as well as base pairing and stacking in nucleic acids (Wolf and Cittadini 2003). In addition, Mg^{2+} is bound to many proteins in cells, including membrane proteins. More than 300 enzymes require Mg^{2+} as a cofactor for their catalytic activity, including those that synthesize and utilize ATP. Examples of these enzymes are given as follows:

1. Glycolysis pathway

Glucose + ATP → Glucose-6-phosphate + ADP	(Hexokinase)
Fructose-6-phosphate + ATP → Fructose-1,6-phosphate + ADP	(Phosphofructose kinase)
1,3-Bis-P-glycerate + ADP ⇌ 3-P-glycerate + ATP	(Phosphoglycerate kinase)
2-Phosphoglycerate ⇌ Phosphoenolpyruvate	(Enolase)
Phosphoenolpyruvate ⇌ Pyruvate	(Pyruvate kinase)

2. Pyruvate dehydrogenase (PDH; active) + ATP → PDH (inactive) + ADP (Pyruvate dehydrogenase kinase)
3. PDH (phospho-enzyme; inactive) + H_2O → PDH (dephospho-enzyme; active) + Pi (Pyruvate dehydrogenase phosphatase)
4. Glycogen metabolism: Glucose-1-phosphate ⇌ Glucose-6-phosphate (Phosphoglucomutase)
5. Gluconeogenesis: Propionate + ATP → Propionyl-CoA + AMP (Acyl-CoA synthetase)
6. Pentose cycle (pentose phosphate pathway)

Glucose-6-phosphate → 6-Phosphogluconolactone	(Glucose-6-P dehydrogenase)
6-Phosphogluconolactone → 6-Phosphogluconate	(Gluconolactone hydrolase)
Ribose-5-phosphate → 5-Phosphoribosyl-1-pyrophosphate (PRPP)	(PRPP synthetase)
Ribose-5-P + Xylulose-5-P ⇌ Sedoheptulose-7-P + Glyceraldehyde-3-P	(Transketoase, vitamin B_6)

7. Fatty acid degradation: Fatty acid + ATP → Fatty acyl-CoA + AMP (Acyl-CoA synthetase)
8. Urea cycle: Citrulline + Aspartate + ATP → Argininosuccinate (AS) + AMP (AS synthase)
9. Pathways for the syntheses of purines and pyrimidines (Many enzymes require Mg^{2+})
10. DNA synthesis: Deoxyribonucleotides → DNA (DNA polymerase)
11. RNA synthesis: DNA → RNA (RNA polymerase)
12. Protein synthesis: Binding of mRNA to ribosomes; stabilization of ribosomes (Initiation factors)

Mg^{2+} conducts electrical (nerve) impulses and alters the firing threshold of neurons in the nervous system. Physiological concentrations of Mg^{2+} block the binding of glutamate and aspartate to, and thus the activation of, N-methyl-D-aspartate (NMDA) receptors in neurons (Morris 1992). Glutamate and aspartate are the principal neurotransmitters for excitatory synaptic transmission in the central nervous system (CNS) of vertebrates. A low concentration of Mg^{2+} in the brain or cerebral spinal fluid (<0.25 mM) causes the NMDA receptors to be activated by glutamate and aspartate and reduces the threshold at which neurons repetitively fire nerve impulses (action potentials), resulting in nervous disorders and, consequently, tetany.

Deficiency symptoms: Mg deficiency (also known as hypomagnesemia) reduces ATP production, the endothelial synthesis of nitric oxide, and antioxidative reactions, as well as feed intake, growth, immunity, and survival of animals. Mammals, birds, and fish with hypomagnesemia also exhibit neuromuscular irritability, tetany, seizures, depression, abnormal carbohydrate and amino acid metabolism, impaired blood flow in the microvasculature, and infertility (Al-Ghamdi et al. 1994; Kubena and Durlach 1990). Grass tetany frequently occurs in ruminants fed Mg-inadequate forages or diets, particularly when ruminal NH_4^+ concentrations are elevated to reduce Mg absorption (Martens and Schweigel 2000). Hypomagnesemia can result in the

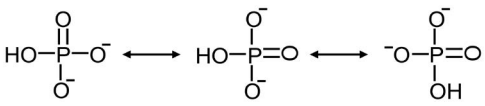
activation of neurons, leading to sustained excitations and muscle cramps. Furthermore, Mg deficiency can induce hypocalcemia and hypokalemia by decreasing PTH activity, the resorption of bone, increasing the calcification of soft tissues, and augmenting the renal excretion of Ca^{2+} and K^{+}. Excessive dietary K interferes with the absorption and metabolism of Mg^{2+} in animals, therefore increasing the risk of hypomagnesemia and tetany. Mg deficiency can be treated by oral administration of Mg (as magnesium citrate, magnesium oxide, magnesium sulfate, or magnesium chloride). In clinics, intravenous Mg treatment is advocated to treat preeclampsia and acute myocardial infarction (Musso 2009).

Excess: Hypermagnesemia is defined as an abnormally high Mg^{2+} concentration in plasma. This metabolic disorder is rare in farm animals but can result from excessive Mg intake, impaired renal excretion of Mg^{2+}, and excessive Mg mobilization in animals. Symptoms of hypermagnesemia include (a) nausea and vomiting; (b) reduced DM digestibility, profuse diarrhea (because of the water flowing into the large intestine), and dehydration; (c) reduced feed intake and impaired growth; (d) muscle weakness and impaired breathing; and (e) low blood pressure, low heart rate, and cardiac arrest (Topf and Murray 2003). Of note, concentrations of Mg^{2+} (as $MgSO_4$) in the ruminal fluid ≥ 320 μg/ml are toxic to ruminal microbes as assessed by the rate of cellulose digestion (Georgievskii et al. 1982). In animals, excessive amounts of dietary Mg^{2+} (e.g., as MgO) can result in digestive dysfunction such as diarrhea, and the strong laxative effects of MgO are often used to treat constipation.

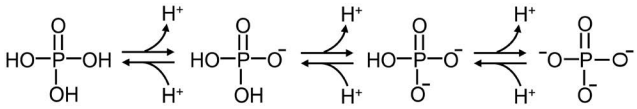
Phosphorus (P)

Phosphorus was discovered from human urine by Hennig Brand in 1669. The name of this mineral is derived from the Greek words *Phôs* (light) and *phorus* (bearer). P is highly reactive and, therefore, is not found as a free element on Earth. Because P tends to combust spontaneously when left alone in air, this mineral is also known as "the Devil's element". In animals and plants, P exists primarily as phosphate [e.g., orthophosphate $(PO_4)^{3-}$ in Ca phosphate and inorganic phosphate (HPO_4^{2-})], which consists of a central atom of P, four atoms of oxygen, and zero to three atoms of hydrogen. The concentration of inorganic phosphate (Pi) is 1.2–2.0 mM in plasma. The total amount of intracellular phosphates (e.g., phosphates in ATP, ADP, AMP, GTP, UTP, creatine phosphate, phosphorylated protein, nucleic acids, and glucose-6-phosphate) is equivalent to 50–75 mM in animal cells. About 85% of the body's phosphates are present in the bones, with about 14% in soft tissues and 1% in the extracellular fluid. Note that animals have requirements for P, but phosphate is the most common form of P in diets and used by organisms.

Phosphate resonates among several forms at neutral pH. A few forms of phosphate are shown below.



Phosphate occurs in equilibrium with H_3PO_4, $H_2PO_4^{-}$, HPO_4^{2-}, and PO_4^{3-}, as below.



The predominant form of phosphate at neutral pH is HPO_4^{2-}. The fully protonated form, which is the predominant form in an environment of low pH, is phosphoric acid (H_3PO_4). In animals, phosphate reacts with Ca^{2+} to form Ca phosphate in bones and is also covalently bound to sugars, proteins, lipids, vitamins, and other organic molecules. Phosphate homeostasis is tightly controlled through the interorgan cooperation of the small intestine, kidneys, and bone.

Sources: Animal products are good sources of P. Although the hydroxyapatite of animal-sourced foods such as meat and bone meal is barely soluble in water at neutral pH, this mineral complex is readily dissolved and dissociated in the gastric fluid (e.g., pH 2–3 in pigs) of the stomach to form phosphate.

Except for seeds, which can have a substantial amount of P, plants and their products (e.g., hays and straws) generally have a low content of P. For example, corn grain and SBM (44% crude protein) contain 0.28% and 0.69% P, respectively. In plants, phosphate is bound to inositol as phytate, which constitutes 60% to 80% of total P in cereals, legumes, nuts, and oilseeds. Insoluble Ca and Mg phytates in cereals and other plant products reduce P bioavailability to animals. Plant-sourced phytate must be hydrolyzed by phytase present in foods and/or derived from gastrointestinal microbes for the utilization of dietary P. The digestibilities of P in plant-sourced foods are generally much lower than those in animal-sourced foods. Thus, phytase is now widely added to swine and poultry diets to increase the bioavailability of phytate P and reduce the amount of supplemental P (Humer et al. 2015; Lee et al. 2021; Philippi et al. 2023). Due to a low amount of P from plant-sourced foods (e.g., roughage and corn grain), dietary P supplementation is common in ruminant, pig, and poultry production systems, as well as for fish and crustaceans fed plant protein-based rations. Ruminants contain ruminal microbial phytase and, therefore, have a greater ability to utilize dietary P than nonruminants. For example, the digestibilities of dietary P in ruminants are 64% for forages and 70% for concentrates (Humer and Zebeli 2015).

The sources of dietary P depend on animal species. For example, strict carnivores obtain P only from animal-sourced foods, whereas omnivores acquire P from both animal- and plant-sourced foods. Most of the animal-sourced feedstuffs have much more bioavailable P than plant-sourced feedstuffs (Kiarie and Nyachoti 2010). Inorganic P in some compounds can be effectively used by all species of animals. Because the cost of inorganic P increased dramatically in recent years (e.g., from \$72.9/10^3 kg in May 2020 to \$345.0/10^3 kg in May 2023; World Bank 2023) and because P is a finite natural resource, adequate knowledge of P utilization by animals is essential to improve its efficiency, economic returns, and sustainability in agriculture.

Absorption and transport: Phosphate in the intestinal lumen is absorbed by the small intestine via three Na^+-dependent phosphate transporters against an electrochemical gradient: Na^+-phosphate cotransporter (NaPi2b), phosphate transporter-1 (PiT1), and phosphate transporter-2 (PiT2; Candeal et al. 2017; Schryver et al. 1972). NaPi2b, the major transporter of phosphate in the gut, transports phosphate across the apical membrane of the enterocyte with a stoichiometry of 3:1 Na^+:HPO_4^{2-} at a relative low K_M of ~10 μM (Sabbagh et al. 2011). The Na^+ concentration in the luminal fluid is greater than that in the cytosol of the enterocyte through the action of the Na^+/K^+-ATPase at the basolateral membrane, thereby providing a concentration gradient to drive Na^+-dependent phosphate absorption. PiT1 and PiT2 preferentially transport monobasic phosphate species with a 2:1 Na^+:$H_2PO_4^-$ stoichiometry (Khanal and Nemere 2008). Upon entering the enterocyte, phosphate is transported as a free anion to its basolateral membrane. At present, details of phosphate efflux across the basolateral membrane of the enterocyte into lamina propria are unknown. However, there is evidence for Na^+-dependent phosphate transport out of the intestinal basolateral membrane (Kikuchi and Ghishan 1987). Basolateral phosphate transport is bidirectional, depending on dietary intake of phosphate (Sabbagh et al. 2011). Phosphate is absorbed into the portal vein as a free anion and is taken up by extraintestinal cells through Na^+-dependent phosphate transporters, NaPi2a, NaPi2c, PiT1, and PiT2. Note that NaPi2a and NaPi2c in the apical membrane of the proximal tubule are responsible for the reabsorption of 80% of filtered phosphate (Sabbagh et al. 2011).

Phosphorus homeostasis in animals is regulated by many factors, including intestinal absorption of dietary phosphate, renal reabsorption and excretion of phosphate, as well as the exchange of phosphate between extracellular and bone storage pools (Marks et al. 2010). Physiological concentrations of calcitriol enhance intestinal phosphate absorption by stimulating the expression of NaPi2b in enterocytes, and in the case of low plasma phosphate concentration, the mobilization of phosphate from the bone matrix (Figure 10.3). In addition, PTH stimulates the release of phosphate from bones (Candeal et al. 2017). In response to hyperphosphatemia or hypocalcemia, the secretion of calcitriol and PTH is enhanced to decrease renal reabsorption of phosphate (Blaine et al. 2015). This physiological response helps to prevent an excessive elevation of plasma phosphate due to bone resorption. In contrast, growth hormone, insulin, insulin-like growth factor 1, and thyroxine increase renal reabsorption of phosphate. Thus, factors that favor muscle protein synthesis usually promote skeletal growth, including mineralization.

The rate of the retention of dietary P in the body of 3-week-old broilers varies widely, from 16% to 72%, depending on the plant-sourced feedstuff (Table 10.12). About 24% and

TABLE 10.12
Retention of Plant-, Animal-, and Inorganic-Sourced Dietary Phosphorus (P) in the Bodies of 3-Week-Old Broilers[a]

Feedstuff	Total P Content (g/kg)	Retention of Source P in Broilers (%)
Plant-Sourced		
Beans	4.9	52
Corn grain	3.0	29
Lupin	3.0	72
Peas	4.1	41
Rapeseed	10.9	33
Rice bran	17.2	16
Soybean (heat-treated)	5.5	54
Soybean meal	7.1	61
Sunflower meal	11.9	38
Wheat	3.4	48
Wheat middlings	10.8	36
Animal-Sourced		
Bone meal	76	59
Fish meal	22	74
Meat meal	29	65
Meat and bone meal	60	66

Inorganic P Source	Total P Content (g/kg)	Retention of Source P in Broilers (%)
Calcium sodium phosphate ($CaNaO_4P$; MW 158.04)	180	59
Defluorinated phosphate [$Ca_4Na(PO_4)_3$; MW 468.04]	182	86
Dicalcium phosphate[b] (anhydrous; $CaHPO_4$; MW 136.06)	197	55
Dicalcium phosphate[b] (MW 136.06)	183	83[†]
Dicalcium phosphate (dihydrous; ($CaHPO_4 \cdot 2H_2O$; MW 172.09)	180	77
Monocalcium phosphate [$Ca(H_2PO_4)_2$; MW 234.05]	226	84
Monodicalcium phosphate [monohydrate; $Ca(H_2PO_4)_2 \cdot H_2O + CaHPO_4 \cdot 2H_2O$]	213	79
Monocalcium phosphate [monohydrate; $Ca(H_2PO_4)_2 \cdot H_2O$; MW 252.07]	237	94
Monodicalcium phosphate [anhydrous; $Ca(H_2PO_4)_2 + CaHPO_4$]	200	80[†]
Monosodium phosphate (NaH_2PO_4; MW 119.98)	224	92

Source: Leske and Coon (2022); Li et al. (2016).

[a] Broilers fed diets containing 1.8 g of calculated retainable P and 5.0 g of Ca per kg diet beginning at 10 days of age. Unless indicated otherwise, P retention in broilers was measured during a 3-day period between 21 and 24 days of age by determining dietary P intake and excreta P output.

[b] The "di" prefix in the common name is used to indicate that the HPO_4^{2-} anion is formed in the molecule after the removal of two protons from H_3PO_4.

59% of dietary P are retained in the body of growing pigs fed diets (flake corn-, tapioca-, sugar-beet pulp-, and straw pellet-based) containing high- and normal-level phytate, respectively (Lee et al. 2021). The rate of the retention of dietary P in the body of growing calves fed low-, medium-, and high-levels of P is 1.6%, 40%, and 38%, respectively (Challa and Braithwaite 1988). In this experiment, young postweaning calves were fed the low-, medium-, and high-P diets supplemented daily with 12.5 g Ca carbonate, 22.0 g dicalcium phosphate, and 22.0 g dicalcium phosphate plus 38.0 g disodium phosphate, respectively. In ruminants with a well-developed rumen that produces phytases to break down the phytate P of plant materials, the rate of digestion of P from these sources is greater as compared to nonruminants. However, the efficiency of utilization of plant-sourced P by ruminants is still lower than that for inorganic P sources. Dietary supplementation with phytase can enhance the digestibility and the utilization efficiency of P in both poultry and swine that are fed plant-based feedstuffs, as well as forage-fed high-producing ruminants (Humer and Zebeli 2015; Philippi et al. 2023). Juvenile largemouth bass and hybrid striped bass fed conventional fishmeal- or soy protein-based diets retain 35–40% (Li et al. 2020, 2021) and 36–42% (He et al. 2023a; Li et al. 2023), respectively, of dietary P in the bodies. The utilization of dietary P is affected by its intake and the intestinal health of animals; the dietary content of protein, minerals (particularly Ca and fluoride), energy, and vitamin D; animal species, disease, environmental temperatures, air pollution, and the quality of drinking water.

Functions: Phosphorus has many biochemical functions in animals (Kornberg et al. 1999). A key role of P is the formation of Ca phosphate from Ca^{2+} and phosphate in the process of biomineralization, which is critical in providing the skeleton of organisms and maintaining body structure. In addition, as a component of nucleotides (ATP, GTP, and UTP), phosphocreatine, nucleic acids (RNA and DNA), phospholipids, sphingosine 1-phosphate, and inositol-1,4,5-trisphosphate, P participates in (a) energy transfer, storage, and utilization; (b) covalent modifications of proteins and enzymes that are critical for the regulation of their biological activities; and (c) the regulation of cellular signaling and physiological responses to hormonal and nutritional alterations. Furthermore, upon their entry into cells or production through metabolism, many low-molecular-weight substrates and metabolites are phosphorylated to prevent their leakage (e.g., phosphate groups of nucleotides, intermediates of glycolysis, pyridoxal phosphate, and 2,3-diphosphoglycerate), thereby maximizing the efficiency of product formation and modulating tissue oxygenation. Finally, phosphate is required as part of a coenzyme (e.g., pyridoxal phosphate) or an activator of an enzyme (phosphate-activated glutaminase and 1α-hydroxylase) to improve the efficiency of enzymatic reactions. Therefore, P occupies the central stage of cell metabolism.

Deficiency symptoms: Phosphorus is the macromineral most likely to be deficient in unsupplemented grazing cattle, and P deficiency is also a major nutritional problem in nonruminants fed grain-based and unsupplemented diets. P deficiencies in animals under range conditions often result from diets with low P but high Ca. The P content of grasses declines rapidly from 0.14% to 0.03% in grazing rangelands of many western U.S. as the forage growing season progresses from August to December and remains low in the winter (Stewart and Page 2020). This long period corresponds to pregnancy in sheep. In animals, most symptoms of P deficiency (also known as hypophosphatemia) are similar to those of vitamin D deficiency. This nutritional problem commonly occurs in grazing livestock receiving no phosphate supplementation, especially in unfertilized tropical and subtropical areas and unfertilized regions of North America, as a result of low P content in grass due to low P content available (e.g., present as tricalcium phosphate) to the local plants (Davidson 1945; McDonald et al. 2021). Note that grasses that have been fertilized with N, P, and K generally contain adequate amounts of P to meet the needs of grazing ruminants; in these cases, P is not the major nutritionally deficient mineral for grazing ruminants if unsupplemented. Feeding forages (e.g., alfalfa) that contain high Ca content but low P content can exacerbate P deficiency in ruminants due to the formation of Ca phosphate. In addition, malnutrition, malabsorption, or disorders affecting renal phosphate reabsorption can result in hypophosphatemia.

Animals with P deficiency exhibit whole-body phosphate depletion, as well as hemolysis, fatigue, reduced myocardial contractility, muscle weakness, respiratory failure, tremors, ataxia, anorexia, nausea, and vomiting (Allen-Durrance 2017). Prolonged P deficiency causes: (a) reduced feed intake; (b) protein malnutrition due to reductions in the absorption, ATP-dependent transmembrane transport, and synthesis of amino acids; (c) the impaired growth and development of bones and the whole body (including skeletal muscle); (d) bone resorption (demineralization); and (e) skeletal defects (e.g., rickets in the young and osteomalacia in adults; Harris 2014). Finally, male and female animals suffer from infertility when their diets are deficient in P (Greene et al. 1985; Morrow 1969). All of these nutritional, metabolic, and reproductive problems can be effectively treated by dietary P supplementation.

Excess: Hyperphosphatemia is defined as an abnormally high phosphate concentration in plasma. In animals, this metabolic disorder can result from excessive phosphate intake, inadequate Ca intake, decreased renal phosphate excretion (e.g., low levels of PTH), or disorders (e.g., tissue injury and excessive bone resorption) that promote the exit of intracellular phosphate to extracellular space. Excessive P can pull Ca^{2+} from bones because PTH signals the bones to release Ca^{2+} into the bloodstream, and can also cause Ca phosphate deposits in vessels and organs due to the excessive formation of the salt. Symptoms of hyperphosphatemia include (a) reduced feed intake and impaired growth; poor digestibility of Fe, Ca, Mg, and Zn; (b) impaired skeletal growth and development; anorexia,

nausea, vomiting, and diarrhea; and (c) low Ca^{2+} concentrations in plasma. Animals with P deficiency also exhibit abnormal neurological function and seizures; cardiovascular dysfunction; the nonskeletal calcification of tissues (especially the kidneys); and acid-base imbalance (i.e., metabolic acidosis) and kidney stone (Ketteler et al. 2016; Spasovski, G. 2015).

Potassium (K)

In 1797, Martin Heinrich Klaproth studied alkali residues of chemical substances and proposed the name "kali" for vegetable alkali. Potassium was discovered in 1807 by Humphry Davy through its isolation from potash (K_2CO_3, a substance from wood ashes). The word "potassium" is derived from *potash*. The chemical symbol of this element, K, is derived from the Latin "kalium" meaning alkali. Potassium is the most abundant cation in the intracellular fluid. The concentration of K^+ is about 140 mM in the intracellular fluid and is about 3.5–5.0 mM in plasma.

Sources: Plants usually contain high levels of K (e.g., 25 g K/kg DM of green grass). In addition, K content is relatively high in meat. Thus, K deficiency in animals is rare. One exception is distillers' grains, with K supplementation being required in these cases. Of note, the soil of certain areas in the world (e.g., Brazil, Panama, and Uganda) contains low concentrations of K, and therefore plants in these regions may be deficient in K (Suttle 2010). In addition, dormant grasses are much lower in K content and may not provide sufficient K to animals.

Absorption and transport: K^+ channels on the apical membranes of the enterocyte, which include K^+/H^+ ATPase, Na^+-Cl^--K^+ transporter, and K^+ conductance channels, are responsible for K^+ absorption by the small intestine. They are widely expressed in both excitable and nonexcitable cells (Heitzmann and Richard 2008). K^+/H^+ ATPase uses ATP to drive the inward flow of K^+ into the enterocyte in exchange for the outward flux of H^+ into the intestinal lumen. Na^+-Cl^--K^+ transporter can bind these three ions and use a Na^+ gradient to transport 1 Na^+, 1 K^+, and 2 Cl^- ions into the cell. K^+ conductance channels use electrochemical energy across the enterocyte membrane as the driving force to transport K^+; these channels are activated by Ca^{2+} or gated by nucleotides, voltage, or specific ligands. Furthermore, K, along with Cl, is absorbed across the apical membrane of the enterocyte or colonocyte through two electroneutral exchangers: K^+/H^+ and Cl^-/HCO_3^-. K^+ is present in the enterocyte as a free cation and is transported out of the cell by the basolateral K^+ channels and the electroneutral K^+-Cl^- cotransporter into the lamina propria. K^+ exits the enterocyte together with Cl^-, with water following these osmolytes. K^+ is absorbed into the portal vein and carried in plasma as a free cation for uptake by extraintestinal cells through K^+ channels and K^+-Cl^- cotransporters.

Functions: The biochemical functions of K are summarized as follows.

Regulation of Intracellular Osmolarity

Because of its abundance inside cells, K^+ plays an important role in regulating intracellular osmotic pressure. Specifically, K^+ contributes to about 50% of intracellular osmolarity in animals. Dietary supplementation with K may be necessary when SBM (rich in K) is replaced with alternative protein sources or when the SBM content is reduced in diets (e.g., those for gestating swine).

Activity of Na^+ Pump and the Functions of the Nerves and Muscle Cells

K^+ is involved in the Na^+ pump activity and in the functioning of the nerves and muscle cells (e.g., cardiac myocytes), as noted previously. Like Na+, K^+ is also used in the conduction of impulses along nerves and muscle cells. This critical role of K^+ is illustrated by hyperkalemic periodic paralysis (HYPP), which is a genetic disease associated with an increase in serum K^+ concentration in humans and horses (Nollet and Deprez 2005).

HYPP disease results from genetic mutations in transmembrane domains III and IV, which make up the fast inactivation gate of $Na_v1.4$ at the neuromuscular junction (Lehmann-Horn and Jurkat-Rott 1999). In patients with genetic mutations of $Na_v1.4$, the Na^+ channel is unable to inactivate. As a result, Na^+ conductance is sustained, and the muscle remains permanently tense. This condition becomes worse in the presence of high concentrations of K^+ in plasma because an elevated level of extracellular K^+ reduces the exit of K^+ from the muscle cells, further prolonging the Na^+ conductance and keeping the muscle in the continuous contraction state. Thus, HYPP is characterized by the hyperexcitability or extreme weakness of skeletal muscle, leading to uncontrolled shaking followed by paralysis. This disease occurs in 2% of Quarter horses with a single stallion ancestor named Impressive (Nollet and Deprez 2005). The HYPP symptoms can be alleviated by decreasing dietary K intake and the concentrations of K^+ in plasma (Kollias-Baker 1999; Reynolds et al. 1998).

Hormone Synthesis

K^+ stimulates the conversion of cholesterol into pregnenolone and the conversion of corticosterone into aldosterone (Chapter 6). As a result, K^+ plays an important role in regulating the synthesis of steroid hormones and glucocorticoids, as well as concentrations of Na^+ in plasma.

H^+-K^+-ATPase

In parietal cells of the stomach, the secretion of H^+ ions into the lumen in exchange for K uptake to generate HCl depends on H^+-K^+-ATPase. Thus, K is required to maintain a low gastric-luminal pH and facilitate protein digestion in animals (Chapter 7).

Deficiency symptoms: A deficiency of potassium (plasma K^+ concentration <3.5 mM), which is known as hypokalemia, results from inadequate dietary intake, gastrointestinal loss (e.g., through vomiting and diarrhea), urinary loss (e.g., diabetic ketoacidosis), hypomagnesemia,

or hyperaldosteronism (Sweeney 1999). Renal K^+-channel efflux is inhibited by Mg^{2+} (Huang and Kuo 2007); therefore, hypomagnesemia results in an increased excretion of K^+ by the kidneys, possibly leading to hypokalemia. In animals, the symptoms of K deficiency include muscle weakness, spasms, tetany, paralysis, numbness (particularly in legs and hands), excessive loss of body water (due to an inability to concentrate urine), low blood pressure, frequent urination, and thirst. Finally, hypokalemia can also cause cardiac rhythm abnormalities, cardiac arrest, and even death in animals. Intravenous and oral administration of K^+, in addition to therapies aimed at correcting the associated endocrine and metabolic diseases, are necessary to treat K deficiency (Jones and Hunt 1983).

Excess: A concentration of K^+ in plasma greater than 5.5 mM is defined as hyperkalemia, which can result from excessive intake, excessive release from cells due to hemolysis and tissue injury, kidney failure (reduced K^+ excretion), hypoaldosteronism (which impairs K^+ excretion in the distal tubule and the collecting duct; common in dogs), and the use of angiotensin-converting enzyme inhibitors (Kovesdy 2017). In animals, the symptoms of hyperkalemia include reduced feed intake and impaired growth; fatigue and muscle weakness; hypomagnesemia and abnormal heart rhythm (arrhythmia), slow heart rate, and cardiac arrest and even death. Hypomagnesemia occurs under hyperkalemic conditions due to the depolarization of the membrane potential of gastrointestinal cells (e.g., ruminal epithelial cells of the rumen) and the subsequent reduction in the absorption of dietary Mg via the electrodiffusion mechanism (Schweigel et al. 1999). The toxicity of K is not likely if adequate drinking water is provided to animals (Greene et al. 1983).

Sodium (Na)

As noted previously for the discovery of K, Martin Heinrich Klaproth studied alkali residues of chemical substances and proposed the name "natron" for mineral alkali in 1797. Na was discovered by Humphry Davy through the electrolysis of caustic soda (NaOH) in 1807. The name of "sodium" is derived from the Latin *sodanum* meaning "headache remedy", and its chemical symbol Na from the Latin *natrium*.

Na is the sixth most abundant element on earth and comprises 2.6% of the earth's crust. This element is very reactive and is seldom found free in nature. NaCl was used by the ancient Egyptians and Chinese to preserve animal- and plant-sourced foods. Na^+ is the principal cation in extracellular fluid. In domestic animals, the concentrations of Na^+ are about 140 mM in plasma or serum and about 14 mM in the intracellular fluid. Na^+ is the fourth most abundant mineral in the animal body (Table 10.7).

Sources: Much of Na is ingested in the form of NaCl (common salt). Most foods of vegetable origin have low Na content, whereas animal products are rich sources of this mineral (Pond et al. 1995). For example, corn grain, SBM, rice bran, and wheat middlings contain only 0.02, 0.02, 0.03, and 0.05% Na, respectively, whereas fishmeal (menhaden) provides 0.40% Na. Thus, Na is usually added as 0.10–0.50% NaCl to corn- and SBM-based diets for poultry and swine, depending on the age of animals as well as the type and content of other feedstuff ingredients. For grazing animals, NaCl is generally supplemented to their diets in free-choice mineral salts.

Absorption and transport: In the lumen of the small intestine, NaCl is ionized to Na^+ and Cl^-. In mammals (e.g., pigs and ruminants), birds, fish, and crustaceans, Na^+ enters the enterocyte from the intestinal lumen via (a) Na^+-glucose cotransporter-1 (SGLT1); (b) Na^+ channels [e.g., AMSC; amiloride-sensitive Na^+ channels (also known as epithelial Na^+ channels, ENaC)]; (c) Na^+-dependent amino acid transporters; and (d) the Na^+-phosphate cotransporter (Carey et al. 1994; Lall and Kaushik 2021; Song et al. 2016; Wright and Loo 2000). Furthermore, Na^+, along with Cl^-, is absorbed across the apical membrane of the enterocyte or colonocyte through two electroneutral exchangers: Na^+/H^+ (NHE2/3) and Cl^-/HCO_3^- (Kato and Romero 2011). The absorption of Na^+ and Cl^- via the transmembrane exchangers does not result in transepithelial currents, which contrasts with other modes of Na^+ and Cl^- transport by the intestine (Gawenis et al. 2002). Electroneutral Na^+ transport (e.g., in the form of NaCl) accounts for most intestinal Na^+ absorption during the period between meals and for ileal Na^+ absorption post-prandially. Na^+, which is present in the enterocyte as a free cation, is transported out of the cell by the basolateral Na^+-K^+-ATPase into the lamina propria (Manoharan et al. 2015). NHE-1, which is present in the basolateral membrane of the enterocyte, plays a role in transporting intracellular Na^+ into the lamina propria in exchange for the entry of H^+ ions. Na is absorbed into the portal vein and carried in plasma as a free cation for uptake by extraintestinal cells through Na^+ channels (Zakon 2012) or transporters (e.g., SGLT2 in kidneys). Intestinal absorption of Na^+ is coupled with water movement and, therefore, plays an important role in preventing diarrhea (Field 2003).

Functions: The biochemical functions of Na are summarized as follows.

Regulation of Extracellular Osmolarity

In animals, the osmolarity of plasma is essentially the same as interstitial osmolarity or intracellular osmolarity. Because of its abundance, NaCl plays an important role in regulating extracellular osmotic pressure. Based on the concentrations of solutes in plasma, its osmolarity is calculated to be 302 mOsmol/L. However, NaCl is not entirely ionized to Na^+ and Cl^- in a physiological solution and has an osmotic coefficient of 0.93. Accordingly, the measured value of plasma osmolarity is 282 mOsmol/L (Guyton and Hall 2000). Of note, Na contributes to about 46% of plasma osmolarity. When the osmolarity of plasma is low, its water enters erythrocytes and other cells within the blood and also moves into interstitial fluid and cells, causing the lysis of blood cells and edema. In contrast, when the osmolarity of plasma is high, water enters from interstitial fluid and

cells into the blood, resulting in cell shrinkage and hypertension. Thus, Na is important for maintaining cell volume and viability, as well as blood volume.

Nutrient Transport Systems

The transport of glucose, amino acids, and various ions (e.g., I^-, Cl^-, and phosphate) across cell membranes requires the cotransport of Na^+ ions. The transport proteins recognize and bind both Na^+ and other specific substrates. ATP is usually required for Na^+-dependent transport processes [e.g., glucose absorption by the small intestine (Figure 10.4)]. The relatively high concentration of extracellular Na^+ is used to drive various nutrient transport systems. Thus, rehydration solutions usually contain NaCl and glucose, and possibly glutamine, glutamate, and glycine (Rhoads 1999; Wang et al. 2013).

Activity of Sodium Pump (Na^+-K^+-ATPase)

This enzyme is a membrane-bound protein and catalyzes the transport of Na^+ ions out of the cell in exchange for K^+ ions into the cell (Chapter 1). In the small intestine, Na-K-ATPase in the basolateral membrane is responsible for maintaining a low Na concentration in the enterocyte that is necessary to promote Na^+-coupled solute cotransport by SGLT1, Na^+-dependent amino acid transporters, and other Na^+-dependent transporters in the apical membrane, as noted previously. Importantly, the Na-K-ATPase is required for the functioning of nerves and muscle cells because the electrical impulses that occur in these cells depend on the relatively high concentrations of extracellular Na and intracellular concentrations of K (Pirkmajer and Chibalin 2016). Thus, an inhibition of Na-K-ATPase ouabain results in reduced ion transport by the small intestine and other tissues (e.g., placenta and endometrium).

Na^+/H^+ Exchange

Na^+/H^+ exchange (NHE), along with HCO_3^--based transport mechanisms, is a major mechanism for regulating intracellular pH in mammalian cells (Kato and Romero 2011). Thus, Na plays a role in regulating intracellular acid-base balance. As noted previously, in the small intestine, different isoforms of the NHE are localized in different membranes of the enterocyte to promote Na^+ absorption.

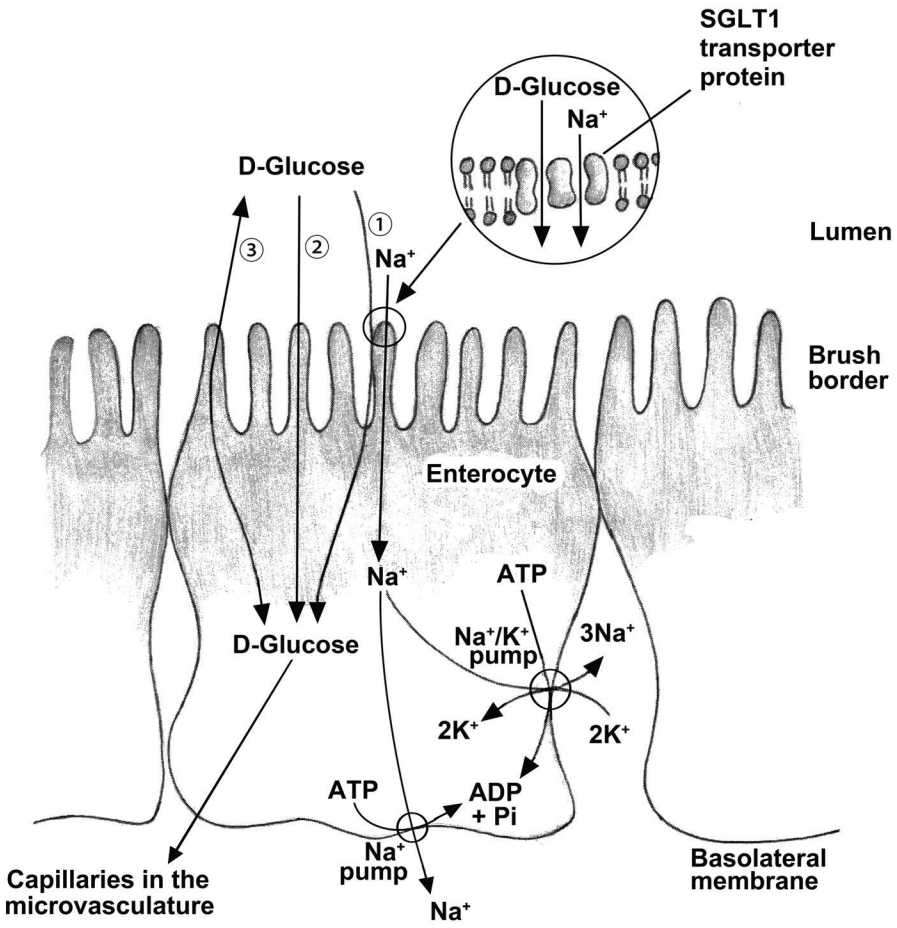


FIGURE 10.4 Intestinal absorption of glucose through Na^+-dependent mechanisms. In the small intestine, Na-K-ATPase in the basolateral membrane is responsible for maintaining a low Na concentration in the enterocyte that is necessary to promote Na-coupled solute cotransport by sodium-glucose cotransporter (SGLT1).

Blood Pressure

Water retention in the body, particularly the blood, is associated with its Na concentration. Concentrations of Na^+ in plasma affect (a) the entry of water into blood vessels by osmosis; (b) the renal reabsorption of Na+ and water; (c) the integrity of red blood cells and vascular endothelial cells; and (d) blood flow and hemodynamics (O'Shaughnessy and Karet 2004).

Action Potentials in Skeletal Muscle

Voltage-gated Na^+-channels provide the biochemical basis for electrical excitability in animals (Zakon 2012). Na^+ plays an important role in the contraction of skeletal muscle, which allows (a) mammals and birds to stand, walk, run, and do other physical work and (b) fish and crustaceans to swim (Figure 10.5). Under normal physiological conditions, the neuromuscular junction in skeletal muscle is activated by signals from the central nervous system. This is followed by the influx of extracellular Na^+ ions into skeletal muscle fibers through a voltage-gated Na^+ channel ($Na_v1.4$). The entry of Na^+ into the muscle fibers depolarizes them, which increases their membrane potential from –90 mV at the resting state to as high as +75 mV. This triggers the efflux of Ca^{2+} from the sarcoplasmic reticulum into the sarcoplasm (cytosol) to cause the contraction of skeletal muscle. To prevent the muscle from being perpetually contracted, the $Na_v1.4$ channel contains a fast inactivation gate to close the Na^+ pore very quickly after it opens, preventing the further entry of Na^+ ions into the cells. To balance intracellular cations, K^+ ions will exit the muscle fibers to repolarize them. This results in the pumping of Ca^{2+} from the contractile apparatus to the sarcoplasmic reticulum, thereby relaxing the skeletal muscle. Thus, Na^+ acts in concert with K^+ and Ca^{2+} to regulate muscle contraction.

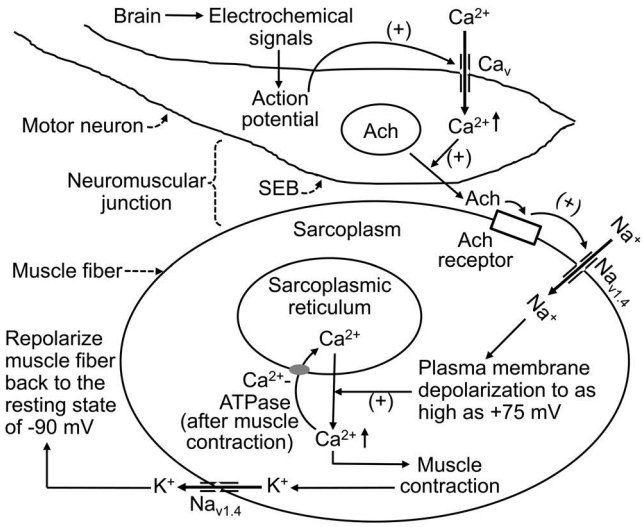


FIGURE 10.5 The role of ion channels in skeletal-muscle contractions. Electrochemical signals from the brain result in an action potential in the motor neuron, which causes Ca^{2+} influx and the releases of acetylcholine (Ach). The neurotransmitter binds to the Ach receptor in the muscle fiber, resulting in the influx of Na^+ through the voltage-gated Na^+ channel ($Na^+_{v1.4}$), depolarization of the muscle plasma membrane, Ca^{2+} efflux from the sarcoplasmic reticulum into the sarcoplasm (cytosol), and muscle contraction. Immediately after a muscle contraction, the sodium pore of the $Na^+_{v1.4}$ channel is closed and the potassium pore of the $Na^+_{v1.4}$ channel is opened to allow for K^+ efflux and repolarization of the muscle fiber to its resting state. Ca_v, voltage-gated Ca^{2+}-channel; $Na^+_{v1.4}$, voltage-gated Na^+-channel; SEB, synaptic end bulb; ↑, increase; and (+) activation.

Regulation of Food Intake

As noted previously, Na^+ is required for the intestinal absorption of dietary glucose, amino acids, and some minerals, thereby affecting the rates of the passage of digesta through the GIT. This, in turn, has a profound effect on food and DM intakes by animals (Chapter 12). In addition, sodium taste receptors (epithelial Na^+ channels) are present in the apical membrane of the taste cells (which are not neurons) in the taste buds of the tongue (Chandrashekar et al. 2010). Interaction of Na^+ with a sodium taste receptor allows Na^+ to move down a concentration gradient into the taste cell. This results in an increase in the intracellular concentration of Na^+ to depolarize the cell membrane, causing the opening of a voltage-gated Ca^{2+} channel on the basolateral membrane of the taste cell for the influx of Ca^{2+}. An increase in intracellular Ca^{2+} concentration causes the release of neurotransmitter molecules (in the form of synaptic vesicles), which are received by receptors on the nearby primary sensory neurons. A resulting electrical signal is transmitted along nerve cells to reach the brain's feeding center, which perceives and interprets the Na stimulus in the tongue. A low intake of dietary Na impairs the activation of Na taste receptors, leading to reduced food intake. However, an excessive intake of dietary Na [e.g., supplementing 0.54% Na as 1.37% NaCl to a corn- and SBM-based diet (containing 0.25% NaCl) for postweaning pigs] reduces food intake by animals (Rezaei et al. 2013). In practice, high levels of NaCl in free-choice protein or energy supplements are used to restrict their consumption by grazing cattle.

Deficiency symptoms: In animals, Na deficiency (also known as hyponatremia) occurs in many parts of the world, particularly in tropical areas of Africa, and the arid inland areas of Australia where pastures contain a low Na content (McDonald et al. 2011). A deficiency of Na results in a decrease in osmotic pressure, dehydration of the body, and hypotension. Symptoms of Na deficiency include reduced feed intake, low BW gain, reduced utilization of digested proteins and energy, nausea, headache, impaired egg production, eye lesions, and reproductive dysfunction (Lien and Shapiro 2007). Severe hyponatremia can cause confusion, seizures, coma, heart failure, and even death (Jones and Hunt 1983). Intravenous or oral administration of NaCl is effective in treating Na deficiency.

Excess: A major concern over Na nutrition in animals is the role of high Na intake in the development of hypertension due to increased water volume in blood vessels. High

concentrations of Na^+ in plasma (also known as hypernatremia) enhance both the passage of water into blood vessels and the circulating levels of angiotensinogen I (Kumar and Berl 1998). The latter undergoes limited hydrolysis in the vasculature of the lungs to become angiotensinogen II, which stimulates the release of aldosterone from the adrenal gland to augment renal reabsorption of Na^+ and water (Figure 10.6). Thus, high concentrations of Na^+ in plasma result in increased urinary losses of Na. In addition, excessive intake of dietary Na reduces the feed intake and growth of animals. Mammals, birds, fish, and crustaceans with hypernatremia exhibit hypertonicity, which causes such severe clinical manifestations as neurological and cardiovascular disorders due to cell shrinking and even death (Lall and Kaushik 2021; Kumar and Berl 1998; Rondon-Berrios et al. 2017). Adequate drinking water should be provided to animals in cases of high Na intake.

Sulfur (S)

A natural form of S known as *shiliuhuang* (a traditional Chinese medicine) has been recognized in China since the 6th century BC. In 1777, Antoine Lavoisier reported that S was an element but not a compound. In 1810, Joseph Gay-Lussac and Louis Thenardand published papers to confirm the elemental nature of S. The name of S is derived from the Latin *sulfur*, meaning "brimstone". Elemental S is a bright and yellow crystal at room temperature. It has a chemical reactivity with all elements, except for gold, platinum, iridium, tellurium, and the noble gases. In nature, S usually exists as sulfide and sulfate minerals.

Sources: Most of the S in animals occurs in organic forms, e.g., in S-containing amino acids (cysteine, cystine, methionine, homocysteine, and taurine), glutathione (GSH), and protein (Hoffer 2002). Disulfide compounds with S–S bonds confer the mechanical strength and insolubility of keratin, which occurs in outer skin, hair, and feathers (Chapter 4). Tissue and milk proteins have a ratio of N to S of ~ 13:1 to 15:1 (g/g). Wool, which is rich in cystine, contains ~4% S. Sulfur is also present in two vitamins (biotin and thiamin), coenzyme A, and chondroitin sulfate (a structural component of cartilage, bone, tendons, and walls of blood vessels). In contrast to animal products, S content is generally lower in most plants (Pond et al. 1995). However, plants grown on soils containing high S content or fertilized with S (e.g., ammonium sulfate) have an adequate or even relatively high amount of S and, in ruminants, may exacerbate a Cu deficiency, especially in the presence of Mo (Hardt et al. 1991). Most natural feedstuffs in some regions of the world (e.g., North America) contain sufficient S to meet the requirements of grazing ruminants (Greene et al. 2016). The concentration of inorganic S in water varies greatly among regions, ranging from <10 mg/L to 75 mg/L.

In the rumen of ruminants, the sources of S for bacteria are either inorganic sulfate or organosulfur substances (e.g., sulfonates, sulfate esters, or S-containing amino acids). The content of S in the diets of most ruminants must be between 0.18% and 0.24% of DM to allow for sufficient synthesis of S-containing compounds by ruminal bacteria (Suttle 2010). Sulfate is transported into the microbes through (a) ATP-binding cassette (ABC)-type transporters and (b) major facilitator superfamily-type transporters (Kertesz 2001). In contrast, ABC-type transporters are responsible for the uptake of all sulfonates and sulfate esters. The synthesis of these transporters is regulated by S supplies. Because of the limited synthesis of S-containing amino acids from S

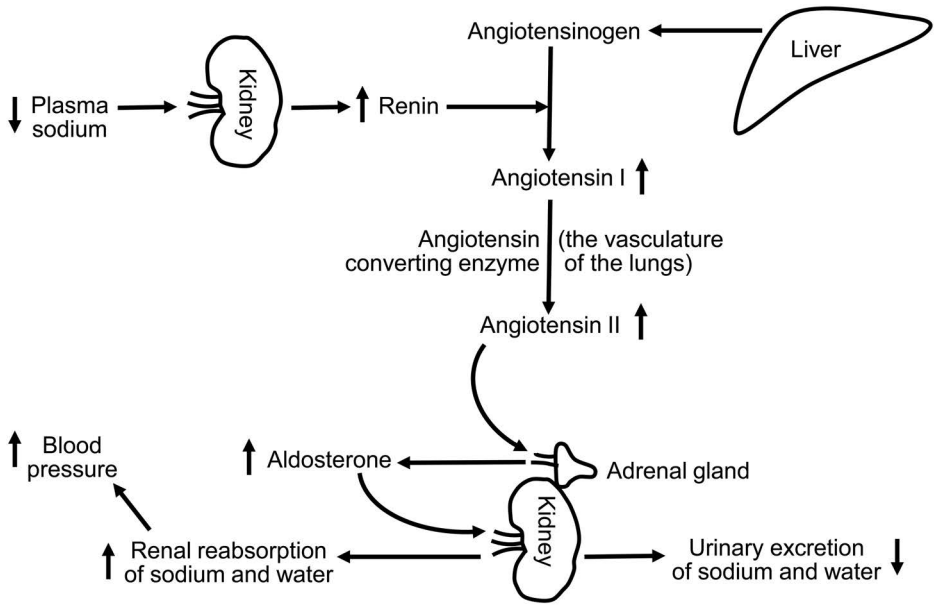


FIGURE 10.6 Regulation of renal water excretion by concentrations of sodium in plasma. When the concentration of sodium in plasma is low, the release of renin by the kidneys is augmented to enhance the production of angiotensin II and aldosterone. The latter promotes renal reabsorption of sodium and water in an attempt to restore the concentration of sodium in plasma to a normal value.

by nonruminants, inorganic S in the diet has virtually no nutritional value for pigs, poultry, dogs, cats, rats, fish, and crustaceans.

Absorption and transport: In animals, the organic forms of S are absorbed by the small intestine as cysteine, methionine, taurine, and GSH, which are transported among different tissues (Chapter 7). In ruminants, inorganic forms of S are used by ruminal microbes to synthesize cysteine and methionine, which are then used by the host (Chapter 7). However, the efficiency of S utilization for microbial synthesis of amino acids and proteins is affected by its sources (Suttle 2010). Absorbed S enters the portal circulation. Large amounts of S are stored as taurine in skeletal muscle, heart, and eyes and as GSH in tissues of the digestive and reproductive systems (Wu 2022).

Functions: Traditionally, little attention has been paid to the nutrition of the S element in nonruminants because these animals ingest S mainly in the form of protein or supplemental S-containing amino acids. In ruminants, S (including supplemental inorganic compounds) is used for microbial synthesis of cysteine and methionine, as noted preciously (Felix et al. 2014). This mineral, in the form of S-containing amino acids, is essential to the life of all organisms. Furthermore, the physiological levels of H_2S, a metabolite of cysteine and a gaseous signaling molecule in animal cells, play an important role in regulating neurotransmission, blood flow, immune response, and nutrient metabolism in the body (Wu 2022).

Deficiency symptoms: In animals, a deficiency of S is generally associated with protein deficiency, as described in Chapter 7. Nonruminants have requirements for dietary S-amino acids, but not the S element. However, both organic and inorganic forms of S can be used by ruminal bacteria, and dietary S is a limiting factor for microbial synthesis of cysteine and methionine (and therefore microbial protein synthesis) in ruminants (Suttle 2010). Replacement of some protein in ruminant rations with non-amino acid nitrogen (e.g., urea) may increase the risk of a deficiency of S because the substituted protein contains methionine and cysteine. For efficient utilization of urea by lactating cows, an N/S ratio (g/g) of 12:1 in the diet is adequate, and a ratio of 10:1 is acceptable for all other kinds of ruminants (NRC 1976). In addition, S supplementation must be considered for ruminant production in regions where S content in soil or plants is low.

Excess: Sulfate concentrations of <600 ppm (mg/L) in drinking water and the S content of <0.4% of DM in diets are generally safe for animals, including swine, poultry, and ruminants (Suttle 2010). Horses and rodents can tolerate 0.5% S (DM basis) in their diets. Because total S content is high (0.4–1.5% of DM) in distiller's dried grains with solubles (DDGS), which is currently often included in ruminant and nonruminant diets, producers are concerned with S toxicity. Excess S in diets and drinking water disturbs the acid-base balance in animals, reduces their feed intake and growth performance, and causes lesions of the gray matter in the brain (Drewnoski et al. 2014). Georgievskii et al. (1982) reported that concentrations of S (as Na_2SO_4) in the ruminal fluid ≥1 mg/ml are toxic to ruminal microbes as assessed by the rate of cellulose digestion. NRC (2005) suggested that the maximum tolerable limit for S in beef cattle diets was 0.3% in diets containing greater than 85% concentrate, or 0.5% in diets containing greater than 40% forages. Pogge et al. (2014) reported that feeding a high-S diet (containing 0.68% S as sodium sulfate) to growing steers for 28 days reduced the absorption of Cu, Mn, and Zn by the small intestine but had no effect on the absorption or retention of Ca, K, Mg, and Na. Ruminants (e.g., cattle, sheep, and goats) consuming elevated levels of S may display "stargazing" as a symptom of polioencephalomalacia due to necrosis of the cerebrocortical region of the brain (Gould et al. 2002). In ruminants, the toxicity of S occurs for the following reasons: (a) the formation of hydrogen sulfite (a toxic agent) by the gastrointestinal flora to kill bacteria and induce excessive production of reactive oxygen species to cause oxidative damage; (b) the production of sulfuric acid to reduce pH in the ruminal fluid and host tissues; and (c) the reduced absorption of other minerals (e.g., Cu, Zn, and Mn) by the small intestine because the ruminal reduction of S produces intermediates that form water-insoluble complexes with the minerals, such as copper sulfide (Cu_2S), zinc sulfide (ZnS), and manganese sulfide (MnS; Drewnoski et al. 2014). Furthermore, excesses of dietary S reduce the bioavailability of Se in both ruminants and nonruminants because Se and S atoms have similar physical and chemical properties. For example, the addition of sulfate to the low-Se diets of pregnant ewes increased the incidence of white-muscle disease in their lambs (Spears 2003). Thus, high S content is a major factor limiting the inclusion of DDGS in the diets of animals (e.g., feedlot cattle), and drinking water can also be a problematic source of S, especially in certain regions of the world (e.g., western ranges in the United States).

MICROMINERALS

Boron (B)

Boron was discovered by Joseph-Louis Gay-Lussac and Louis-Jaques Thénard (French chemists) and independently by Humphry Davy (a British chemist) in 1808. These scientists isolated boron by combining boric acid [$B(OH)_3$ or H_3BO_3] with K. The name of Boron is derived from the Arabic word *buraq* (meaning "white") and the Persian word *burah* (meaning "borax"). Boron is not a metal, but is a bioactive element and has an oxidation state of +3. The chemical properties of boron resemble those of Si (Cotton and Wilkinson 1988). In nature, boron is not present in an elemental form but exists predominantly as boric acid and the salts of polyboric acids (e.g., sodium tetraborate decahydrate, $Na_2B_4O_7{\cdot}10H_2O$, also called borax).

Sources: Boron is widely present in natural water, mainly as undissociated boric acid with some borate ions. Concentrations of boron in groundwater range widely from

TABLE 10.13
Content of Microminerals in the Whole Bodies of Animals

Micromineral	Content (mg/kg body weight)	Micromineral	Content (mg/kg body weight)
Iron	30–80	Cobalt	0.02–0.10
Zinc[a]	8–45	Nickel	0.04–0.05
Copper	2–3.5	Selenium	0.02–0.025
Molybdenum	1–4	Boron	<0.02
Manganese	0.48–0.56	Bromine	<0.02
Iodine	0.2–0.5	Vanadium	<0.02

Sources: Georgievskii et al. (1982); Mertz (1987).

[a] The content of zinc in the body varies markedly with animal species and physiological states, such as 8.5 mg/kg BW in newborn calves, 20 mg/kg BW in newborn piglets, and 45 mg/kg BW in laying hens (Georgievskii 1982; Herzig et al. 2009).

<0.3 to >100 mg/L, depending on the regions of the world (WHO 2003). The richest sources of boron are fruits, vegetables, the edible seeds (pulses) and leaves of legumes, and nuts. Meats, fish, dairy products, and most grains (e.g., corn and rice) are poor sources of this mineral. Boron occurs primarily as boron oxides (e.g., B_2O_3) and borate anions complexed with Ca^{2+} and pectin in plants and as Ca salts in the bones of animals. The content of boron in the bodies of animals is generally <0.02 mg/kg BW (Table 10.13). Concentrations of boron in the serum of healthy animals range from 6 to 100 μg/100 ml, depending on species and nutrient intake (Table 10.14).

Boric acid is not a proton donor. However, it accepts a hydroxyl ion (a Lewis acid) to form a tetrahedral anion $[B(OH)_4^-]$ (Hunt 2008). At pH 1, $B(OH)_3$ prevails as the dominant species [i.e., ~100% $B(OH)_3$]; at pH 9.25, the molar ratio of $B(OH)_3$ to $B(OH)_4^-$ is 1:1 (i.e., 50% each); and at pH 11, there is little or no $B(OH)_3$ in an aqueous solution (i.e., ~0%). Thus, within the lumen of the GIT, most ingested boron exists as boric acid for absorption.

$$B(OH)_3 + 2H_2O \rightleftharpoons H_3O^+ + B(OH)_4^- \quad [pK_a = 9.25 \text{ at } 25°C]$$

Absorption and transport: Following inhalation, boron is absorbed by the lungs. Dietary boron is rapidly and effectively absorbed by the small intestine via NaBC1 (Na^+-driven boron channel-1), with a $2Na+/1B(OH)_4^-$ stoichiometry (Liao et al. 2011; Park et al. 2004). In addition,

TABLE 10.14
Concentrations of Microminerals in the Serum of Animals

Animal	Fe	Cu	Zn	Iodine	Mn	Mo	Co	Borum	Se
					μg/100 ml				
Cat	59–169	33–105	50–110	—	3–5	—	2.3–2.8	50	28–64
Calf	120–160	80–120	100–150	6.1–7.4	3–5	1.0	1.0	36	14–22
Chicken[a]	102–516[a]	20–30	150–210	5–10	1.5–2.4	1.0–3.4	0.5–0.8	80	4–6
Cow (dairy)	100–184	50–120	80–150	4–8	1.5–2.5	0.3–0.6	0.5–0.7	52	4–8.5
Dog	97–263	42–100	70–200	4–9.2	0.4–0.74	0.55–1.2	0.1–0.3	10–40	16–45
Goat	90–195	102–203	70–130	8–10	1–2	1.3	0.1–0.35	12	6–16
Horse	110–200	100–190	60–70	2–4	1.7–2.5	2–5	0.7–2.2	6–80	14–24
Pig	56–195	100–250	65–160	5–8	4–5	0.6–1.4	1.7–4.0	33	10–20
Rat	152–220	67–120	100–115	4–5	2.5–4	0.5–2.5	2.7	20–100	44
Sheep	100–150	60–100	100–120	4–8	4–5	2.8–3	0.5–1.0	100	5.5–24

Source: Altunok et al. (2007); Armstrong et al. (2000); Cedeño et al. (2020); Dalia et al. (2017); Fu et al. (2022); Georgievskii et al. (1982); Giannetto et al. (2022); Huck and Clawson (1976); Hunt (1989); Kaneko (1980); Kincaid and Hodgson (1989); Laboklin (2024); Miyamoto et al. (2000); Mondal et al. (2010); Pilarczyk et al. (2010); Read et al. (1990); Ronis et al. (2015); Schweinzer et al. (2017); Smith and Sherman (2009); Yörük et al. (2007).

[a] Concentrations of iron in the serum of chickens (μg/100 ml) are: growing chicks = 102; nonlaying hens = 158; laying hens = 516.

Note: —, data are not available.

aquaporins (AQPs) 3, 7, 8, 9, and 10 can transport boric acid but not borate ions. Inside the enterocyte, boron exists as both boric acid and borate ions, which exit the cell across its basolateral membrane via NaBC1 and the AQPs. About 85% of ingested boron is absorbed into the portal circulation. Boron is transported in plasma mainly as uncharged boric acid in an unbound form (EPA 2004). Extraintestinal tissues, e.g., kidneys (Liao et al. 2011) and bones, take up boron from plasma via NaBC1 (a ubiquitous electrogenic Na^+-coupled borate transporter) and the AQPs (Park et al. 2004). Absorbed boron is excreted primarily in the urine as boric acid, and minor elimination pathways include feces, saliva, and sweat. In animals, boron is not absorbed across the intact skin.

Functions: Boric acid and borate can interact reversibly with organic molecules containing *cis*-hydroxyl groups, including AMP, pentoses (e.g., ribose and apiose), polysaccharides, pyridoxine, pyrimidine nucleotides, riboflavin, and vitamin C, thereby affecting cell metabolism (Uluisik et al. 2018). Thus, boron stimulates the synthesis of 17β-estradiol, testosterone, 25-hydroxy-vitamin D_3, calcitonin and osteocalcin, thereby playing an important role in reproduction, Ca and Mg metabolism, bone structure and mineralization, and muscle strength (Harris 2014; Nielsen 2008). In addition, boron plays an essential role in osteoblast growth and in maintaining the normal structure and function of bones and joints (Newnham 1994). Furthermore, there is evidence that boron is essential for the growth of bones and other connective tissues and that it stimulates intestinal Mg^{2+} absorption, reduces the production of inflammatory molecules, improves neurological function, and enhances wound healing in animals (Pizzorno 2015).

Deficiency symptoms: Boron is widely present in plant- and animal-sourced feedstuffs, as well as drinking water, and its deficiency is therefore rare in livestock, poultry, fish, and crustaceans under practical feeding conditions. However, boron deficiency in animals can be induced under experimental conditions. Dietary boron deficiency results in growth restriction, a loss of bone mass (osteoporosis), decreased electrical conductivity in the brain, impaired reproductive function, and oxidative stress in animals (Pizzorno 2015). For example, in chicks with a marginal intake of vitamin D, dietary deprivation of boron exacerbated gross bone abnormalities, impaired the initiation of cartilage calcification, and decreased chondrocyte density in the zone of the growth plate (Nielsen 2008). In addition, compared with a diet containing 0.31–1.85 mg boron/kg, boron deprivation or a low boron content in diets (0.045–0.062 mg/kg) resulted in testicular atrophy, low sperm counts, and sperm abnormality in male frogs, as well as ovarian atrophy and impaired oocyte maturation in female frogs (Fort et al. 2002). Furthermore, impaired embryogenesis and high rates of embryonic mortality occurred in zebrafish fed a boron-depleted brine shrimp diet and maintained for six months in water containing only 1.1 μg boron/L, compared to those maintained in water containing about 490 μg boron/L (Rowe and Eckhert 1999). At present, the biochemical mechanisms responsible for the beneficial effects of boron in improving bone growth and reproductive performance in animals are unknown.

Excess: High concentrations of boron bind NAD^+ and *S*-adenosylmethionine and also inhibit oxidoreductases, xanthine oxidase, glyceraldehyde-3-phosphate dehydrogenase, and aldehyde dehydrogenase (Hunt 2012). Thus, excess boron in diets and the environment is toxic to animals, as shown for rats (the dietary intake of 17.5 mg boron/kg BW/day; Kabu and Akosman 2013). Symptoms include low appetite, nausea, and weight loss; testicular cell damage and atrophy; infertility in males and females; oxidative stress and damages to tissues (e.g., the brain, liver, and kidneys); impaired growth of bones; and decreases in hemoglobin levels and splenic hematopoiesis.

Bromine (Br)

Br was independently discovered from a mineral water spring by Carl Jacob Löwig in 1825 and from seaweed by Antoine Balard in 1926. The name of Br is derived from the Ancient Greek "stench" based on its disagreeable smell. Its chemical properties are intermediate between those of Cl and I. Br is less reactive than Cl but more reactive than I. Chemically, Br is a strong oxidizing agent, reacting with many elements to complete its outer shell. Because the elemental Br is very reactive, it usually occurs as compounds [e.g., hydrogen bromide (HB) and sodium bromide (NaB)] in nature.

Sources: Seaweed is a rich source of Br. Marine organisms are the main source of organobromine compounds, which are synthesized by a unique algal enzyme, vanadium-dependent bromoperoxidase (Carter-Franklin and Butler 2004). Plants also contain this mineral. Bromide is present in groundwater at various concentrations (from <0.05 to 11 mg/L), depending on the regions of the world (Brindha and Elango 2013). Of note, the Dead Sea, in which plants and animals cannot survive, contains 0.5% bromide ions (Kesner 1999).

Absorption and transport: Following inhalation, bromine is absorbed by the lungs. Dietary Br is rapidly and effectively absorbed as bromide by the small intestine. There is evidence that bromide shares the Cl^- and I^- transporters for absorption from the intestinal lumen into the enterocytes (Abraham 2005; Mahajan et al. 1996). Bromide occurs inside the enterocyte as free ions and exits the cell across its basolateral membrane possibly via anion exchangers. Absorbed bromide enters the portal circulation, and bromine crosses blood cell membranes as an electrically neutral molecule. Bromide is transported as free ions in plasma. Extraintestinal tissues take up bromide possibly via Cl^- channels (e.g., Na^+-K^+-$2Cl^-$ cotransporters) and I^- transporters. In tissues, bromine is rapidly converted into bromide.

Functions: In eosinophils, peroxidase (a haloperoxidase) preferentially uses bromide over Cl^- as the cofactor to produce hypobromite (hypobromous acid), which kills multicellular parasites and some bacteria. In addition, animal studies indicate that Br is essential for the cross-linking

(maturation) of type IV collagen, which is predominantly a basal lamina component in arterioles, venules, and capillaries (McCall et al. 2014).

Deficiency symptoms: Because Br is present in feeds and drinking water, its deficiency is rare in mammals, birds, fish, and crustaceans under practical feeding conditions. Under experimental conditions, the dietary deficiency of Br impairs immunity, as well as the growth and function of the connective tissue in animals. For example, compared to goats fed a 20-mg Br/kg diet, goats fed a 0.8-mg Br/kg diet exhibited growth depression, reduced fertility, increased abortions, and reduced life expectancy, as well as structural abnormalities of the thyroid gland, heart, lungs, pancreas, and ovaries (Ceko et al. 2016).

Excess: Excess Br is an extreme irritant. In addition, it rapidly reacts with water to form hypobromous acid (HOBr) and hydrobromic acid (HBr). These products have deleterious effects on tissues. Furthermore, because Br shares the same transporter with I for intestinal absorption and transport by extraintestinal tissues, Br competes with I for metabolic pathways. Thus, excess Br interferes with the uptake and utilization of I by the thyroid gland for the synthesis of thyroid hormones. DNA damage in cells, neurological dysfunction, skin defects, and renal failure also occur in animals with chronic exposure to excess Br (Abraham 2005). Br is used as an antidote of I poisoning.

$$Br_2\,(\text{Bromine gas}) + H_2O \rightleftharpoons \text{Hypobromous acid (HOBr)} + \text{hydrobromic acid (HBr)}$$

Chromium (Cr)

Cr was discovered by Louis-Nicolas Vaquelin in 1797 through chemical studies of Siberian red lead (also known as crocoite) that was subsequently identified as (PbCrO4). He isolated Cr in 1798 by heating chromium oxide (CrO3) in a charcoal oven. Cr is relatively abundant in the earth's crust and naturally occurs in the form of compounds.

The name of Cr is derived from the Greek *chrōma*, meaning "color". Many Cr compounds are intensely colored, with Cr being in different oxidation states. Cr and Cr^{3+} are not considered toxic, but hexavalent Cr [Cr^{6+}, $Cr_2O_7^{2-}$], such as a chromate or dichromate, is a powerful oxidant and highly soluble in water. Almost all Cr is obtained from the single commercially viable ore chromite, chromium oxide ($FeCr_2O_4$). Biological roles of Cr were unknown until Schwarz and Mertz (1959) discovered that a dietary deficiency of Cr impaired glucose utilization in rats.

Sources: Seafoods, meats, liver, egg yolk, yeast, nuts, legumes, potatoes, and green leafy vegetables are good sources of Cr^{3+}.

Absorption and transport: In the stomach's acidic environment, the hexavalent form of Cr is reduced to Cr^{3+}. In the small intestine, the trivalent Cr is absorbed into the enterocytes via passive diffusion (Dowling et al. 1989). In the enterocytes, Cr is bound to chromodulin, which is a low-molecular-weight oligopeptide (Gly-Cys-Asp-Glu) synthesized by nearly all tissues, particularly the liver (Vincent 2000). One molecule of chromodulin binds four equivalents of chromic ions. Discharge of chromium-containing vesicles may be responsible for the export of Cr out of the enterocyte. Only 0.4–2.8% of ingested Cr is absorbed by animals (Harris 2014). Absorbed Cr enters the portal circulation. In the blood, Cr is carried by transferrin and albumin for transport to tissues, which take up Cr via transferrin receptor-mediated endocytosis. Cr is bound to chromodulin for storage in the cells.

Functions: In skeletal muscle, heart, adipocytes, and hepatocytes, Cr^{3+} binds tightly to chromodulin (an oligopeptide), forming the chromium-chromodulin complex. The latter binds the insulin receptor, thereby maintaining and amplifying the tyrosine kinase activity of the insulin receptor, as well as activating GLUT4 trafficking and AMPK (Hoffman et al. 2014; Vincent 2000). Thus, Cr plays an important role in regulating the metabolism of glucose and lipids in animals, including the stimulation of glucose uptake and utilization by skeletal muscle as well as fatty acid and cholesterol synthesis in the liver.

Under practical feeding conditions, dietary supplementation with organic Cr (e.g., 0.2 ppm) can enhance the number of live-born piglets in sows (Hagen et al. 2000), as well as the growth performance of pigs from weaning to market weight and their meat quality (He et al. 2023b). Similar results have been reported for the growth performance and meat quality of poultry (Feng et al. 2021). Some, but not all, studies with cows indicated that dietary Cr supplementation enhanced DM intake and immunity during the transition period and also milk production in the early, mid, and late stages of lactation (Lashkari et al. 2018). This discrepancy may be due, in part, to the composition of feed ingredients used in studies. For example, Sadri et al. (2009) reported that supplementation with Cr-methionine (~10 mg Cr/day) increased prepartum DM intake when barley, but not corn, was used as the grain source. Nonetheless, Cr picolinate (Phibro Animal Health) has been permitted by the U.S. Food and Drug Administration (FDA) for supplementation in swine diets at a level up to 0.20 ppm Cr since 1996. In addition, the FDA has permitted Cr propionate (Kemin Industries) for supplementation in swine diets at a level of 0.20 ppm Cr since 2000 and cattle diets at a level of up to 0.50 ppm Cr since 2009. Furthermore, Cr propionate was approved by the FDA as a feed additive to the diets of broilers in 2016, horses in 2019, and turkeys in 2024.

Deficiency symptoms: In animals, the insufficient intake of dietary Cr is associated with reduced insulin sensitivity, elevated glucose concentrations in plasma, and dyslipidemia. Thus, Cr deficiency can predispose individuals to metabolic syndrome. Animals (including humans) maintained on chromium-free total parenteral nutrition support have a high risk for the development of Cr deficiency (Wallach 1985).

Excess: Cr toxicity in animals can occur after acute or chronic ingestion of excess Cr (Harris 2014). Symptoms include elevated concentrations of 8-hydroxydeoxyguanosine

(an indicator of DNA damage) and peroxidized lipids in plasma. Cultured cells exposed to toxic Cr levels exhibit chromosomal damage, DNA mutation, mitochondrial damage, oxidative stress, and induction of apoptosis.

Cobalt (Co)

Georg Brandt is credited with discovering Co in 1735. Its name is derived from the German word *Kobalt,* meaning "goblin", a superstitious term used for the ore of Co. However, centuries earlier, Co was used in Egyptian sculptures and Persian jewelry. The discovery that Co is present in the corrin ring of vitamin B_{12} pointed to an important role for this mineral in animal nutrition.

Sources: Seafoods (e.g., clams, oysters, scallops, and shrimp), meats (beef, pork, and poultry), liver, milk, yeast, and leafy vegetables are good sources of Co. The content of Co in plants varies with their species and the content of Co in soil. The inorganic forms of Co include cobalt arsenide, cobalt arsenosulfide, cobalt oxide, cobalt sulfide, cobalt chloride, and cobalt sulfate (WHO 2006). The organic form of Co in plants and animals is primarily vitamin B_{12}. Ruminants obtain Co from both forages and soil. However, the content of Co in plants depends on their species (more Co in legumes than in cereals), soil type, and vegetative stages.

Absorption and transport: Inorganic Co^{3+} and Fe^{2+} have identical electronic configurations and share a common intestinal absorptive pathway (Naylor and Harrison 1995). Fe, which is usually more prevalent than Co in foods, out-competes Co for intestinal absorption. The absorption of Co as vitamin B_{12} occurs via complex pathways involving specific binding proteins, as described in Chapter 9. Absorbed Co enters the portal circulation. In the blood, vitamin B_{12} and its related metabolites are carried, respectively, through albumin and specific binding proteins into tissues, which take them up through Fe and vitamin B_{12} transporters, respectively. Assimilation of dietary Co by nonruminants is usually low (e.g., birds 3–7%; pigs 5–10%; and horses 15–20%; Georgievskii et al. 1982).

In the rumen, bacteria take up Co through Co transporters (COTs) 1 and 2 (Kobayashi and Shimizu 1999), as well as an ABC-type Co transport system (Cheng et al. 2011). COT1 may play an important role in alleviating the toxicity of excess Co via its intracellular sequestration or compartmentalization in microbes. COT2, also known as GRR1, is responsible for glucose-dependent divalent cation transport. Bacteria use Co to synthesize vitamin B_{12}.

Content in the body and tissues: The content of Co in the body of animals (μg/kg BW) is as follows: adult cows, 83; newborn pigs, 110; and newly hatched chicks, 60 (Georgievskii et al. 1982). The content of Co in the tissues (μg/kg fresh weight) of farm animals is as follows: liver, 30–100; kidneys, 20–30; bones, 20–30; heart, 12–35; spleen, 20–40; skeletal muscle, 3–7; and pancreas, 10–30. For example, the content of Co in tissues (μg/kg DM) of sheep fed a Co-adequate regular diet is as follows: liver, 150; spleen, 90; kidneys, 250; heart, 60; and pancreas, 110 (Georgievskii et al. 1982).

Functions: Methyl cobalamin and 5'-deoxyadenosyl cobalamin are required by methionine synthetase and methylmalonyl-CoA mutase as cofactors, respectively (Chapter 9). Methionine synthetase plays an important role in reducing the concentrations of homocysteine (an oxidant) in the liver and plasma, whereas methylmalonyl-CoA mutase is essential for the synthesis of glucose from propionate. The latter is crucial in maintaining glucose homeostasis in ruminants. In eukaryotes and prokaryotes, Co is a cofactor of methionine aminopeptidase, which removes N-terminal methionine from polypeptides to render them biologically active (Arfin et al. 1995). In yeast, methionine aminopeptidase uses either Co or Zn as a cofactor.

Deficiency symptoms: Various parts of the world have a low content of Co in the soil, and its forage vegetation is deficient in Co (<0.88 mg/kg DM; Georgievskii et al. 1982). Animals grazing in these regions have a low amount of Co in the body (30–60 μg/kg BW). Symptoms of Co deficiency in animals are similar to those of vitamin B_{12} deficiency (Chapter 9). Co deficiency can be treated by dietary supplementation with Co and vitamin B_{12} in ruminants and nonruminants, respectively.

Excess: Toxic threshold levels of dietary Co intake differ among animals (mg/kg BW/day): 0.5 for calves, 1.0 for cows, 2–3 for sheep, and 3.0–3.5 for chicks (Georgievskii et al. 1982). Growing-finishing pigs can tolerate up to 200 ppm Co in corn- and SBM-based diets containing 82 to 178 ppm Fe (Huck and Clawson 1976). Co excess results in reduced feed intake, growth restriction, dermatitis, cardiomyopathy, and goiter (Harris 2014). The latter occurs because Co reduces the uptake of I and, therefore, thyroid hormone synthesis by the thyroid gland.

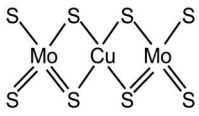
Copper (Cu)

Cu was discovered in the Middle East around 9000 B.C. It is a reddish-orange, soft, malleable, and ductile metal. Cu is involved in oxidation-reduction reactions and occurs in the cuprous (Cu^{+}) and cupric (Cu^{2+}) states. Cu does not react with water, but it does slowly react with atmospheric oxygen to form a layer of brown-black copper oxide. O'Dell et al. (1961) found that dietary Cu deficiency resulted in anemia, reduced growth, and aortic rupture in chicks. This seminal work extended the physiological role of Cu beyond its action as an electron donor or acceptor in the mitochondrial electron transport system.

Sources: Cu is present in most foods. Seeds, seed byproducts, nuts, whole grain cereals, legumes, soybean products, dark chocolate, and leafy green vegetables are good sources of Cu. The content of Cu in plants is greatly affected by Cu content in soil. Meats (including pork, poultry, and beef), liver, and eggs are rich in Cu. In contrast, milk contains relatively low concentrations of Cu. Feed additives of Cu include copper sulfate and copper proteinate.

Absorption: In the stomach, Cu salts are dissociated to Cu ions, a small amount of which is absorbed by the gastric epithelial cells. In the lumen of the small intestine and the apical membranes of its enterocytes, Cu^{2+} is reduced to Cu^{1+} by Cu reductase (a member of the family of metalloreductases called Steap proteins) and vitamin C; both Cu^{1+} and Cu^{2+} are absorbed into the enterocyte through Cu transporter-1 and DCT1, respectively. Inside the enterocyte, Cu is transported by GSH to metallothionein for storage and by three Cu chaperones to its target sites: by Cox-17 to mitochondria, by Cu chaperone for cytosolic superoxide dismutase (CCS) to the enzyme, and by ATOX-1 to ATP7a. The latter is a Cu-ATPase that is embedded in the surface of vesicles of the trans-Golgi network (TGN). The TGN vesicles move Cu to the basolateral membrane of the enterocyte for discharge into the lamina propria, with the exit being powered by ATP hydrolysis. Dietary Cu is absorbed primarily by the upper small intestine into the portal vein.

Many substances present in the diet affect the bioavailability of Cu. Specifically, Cu from inorganic compounds is poorly absorbed in ruminants, with only 5–10% of ingested Cu being absorbed and retained. In nonruminants (e.g., swine and poultry), the absorption of inorganic Cu is much higher (Table 10.10). In both ruminants and nonruminants, most of the fecal Cu is unabsorbed dietary Cu, but some fecal Cu is derived from biliary excretion from the liver. The bile is the major pathway for the secretion of endogenous Cu. Dietary Mo also interacts with S to affect Cu absorption and retention in the body. Specifically, sulfite (formed from sulfate or organic S compounds by microbes) reacts with Mo to form thiomolybdate, which then binds with Cu to form an insoluble copper thiomolybdate (tetrathiomolybdate, $CuMo_2S_8$), thereby limiting the absorption of dietary Cu (Pitt 1976). Tetrathiomolybdate may also affect Cu metabolism in animals. Note that sulfide is produced by ruminal microbes through the reduction of sulfate and the degradation of S-amino acids (Spears 2003). The presence of Na_2S and roxarsone as well as excessive cysteine and vitamin C in diets reduce the intestinal absorption of Cu in nonruminants (Baker 2001), but related data on ruminants are not available.



Tetrathiomolybdate

Baker (1999) reported that cupric oxide (CuO), which contains 80% Cu and, therefore, occupies less "space" in a trace-mineral premix, is not soluble in water and is unavailable for absorption by the small intestine. The bioavailability of Cu in CuO is not significantly different from zero in nonruminants. Good sources of utilizable forms of Cu in animal diets are Cu_2O (88% Cu), CuCl (64.2% Cu), $CuCO_3 \cdot Cu(OH)_2$ [known as alkaline Cu carbonate; 57% Cu], $CuCl_2$ (47.3% Cu), cupric acetate (35.0% Cu), and $CuSO_4 \cdot 5H_2O$ (25.5% Cu; Cromwell et al. 1998; Suttle 2010).

Suckling rats at 4 and 16 days of age absorb 100% and 63% of ionic Cu, respectively, and 93% and 44% of biliary Cu, respectively (Mistilis and Mearrick 1969). The high rate of Cu absorption during the first days after birth is likely mediated by the pinocytosis of protein-bound Cu. Interestingly, 21- to 28-day-old rats weaned at 20 days of age absorbed only ~10% of dietary Cu, possibly due to a cortisol-mediated decrease in intestinal Cu transport (Mistilis and Mearrick 1969). Thus, both age and weaning affect intestinal digestion and absorption of Cu. Likewise, lambs absorb 71% and 47% of Cu in milk at 28 and 14 days before weaning but only 11% of dietary Cu at 15–42 days after weaning possibly due to complex interactions of Cu with fiber and other minerals in the ruminal environment (Suttle 1975). Thus, adult sheep retain only a very small proportion (3–5%) of the ingested Cu. In postweaning pigs and post-hatching poultry fed corn- and SBM-based diets, the absorption rate of Cu in $CuSO_4$ is low to medium, ranging from 30% to 55% depending on the dietary content of phytate, Fe, Zn, Mo, and other microminerals that can have antagonisms with Cu (Baker et al. 2003; Ketata et al. 2024). Although vitamin C can facilitate the conversion of Cu^{2+} into Cu^{1+} in the lumen of the small intestine for absorption, an excess of this vitamin is known to reduce the solubility, concentration in the luminal liquid phase, and absorption of dietary Cu (Van den Berg et al. 1994). Additionally, Cu ions, along with Zn and Mo, can be trapped as insoluble hydroxide precipitates in the lumen of the gut and, therefore, are unavailable for intestinal absorption. This explains why the bioavailability of Cu in plant-sourced feedstuffs fed to postweaning mammals and post-hatching poultry is not high. In both nonruminants and preruminants, moderate excesses of dietary Fe do not affect intestinal Cu absorption (Baker 2001; Spears 2003). However, in ruminants, moderate excesses of dietary Fe substantially reduce intestinal Cu absorption due to complex interactions of minerals in the rumen (Spears 2003).

Transport in the blood: In the portal blood, 90% of Cu is bound to plasma ceruloplasmin (binding primarily Cu^{2+}) for transport to tissues, and the remaining Cu is bound to plasma albumin. Ceruloplasmin, which was discovered in the 1940s by Holmberg and Laurell (1948), is a single polypeptide chain (MW 132,000) synthesized and released by the liver. The large size of this protein prevents its entry into the glomerular filtrate, and thus the loss of the bound Cu in the urine. Absorbed Cu enters the portal circulation. About 75% of Cu in the portal plasma is taken up by the liver via CTR1, and the remainder moves into the peripheral circulation. Within the liver, 20% of the extracted Cu are excreted as a component of the bile into the GIT, and 80% is exported to the periphery circulation for distribution to extrahepatic tissues. The trafficking of Cu in hepatocytes is essentially the same as that in enterocytes, except that (a) hepatocytes express ATP7b (a Cu-ATPase) rather than ATP7a; (b) the ATP7b releases Cu into the bile and the blood; and (c) hepatocytes also release Cu in its ceruloplasmin-bound form into the blood. Before being taken up into peripheral

TABLE 10.15
Copper Metalloenzymes and Binding Proteins

Enzyme or Protein	Function
Amine oxidase	Catabolism of amines including histamine and polyamines
Ascorbate oxidase	Converts L-ascorbate and O_2 into dehydroascorbate and H_2O
Cytochrome c oxidase	Mitochondrial respiratory chain
Cytochrome oxidase	Mitochondrial respiratory chain
Dopamine ß-hydroxylase	Conversion of dopamine into norepinephrine
Ceruloplasmin (a ferroxidase)	Multi-copper oxidase in plasma
Hephaestin (a ferroxidase)	Multi-copper oxidase that facilitates the export of Fe across the basolateral membrane of the enterocyte; essential for the intestinal absorption of dietary Fe into the bloodstream
Lysyl oxidase	Hydroxylation of lysine residues in collagen
Nitrite reductase (Cu, bacteria)	NO_2^- (nitrite) + $2e^-$ + $2H^+$ → NO (nitric oxide) + H_2O
PAM	Amidation of the glycine residue in neuropeptides
Superoxide dismutase (cytosol)	Requires Cu^{2+} and Zn^{2+}. Removal O_2^-
Tyrosine oxidase	Melanin synthesis
Uricase (not in humans)	Uric acid → Allantoin

Note: PAM, peptidylglycine α-amidating monooxygenase

tissues, plasma Cu^{2+} concentration is reduced by vitamin C to Cu^+, which is then transported across the plasma membrane of animal cells via CTR1. Within animal cells, Cu is stored in a metallothionein-bound form (Table 10.15).

Content in the body and tissues: About two-thirds of the body's Cu is present in the skeleton and muscles. Specifically, the content of Cu in the tissues of farm animals (mg/kg fresh weight) is as follows: whole blood, 0.8–1.2; liver, 8–100; spleen, 1.2–12; kidneys, 2–4; heart, 3–4; skeletal muscle, 2–3; brain, 0.5–5.3; and bones, 3.7–40, depending on dietary Cu intake (Georgievskii et al. 1982). The concentrations of Cu in plasma (mg/L) are as follows: dogs, 0.67; pigs, 2.15; cattle, 0.91; and sheep, 1.15. Because of an increase in its mobilization from tissues, concentrations of Cu in serum are elevated when animals are subjected to infectious diseases and other stressful conditions (Orr et al. 1990).

Concentrations of Cu in plasma or serum are tightly regulated by the dietary intake of Cu and Zn, the rates of absorption of Cu by the small intestine through specific transporters, concentrations of Cu-binding and transport protein (ceruloplasmin) in plasma, and abundances of the intracellular Cu-binding protein (metallothionein) and Cu-containing enzymes (e.g., cytochrome oxidases and superoxide dismutase). In response to low Cu intake, the rates of intestinal Cu absorption and Cu mobilization from tissues (e.g., liver) are increased, but the rates of Cu excretion through the intestine and kidneys are reduced to compensate for the dietary deficiency. Thus, mild or moderate deficiencies of dietary Cu may not affect its circulating levels in animals. Conversely, animals can adapt to an increase in dietary Cu intake by reducing the rates of its intestinal absorption (due to reduced expression of intestinal Cu transporters) while increasing the rates of both Cu storage in tissues and Cu excretion (due to increased secretion of Cu from the liver into the bile) to maintain homeostasis. Furthermore, the mobilization of Cu from ceruloplasmin and tissues for its entry into the blood is stimulated under the conditions of inflammation and infections as a defense mechanism against invading pathogens. Therefore, concentrations of Cu in the liver, but not in plasma or serum, are a good measure of Cu nutritional status in animals. Under conditions of severe Cu deficiency, concentrations of Cu in plasma or serum may be substantially reduced.

Functions: As a cofactor of enzymes, Cu participates in redox and hydroxylation reactions with important physiological functions. These roles of Cu are highlighted in the following sections.

Mitochondrial electron transport system. The oxidation state of Cu can shift between Cu^+ and Cu^{2+}. Thus, Cu acts as an electron donor or acceptor in cytochrome c and cytochrome oxidase of the mitochondrial electron transport system. These oxidation-reduction reactions are essential for ATP production in animals.

Growth and Development of Connective Tissue

Cu is an essential cofactor of lysyl oxidase (Table 10.15). This enzyme catalyzes the cross-linking of collagen and elastin that is essential for the formation of mature extracellular proteins in connective tissue. Thus, through the action of lysyl oxidase, Cu maintains the strength of connective tissue in various organs (e.g., the heart, skeletal muscle, and blood vessels) and supports bone formation.

Antioxidative Reactions

Cu is a cofactor of a cytosolic antioxidant enzyme, Cu-Zn superoxide dismutase Zn [Cu,Zn-SOD] (Table 10.15). This

enzyme catalyzes the conversion of the superoxide (O_2^-) free radical into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2). The latter is degraded by catalase to form water. High levels of superoxide, which is produced by reactions involving oxygen metabolism, irreversibly oxidize macromolecules and cell membranes, resulting in cell and tissue damage. Therefore, Cu, like Zn, is required for antioxidant defense in animal cells.

$$O_2^- + Cu^{2+} \rightarrow Cu^+ + O_2$$
$$O_2^- + Cu^+ + 2\ H^+ \rightarrow H_2O_2 + Cu^{2+}$$
$$\text{Net reaction: } 2\ O_2^- + 2\ H^+ \rightarrow O_2 + H_2O_2$$

Fe Metabolism

Ferroxidase oxidizes Fe^{2+} to Fe^{3+}, which is carried by transferrin in the blood to tissues, such as the liver and bone marrow. Four different proteins that possess ferroxidase activity require Cu as their cofactor for their enzymatic activities in animals: the circulating ceruloplasmin, the membrane-bound ceruloplasmin (also called glycosylphosphatidylinositol-anchored ceruloplasmin), hephaestin (in the intestine, spleen, placenta, and kidneys), and zyklopen (in multiple tissues, including the placenta and mammary gland). Cu deficiency results in Fe overload in selected tissues, including the intestine, liver, brain, and retina. This is consistent with the notion that Cu plays an important role in Fe metabolism in animals.

Other Enzymes

Some enzymes also depend on Cu for their biological activities (Table 10.15). For example, as a cofactor of dopamine ß-hydroxylase, Cu is required for the synthesis of norepinephrine (a neurotransmitter) from dopamine (Figure 10.7) and, therefore, for neurological function (Mertz 1987). Likewise, tyrosine oxidase depends on Cu for enzymatic activity to catalyze melanin synthesis from tyrosine; thus, Cu is necessary for pigment formation. In addition, ascorbate oxidase converts L-ascorbate and O_2 into dehydroascorbate and H_2O, thereby participating in vitamin C metabolism. Furthermore, Cu is the cofactor of peptidylglycine α-amidating monooxygenase, which amidates the glycine residues in neuropeptides to render the peptides more hydrophilic and biologically active. These signaling peptides are crucial for neurological development and function.

$$\text{Peptide-C(O)NHCH}_2\text{CO}_2^- + O_2 + 2\ [H] \rightarrow$$
$$\text{Peptide-C(O)NH}_2 + \text{CH(O)CO}_2^- + H_2O$$

Antimicrobial Effects

Cu has an antimicrobial effect that inhibits the growth of molds, fungi, algae, and harmful microbes (Dollwet and Sorenson 1985). Indeed, Cu had been used as an antibiotic long before the concept of microbes became understood in the nineteenth century. The underlying mechanisms may include structural alteration and dysfunction of proteins in

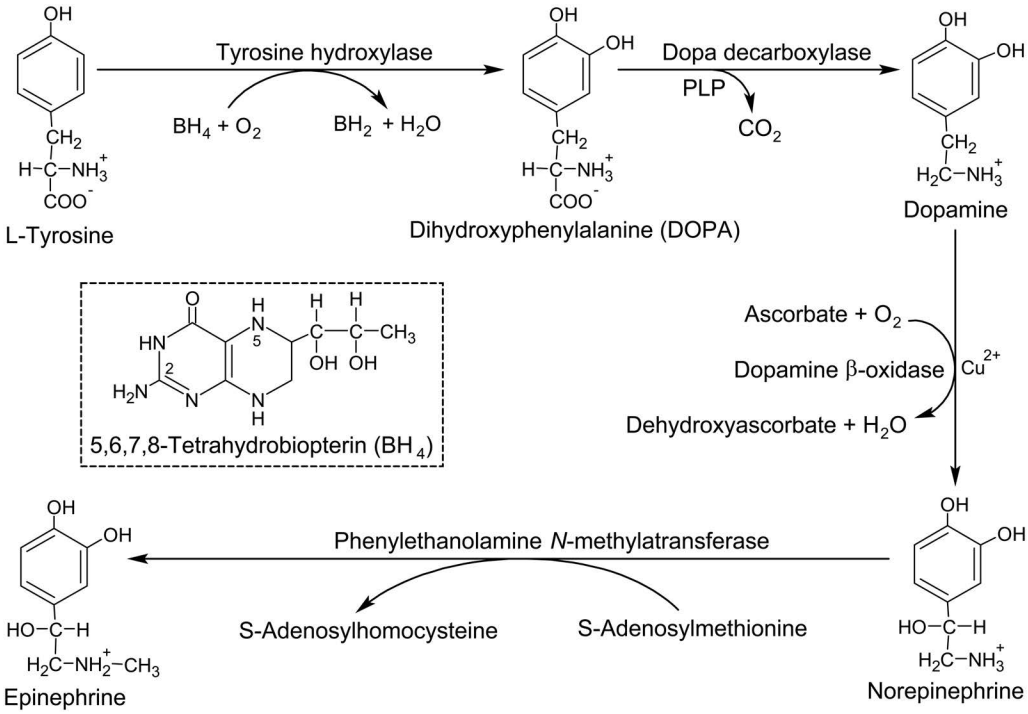


FIGURE 10.7 The synthesis of norepinephrine from dopamine via copper-dependent dopamine ß-hydroxylase in animals. Norepinephrine is both a hormone and a neurotransmitter. This pathway occurs in (a) the adrenal medulla, which releases norepinephrine into the blood as a hormone; and (b) noradrenergic neurons of the central and sympathetic nervous systems, which use norepinephrine as a neurotransmitter.

bacteria and viruses; production of hydroxyl radical and radical-Cu complexes to inactivate microbes; interference of microbial metabolism; antagonizing the actions of other essential elements, such as Zn and Fe; and interaction with lipids to cause their peroxidation and opening holes in the cell membranes of bacteria. Of note, dietary supplementation with either copper sulfate or tribasic copper chloride (Cu_2OH_3Cl) [200 ppm Cu] prevents diarrhea and improves growth in postweaning piglets (Cromwell et al. 1998).

Pigmentation

Cu is a reddish-brown color, which contributes to the pigment of feathers in certain birds. For example, turacin, a red porphyrin pigment in the feathers of the bird family Musophagidae, the turacos, contains 7% Cu (Blumberg and Peisach 1965). The porphyrin (uroporphyrin III) is insoluble in water, but is soluble in an alkaline solution. In crustaceans (e.g., shrimp, crabs, and lobsters), Cu is an essential component of hemocyanin (a copper-containing protein), which accounts for 90–95% of total plasma proteins in their hemolymph. Hemocyanin is not typically found inside hemocytes but is suspended in the hemolymph to carry oxygen to tissues (Conant et al. 1933). Oxygenated hemocyanin (oxyhemocyanin) has an intense blue color, whereas deoxygenated hemocyanin (deoxyhemocyanin) is colorless. In response to infections, some hemocyanin in plasma is internalized by hemocytes via endocytosis. In addition to its primary function in oxygen transport, hemocyanin plays an important role in invertebrate innate immunity through the following mechanisms: (a) possessing phenoloxidase-like enzymatic activity to convert tyrosine into melanin, which acts as a barrier against pathogens, aids in their encapsulation and killing, and facilitates wound healing; (b) interacting with innate immune receptors (e.g., Toll-like receptors) to produce cytotoxic molecules and activate proinflammatory responses that help eliminate the pathogens; (c) killing microbes through agglutination and the production of antimicrobial peptides; and (d) inhibiting viral replication via binding to specific viral surface glycoproteins, thereby preventing the entry of the virus into host cells.

Heme Synthesis

Cu is a cofactor of cytochrome oxidase, which plays an important role in the reduction of Fe^{3+} to Fe^{2+} in the mitochondrial electron transport system. Ferrochelatase requires Fe^{2+}, but not Fe^{3+}, as a cofactor to catalyze the formation of heme from protoporphyrin IX. In addition, ferrochelatase depends on Cu for enzymatic activity (Wagner and Tephly 1975). Thus, the rate of heme synthesis positively correlates with cytochrome oxidase activity in cells (including hepatocytes), and dietary Cu deficiency impairs heme synthesis in animals.

Deficiency Symptoms

Dietary Deficiency of Cu

Any animal is deficient in Cu when its diet contains inadequate Cu. Abnormal wool (easily broken) occurs in sheep raised on a diet deficient in Cu, as occurs in Australia, where the soil has a low Cu content. This nutritional disease can be treated by increasing dietary intake of Cu. Cu deficiency in animals (particularly sheep) can also be triggered by the high intake of dietary Mo (Dick 1952). Much evidence shows that Cu deficiency is widespread in grazing ruminants, particularly in tropical regions, because of the low Cu content in diets and the relatively high content of antagonistic minerals (e.g., Mo, S, and Fe; Suttle 2010). Likewise, nonruminants, which are fed corn- and SBM-based diets without Cu supplementation and housed in confined pens, are susceptible to Cu deficiency. General metabolic disorders and diseases resulting from dietary Cu deficiency include reduced feed intake and growth restriction; impaired Fe utilization and anemia; oxidative stress; connective tissue degeneration, bone mineral loss, and cardiovascular dysfunction; thinning and weak hair, skin abnormalities, and pale skin pigment; impaired immunity; demyelination of the central nervous system, destruction of the cerebral white matter, lack of coordination of the legs, seizures, as well as lesions of the brain stem and spinal cord motor tracts (Jones and Hunt 1983).

Genetic Diseases

Severe defects in Cu transport occur in two genetic diseases: Wilson's disease (which was first recognized by an English physician in 1906) and Menkes disease (which was identified by John Menkes in 1960). Both diseases result from mutations in a gene encoding a Cu-binding P-type ATPase and are characterized by a low concentration of Cu in plasma.

1. *Menkes disease*
   In normal subjects, a Cu-binding P-type ATPase is expressed in all tissues, except the liver. This enzyme is responsible for directing the efflux of Cu from cells. In Menkes disease, the efflux of Cu from the basolateral membrane of the enterocyte into the portal vein is impaired (Figure 10.8). This results in (a) the accumulation of Cu in the intestine and the kidney; (b) decreases in concentrations of Cu in the liver, serum, and brain, as well as ceruloplasmin concentration in serum; (c) a deficiency of Cu-dependent enzymes; and (d) abnormal (easily broken) and steely hair (kinky hair; Patel et al. 2017). Despite the accumulation of Cu in the intestine and kidneys, the activities of many Cu-dependent enzymes are reduced because of a defect in the incorporation of Cu into the apoenzymes. Menkes disease is X-linked and affects only male infants. Infants with this disease exhibit disorders in the nervous system (mental dysfunction), connective tissue, and vasculature, as well as hypotonia, seizures, and failure to thrive. No effective treatment is available for treating Menkes disease. However, treatment with daily injections of Cu may ameliorate the symptoms of Menkes disease (Kaler et al. 2008).

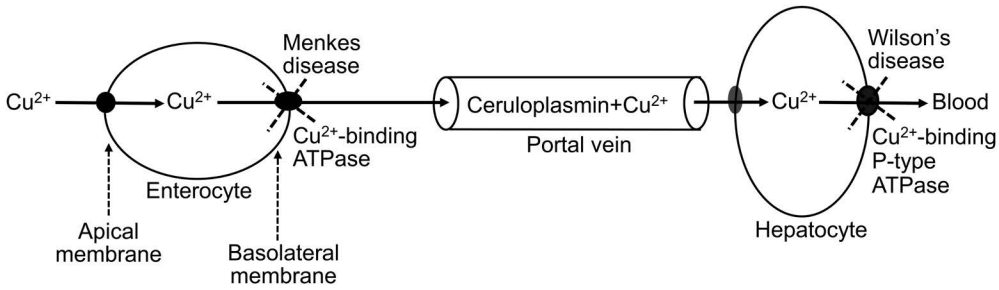


FIGURE 10.8 Menkes disease and Wilson's disease. In Menkes disease, the efflux of copper from the basolateral membrane of the enterocyte into the portal vein is impaired because of a deficiency in Cu-binding ATPase. In Wilson's disease, the efflux of copper out of hepatocytes into the blood is impaired because of a deficiency in Cu-binding P-type ATPase (ATP7b).

2. *Wilson's disease*
   The liver has a different type of Cu-binding P-type ATPase (ATP7b), which is responsible for directing the efflux of Cu out of hepatocytes into the blood. In Wilson's disease, this ATPase is defective in (a) the liver, which inhibits Cu efflux from the liver into the bile or blood circulation (Figure 10.8); and (b) the kidneys, brain, placenta, red blood cells, and retina. This results in (a) the accumulation of Cu in the liver, brain, kidney, red blood cells, retinal cells, and urine; and (b) decreases in serum Cu^{2+} and ceruloplasmin concentrations. The consequences are hepatic and neurological damage. Patients with Wilson's disease also have a green or golden pigmented ring around the corner of their eyes, due to the deposition of Cu in the cells (Rodriguez-Castro et al. 2015). The treatment of Wilson's disease may include low dietary Cu along with lifelong administration of D-penicillamine (a cysteine analogue), which chelates Cu and depletes the body of the excess of this mineral (Rodriguez-Castro et al. 2015). Zn salts are also supplemented to affected patients to attenuate the intestinal absorption of Cu and induce expression of metallothionein to bind Cu, thereby reducing the concentration of free Cu in the blood.

Excess: Cu is a strong oxidizing agent. Thus, its excess results in stomach pains, nausea, and diarrhea, as well as tissue injury (including oral lesions in response to excess $CuSO_4$ intake) and disease in animals (Formigari et al. 2007). After entering the blood, Cu partitions into red blood cells and plasma, with a 5- to 10-fold greater increase in its concentration in red blood cells than in plasma (Jones and Hunt 1983). Excess Cu binds proteins and induces oxidative damage to the proteins (including hemoglobin in red blood cells that carry oxygen throughout the body) and those in plasma and other tissues (such as the liver, kidneys, and small intestine). Thus, the high dietary intake of Cu is toxic to animals.

Species Differences in Sensitivity to Nutritional Cu Toxicity

There are two forms of Cu toxicity: acute or chronic Cu toxicity. Acute Cu toxicity results from exceedingly high consumption of Cu from diets (e.g., 20–100 mg/kg diet in sheep and young calves; and 200–800 mg/kg diet in adult cattle; Jones and van der Merwe 2008). Chronic Cu toxicity occurs when high amounts of Cu at doses below the acutely toxic level are consumed over a period of time (e.g., Cu levels in diets >25 ppm; a ratio of Cu to Mo >10:1). Because Cu can be stored in the liver for an extensive period (e.g., up to 18 months), the current and past feeds may be the sources of toxicity. Among domestic animals, sheep are most susceptible to chronic Cu toxicity (Todd 1969). This is because ovine hepatocytes have a high affinity for Cu and excrete Cu into the bile at a low rate, resulting in an accumulation of Cu in the cells. Goats differ from sheep in their sensitivity to high Cu diets, but are more similar to cattle. Goats and cattle can tolerate higher Cu intake than sheep, but are more sensitive to high Cu diets than swine and poultry.

Cu toxicity is relatively uncommon in nonruminant farm animals (swine and poultry). They can tolerate excess dietary Cu better than ruminants. As stated previously, Cu has an antimicrobial effect that inhibits the growth of bacteria, fungi (e.g., molds), algae and other harmful microbes, and its dietary supplementation is effective in enhancing the growth of nonruminants. Swine and poultry can be fed up to 250 and 400 mg Cu (as $CuSO_4$) per kilogram of diet, respectively, to improve their intestinal health and feed efficiency.

Symptoms of Nutritional Cu Toxicity and Its Treatment

Animals with nutritional Cu toxicity exhibit elevated Cu concentration in serum (>2.0 ppm), anorexia, pallor, weakness, recumbence, red or brown urine, anemia, brown or dark blood, and pink serum. Untreated animals are often found dead, and their necropsy can show hepatocellular injury, bile duct occlusion, icterus (jaundice), and blue kidneys. Icterus (e.g., in the sclera of the eye) is caused by the disintegration of blood corpuscles (Jones and Hunt 1983). If Cu concentrations in serum are <2.0 ppm, a possibility of Cu toxicity cannot be excluded because most Cu is stored in the liver and a change in serum Cu concentration is often transient.

Cu toxicity in animals can be treated. First, dietary Cu content must be reduced substantially. Second, when animals (e.g., sheep) show symptoms of Cu toxicity, they

can receive oral administration of either D-penicillamine (50 mg/kg BW) once a day for 6 days or Mo (10 mg/kg BW twice daily), as noted previously. Third, the safe levels of thiomolybdates and/or S can be either supplemented into diets to inhibit the intestinal absorption of Cu or administered intravenously [e.g., 0.83 mg Mo (as trithiomolybdate) per kilogram of BW] into animals to decrease the uptake of plasma Cu by tissues, thereby preventing Cu poisoning symptoms (Wang et al. 1992). Fourth, cysteine can chelate Cu, thereby reducing the absorption and toxicity of excess dietary. For example, dietary supplementation with cysteine (1 or 5 g/kg diet) alleviates the toxicity of Cu (induced with the addition of 1 g Cu [as CuSO4]/kg diet) in growing chicks more effectively than dietary supplementation with either vitamin C (1 g/kg diet) or Zn (1 g/kg diet) from ZnO (Persia et al. 2003). Dietary supplementation with a lower dose of cysteine (0.5 g/kg diet) together with vitamin C (1 g/kg diet) plus Zn (1 g/kg diet) from ZnO is also effective in reducing the toxicity of dietary excess Cu (Persia et al. 2003).

Fluorine (F)

In 1529, Georgius Agricola (a German mineralogist) discovered F through his studies of the compound called fluorspar [now known as Ca fluoride (CaF_2)] that was added to metal ores to lower their melting points and help metals fuse. Andreas Marggraf published the first scientific paper about the fluorspar stones in 1770, and Karl W. Scheele first identified F as a constituent of HF in 1771. The F element was first isolated in 1886 by French chemist F. Henri Moissan, who was awarded the Nobel Prize in Chemistry in 1906 for his work. The name of F is derived from the Latin word *fluere*, meaning "to flow".

F is the lightest halogen. As the most electronegative element, F is extremely reactive toward H^+ at a low pH, and toward Ca^{2+} and Mg^{2+} at neutral pH. F also reacts with all other elements except for argon, neon, and helium. The molecular form (i.e., F_2) of F is composed of two F atoms and is a highly toxic, reactive, yellowish-green gas with a pungent odor. This gas exists naturally, and is reported as being trapped inside a dark purple rock called antozonite [a CaF_2 product (a fluorite)], which was historically also known as Stinkspar and fetid fluorite (Schmedt auf der Günne et al. 2012). The fluoride ion (F^-) is the anion of F and is present in some chemical compounds such as HF, Ca fluoride (CaF_2), sodium fluoride (NaF), and sodium fluorosilicate ($Na_2[SiF_6]$). In terms of charge and size, F^- resembles the hydroxide ion (OH^-). When HF is dissolved in water, it is called hydrofluoric acid. In an aqueous solution at neutral pH, HF is essentially completely ionized to H^+ and F^-. At low pH (e.g., pH 2–3), H^+ readily reacts with F^- to form HF. In cells with a pH of 7.0, F^- reacts with Ca^{2+} to form Ca fluoride, which combines with phosphate to form fluorapatite [$FCa_5(PO_4)_3$]. The latter is present in the bones and teeth (Nielsen 2009). In the animal body, about 99% of F^- is found in bones and teeth, with the rest in soft connective tissue and other cell types.

Sources: F^- is widely present in plant- and animal-sourced feedstuffs, as well as ground-surface water. Naturally occurring F^- concentrations in surface waters are generally low (<0.5 ppm), but a wide range of F^- (0.1 to 6 ppm) has been reported in groundwater depending on the locations (EFSA 2013). Seawater contains F^- at the concentrations of 1.2 to 1.5 ppm.

Absorption and transport: In the gastric lumen with a low pH, F^- reacts with H^+ to form HF, which is taken up by the stomach via nonionic carrier-mediated diffusion (Barbier et al. 2010). In the small intestine, F^- is transported into the enterocyte across its apical membrane by a transmembrane carrier (likely an F^-/H^+ cotransporter) and possibly by an anion exchanger. In the enterocyte, F^- binds to Ca^{2+} and Mg^{2+} to form Ca and Mg ionospheres, respectively. F^- exits the cell across its basolateral membrane via the export Fluc protein, an F^- carrier (also known as FEX, F^- export protein), which is a single-barreled transmembrane channel in animals (Berbasova et al. 2017). Absorbed F^- enters the portal circulation. In the blood, F^- binds to Ca^{2+} or Mg^{2+} for transport into tissues, which take up (a) free F^- via an F^- channel and (b) the Ca^{2+} or Mg^{2+}- complexed F^- via Ca^{2+} and Mg^{2+} channels, respectively.

In animals, the nonionic HF is more readily transported across cell membranes than F^-. The absorption of F^- by the GIT is very rapid. About 40% and 45% of ingested F^- are absorbed by the stomach and the small intestine, respectively (Barbier et al. 2010). High dietary content of Ca^{2+} and Mg^{2+}, which form insoluble salts with F^-, can reduce the absorption of F^- by the small intestine.

Functions: F^- is an integral component of calcified tissues. This mineral enhances Na^+-dependent phosphate transport by osteoblasts (Selz et al. 1991) and tyrosine kinase activity in these cells (Burgener et al. 1995) to promote their proliferation and bone formation. Adequate intake of F^- is essential for the hardening, normal structure, and strength of bones and teeth. An adequate intake of F^- from all sources (including nondietary sources) is 0.05 mg/kg BW/day for both children and adults (including pregnant and lactating women). Humans can tolerate the intake of 0.2 mg F^-/kg of BW/day (Baines et al. 2017).

Deficiency symptoms: Because diets and drinking water with added iodine monofluoride usually contain F^-, its deficiency is rare in animals under practical feeding conditions. However, farm sources of water may not provide adequate F^-. In cases of F^- deficiency induced under experimental conditions (e.g., <12 ppm F^- in diets for dairy cows), deficiency symptoms include tooth decay and dental caries, as well as possibly osteoporosis (Nielsen 2009). The latter is characterized by a decrease in bone mass and an increase in bone fragility.

Excess: Mechanisms for the toxicity of F and F^+ are clearly understood (Kabir et al. 2020). Fluorine gas is a powerful oxidizing agent; therefore, it is extremely toxic and often fatal by inhalation and skin absorption. In addition, F^- is an inhibitor of many metal-dependent and independent enzymes such as enolase, aconitase,

pyrophosphatases, the membrane-bound, proton-pumping H^+-ATPase, acid phosphatase, acetylcholinesterase, catalase, cytochrome c oxidase, glucose-6-phosphatase, GSH peroxidase, lactate dehydrogenase, protein phosphatase, and superoxide dismutase (Strunecka and Strunecky 2020; Whitford 1996). These enzymes are involved in glycolysis, the Krebs cycle, the mitochondrial electron transport system, gluconeogenesis, antioxidative defense, the hydrolysis of pyrophosphate, neurotransmission, and redox balance. Thus, excess F^- enhances the production of reactive oxygen species by cells, reduces the intracellular concentrations of GSH, and stimulates the release of cytochrome c from the mitochondria of cells, resulting in oxidative stress and cell death (Barbier et al. 2010).

Acute and chronic F or F^+ poisoning occur when animals (including humans) are exposed to indoor or outdoor air with excess F or F^+ due to the burning of the coal that contains high F levels, or drink water (including groundwater) and consume food that contains elevated levels of F^-. Chronic exposure to high levels of F^- (e.g., >93 ppm F^- in diets for dairy cows) affects: (a) the formation of enamel (the outer covering of the tooth), leading to dental fluorosis that is characterized by mottled, discolored, and porous enamel; and (b) osteoblast growth and structure, causing osteoporosis in bones (Jones and Hunt 1983). Endemic fluorosis in both teeth and bones occurs in dogs, beef cattle, sheep, and lactating cows due to their chronic exposure to F^- in the neighborhood of F industries (Krishnamachari 1987). In humans, symptoms of F^- toxicity may appear with an intake of 3 mg F^-/kg of BW, including tooth discoloration, tooth decay, physical weakness, neurological disorders, high blood pressure, acne, seizures, nausea, vomiting, diarrhea, and abdominal pain. Death may result from the ingestion of 32 and 16 mg F^-/kg of BW in an adult and a child, respectively. In animals (including humans), the toxicity of F^- can be treated by (a) oral administration of dilute Ca hydroxide or Ca chloride to prevent intestinal F^- absorption; and (b) intramuscular injection of Ca-gluconate to increase Ca^{2+} concentrations in plasma, which in turn block F^- uptake by bones and other tissues.

Iodine (I)

Iodine was discovered by the French chemist Barnard Courtois in 1811. Three years later, Humphry Davy used the word "iodine" to describe this mineral. Its name is derived from the Greek *ἰωδης,* meaning "violet-colored". Iodine exists as a gas (I_2) or as salts (e.g., KI and NaI) and has many oxidation states, including iodide (I^-), iodate (IO_3^-), and the various periodate anions [e.g., metaperiodate (IO_4^-) and orthoperiodate (IO_6^{5-})]. In animals, about 70–80% of iodine is present in the thyroid gland, with the remainder being mostly in skeletal muscle. As early as 1850, Jean Boussingault found that dietary iodine was effective in preventing and treating goiter.

Sources: Trace amounts of iodine are present in plants. It occurs mainly as inorganic I^-. For example, plants grown in iodine-deficient soils have as low as 10 μg/kg DM, whereas plants grown in iodine-rich soils contain 1 mg/kg DM. The richest sources of iodine are seafood, mushrooms, and sunflower seeds. Eggs, freshwater fish, meats, liver, peanuts, and bone meal are good sources of this mineral. Grains, vegetables, and nuts contain a low amount of iodine. In areas where goiter is endemic and the consumption of seafood is limited, diets for humans are usually supplemented with iodine in the form of iodized salt. The hydrogen iodide derivative of ethylenediamine [ethylenediamine dihydriodide (EDDI)] is the main supplement to livestock and poultry diets. EDDI can be added to diets to prevent foot rot in cattle (Berg et al. 1984).

Absorption and transport: Dietary iodine is converted into the I^- ion before it is absorbed by the stomach (a minor site of I^- absorption) and the small intestine (the major site of I^- absorption) via the Na^+/I^- symporter (NIS, also known as Na^+/I^- cotransporter, a glycoprotein) into the enterocyte (Nicola et al. 2009). In rats, 15% of the I^- (as KI) placed in the stomach and 49% of the I^- (as KI) added to the duodenum are absorbed within 1 h (Small et al. 1961). Note that NIS is not present in the basolateral membranes of the enterocytes, and it is likely that I^- exits the cells via facilitated passive diffusion into the lamina propria. The Na^+ gradient drives the entry of extracellular I^- into the cell, with a $2Na^+/1I^-$ stoichiometry (Eskandari et al. 1997). I^- exits the enterocyte across its basolateral membrane into the lamina propria and enters the portal circulation. To date, a vesicle or basolateral membrane transporter of I^- has not been identified. In the blood, I^- is bound to albumin and is taken up by tissues, such as the liver and thyroid gland, through NIS (Nicola et al. 2015). About 85% of absorbed I^- is utilized by the thyroid gland.

NIS expression and activity are subject to nutritional regulation (Nicola et al. 2015). For example, the high dietary intake of iodine or intracellular I^- concentrations in enterocytes reduces NIS-mediated uptake of I^- through several posttranscriptional mechanisms: (a) reduced expression of the NIS gene; (b) increased degradation of the NIS protein; and (c) reduced mRNA levels for NIS due to a reduction in its stability. To date, there are no experimental data regarding hormonal regulation of NIS expression in the small intestine. However, there is a suggestion that thyroid hormones enhance intestinal NIS expression to maximize dietary I^- absorption (Nicola et al. 2015).

Content in the body and tissues: The content of iodine in mammals is 35–200 μg/kg BW and varies with dietary iodine intake. A 100-kg pig and a 500-kg cow contain 4.5 and 17 mg iodine, respectively (Georgievskii et al. 1982). In animals fed iodine-adequate diets, the distribution of iodine in the body (%) is as follows: thyroid gland, 70–80; skeletal muscle, 10–12; hide, 3–4; bones, 3; and other organs, 5–10. The content of iodine in tissues (μg/100 g fresh weight) of calves is as follows: thyroid gland, 24–38; whole blood, 6.3–7.8; serum, 6.1–7.4; hair, 1.7–3.2; hide, 2.7–4.0; liver, 8.0–8.1; kidneys, 7.0–7.2; and lungs, 5.0–5.2 (Georgievskii et al. 1982).

Functions: Iodine plays an important role in the synthesis of thyroid hormones (Leung and Braverman 2014). This metabolic pathway begins with a protein called thyroglobulin. Its tyrosine residues are iodized by I^+, which is formed from I^- by H_2O_2-dependent thyroperoxidase. Thyroxine- and triiodothyronine-containing thyroglobulin enters the lysosome for proteolysis to release thyroxine and triiodothyronine. In plasma, 99% of these two hormones are bound to the thyroid-binding proteins. The thyroid hormones regulate the metabolism of protein, lipids, and glucose, as well as basal metabolism, and are essential to proper development and differentiation of all animal cells that are required for maturation of tissues (particularly the brain, heart, skeletal muscle, small intestine, kidneys, and lungs; Nicola et al. 2015).

Deficiency symptoms: In animals, an insufficient intake of dietary iodine reduces the synthesis of thyroxine and triiodothyronine, leading to hypothyroidism. The deficiency of thyroid hormones impairs negative feedback control of pituitary gland activity, resulting in increased production of thyroid-stimulating hormone. The latter causes the thyroid to enlarge with the onset of the disease called goiter as an attempt by the thyroid gland to trap more I^- and compensate for the deficiency of iodine (Dumont et al. 1992). In addition, animals with I deficiency exhibit dry and scaly skin, stunted growth, excess fat deposition, retarded psychomotor development, impaired reproduction, and neurological dysfunction (Zimmermann 2009). These adverse effects occur at all stages of the life cycle and, when occurring in utero, can have transgenerational impacts. Metabolic disorders resulting from dietary iodine deficiency can be prevented and treated through dietary supplementation with iodine.

Excess: Like dietary iodine deficiency, the chronic overconsumption of iodine also results in hypothyroidism and goiter (Leung and Braverman 2014). Such a finding, known as the Wolff–Chaikoff effect, was initially reported by Wolff and Chaikoff (1948). The underlying mechanisms include (a) the production of several substances (e.g., intrathyroidal iodolactones, iodoaldehydes, and iodolipids) that inhibit thyroid peroxidase activity; (b) reduced activity of intrathyroidal deiodinase; and (c) reduced abundance of NIS in the apical membrane of enterocytes and the basolateral membrane of thyroid follicular cells due to reduced gene expression (Nicola et al. 2015).

Excess iodine is uncommon in livestock, poultry, fish, and crustacean nutrition. However, this nutritional problem can occur with high supplemental doses of iodine. Excessive intake of dietary iodine [e.g., the I content (from KI) of ≥600 mg/kg diet in chicks] causes the thyroid gland to become overactive and produce excess thyroid hormones (hyperthyroidism). Affected animals lose BW (particularly muscle, bone, and adipose tissue) and suffer from neurological dysfunction, growth depression, and reduced feed efficiency (Jones and Hunt 1983; Leung and Braverman 2014). In chicks, the toxic effect of supplemental iodine (1000–1500 mg/kg diet) could be fully reversed by the dietary addition of 50 or 100 mg Br (provided as NaBr)/kg diet (Baker et al. 2003). This is because bromide, like thiocyanate, is an inhibitor of I^- transport and utilization by the small intestine and thyroid gland (Abraham 2005).

Iron (Fe)

There is a rich history of work on Fe (Sheftel et al. 2012). Fe objects were found in Egypt around 3500 BC. However, René Antoine Ferchault de Réaumur was the first person to write about the various types of Fe when he published his book in 1722. On Earth, most of Fe exists in the Fe^{3+} state. Fe is the most abundant trace mineral in animals (Table 10.13) and occurs primarily in the Fe^{2+} state. Of note, ~65% of the whole-body Fe is contained within the hemoglobin of erythrocytes (Wallace 2016). Except for newborn pigs, which have a relatively low amount of Fe at birth, neonates contain relatively high levels of Fe, about 70 mg per kilogram of BW. In mammals, birds, and fish, almost all Fe (>90%) is present in protein-bound forms as Fe, oxo–diiron (Fe–O–Fe), oxo–iron–zinc (Fe–O–Zn), Fe–S clusters, and heme (Ward and Kaplan 2012). Free Fe concentrations in healthy animals are particularly low for two reasons: (a) Fe^{2+}, and to a lesser extent Fe^{3+}, are toxic to cells and animals; and (b) Fe^{3+} is barely soluble in water at neutral pH. In contrast to Fe^{3+}, Fe^{2+} is soluble in water at acidic or neutral pH.

Sources: Fe is widely distributed in foods, but its content varies greatly. Concentrates, green leafy materials, and roughage generally provide sufficient quantities of Fe for farm animals (McDonald et al. 2021). Leguminous plant species have more Fe (200–400 ppm on a DM basis) than grasses (~40 ppm). Ferritin, which is a protein complexed with Fe^{3+}, is a major form of Fe in food legumes such as soybeans (Theil 2004). Cereal grains are also good sources of Fe (30–60 ppm). However, the bioavailability of Fe in plant-sourced foods is generally low for mammals, birds, and fish (Haider et al. 2018). Animal products contain more bioavailable Fe in organic forms (e.g., heme and nonheme Fe proteins) than plant products that contain mainly nonheme Fe. In animals, each heme (MW 616.5) contains one iron (MW 55.845). Note that a relatively small amount of leghemoglobin (a heme protein) occurs in plants such as soybean beans (Kundu et al. 2023) and bacteria (Andrews et al. 2003). Feed additives are the major source of Fe in plant-based diets. At pH 6 and 8, Fe salts have a low solubility of 5.9%, but Fe-peptide complexes are highly soluble (>90%).

Absorption: In the stomach, Fe binds to a glycoprotein called gastroferrin, which is secreted by the oxyntic cells of the gastric mucosa; the Fe-complexed gastroferrin flows into the duodenum along with food. In the lumen of the small intestine, Fe^{3+} is reduced to Fe^{2+} by vitamin C or duodenal cytochrome *b* (DCYTB, also called duodenal ferric reductase) on the apical membrane surface. DCYTB is a transmembrane oxidoreductase that utilizes intracellular vitamin C as an electron donor to reduce extracellular Fe^{3+} to Fe^{2+} (Asard et al. 2013). Subsequently, Fe^{2+}

is taken up into the enterocyte through the apical-membrane divalent cation transporter-1 [DCT1; also known as divalent metal ion transporter-1 (DMT1) and natural resistance-associated macrophage protein-2 (NRAMP2)]. DCT1 also transports Zn^{2+}, Cu^{2+}, and Mn^{2+} but plays only a minor role in transporting Ca^{2+} or Mg^{2+}. Upon entering the enterocyte, Fe^{2+} binds to the paraferritin complex (a protein complex) and is then transferred to the basolateral membrane for exit into the lamina propria via ferroportin-1 (Figure 10.9). Because ferroportin only recognizes Fe^{2+}, Fe^{3+} must be reduced (e.g., by vitamin C) to Fe^{2+} in the paraferritin complex (Ward and Kaplan 2012). This protein complex does not store Fe, but only mediates the passage of Fe to the basolateral membrane of the enterocyte for export. Finally, Fe^{3+} that is bound to mucins in the lumen of the small intestine enters the enterocyte through the mobilferrin-mediated Fe^{3+} pathway. In this pathway, β_3-integrin facilitates the influx of Fe^{3+} into the cell. Within the cytosol of the enterocyte, mobilferrin, which is localized in vesicles near the apical membrane in a complex linked to β_3-integrin and gastroferrin, shuttles Fe^{3+} from β_3-integrin to gastroferrin. Eventually, Fe^{3+} is transferred to the paraferritin complex.

Heme Fe (heme-Fe^{2+}), which is bound to the heme's porphyrin ring, is absorbed as an intact complex from the small-intestinal lumen into the enterocyte via its apical membrane heme carrier protein-1 (HCP1). Note that the HCP1-mediated Fe transport functions independently of the putative heme receptor and receptor mediated heme endocytosis, which was proposed in the 1970s for heme uptake by the small intestine of both pigs and humans (Grasbeck et al. 1979). Once inside the enterocyte, heme is degraded by heme oxygenase to release Fe^{2+} and biliverdin. Fe^{2+} binds to the paraferritin complex.

Intracellular Fe^{2+} exits the enterocyte across its basolateral membrane via ferroportin-1. Hephaestin, which is a transmembrane protein in the basolateral membrane of the enterocyte, facilitates the basolateral export of Fe (Vulpe et al. 1999). The abundance of ferroportin in enterocytes is regulated by a 25-amino acid polypeptide called hepcidin, which is synthesized by hepatocytes (Ward and Kaplan 2012). Hepcidin inhibits ferroportin activity by promoting the trafficking of ferroportin from the plasma membrane into the cytosol and then the degradation of ferroportin. The hepatic expression of hepcidin is upregulated by two proteins [human hemochromatosis protein (HFE) and hemojuvelin (HJV)] in response to high Fe stores in the liver, thereby reducing intestinal Fe absorption (Barton et al. 2015). Under inflammatory conditions, plasma hepcidin concentration is abnormally high, the hepatic expression of hepcidin is increased, the intestinal absorption of Fe is reduced, and Fe is trapped within macrophages and the liver (Ganz 2003). This results in anemia due to a low concentration of Fe in erythrocytes. In contrast, when hepcidin concentration in plasma is abnormally low (e.g., in hemochromatosis, a hereditary disease associated with an excessive accumulation of Fe in plasma), the circulating levels of Fe are high primarily because of an increase in ferroportin-mediated efflux of Fe from cells (including enterocytes, hepatocytes, and immunocytes).

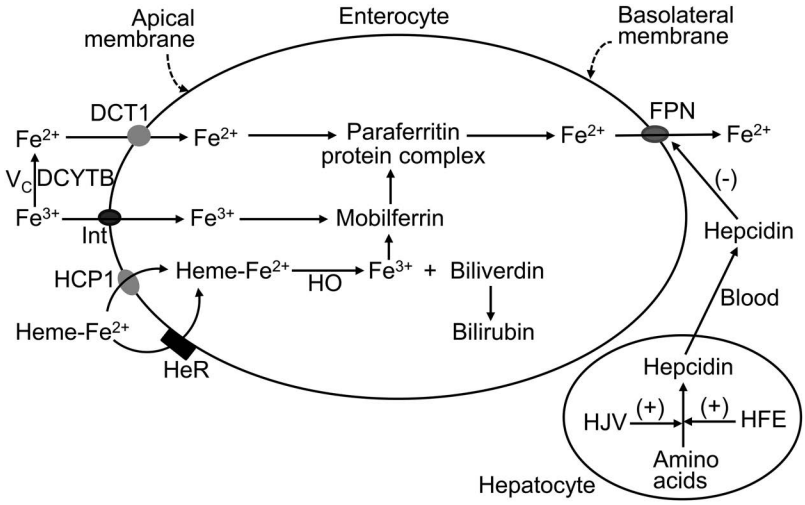


FIGURE 10.9 Intestinal absorption of dietary iron. In the lumen of the small intestine, Fe^{3+} is reduced to Fe^{2+} by vitamin C (V_c) or duodenal cytochrome *b* (DCYTB) on the apical membrane surface. Inorganic Fe^{2+} is absorbed by the enterocyte via divalent cation transporter-1 (DCT1), whereas Fe^{3+} binds mucins and then enters the cell via β_3-integrin (Int). Heme-Fe^{2+} is taken up by the enterocyte through heme carrier protein-1 (HCP1) or a heme receptor (HeR)-mediated endocytosis. Inside the enterocyte, heme-Fe^{2+} is oxidized to Fe^{3+} by heme oxygenase (HO). Fe^{3+} is reduced to Fe^{2+}, which is transported by the paraferritin protein complex to the basolateral membrane and exits the cell via ferroportin (FPN). FPN is inhibited by hepcidin (a 25-amino acid polypeptide), which is synthesized from amino acids in hepatocytes. Hepatic synthesis of hepcidin is activated by human hemochromatosis protein (HFE) and hemojuvelin (HJV). Under inflammatory conditions, hepcidin production is elevated to reduce intestinal iron absorption and result in anemia. (+), activation; (–), inhibition.

The efficiency of absorption of dietary Fe by the small intestine varies markedly with its sources and its content. For example, the efficiency of Fe absorption in animals is greater when fed a low-Fe diet than an Fe-adequate or excess diet, e.g., 40% and 15% of true Fe absorption in calves fed liquid diets containing 40 and 100 mg Fe/kg, respectively (ARC 1980). In addition, dietary supplementation with vitamin C, cysteine, and organic acids (e.g., citrate and lactate) can double the efficiency of Fe absorption (Baker 2001). In contrast, the presence of large amounts of phytate, oxalates, Zn, or Mn can reduce the efficiency of Fe absorption by 50% (Baker 2001). The bioavailability of Fe in diets should be considered in their formulations.

Transport in the blood: Absorbed Fe^{2+} enters the portal circulation. In the blood, Fe^{2+} is rapidly oxidized to Fe^{3+} by ferroxidase II, and Fe^{3+} is transported by binding to transferrin [Fe-binding protein (a glycoprotein) released by hepatocytes], which contains two sites for binding Fe^{3+} ions but binds Fe^{2+} weakly (Harris 2014). Of note, transferrin binds Fe^{3+} tightly but reversibly. Transferrin receptors on the plasma membrane of extraintestinal cells bind transferrin for its uptake into the cytoplasm via endocytosis. The entrapped transferrin merges with an endosome, where Fe^{3+} is reduced to Fe^{2+} by STEAP3 [six transmembrane epithelial antigens of the prostate protein-3; a metalloreductase (Sendamarai et al. 2008)]. Fe^{2+} exits the endosome, via DCT1, into the cytosol, where Fe^{2+} binds two proteins (ferritin and hemosiderin) for storage. Hemosiderin is a water-insoluble protein complex and is typically found in lysosomes. During pregnancy, uteroferrin (a progesterone-induced Fe-binding protein) plays an important role in the storage and transport of Fe in the conceptus (Bazer et al. 1991; Roberts et al. 1986).

Content in the body and tissues: The content of Fe in animals (mg/kg BW) is as follows: cows, 60; horses, 66; sows, 50; 0- to 35-day-old pigs, 36–40; chickens, 80 (Georgievskii et al. 1982). The concentration of Fe in plasma (mg/L) of animals is as follows: adult beef cattle, 1.4–1.5; calves, 1.2–1.6; chickens, 1.0 (nonlaying hens) to 5.2 (laying hens); mice, 0.9; pigs, 1.23; sheep, 1.52–1.82. Its concentrations in serum are shown in Table 10.14. Of note, the concentrations of microminerals (including Fe) in serum or plasma are generally not good indicators of their nutritional status in animals because microminerals are sequestered within cells and are released from the cells into plasma under various physiological and pathological conditions. The content of Fe in the tissues of mammals (mg/100 g fresh weight) is as follows: whole blood, 20–45; spleen, 20–40; liver, 10–20; kidneys, 4–6; heart, 4–8; skeletal muscle, 1.5–3; brain, 2–2.5; and bones, 3.5–4 (Georgievskii et al. 1982).

Functions: Fe is essential for erythropoiesis (the formation of red blood cells), which occurs in fetal livers (mammals), erythroid cells (embryos of birds), red bone marrow (postnatal mammals and birds), and kidneys of fish (Bruns and Ingram 1973; Palis and Segel 1998; Roberts et al. 1986).

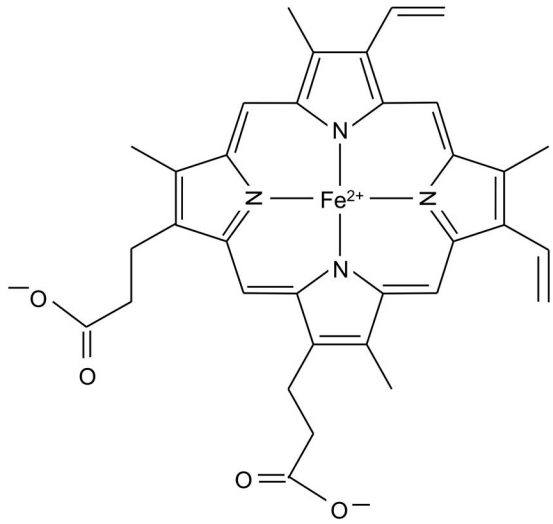


FIGURE 10.10 The structure of heme. The pyrrole rings and methylene bridge carbons are coplanar, and the iron atom (Fe^{2+}) resides in almost the same plane. The fifth and sixth coordination positions of Fe^{2+} are directed perpendicular to, and directly above and below, the plane of the heme ring. Thus, heme acts as a chelator of iron. Heme is synthesized in the liver from succinyl-CoA and glycine.

Fe is a component of heme (Figure 10.10), with the ratio of Fe to heme being 1:1 (mol/mol). Heme proteins include hemoglobin and myoglobin (which are used for O_2 binding, transport, and storage), metalloenzymes, and cytochromes. Furthermore, Fe is required for the activities of some nonheme enzymes (Sheftel et al. 2012). Fe also participates in oxidoreduction reactions. In cytochromes, the oxidation and reduction of the Fe atom are essential to their biological function. In contrast, oxidation of Fe^{2+} to Fe^{3+} in hemoglobin or myoglobin inhibits their biological activity.

Hemoglobins

Concentrations of hemoglobins in the blood of healthy animals are 100–140 g/L (Fox et al. 2002; Sheftel et al. 2012). These proteins contain 60% of the body's Fe in horses, 68% in dogs and cattle, 73% in rats, and 80% in pigs (Georgievskii et al. 1982). In mammals, birds, and teleost fishes, hemoglobins are tetrameric proteins (MW, 64,500): HbA (normal adult hemoglobin, $\alpha_2ß_2$), HbF (fetal hemoglobin, $\alpha_2\gamma_2$), and HbS (sickle cell hemoglobin, α_2S_2, on residue 6 of the ß-chain, Val replaces Glu; Thom et al. 2013). Each subunit of hemoglobin has one heme. By weight, the content of heme in hemoglobin is 3.82%, and 1 g of hemoglobin contains 3.46 mg of Fe (or 0.346% Fe by weight). Hemoglobin represents more than 95% of the proteins of the red blood cell (ARC 1981; Sheftel et al. 2012). Hemoglobins of vertebrate erythrocytes transport (a) O_2 from the respiratory organ to peripheral tissues; (b) CO_2 and protons from peripheral tissues to the lung for subsequent excretion; and (c) nitric oxide (NO), which is synthesized from arginine in almost all cell types (Glanz 1996).

2,3-Bisphosphoglycerate, an intermediate in glycolysis, stabilizes the tetrameric structure of hemoglobin (Arnone 1972), indicating another important function of glycolysis in the functions of red blood cells.

Myoglobin

Myoglobin is a single polypeptide chain (MW 16,900). It contains 10% of the body's Fe in dogs and cattle and 20% in rats and horses (Georgievskii et al. 1982). Myoglobin is a minor protein in muscle cells and is used for the short-term storage of oxygen. In diving mammals such as dolphins and seals, the myoglobin content of skeletal muscle can reach 3–8% of total protein.

Cytochromes

Cytochromes are Fe-containing heme proteins (Georgievskii et al. 1982). Several cytochromes occur in the respiratory chain, i.e., cytochromes b, c_1, c, and aa_3 in animal cells. Cytochrome aa_3 is also known as cytochrome oxidase, which is the terminal component of the mitochondrial electron transport system. Cytochromes b, c_1, and c are also classified as dehydrogenases, which are involved as carriers of electrons from flavoproteins to cytochrome aa_3 (cytochrome oxidase). Cytochromes P450 and b_5 are involved in the microsomal hydroxylation of many drugs, pesticides, carcinogens, and other xenobiotics via the cytochrome P450 monooxygenase systems. Cytochrome b_5 is involved in the microsomal Δ^9 desaturation of long-chain fatty acids. In bacteria, NO reductase (heme-cytochrome c) converts NO into nitrous oxide (N_2O), and nitrite reductase (heme-cytochrome c) catalyzes the formation of NH_3 from NO_2^- (Averill 1996).

$$2NO + 2e^- + 2H^+ \left(\text{from 2 reduced cytochrome c}\right) \rightarrow N_2O + H_2O + 2 \text{ Oxidized cytochrome c}$$

$$\left(\text{NO reductase containing cytochrome c-heme, bacteria}\right)$$

$$NO_2^- + 6e^- \left(\text{from 6 reduced cytochrome c}\right) + 7H^+ \rightarrow NH_3 + 6 \text{ Oxidized cytochrome c} + 2H_2O$$

$$\left(\text{nitrite reductase containing cytochrome c-heme}\right)$$

Heme Enzymes

Heme enzymes depend on heme for their biological activities (Poulos 2014). They include peroxidase, catalase, prostaglandin endoperoxide synthase, guanylate cyclase, and myeloperoxidase in animals (Table 10.16). Bacteria contain these enzymes, as well as nitrite reductase. These enzymes have important physiological and immunological functions. For example, NO increases cGMP production by activating guanylate cyclase, whereas hypochlorous acid (HOCl, a strong oxidant) is used by phagocytic cells to kill pathogenic microbes. This may explain, in part, why severe Fe deficiency is associated with increased incidence of infections.

Heme-binding protein: Hemopexin is a heme-binding protein synthesized by hepatocytes (Ascenzi and Fasano 2007). This protein can bind free heme in the hepatocytes and plasma. Therefore, hemopexin plays an important role in heme metabolism and transport.

Nonheme Fe Enzymes and Proteins

Animals contain many nonheme Fe enzymes (Table 10.16). The Fe in these enzymes is generally tightly bound to the S atoms of cysteine residues. Hence, these enzymes are called "Fe-S proteins", with the Fe-S center being 2Fe-2S, 4Fe-4S, or 3Fe-4S. These proteins are located in mitochondria, cytosol, and nucleus to exert their diverse functions (Stehling and Lill 2013).

Fe Enzymes and Proteins Containing Neither Heme nor the Fe-S Center

Prolyl hydroxylase (Fe^{2+}), lysyl hydroxylase (Fe^{2+}), and lipoxygenase (Fe^{2+}) do not contain heme or the Fe-S center. However, these enzymes require Fe^{2+} for their catalytic activities. Lipoxygenase oxidizes lipids into hydroperoxide, endoperoxide, and peroxyl free radicals. In animals, Fe-binding proteins that do not contain heme or the Fe-S center include transferrin (Fe-binding protein in blood), uteroferrin (Fe-binding protein in the porcine conceptus), and lactoferrin (Fe-binding protein in milk). In bacteria, nitrogenase (Fe and Mo) reduces N_2 to NH_3.

Fenton reaction: The Fenton reaction is named after Henry John Horstman Fenton, who discovered the formation of a reactive oxygen species from H_2O_2 and ferrous Fe in 1894. This reaction, which results in the generation of the hydroxyl radical ($OH^\bullet$) and oxidative stress, is activated under conditions of inflammation (increased intracellular concentrations of Fe) and tissue injury (increased extracellular concentrations of Fe) in animals (Hanschmann et al. 2013).

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^\bullet$$

Fe^{3+} released from oxidized hemoglobin and myoglobin proteins can oxidize low-density lipoproteins, thereby possibly playing a key role in atherosclerosis (Umbreit 2007). In addition, the oxidation of heme proteins to release heme and the subsequent oxidation of matrix lipids by the hemin Fe^{3+}-derived radicals can adversely affect the quality, odor, and flavor of meats (Grunwald and Richards 2006). Interestingly, trout and perch hemoglobins are oxidized much more rapidly than bovine and avian hemoglobins (Richards et al. 2002; Richards and Dettmann 2003). Preventing oxidative stress has important implications for both health and the food industry.

Interaction with Cu: Both Fe and Cu have two oxidation states and are highly redox active. Thus, it is not surprising that Fe-Cu interactions exist in animal cells (Gulec and Collins 2014). The first nutritional evidence was provided in the 1920s by Conrad Elvehjem and colleagues that rats with anemia induced by dietary Fe deficiency required dietary provision of both Fe and Cu to restore the concentration of hemoglobin in the blood (Hart et al. 1928). When

TABLE 10.16
Heme and Nonheme Iron Enzymes

Enzyme	Function
Heme enzyme	
Catalase	$2H_2O_2 \rightarrow 2H_2O + O_2$
Guanylate cyclase	GTP → cGMP + PPi
Indoleamine 2,3-dioxygenase (IDO)	Tryptophan (Trp) catabolism (Trp → *N*-formylkynurenine)
Myeloperoxidase	$H_2O_2 + Cl^- \rightarrow H_2O + HOCl$ (Hypochlorous acid)
Nitrite reductase (heme, bacteria)	NO_2^- (nitrite) + $2e^- + H^+ \rightarrow$ NO (nitric oxide) + H_2O
Oxygen reductase (heme, bacteria)	$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$
Peroxidase	ROOR' + $2e^- + 2H^+ \rightarrow$ ROH + R'OH (e.g., $H_2O_2 + AH_2 \rightarrow 2H_2O + A$)
Prostaglandin endoperoxide synthase	Arachidonic acid + $2O_2 \rightarrow$ Prostaglandin H_2 (PGH_2)
Tryptophan 2, 3-dioxygenase (TDO)	Trp catabolism (Trp → *N*-formylkynurenine)
Nonheme iron enzyme (iron-sulfur proteins)	
Aconitase	Citrate → Isocitrate (the Krebs cycle)
Adrenodoxin	Cholesterol → Steroid hormones
Coenzyme Q reductase	Mitochondrial respiratory chain
Δ^9-Desaturase	Synthesis of monounsaturated fatty acids
Dihydroxybiphenyl dioxygenase	Estradiol deoxygenation
Ferredoxin hydrogenase (bacteria)	H_2 + 2Oxidized ferredoxin $\rightleftharpoons 2H^+$ + 2Reduced ferredoxin
[Fe]-Hydrogenase (bacteria)	$H_2 \rightleftharpoons 2H^+ + 2e^-$
[Fe-Fe]-Hydrogenase (bacteria)	$H_2 \rightleftharpoons 2H^+ + 2e^-$
[Ni-Fe]-Hydrogenase (bacteria)	$H_2 \rightleftharpoons 2H^+ + 2e^-$
Hydrogenase (bacteria)	H_2 + $NAD(P)^+$ + 2Oxidized ferredoxin (Fd) $\rightleftharpoons H^+$ + NAD(P)H + 2Reduced Fd
3-Hydroxyanthranilate dioxygenase	Trp catabolism (3-Hydroxyanthranilate → ACMS)
Lipoxygenases	Hydroperoxidation
NADH dehydrogenase	Mitochondrial respiratory chain
Phenylalanine hydroxylase	Hydroxylation of phenylalanine to tyrosine
Proline hydroxylase	Hydroxylation of proline residues in collagen
Ribonucleotide reductase	RNDP → Deoxy-RNDP (DNA synthesis)
Succinate dehydrogenase	Succinate + FAD $\rightleftharpoons$ Fumarate + $FADH_2$ (the Krebs cycle)
Xanthine dehydrogenase	Xanthine → Uric acid (NAD-dependent, purine degradation)

Notes: ACMS, 2-amino-3-carboxymuconate semialdehyde; Deoxy-RNDP, deoxy-ribonucleotide diphosphate (dADP, dUDP, dGDP, and dCDP), which are converted to dATP, dTTP, dGTP, and dCTP for DNA synthesis; RNDP, ribonucleotide diphosphate (ADP, UDP, GDP, and CDP).

dietary Fe is deficient, Cu uptake by enterocytes and hepatocytes is elevated, resulting in increased concentrations of Cu in these cells (Gulec and Collins 2014). Accumulation of Cu in the liver can stimulate the synthesis and release of ferroxidase, which catalyzes the oxidation of Fe^{2+} to Fe^{3+} in plasma, thereby impairing the export of Fe from hepatocytes into the blood. When dietary Cu is deficient, Fe is accumulated in the liver (Williams et al. 1983) because the export of this mineral from hepatocytes into the blood is impaired due to the hepcidin-mediated internalization of ferroportin from the plasma membrane into the cytosol (Kono et al. 2010). Because the Fe absorbed from the intestine is retained by the liver, the plasma concentration of Fe is reduced in Cu-deficient animals, thereby limiting Fe supply to the bone marrow and causing anemia (Reeves and DeMars 2006).

Deficiency symptoms: In animals, Fe deficiency results from the inadequate intake and impaired absorption of dietary Fe, the impaired efflux of Fe from the liver, the reduced availability of Fe-binding and transport proteins due to protein malnutrition, as well as the antagonism of Fe absorption and transport by other divalent metals (Sheftel et al. 2012). Gestating mammals are highly sensitive to Fe deficiency because of their rapidly expanding body fluid volume and rapid fetal growth (Wu et al. 2012). Likewise, compared with adults, neonates are more vulnerable to Fe deficiency due to their rapid tissue growth. Nursing animals consuming milk born from Fe-deficient dams are at great risk for Fe deficiency if not provided with an exogenous source of Fe because milk is very low in Fe. Low appetite, reduced growth, anemia, reduced blood flow, and impaired immune function are symptoms of Fe deficiency. Because

TABLE 10.17
Content of Iron in the Hemoglobin and Whole Bodies of Young Pigs

		Hemoglobins in the Body		Iron in Hemoglobin		Iron in the Body	
Age (days)	Body Weight (BW, (kg)	Amount (g)	Content (g/kg BW)	Amount (mg)	Content (mg/kg BW)	Amount (mg)	Content (mg/kg BW)
0	1.3	12.3	9.46	41.8	32.2	52.3	40.2
7	2.7	28.8	10.7	97.9	36.3	122.4	45.3
14	4.2	38.4	9.14	130.6	31.1	163.3	38.9
21	6.0	52.1	8.68	177.1	29.5	221.4	36.9
28	7.8	66.1	8.47	224.7	28.8	280.9	36.0
35	9.8	80.9	8.26	275.1	28.1	343.9	35.1

Adapted from Georgievskii et al. (1982).

of the essential roles of Fe in oxygen transport and storage, as well as energy metabolism, animals with Fe deficiency exhibit extreme fatigue, weakness, shortness of breath, dizziness, pale skin, chest pain, fast heartbeat, cold feet, and unusual cravings for dirt (Haider et al. 2018; Theil 2004).

Among farm animals, piglets reared in confinement or entirely dependent on unsupplemented milk- or plant-based diets are particularly susceptible to anemia (Pond et al. 1995). Pigs are born with a limited Fe reserve and grow very rapidly during the neonatal period (Table 10.17), but sow's milk provides <8 mg of Fe per day for a 2-kg piglet (compared with its requirement for 70 mg Fe per day; Georgievskii et al. 1982). Thus, newborn piglets must receive an intramuscular injection of 200 mg Fe (in the form of an Fe-dextran complex) within the first few days after birth. In contrast, anemia is not common in lambs and calves because they usually receive supplemental feeding. The amount of 25–30 mg soluble Fe/kg dietary DM is sufficient for the appetite, growth, and development of preruminants (Bremner et al. 1976).

Excess: Fe toxicity is not a common problem in farm animals but may result from a prolonged period of oral administration of Fe (Pond et al. 1995). However, a flow of large amounts of erosive material (containing Fe, Cu, and toxic heavy metals) to farms during flooding may cause Fe toxicity and deaths in both ruminants and nonruminants. Likewise, a concentration of Fe as low as 0.1 ppm in the surrounding water can damage the gills of fish and crustaceans, leading to secondary bacterial, fungal, and viral infections (Dryden Aqua 2024). Deaths occur in some aquatic animals (including brown trout, mountain whitefish, boreal toad tadpoles, oligochaete worms, and planarians) when exposed to 0.5 ppm Fe^{3+} in their surrounding water for >30 days (Cadmus et al. 2018). Dietary content of Fe at 5 g/kg DM is toxic to 20-kg pigs as indicated by anemia, diarrhea, reduced feed intake, reduced weight gain, and hypothermia, whereas the intramuscular administration of a single dose of 0.6 g Fe (as ferrous sulfate) per kilogram of BW to 20-kg pigs causes death (Georgievskii et al. 1982). Chronic Fe toxicity results in (a) Cu and P deficiencies; (b) reduced Co absorption; (c) stomach pain and gastrointestinal dysfunction (because Fe is corrosive to the lining of the GIT); (d) nausea and vomiting; (e) depression, reduced feed intake, and impaired growth; (f) the excess generation of reactive oxygen species; (g) oxidative stress and damage to tissues (e.g., liver and brain); and (h) cardiomyopathy, hypotension, and seizures (Georgievskii et al. 1982; Gozzelino and Arosio 2016; Papanikolaou and Pantopoulos 2005). Animals with Fe toxicity may die due to hepatic or respiratory failure (Papanikolaou and Pantopoulos 2005).

Chelating agents are used to treat Fe toxicity. For example, deferoxamine [also known as desferioxamine, a natural product isolated from Actinomycetes (*Streptomyces pilosus*)] strongly binds (a) free Fe^{3+} at an acidic pH and (b) free Fe^{2+} at pH 7.0, yielding ferrioxamine (a water-soluble complex) in both cases (Goodwin and Whitten 1965). Specifically, the formation of ferrioxamine from desferioxamine and Fe^{3+} is maximum (100%) at pH 2.5–5.8 and decreases gradually to 60% with deceasing pH from 5.8 to 8. In contrast, the formation of ferrioxamine from desferioxamine and Fe^{2+} increases gradually from 5% to maximum (100%) with increasing pH from 2.4 to 7.0 and remains maximally bound at pH 8.0. Ferrioxamine in plasma is readily excreted in the urine. Intravenous, intramuscular, or subcutaneous administration of desferioxamine can remove excess Fe from the body (Valentovic 2008). Additionally, adding deferoxamine (which is poorly absorbed by the GIT) to high-Fe diets may alleviate or prevent Fe toxicity.

Zn^{2+} shares the same transporter (DCT1) with Fe^{2+} in enterocytes and extraintestinal cells. Thus, elevated but safe levels of Zn in diets (e.g., 150 ppm; i.e., 10 times the requirement in chicks; Parsons et al. 1989) may reduce the absorption and distribution of excess Fe in the body. However, there is little information about the role of dietary Zn supplementation to treat dietary Fe toxicity.

Manganese (Mn)

Mn was proposed as a new element by Carl W. Scheele (a Swedish chemist) in 1774. However, Johan Gahn (also a

Swedish chemist) is generally recognized for its discovery late that year, when he isolated Mn from the mineral pyrolusite by heating it with charcoal. Mn derives its name from the Greek *magnesia*, a region in southeastern Greece. The term "manganese" was first used in 1779 by Louis Guyton de Morveu to describe this elemental mineral. Both Mn and Mg are divalent metals, but they differ in chemical and biochemical properties. The nutritional study of Kemmerer et al. (1931) led to the discovery that dietary Mn is required for ovulation in female mice. Subsequent studies also found that Mn is essential for the growth, lactation, and fertility of both ruminants (Hidiroglou 1979) and swine (Grummer et al. 1950). These investigations highlight an important role for minerals in animal production.

Sources: Mn is present naturally in ores as oxides, hydroxides, carbonates, and silicates. Animal products, as well as plant foods (e.g., nuts, green leafy vegetables, and most whole grains), are good sources of Mn. However, corn contains a very low content of this mineral. The concentrations of Mn in water and forages vary with location.

Absorption and transport: Less than 10% of ingested Mn (e.g., 3–5% even for $MnSO_4 \cdot H_2O$ in poultry) and <1% are absorbed by the GIT of nonruminants and ruminants, respectively (Baker 2001; Spears 2003). In the small intestine, Mn^{2+} is absorbed into enterocytes primarily by DCT1 (Bai et al. 2012). ZIP8 may also play a role in the transport of Mn^{2+} across the apical membrane of the cells. Within enterocytes, Mn^{2+} is transported into its target sites by a Golgi transporter PMR1P (Mn-transporting ATPase) and vesicles. Both PMR1P and vesicle discharge mediate the exit of Mn^{2+} across the basolateral membrane of the enterocyte into the lamina propria. Dietary intake of divalent metals, such as Fe^{2+}, Ca^{2+}, Cu^{2+}, Mg^{2+}, and Zn^{2+} negatively inhibits Mn^{2+} absorption by the small intestine because they compete for DCT1, possibly with Fe^{2+} being the strongest inhibitor. Absorbed Mn enters the portal circulation. During transport in the blood, Mn^{2+} is carried to peripheral tissues by transferrin (~50%) as well as albumin (~5%), α2-macroglobulin, heavy γ-globulins, lipoproteins, citrate, and carbonate (Davidsson et al. 1989; Scheuhammer and Cherian 1985). Extraintestinal tissues take up Mn^{2+} by specific transporters. NRAMP1 is the major transporter of Mn^{2+} in macrophages and monocytes. Under practical feeding conditions, moderate excesses of Fe or Co in corn- and SBM-based diets have only minimal effects on Mn absorption, but excesses of dietary inorganic or phytate P substantially reduce Mn absorption in swine and poultry (Baker 2001). Removal of all fiber and phytate from diets for nonruminants can nearly double the efficiency of Mn absorption. In animals, biliary secretion (and thus fecal output) is the main pathway for Mn excretion.

Content in the body and tissues: Newborn calves, newborn piglets, and newly hatched chicks contain 65–70 mg, 0.65–0.7 mg, and 10–20 μg Mn, respectively (Georgievskii et al. 1982). Concentrations of Mn in the whole blood and the plasma of cattle are 50–100 and 5–6 μg/L, respectively. The content of Mn in tissues of animals (μg/100 g fresh weight) is as follows: liver, 200–300; kidneys, 120–150; bones, 300–370; pancreas, 140–160; spleen, 20–25; heart, 25–30; brain, 34–40, and skeletal muscle, 15–25. The distribution of Mn in the body (%) is as follows: bone, 55–57; liver, 17–18; skeletal muscle, 10–11, hide, 5–6; and other organs, 10–13 (Georgievskii et al. 1982).

Functions: Mn^{2+} is required for some enzymes involved in amino acid and glucose metabolism, ammonia detoxification, antioxidative reactions, bone development, and wound healing (Table 10.18). For example, Mn^{2+} is an essential cofactor for arginase, which hydrolyzes arginine into ornithine (a precursor of polyamines and proline) plus urea (Brook et al. 1994). Likewise, Mn^{2+} regulates the expression of Mn^{2+}-containing superoxide dismutase (an antioxidative enzyme) in animal tissues (Luo et al. 2007). In addition, glycosyltransferase catalyzes the synthesis of proteoglycans that are needed for the formation of cartilage and bone. Furthermore, the catalytic center of prolidase, which catalyzes the hydrolysis of proline- or hydroxyproline-containing dipeptides, contains Mn^{2+} (Besio et al. 2013).

Deficiency symptoms: In animals, Mn deficiency results in low appetite and growth restriction; abnormal metabolism of amino acids, glucose, and lipids [e.g., hyperammonemia, impaired gluconeogenesis, and reduced plasma

TABLE 10.18
Manganese-Dependent Enzymes in Animals

Enzyme or Protein	Function
Arginase	Arginine + H_2O → Ornithine + Urea
Acetyl-CoA carboxylase	Enz-biotin + HCO_3^- + ATP → Enz-biotin-COO^- + ADP + Pi
Glutamine synthetase	Glutamate + NH_4^+ → Glutamine
Glycosyltransferase	Synthesis of proteoglycans
Manganese superoxide dismutase	$2O_2^-$ + $2H^+$ → H_2O_2 + O_2 (mitochondria)
Phosphoenolpyruvate carboxykinase (PEPCK)	Oxaloacetate + GTP → Phosphoenolpyruvate + GDP + CO_2
Prolidase	Hydrolysis of proline- or hydroxyproline-containing dipeptides
Propionyl-CoA carboxylase	Enz-biotin + HCO_3^- + ATP → Enz-biotin-COO^- + ADP + Pi
Pyruvate carboxylase	Pyruvate + HCO_3^- + ATP → Oxaloacetate + ADP + Pi

high-density lipoprotein (HDL) levels]; skeletal and bone defects (i.e., bone malformation in calves and piglets); oxidative stress and reduced insulin sensitivity; scaly skin and dermatitis; degeneration of reproductive tracts and reduced reproductive function in both males and females; and impaired immunity (Georgievskii et al. 1982; Harris 2014). Young birds also exhibit perosis. These abnormalities can be treated by dietary supplementation with Mn.

Excess: High concentrations of Mn in tissues, particularly the brain, result in neurological disorders in animals. Symptoms of Mn toxicity include reduced feed intake and growth restriction; headaches, violent psychotic behavior, and Parkinson-like symptoms; muscle rigidity, leg cramps, and irritation (Santamaria and Sulsky 2010). Excessive Mn (e.g., 250–500 mg/kg diet for sheep and 2.6–3 g/kg diet for calves; or 0.32 mg/ml in ruminal fluid) adversely alters the microbial population, concentrations of total short-chain fatty acids, and the ratio of propionate to total short-chain fatty acids in the rumen, while reducing growth and the concentration of hemoglobin in blood (Georgievskii et al. 1982). The risk of Mn neurotoxicity is increased in animals receiving total parenteral nutrition.

Molybdenum (Mo)

Mo was discovered by Carl Welhelm Scheele in 1778. However, he did not obtain the pure element of Mo. In 1788, Peter Jacob Hjelm first described the isolation of Mo from molybdenite. Mo derives its name from this mineral. Mo can occur as Mo^{4+}, Mo^{5+}, and Mo^{6+}. The most common form of Mo is molybdate (MoO_4^-). Tungsten, which is similar to Mo in chemical structure, competes with Mo for binding to molybdopterin (a pterin that is eventually complexed with Mo).

Sources: Seafoods, meats, milk and dairy products, egg yolk, legumes (e.g., beans and peas), leafy vegetables, and whole grains are good sources of Mo. Feed additives of Mo include molybdenum chloride, sodium molybdate, molybdenum glycinate, and molybdenum amino acid chelate.

Absorption and transport: Mo is absorbed by the stomach and small intestine through an active, carrier-mediated process, which is inhibited by sulfate (Mason and Cardin 1977). This may explain why high dietary sulfate content reduces the absorption and retention of dietary Mo in ruminants and why these animals are more susceptible to Mo toxicity when dietary sulfate is reduced to sulfide in the rumen (Suttle 2010). Mo exits the enterocyte across its basolateral membrane via unknown transporters, which may include multidrug resistance-associated proteins 3 and 4 (MRPs 3 and 4) for transporting GSH conjugates. Molybdate (MoO_4^{2-}) and sulfate (SO_4^{2-}) may share the same transporter because they exhibit mutually competitive inhibition for absorption by the small intestine. Absorbed Mo enters the portal circulation and is transported in the blood either as the free hexavalent molybdate anion (MoO_4^{2-}) in plasma or attached to proteins in red blood cells. Mo is taken up by tissues through yet unknown transporters, which may include a Na^+-dependent active transporter. About 90% and 10% of absorbed Mo are excreted in the urine and liver secretions, respectively.

Content in the body and tissues: The blood of animals contains 10–20 μg Mo/L, with 70% of Mo being in erythrocytes and 30% in plasma (Georgievskii et al. 1982). Thus, concentrations of Mo in plasma are 3–6 μg/L. The content of Mo in tissues (mg/kg fresh weight) of animals fed Mo-adequate diets is as follows: bones, 15–40; liver, 2–13; kidneys, 2–3.4; spleen, 0.4–1.2; skeletal muscle, 0.4–1.2; heart, 0.5–0.8; brain, 0.1–0.15; and hide, 2.5–5.0 (Georgievskii et al. 1982). The distribution of Mo in the body of animals (%) is as follows: bones, 60–65; hide, 10–11; wool (sheep), 5–6; skeletal muscle, 5–6; liver, 2–3; other tissues, 9–18 (Georgievskii et al. 1982).

Functions: Three Mo-dependent enzymes require Fe as Fe-S centers or heme for intra-electron transfer, and one Mo-dependent enzyme uses NAD^+ as the electron acceptor. These enzymes in animal cells, which are indicated in the following reactions, play an important role in purine catabolism and uric acid synthesis, aldehyde detoxification, and the metabolism of S-containing amino acids. As noted previously, Mo can be used to treat Cu toxicity in animals.

$\text{Hypoxanthine} + O_2 + H_2O \rightarrow \text{Xanthine} + H_2O_2$	(Xanthine oxidase, Mo, Fe/S)
$\text{R-CHO} + O_2 + H_2O \rightarrow \text{R-COO}^- + H_2O_2 + H^+$	(Aldehyde oxidase, Mo)
$SO_3^{2-}(\text{sulfite}) + H_2O \rightarrow SO_4^{2-}(\text{sulfate}) + 2H^+ + 2e^-$	(Sulfite oxidase, Mo, heme-Fe)
$\text{Xanthine} + NAD^+ + H_2O \rightarrow \text{Uric acid} + NADH + H^+$	(Xanthine dehydrogenase, Mo, NAD^+)
$NO_3^- + NADPH + H^+ \rightarrow NO_2^- + NADP^+ + H_2O$	(Nitrate reductase, Mo; bacteria)
$N_2 + 8e^- + 8H^+ \rightarrow H_2 + 2NH_3$	[Nitrogenase (Fe and Mo, or V); bacteria]

Mo plays an important role in the utilization of nitrate and nitrogen gas by bacteria through the following two Mo-dependent reactions. Nitrate reductase, which is a transmembrane respiratory enzyme in bacteria, fungi, and yeast, contains FAD and heme-Fe. Nitrogenase contains a nonheme Fe-S center.

$$\text{Nitrate reductase: } NO_3^-(\text{nitrate}) + NADH + H^+ \rightarrow NO_2^-(\text{nitrite}) + NAD^+ + H_2O$$

$$\text{Nitrogenase: } N_2 + 8H^+ + 8e^- + 16\text{Mg-ATP} \rightarrow 2NH_3 + H_2 + 16\text{Mg-ADP} + 16\text{Pi}$$

Deficiency symptoms: Mo deficiency is rare in farm animals under practical production conditions. Animals with experimentally induced Mo deficiency exhibit elevated concentrations of xanthine and sulfite in plasma, as well as an abnormal heart rhythm and neurological dysfunction (Harris 2014).

Excess: High levels of Mo, which can result in Mo toxicity (known as molybdenosis), interfere with the absorption and utilization of the dietary Cu, resulting in anemia, diarrhea, fatigue, and exhaustion in animals. There are reports that 200 ppm Mo in diets or 5 mg Mo/kg BW is safe for steers (Kessler et al. 2012).

Nickel (Ni)

Ni was discovered by Axel Fredrik Cronstedt in 1751. However, its use for making bronzes and Ni-Cu alloys dates back as far as 3500 BCE. The name of this mineral is derived from the German word *kupfernickel*, which means Devil's copper or St. Nicholas's (Old Nick's) copper. The most common oxidation state of Ni is +2 and is chemically active. Ni^{2+} forms compounds with many anions, including sulfide, sulfate, carbonate (CO_3^{2-}), hydroxide, carboxylates, and halides.

Sources: Ni is widely spread in plants (Nielsen 1987). Nuts (including peanuts) and leguminous seeds are good sources of Ni. Most plant-sourced feedstuffs contain this mineral. Common forages contain 0.5–3.5 mg Ni per kilogram of DM. In contrast, animal products are poor sources of Ni.

Absorption and transport: Dietary Ni is absorbed by the small intestine via DCT1. In rats with a mutation in the DCT1 protein, there is no intestinal transport of Ni via the apical membrane of the enterocyte (Knöpfel et al. 2005). Ni exits the enterocyte across its basolateral membrane via yet unidentified transporter(s) for passive diffusion. Less than 10% of ingested Ni is absorbed into the portal circulation, and the remainder is excreted in the feces (Nielsen 1987). In the blood, this mineral is transported by albumin to tissues, which take up Ni via clathrin-mediated endocytosis (Muñoz and Costa 2012).

Functions: The role of this mineral in microbial metabolism is well known. For example, by serving as a cofactor of nine bacterial enzymes: urease, superoxide dismutase, glyoxalase I, acireductone dioxygenase, [NiFe]-hydrogenase, carbon monoxide dehydrogenase, acetyl-CoA A synthase/decarbonylase, methyl-coenzyme M reductase, and lactate racemase (Boer et al. 2014), Ni affects the metabolism of amino acids, carbohydrates, and lipids in intestinal bacteria. Thus, Ni plays an important role in the utilization of nonprotein nitrogen and urea recycling in ruminants. Ni is selected by these diverse enzymes because of its plasticity in coordination and redox chemistry (Ragsdale 2009). In animal cells, Ni activates heme oxygenase to produce carbon monoxide, a gaseous signaling molecule (Sunderman et al. 1983). Results from some animal studies have shown that Ni acts in synergy with vitamin B_{12} to stimulate DNA synthesis and hematopoiesis in bone marrows (Nielsen 1991). Furthermore, a dietary deficiency of Ni impairs the growth of several animal species, including chicks, cows, goats, pigs, rats, and sheep (Nielsen 1991).

Deficiency symptoms: Because of its relatively high content in plant feedstuffs and its presence in animal-sourced products and drinking water, Ni is normally not deficient in livestock, poultry, fish, or crustaceans under practical feeding conditions. When Ni deficiency was experimentally induced in six animal species (cattle, chicks, goats, pigs, rats, and sheep), they exhibited growth restriction, impaired hematopoiesis, reduced hematocrit, oxidative stress, or structural abnormalities of tissues (Nielsen 1987). These deficiency symptoms can be fully alleviated by dietary supplementation with Ni.

Excess: Toxic levels of Ni inhibit dioxygenases, including histone demethylases. Excess dietary intake of Ni (e.g., ≥250 mg/kg diet for chicks, dogs, ducks, mice, monkeys, rabbits, pigs, and rats) results in toxic effects (Nielsen 1987). Symptoms of Ni toxicity include depressed food intake partly due to low palatability, growth restriction, diarrhea, dark-colored feces, coarse and shaggy hair, vomiting, anemia, and tissue (e.g., liver, lung, and skin) lesions. Of note, cattle (including lactating cows and dairy calves) are less sensitive to Ni toxicity because dietary supplementation with ≤ 250 mg Ni/kg does not affect feed consumption, milk production (cows), or growth (calves) (O'Dell et al. 1970a,b). In animals, high dietary Ni reduces the intestinal absorption of Fe, Cu, Zn, and Mg and consequently exacerbates their dietary deficiencies. High dietary intake of vitamins or protein may alleviate Ni toxicity in animals, including rats and chicks (Nielsen 1987).

Selenium (Se)

Se was discovered by Jöns Jacob Berzelius in 1817 as a nonmetal element that was present in S-containing compounds. Its name is derived from the Greek word *selene*, meaning "moon". Because the chemical property of Se is similar to that of S (e.g., nearly identical atomic radius), Se can partly replace S in methionine and cysteine, as well as sulfide ores. Thus, the inorganic form of Se is present in metal sulfide ores, whereas the organic form of Se occurs in certain amino acids (e.g., selenocysteine and selenomethionine). Se was initially thought to be a toxic metal until Schwarz and Foltz (1957) discovered that a dietary deficiency of Se induced liver necrosis in rats.

Like Cr, Se is a dietary mineral that is regulated by the U.S. FDA because of its historical recognition as a toxic substance. In 1929, the U.S. Department of Agriculture (USDA) identified Se as a toxic substance in animal feeds. However, based on the results of subsequent studies, the FDA approved the addition of Se as a nutritional supplement to swine and certain poultry diets in 1974 and to ruminant diets in 1979 (Ullrey 1980). The inability to supplement Se-deficient diets prior to FDA approval had cost the U.S. animal industry hundreds of millions of dollars.

Sources: Se content in concentrate feeds varies greatly with plant species (e.g., 0.1 mg to 4 g Se per kilogram of DM), soils (generally <1 mg to 100 mg Se per kilogram of soil), and seasons (Georgievskii et al. 1982). Some regions of the world are deficient, but some are rich, in this mineral. The concentrations of Se in groundwater range from <3 to 340 μg/L (Bajaj et al. 2011; Naftz and Rice 1989).

The dominant forms of Se in feeds and forages are seleno-methionine, and seleno-cysteine. Seafoods, meats, liver, milk, yeast, nuts, and green leafy vegetables are good sources of this mineral. Organic forms of Se are better utilized by animals, as compared with inorganic forms (Bodnar et al. 2016). In inorganic forms, Se exists as selenious (H_2SeO_3) and selenic (H_2SeO_4) acids, and their salts are called selenites and selenates, respectively. Se can also occur as H_2Se (hydrogen selenide) and $(CH_3)_3Se^+$ (trimethylselenonium ion).

The content of Se in soils (and thus plants) differs substantially worldwide (NRC 1983). Se-deficient regions in the United States are the Northwest, the Northeast, the Atlantic coastal area, Florida, and areas surrounding the Great Lakes (with Se content in most of the soils from these regions being <0.5 ppm). Se-excessive regions in the United States are South Dakota, Wyoming, Montana, North Dakota, Nebraska, Kansas, Colorado, Utah, Arizona, and New Mexico (e.g., the mean Se content in soils from the western United States being 4.5 ppm), producing plants with high Se levels that may be toxic to livestock. In Canada, in almost all regions of the east and north of the Great Lakes, in the northern regions of the prairie provinces, and in parts of the Rocky Mountains, Se deficiencies are prevalent, but there is toxic vegetation in extensive areas of Alberta, Saskatchewan, and Manitoba. In China, the northeastern and the southwestern regions are deficient in Se (with the Se content in soils being <0.125 ppm), but there are also Se-excessive regions such as Enshi (Hubei) and Ziyang (Sichuan) where the mean Se content in soils is 27.8 and 17.3 ppm, respectively (Dinh et al. 2018). In addition, low-Se soils can contribute to Se deficiencies in unsupplemented grazing livestock raised in some regions of Australia, New Zealand, Scotland, Finland, Sweden, Austria, Germany, France, Western Russia, Turkey, and Greece (NRC 1983).

Absorption and transport: In ruminants, most dietary selenate (SeO_4) is reduced to selenite (SeO_3) in the rumen, and the remaining selenate enters the small intestine for absorption (Weiss 2005). About 30–40% of selenite can be further converted by ruminal microbes to insoluble forms of selenium that escape to the large intestine for excretion. Organic forms of Se complexed with amino acids (e.g., methionine and cysteine) in feeds can be either directly incorporated into microbial proteins (~10%) or released after the degradation of the amino acids in the rumen.

In both ruminants and nonruminants, selenate is absorbed rapidly by the small intestine via the apical Na^+-sulfate cotransporters 1 and 2, whereas selenite is taken up into the enterocyte via facilitated diffusion (Burk and Hill 2015). The absorption of selenite varies from 50% to 90%, and the absorption of selenate is nearly complete. Organic forms of dietary Se complexed with methionine or cysteine are fully transported into enterocytes via the transporters of these amino acids. Thus, the absorption of enteral Se by the small intestine is relatively efficient compared to many other microminerals. For example, the true absorption of Se in diets supplemented with inorganic Se is about 50% in dairy cows, goats, and sheep; the true digestibilities of Se from Se-methionine (measured in goats) and Se-yeast (measured in sheep) are 65% and 44%, respectively (Weiss 2005); and 63% of orally administered inorganic Se is absorbed by the small intestine of swine (Baker 2001). Inside enterocytes, inorganic Se is incorporated into selenoprotein P (Sepp1). The latter is exported across the basolateral membrane of the cells into the lamina propria via exocytosis. The absorbed inorganic Se enters the portal circulation as Sepp1, which is subsequently taken up by the liver via Sepp1 receptor (apolipoprotein E receptor-2)-mediated endocytosis. In hepatocytes, Sepp1 is degraded through proteolysis to release Se, which is used for the synthesis of selenoproteins, including liver-type Sepp1. The latter is exported from the liver into the blood via receptor-mediated exocytosis. In systemic circulation, Sepp1 is the major transport form of the diet-derived inorganic Se. Sepp1 in the blood is taken up by extrahepatic tissues via Sepp1 receptor-mediated endocytosis. Intracellular catabolism of Sepp1 releases Se, which then participates in biochemical reactions.

Dietary seleno-methionine and seleno-cysteine (the organic forms of Se in which the S atom is replaced by Se) are absorbed by the enterocyte via methionine and cysteine transporters, respectively (Chapter 7). These two amino acids exit the enterocyte across its basolateral membrane into the lamina propria via methionine and cysteine transporters, respectively. The absorbed organic Se enters the portal circulation as seleno-methionine and seleno-cysteine, which are subsequently taken up by the liver via methionine and cysteine transporters, respectively. In hepatocytes, these two amino acids are degraded to release Se, which is used for the synthesis of selenoproteins, including liver-type Sepp1. The metabolic fate of Sepp1 is the same as described previously. Either high or low intakes of dietary Ca or excesses of dietary S or Fe reduce the intestinal absorption of Se in ruminants, including lactating dairy cows and calves (Spears 2003). Similarly, cyanogenetic glycosides (in certain legumes) are metabolized in the rumen to cyanide, which reduces the absorption of Se by the small intestine, leading to a much lower nutritional status of Se in the whole body, as reported in ewes fed a white-clover variety (Spears 2003).

Content in the body and tissues: Georgievskii et al. (1982) reported the content of Se in the bodies and tissues of animals. The concentration of Se in the whole blood is 50–180 μg/L, with about 70% of Se being present in erythrocytes and 30% in plasma. The concentrations of Se in tissues (μg/100 g fresh weight) of sheep fed a diet containing 0.3 ppm Se are as follows: kidneys, 78; liver, 19; pancreas, 14; spleen, 12; heart, 9.7; skeletal muscle, 8.9; lungs, 8.4; brain, 6.9; hooves, 2.7; and hair, 21. The concentrations of Se in tissues (μg/100 g fresh weight) of sheep fed a diet containing 2–4 ppm Se are as follows: kidneys, 87; liver, 60; pancreas, 39; spleen, 30; heart, 30; skeletal muscle, 23; lungs, 23; brain, 26; hooves, 72; and hair, 49. The increases

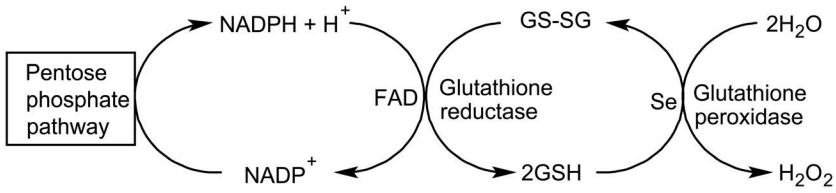


FIGURE 10.11 Role of glutathione in anti-oxidative reactions in cells. Selenium (Se) is a cofactor of glutathione peroxidase (4 Se atoms/protein). Thus, a deficiency of Se results in oxidative stress in animals.

in Se concentrations in response to its dietary intake are not uniform throughout the tissues. The content of Se in farm animals is 20–25 μg/kg BW and varies with dietary Se content. The distribution of Se in the body (%) is as follows: skeletal muscle and heart, 50–52; bones, 10; liver, 8; hide, hair and horny tissues, 14–15; and other tissues, 15–18.

Functions: Se is a cofactor of GSH peroxidase [4 Se atoms/protein] (Figure 10.11) and many other selenoproteins with redox signaling functions (Table 10.19). For example, thioredoxin converts oxidized peroxiredoxin and oxidized protein into reduced peroxiredoxin and reduced protein, respectively, whereas glutaredoxin reduces H_2O_2, lipid hydroperoxide, and oxidized protein (Figure 10.12). Thus, this mineral plays an important role in antioxidative reactions (Hanschmann et al. 2013). In addition, Se can regulate the expression

TABLE 10.19
Seleno-Enzymes and Proteins in Animals

Enzyme or Protein	Function
Enzyme	
Classical GSH peroxidase (GPX1)	$2GSH + H_2O_2 \rightarrow GS–SG + 2H_2O$
Gastrointestinal GSH peroxidase (GPX2)	$2GSH + H_2O_2 \rightarrow GS–SG + 2H_2O$
Plasma GSH peroxidase (GPX3)	$2GSH + H_2O_2 \rightarrow GS–SG + 2H_2O$
Phospholipid-hydroperoxide (PLH) GSH peroxidase (GPX4)	$2GSH + PLH \rightarrow GS–SG + Lipid + 2H_2O$
Iodothyronine 5'-deiodinase-1 (liver, kidney)	Thyroxine (T_4) → 3,5,3'-Triiodothyronine (T_3)
Iodothyronine 5'-deiodinase-2 (thyroid gland, muscle, heart)	Thyroxine (T_4) → 3,5,3'-Triiodothyronine (T_3)
Iodothyronine 5'-deiodinase-3 (brain, fetal tissues, placenta)	Thyroxine (T_4) → 3,5,3'-Triiodothyronine (T_3)
Thioredoxin reductase (containing FAD^+)	$OTR + NADPH + H^+ \rightarrow RTR + NADP^+$
Methionine sulfoxide (MSO) reductase	$MSO(Protein) + NADPH + H^+ \rightarrow Methionine(Protein) + NADP^+ + H_2O$
Protein	
Selenoprotein P (plasma)	A secreted, antioxidative glycoprotein
Selenoprotein W (initially identified in sheep muscle and heart)	An antioxidative protein

Note: GSH, reduced glutathione; GS-SG, oxidized glutathione; OTR, oxidized thioredoxin; RTR, reduced thioredoxin

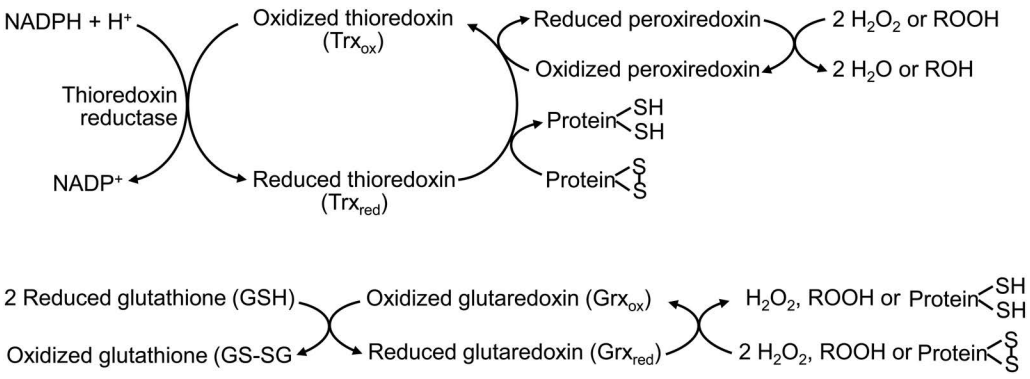


FIGURE 10.12 Roles of thioredoxin, peroxiredoxin, and glutaredoxin in anti-oxidative reactions in animals. LOH, lipid; LOOH, lipid hydroperoxide. Reduced thioredoxin catalyzes the conversion of oxidized peroxiredoxin and oxidized protein into reduced peroxiredoxin and reduced protein, respectively. Reduced glutaredoxin catalyzes the conversion of H_2O_2, lipid hydroperoxide, and oxidized protein into H_2O, lipid hydroperoxide, and reduced protein, respectively.

of genes related to antioxidative defense in animal cells (Burk and Hill 2015).

Deficiency symptoms: Se deficiency results in oxidative stress and tissue damage in animals. In animals, deficiency symptoms include (a) enhanced hemolysis; (b) white muscle disease (gross skeletal-muscle degeneration and loss of pigmentation); (c) Keshan disease (named after a region in Northeast China), which is characterized by poor blood circulation, cardiomyopathy, and myocardium necrosis; (d) Kashin–Beck disease (a degenerative osteoarthritis with skeletal deformations and dwarfism); (e) impairment of cell-mediated and humoral immune responses; (f) male infertility; and (g) increased the risk of cancer development (Hanschmann et al. 2013; Harris 2014; Wrobel et al. 2016). Se deficiency can be treated by dietary supplementation with Se.

Excess: Although Se is essential for health, high doses of Se induce oxidative stress. Therefore, excess Se is highly toxic to mammals, birds, fish, and crustaceans. Se toxicity (selenosis) in animals can occur after acute or chronic ingestion of excess Se (Georgievskii et al. 1982). Symptoms include nausea and vomiting; reduced food intake and growth restriction; nail discoloration, brittleness, and loss; hair loss; fatigue; irritability; neurological dysfunction; garlic-like breath odor (due to the volatile metabolite dimethylselenide); and cirrhosis of the liver, pulmonary edema, or even death (Wrobel et al. 2016). In animals, plasma Se concentrations 10 to 15 times greater than normal values can be fatal (NRC 2005). Because Se poisoning does not have an antidote, recognizing symptoms is crucial for the effective management of affected subjects. Based on clinical studies, chelation or emesis is not recommended for treatment of Se toxicity. However, once the cause has been identified, it is essential to prevent further exposure.

Silicon (Si)

Si-containing rocks were known to ancient civilizations. Johann Heinrich Pott recognized silica as an "earth" in the mid-18th century. Si was discovered by Jöns Jakob Berzelius (a Swedish chemist) in 1823 by heating potassium chips in a silica container. The name Si is derived from the Latin word *silicis*, meaning "flint". It is the seventh-most abundant element in the universe and the second-most abundant element in the earth's crust, only after oxygen. Si has great chemical affinity for oxygen and forms long or circular chains with oxygen atoms or the "–OH" group. In addition, Si has a strong anion character to bind Ca^{2+}, Fe^{2+}, Mg^{2+}, and Mn^{2+}, as well as Al. In animals, this mineral forms complexes with collagen for biomineralization in bones. Although Si is present in all tissues, it is most prevalent in bones and other connective tissues, including skin and arteries.

Sources: Si is widely present in plant- and animal-sourced feedstuffs. Silica content in plants is generally higher than that in animals. For example, whole grasses and cereals contain 30–60 g silica per kilogram of DM. However, fruits and bone-free animal products contribute little silica to diets. In nature, silica exists as silicon dioxide (SiO_2, also known as silica) and silicates (silicic acid esters). Silicon dioxide is poorly soluble in water. In neutral water, silicates are broken down to orthosilicic acid [$Si(OH)_4$], which is the major Si species absorbed by the small intestine. A synthetic compound consisting of Si is silicone, which is a polymer with a silicon-oxygen-silicon (Si-O-Si) backbone and is not absorbed by the intestine due to its poor solubility in water.

Absorption and transport: In animals, dietary silica as $Si(OH)_4$ is absorbed by the small intestine via (a) NaPi2b, a known Na^+-phosphate cotransporter (Ratcliffe et al. 2017); and (b) aquaporins (AQPs) 3, 7, and 9 (Garneau et al. 2015). $Si(OH)_4$ exits the enterocyte across its basolateral membrane into the lamina propria. Basolateral $Si(OH)_4$ transporters may include those for passive diffusion, AQP8, and a Na^+-dependent transporter for the phosphate export. Absorbed silica enters the portal circulation. In the blood, $Si(OH)_4$ is not bound to proteins and is taken up by extraintestinal tissues possibly via (a) tissue-specific AQPs 3, 7, 9, and 10 (Garneau et al. 2015); and (b) possibly NaPi2a and NaPi2c, as reported for phosphate ions. In the extracellular matrix of many tissues, including the aorta, trachea, tendon, bone, and skin, $Si(OH)_4$ is largely bound to glycosaminoglycans (Price et al. 2013).

Dietary forms of Si affect its absorption by the small intestine. About 50–60% of the soluble $Si(OH)_4$ in drinking water and ingested green beans is absorbed by the small intestine (Sripanyakorn et al. 2009). In contrast, only 2% of the Si in ingested bananas, where Si forms large complex polymers, is taken up by the gut (Price et al. 2013). Silica nanoparticles can be absorbed through the intestine, but particle diameter and surface properties are major determinants of the rates of their absorption.

Functions: Si enhances the activities of (a) prolyl hydroxylase for post-translational hydroxylation of proline residues in collagen and, therefore, the maturation of this protein; (b) alkaline phosphatase to dephosphorylate compounds under alkaline conditions; and (c) acid phosphatase to dephosphorylate compounds under acidic conditions (Price et al. 2013). This mineral also stimulates the synthesis of type-1 collagen and elastin, osteoblast differentiation, and the incorporation of Ca^{2+} and phosphate into bones. Furthermore, Si binds to glycosaminoglycans and plays an important role in the formation of cross-linkages between collagen and proteoglycans (Sripanyakorn et al. 2009). Collectively, Si is essential for the formation and rigidity of bones, as well as the maintenance of the normal structure of connective tissues.

Deficiency symptoms: Because silica is widely present in feeds and drinking water, its deficiency is rare in mammals, birds, fish, and crustaceans under practical feeding conditions. Under experimental conditions, dietary Si deficiency has the following adverse effects: (a) reduces the content of collagen, glycosaminoglycan, articular cartilage, hexosamines, and hydroxyproline in bones; and (b) impairs bone calcification and formation, connective tissue

development, cardiovascular function, and wound healing (Schwarz 1978). A lack of Si in purified diets reduced the average daily weight gain of 1- to 21-day-old chicks by 50% (Carlisle 1972) and caused bone abnormalities (Carlisle 1980). Similar results were reported for young rats (Schwarz and Milne 1972). The growth depression and impaired bone biomineralization of Si-deficient chicks and rats can be prevented by dietary supplementation with Si (e.g., 100 ppm Si as $Na_2SiO_3 \cdot 9H_2O$; Carlisle 1972, 1980; Schwarz and Milne 1972).

Excess: In animals, the inhalation of SiO_2 results in irritation, fibrogenic reaction, and phagocytosis of silica particles by alveolar macrophages in the lungs (Jones and Hunt 1983). The engulfed silica fuses with the lysosomes, resulting in damage to the lysosomal membrane and the subsequent release of lysosomal enzymes to kill the phagocytes. Free SiO_2 released from the lysed cells is engulfed again to initiate another vicious cycle to eventually damage the lungs. This disease is called silicosis. Oral intake of silica from naturally occurring foods generally does not pose a risk of adverse effects (Harris 2014; Sripanyakorn et al. 2009). However, the chronic intake of excess SiO_2 or other silica compounds (e.g., magnesium trisilicate) can result in its deposits in the renal tubules to develop a disease called calculi.

Tin (Sn)

The discoverer and discovery date of tin are unknown. However, the peoples of Egypt, Mesopotamia, and the Indus Valley started to use this mineral for manufacturing alloys (e.g., bronze) around 3000 BC. The name "tin" is derived from the Latin word *stannum*, which means an alloy of silver and lead. Tin has two main oxidation states, Sn^{2+} and Sn^{4+}. Tin is widely used to plate steel cans for food-processing equipment (e.g., cooking utensils) and food containers. Cassiterite (SnO_2, Sn^{4+}), the naturally occurring tin oxide form of tin that is soluble in acids but insoluble in water, is the most important raw material for chemical research and the commercial production of tin. The salts of tin (e.g., $SnCl_2$ and SnF_4) are soluble in water.

Sources: Tin is present at very low concentrations in plant- and animal-sourced feedstuffs, as well as drinking water. Dissolved in water, tin generally occurs as $SnO(OH)_3^-$. Concentrations of tin in the water of rivers are usually 6–10 ng/L, with much higher concentrations of up to 300 ng/L in some regions, such as the Rhine (WHO 2004). In most unprocessed foods, the content of tin (mainly as Sn^{4+}) is generally <1 mg/kg DM, but higher content is present in canned foods due to dissolution of the tin coating or tin plate. Stannous fluoride (tin fluoride, SnF_4) is used as a vehicle for F^- to improve oral health.

Absorption and transport: Little is known about the cellular and molecular characteristics of intestinal tin absorption in animals. Dietary tin (+2 and +4) is absorbed by the small intestine via a passive transporter. Only a small amount of dietary tin (3–40% depending on content) is absorbed into the portal circulation, and the remainder is excreted in the feces (Salant et al. 1914). Through the blood circulation, tin is transported to tissues, which take up tin via yet unknown transporters. In animals, tin occurs mainly in bones and the skin (also feathers in birds), and to a much lesser extent, other tissues (e.g., liver, kidneys, lungs, spleen, and lymph nodes; Johnson and Greger 1985; Salant et al. 1914). During intestinal absorption, transport in the blood, and storage in tissues, tin does not undergo change in its oxidation state.

Deficiency symptoms: Because tin is present in feeds, its deficiency is rare in mammals, birds, fish, and crustaceans under practical feeding conditions. However, tin deficiency in animals can be induced under experimental conditions. Schwarz et al. (1970) reported that rats fed a tin-free purified diet exhibited poor growth and loss of hair. Addition of tin compounds (e.g., stannic sulfate and potassium stannate) supplying 1–2 mg tin/kg diet increased growth rates of the rats by 50–60%, compared with tin-deficient rats because the experimental diet did not contain adequate riboflavin. Compared to rats fed a diet containing 2 mg tin/kg, rats fed a diet containing 17 μg tin/kg exhibited poor growth and decreased food efficiency (Yokoi et al. 1990). Dietary supplementation with tin fully restored the normal rates of food intake and growth in the animals.

Excess: Excess tin causes metabolic and physiological abnormalities in animals. For example, the activity of δ-aminolevulinic acid dehydratase in the erythrocytes of rats was reduced by 45% in response to high tin intake (Johnson and Greger 1985). In addition, compared with the control, dietary supplementation with 720 mg tin/kg diet to growing chickens decreased BW gain, feed intake, and feed efficiency, as well as the content of hemoglobin, red blood cells, and hematocrit in the blood (Sun et al. 2014). Furthermore, oral administration of excess stannous chloride [also known as tin chloride ($SnCl_2$); 30 mg tin/kg every 12 h] for 3 days reduced intestinal Ca^{2+} absorption (Yamaguchi et al. 1979). Finally, activities of antioxidative enzymes (e.g., GSH peroxidase and superoxide dismutase) in tissues are reduced by a high intake of dietary tin (Sun et al. 2014).

Vanadium (V)

Vanadium was discovered in Mexico by Andrés Manuel del Rio (a Spanish chemist) in 1801 and was confirmed by Nils Gabriel Sefström (a Swedish chemist) in 1830. The metal was isolated by Henry Enfield Roscoe in 1867 by combining vanadium trichloride (VCl_3) with hydrogen gas (H_2). This element was named for Vanadis, the Scandinavian goddess of beauty, due to the multiple valance states (–3 to +5) of the element. The elemental metal is rarely found in nature, and V exists as complexes with oxygen, nitrogen, and other elements (Crans et al. 2004). V^{2+} compounds are reducing agents, and V^{+5} compounds are oxidizing agents. V^{+4} compounds exist as vanadyl derivatives that contain the VO^{2+} center.

Vanadium with the valance states of +4 and +5 is more relevant to biology (Tsiani and Fantus 1997). In nature, vanadium coordinates with oxygen to form an oxyanion. Vanadates (VO_4^{2-}) are similar to molybdates (MoO_4^{2-}) and phosphates (HPO_4^{2-}) in chemical structure.

Sources: Vanadium is present in plant- and animal-sourced foodstuffs, as well as drinking water. The average concentration of vanadium in fresh water, groundwater, and potable water is 0.5 μg/L, with the highest concentrations being up to 130 μg/L in volcanic areas (Rehder 2015). Some mineral water springs contain high concentrations of vanadium ions (e.g., 54 μg/L in springs near Mount Fuji; Rehder 2008). The average content of vanadium in foods (where vanadium is mainly present in the form of the vanadyl species) is 5–30 μg/kg DM (Rehder 2015). Rich food sources of this mineral are shellfish, whole grains, and black pepper. Meats, dairy products, legumes, and fruits contain a low amount of vanadium. In animal diets, vanadium occurs mainly as VO^{2+} (vanadylate, V^{4+}) and HVO_4^{2-} (vanadate, V^{5+}). The air and the stainless-steel equipment used for feed processing may also be sources of vanadium.

Absorption and transport: Absorption of dietary vanadium by the small intestine is mediated by transmembrane carriers (Nielsen 1995). In the lumen of the stomach, V^{5+} is reduced by vitamin C to V^{4+}, which is less toxic than the former. V^{4+} enters the duodenum. In the small intestine, V^{4+} and V^{5+} are absorbed by phosphate and other anion transporters, and also possibly via transferrin and other pathways for nonheme Fe. The rate of V^{5+} transport by enterocytes is 3 to 5 times greater than that of V^{4+} (Nielsen 1987). Thus, the amount of dietary vanadium that is absorbed by animals depends on gastric conditions and the time of the transit of food through the stomach. Upon entering the enterocyte, vanadium binds to ferritin. Vanadium exits the enterocyte across its basolateral membrane via PO_4^{3-} transporters for VO_4^{3-} and nonheme Fe export pathways. Absorbed vanadium enters the portal circulation. In the blood, this mineral is transported mainly as V^{4+}-transferrin and V^{4+}-ferritin complexes in plasma and as the V^{4+}-hemoglobin complex within erythrocytes. Vanadium exits the capillaries of the microvasculature into the interstitial fluid, where V^{4+} is the predominant form of vanadium. Extraintestinal tissues take up V^{4+} and V^{5+} via anion transporters, which may include phosphate transporters and other anion transporters (e.g., Cl^-/HCO_3^- exchangers and divalent anion-Na^+ cotransporter). In addition, the transferrin pathway may also be responsible for the uptake of vanadium by peripheral tissues and cells (Yuliani et al. 2024). Within animal cells, V^{5+} is reduced by vitamin C, GSH, and NADH to V^{4+}, which is the primary form of vanadium. Ferritin may be the major intracellular binding protein for V^{4+} and V^{5+}.

Less than 5% of ingested vanadium is absorbed by the intestine of fed animals, and the remainder is excreted in the feces. For example, only 0.34% of ingested vanadium as ammonium metavanadate was absorbed by the intestine of fed sheep (Hansard et al. 1982), and 2.6% of ingested vanadium as V_2O_5 (vanadium pentoxide) was absorbed by fed rats (Conklin et al. 1982). In contrast, in fasted rats, 30% of orally administered vanadium as Na_3VO_4 was absorbed by the small intestine, with the remainder being recovered in the feces (Wiegmann et al. 1982). Thus, the rate of the intestinal absorption of vanadium varies greatly with animal species, nutritional state, and dietary composition. Under fed conditions, only a small amount of dietary vanadium is available to animals.

Functions: The role of vanadium as an essential cofactor of bromoperoxidase in marine algae has stimulated great interest in its biological function (Butler 1998). In this reaction, $R\text{-}H + Br^- + H_2O_2 \rightarrow R\text{-}Br + H_2O + OH^-$, the brominated hydrocarbon product is essential for algal growth and survival. To date, a vanadium-dependent enzyme has not been identified in animal cells. Pharmacological but safe doses of vanadium (0.1 mg V_2O_5/ml in drinking water for 14 days) reduce intestinal transport of glucose in both normal and diabetic rats (Madsen et al. 1993), which likely results from an inhibition of Na^+/K^+-ATPase activity in the basolateral membrane of enterocytes (Hajjar et al. 1987). Furthermore, vanadium enhances insulin action in animal cells through stimulating the phosphorylation of the insulin receptor, thereby augmenting glucose utilization by skeletal muscle, heart, and adipose tissue (Crans et al. 2004).

Deficiency symptoms: Because vanadium is widely present in feeds and drinking water, its deficiency is rare in animals under practical feeding conditions. Under experimental conditions, dietary vanadium deficiency reduces growth and reproductive performance, as reported for rats fed a vanadium-free purified diet (Schwarz and Milne 1971). The addition of vanadium to the diet enhanced chicken growth by over 40%. Vanadium deficiency in chickens also decreases feather growth and bone abnormalities in the tibia (Mertz 1974). Furthermore, Anke et al. (1990) found that goats fed a vanadium-deficient diet (10 μg vanadium/kg) had a higher abortion rate and produced less milk during the first 56 days of lactation, compared with goats fed a diet containing 2 mg vanadium/kg. In addition, kids from vanadium-deprived goats exhibited a high rate of mortality, skeletal deformations in the forelegs, and swollen tarsal joints of the forefeet (Anke et al. 1990).

Excess: Toxic levels of dietary vanadium (mg/kg diet) differ among animals: rats, 25; growing chicks, 30; laying hens, 20; sheep, 400 (Nielsen 1987). High concentrations of vanadium inhibit the activities of alkaline, acid, and protein phosphatases, as well as protein phosphorylases, diphosphoesterase, ribonuclease, and Na^+/K^+-ATPase, with Ki values being 0.4 to 2.5 μM (Crans et al. 2004). Thus, excess vanadium interferes with cell signaling transduction and metabolism. There are reports that 200 ppm ammonium metavanadate in drinking water impairs male fertility and enhances embryonic death in rats (Morgan and El-Tawil 2003). In addition, excess vanadium in diets results in (a) gastrointestinal discomfort, reduced food intake, diarrhea, and growth restriction; (b) green coloring of the tongue; (c) oxidative stress; and (d) renal failure, respiratory

dysfunction, and cardiovascular defects (Assem and Levy 2009). Vanadium toxicity can be alleviated by dietary EDTA, chromium, protein, Fe^{2+}, and NaCl (Nielsen 1987).

Zinc (Zn)

Pure metallic Zn was discovered in 1746 by the German chemist Andreas S. Marggraf. Its name is derived from the German word *Zinke* meaning "spiked" for the shape of Zn crystals. However, centuries earlier, Zn ores were used for making brass (a mixture of Cu and Zn), which was found between 1400–1000 BC in Palestine. Through nutritional studies in the 1930s, Conrad Elvehjem and colleagues discovered that Zn was essential for rat growth (Todd et al. 1934). Two decades later, Zn deficiency due to the consumption of phytate-rich unleavened bread was found to be associated with a type of human dwarfism in Egypt (Prasad et al. 1963). To date, Zn is routinely included in the diets of mammals, birds, fish, and crustaceans.

Zn does not donate or accept electrons, and therefore Zn is not involved in coupled oxidation-reduction reactions. However, Zn^{2+} has strong attraction to electron pairs (Georgievskii et al. 1982). In the plasma and intracellular fluid, free Zn reacts rapidly with water to yield an insoluble zinc hydroxide:

$$Zn^{2+} + H_2O \rightarrow Zn(OH)_2 \downarrow + 2H^+.$$

Therefore, proteins are required to transport Zn in animals. In animals, the whole-body content of Zn ranks second among trace minerals behind Fe (i.e., 2–2.5 g of Zn/70 kg BW), with most of Zn being present in skeletal muscle (47%), bone (29%), skin (6%), liver (5%), brain (1.5%), kidneys (0.7%), and heart (0.4%; Harris 2014). Zn is rich in the seminal fluid of males. In all cells, Zn exists primarily as a complex with proteins and nucleic acids.

Sources: Calamine, a historic name for an ore of Zn, is a rich source of zinc carbonate ($ZnCO_3$, smithsonite) and zinc silicate [$Zn_4Si_2O_7(OH)_2 \cdot H_2O$, hemimorphite]. Seafood (e.g., oysters, crab, lobster, and other shellfish), meat, eggs, dairy products, fish meal, yeast, seeds, nuts, and legumes (cooked dried beans and peas), as well as the bran and germ of cereal grains, are good sources of Zn (Harris 2014). The source of zinc is critical; chick growth was reduced with 1 g of Zn/kg of diet from Zn sulfate ($ZnSO_4$), but not with the same level of Zn from Zn oxide (ZnO) (Persia et al. 2003). Most practical diets that contain a well-balanced mineral premix can supply sufficient Zn for farm animals. Forage-based diets for ruminants and horses are generally deficient in Zn, and, therefore, are usually supplemented with a free-choice mineral premix (Greene 2000).

Absorption and transport: In gastric fluid with an acidic environment, Zn can exist as a free ion. In the lumen of the small intestine with a neutral or slightly alkaline solution, Zn binds to gastroferrin to increase its solubility. Zn is absorbed into the enterocyte via the apical membrane transporters ZIP4 (a member of the Zn family of transporters) and DCT-1 (Hojyo and Fukada 2016; Hampton et al. 1976). Expression of ZIP4 is stimulated by dietary Zn intake. Upon entering the enterocyte, Zn binds primarily to a cytosolic protein called cysteine-rich intracellular protein and possibly a nonspecific binding protein (NSBP) for transport to the basolateral membrane. This mineral may also bind to cytosolic metallothionein for temporary storage in enterocytes. Zn exits the enterocyte across its basolateral membrane into the lamina propria via Zn transporter-1, then enters the portal circulation. Intestinal absorption of Zn is reduced by high dietary levels of various minerals (e.g., Ca, phosphorus, and Cu) and phytate possibly due to the formation of insoluble complexes and competition for absorption sites in the GIT. In farm animals (e.g., swine and ruminants), 98% and 2% of the total Zn absorption occurs in the small intestine and the large intestine, respectively (Georgievskii et al. 1982).

Absorbed Zn enters the portal circulation. In plasma, about 60%, 30%, and 10% of Zn bind to albumin, α_2-macroglobulin, and other serum factors, respectively (Harris 2014). Free Zn is present in serum at about 0.1 μM. Zn in the blood is taken up by the liver via ZIP2/3, and exits this organ via ZnT-1 into the systemic circulation. Various other organs take up Zn via ZIPs (e.g., kidneys, ZIP4; pancreas, ZIP1; spleen and bone marrow, ZIP2/3; Dempski 2012). There is evidence that voltage-gated Ca^{2+} channels take part in the transport of Zn^{2+} into animal cells (Bouron and Oberwinkler 2014). Once entering the cells, Zn is assimilated into various metalloproteins including metallothionein. Metallothionein is a small protein (MW, 10,000), and about 1/3 of its amino acid residues are cysteine. Metallothionein may act as a reservoir for Zn and as a detoxifying agent for toxic heavy metals, such as Cd.

The BW gain and the accumulation of Zn in bone are effective measures of dietary Zn bioavailability in animals. Some of the factors that affect Fe absorption also influence Zn absorption. The efficiency of absorption of Zn by the small intestine decreases with increasing dietary Zn intake (Ketata et al. 2024). Diseased conditions (e.g., trauma and infection), stress (e.g., heat stress), and excessive dietary Ca substantially reduce Zn absorption (Baker 2001). Interestingly, excesses of dietary Zn can exacerbate Cu and Fe deficiency states, but excesses of dietary Cu or Fe have only minimal effects on Zn absorption or utilization in nonruminants. Thus, transmembrane transporters for cationic minerals in enterocytes or extraintestinal cells appear to prefer Zn over Cu and Fe as substrates.

Content in the body and tissues: A newborn calf, newborn piglet, and newly hatched chick contain 500 mg, 24–25 mg, and 0.35–0.4 mg Zn, respectively (Georgievskii et al. 1982). The content of Zn in tissues (mg/100 g fresh weight) of adult mammals is as follows: liver, 4–8; kidneys, 1.3–1.8; spleen, 1.5–2.0; heart, 1.4–1.8; skeletal muscle, 0.8–1.2; brain, 0.8–1.3; bones, 6–12; and hide, 2–3. The content of Zn in tissues (mg/100 fresh weight) of 22-kg pigs is as follows: skeletal muscle, 2.2; bones, 5.0; liver, 7.7; whole blood, 0.38; heart, 1.7; hide, 1.2; stomach, 2.1; small intestine, 1.8; and colon, 1.8 (Georgievskii et al. 1982). The concentrations of Zn in the whole blood and plasma are 2.5–6.0 and 1–2 mg/L, respectively.

Concentrations of Zn in plasma or serum are tightly regulated by the dietary intake of Zn and Cu, the rates of absorption of Zn by the small intestine through specific transporters, and the abundance of the intracellular Zn-binding protein (metallothionein). In response to low Zn intake, the rates of intestinal Zn absorption and Zn mobilization from tissues (e.g., liver) are increased, but the rates of Zn excretion through the intestine and kidneys are reduced to compensate for the dietary deficiency. Thus, the circulating levels of Zn may not decrease when animals experience mild or moderate deficiencies of Zn. Conversely, animals can adapt to an increase in dietary Zn intake by reducing the rates of its intestinal absorption while increasing the rates of Zn storage in tissues and excretion to maintain homeostasis. Therefore, concentrations of Zn in the liver, but not in plasma or serum, are a good measure of Zn nutritional status in animals. Under conditions of severe Zn deficiency, concentrations of Zn in plasma or serum may be substantially reduced.

Functions: There are about 100 Zn enzymes associated with the mammalian genome (Harris 2014). Examples of Zn-dependent enzymes and Zn-binding proteins are summarized in Table 10.20. To maintain a Zn-finger structure (a structural configuration that contains Zn ions to stabilize

TABLE 10.20
Zinc-Dependent Enzymes and Binding Proteins in Animals

Enzyme or Protein	Function
Alanyl-glycine dipeptidase	Hydrolysis of alanyl-glycine dipeptide
Alcohol dehydrogenase	Alcohol catabolism
Alkaline phosphatase	Hydrolyzes phosphate groups
Aminolevulinic acid dehydrogenase	Biosynthesis of heme
Aminopeptidase (Zn^{2+}, Co^{2+}, Mo^{2+})	Hydrolysis of polypeptides
Angiotensin-converting enzyme	Regulation of Na balance and blood pressure
Carbonic anhydrase	$CO_2 + H_2O \rightarrow H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$
Carboxypeptidase A	Digestion of dietary protein
Carboxypeptidase B	Digestion of dietary protein
Carnosidase (Zn^{2+}, Mn^{2+})	Carnosine + H_2O → L-Histidine + β-Alanine
Component 9 of complement	Immune system
Dehydroquinate synthase	Microbial synthesis of aromatic amino acids
DNAase	DNA degradation
DNA polymerase	DNA synthesis from deoxyribonucleotides
Enolase (Zn^{2+}, Mg^{2+}, Mn^{2+})	2-Phospho-D-glycerate ⇌ Phosphoenolpyruvate + H_2O
Fructose-1,6-bisphosphatse	Gluconeogenesis
Galactosyltransferase complex	Synthesis of lactose in lactating mammary glands
Glutamate dehydrogenase	Glutamate + NAD^+ ⇌ NH_4^+ + α-Ketoglutarate + NADH + H^+
Glycyl-glycine dipeptidase	Hydrolysis of glycyl-glycine
Glycyl-leucine dipeptidase (Zn^{2+}, Mn^{2+})	Hydrolysis of glycyl-leucine
Glyoxalase	Detoxification of aldehydes
Insulin in secretory vesicles	Used for stabilizing insulin
Lactate dehydrogenase	Pyruvate + NADPH + H^+ ⇌ Lactate + NAD^+
Mannosidase	Hydrolysis of mannose
Metallothionein	Storage or detoxification of zinc
5'-Nucleotidase	Cleavage of phosphate from nucleoside 5'-monophosphates
*Poly(ADP-ribose)polymerase	Repairs DNA damage
Protein kinase C	Cell signal transduction
Phospholipase C	PIP_2 → 1,2-Diacylglycerol + IP_3
*Retinoic acid receptor	DNA binding and genetic regulation
RNAase	RNA degradation
RNA polymerase	RNA synthesis from ribonucleotides
*Steroid hormone receptor	DNA binding and genetic regulation
Superoxide dismutase (cytosol)	Removal of O_2^-
Thymulin	Hormone of the immune system
*Transcription factors	Regulation of synthesis of many mRNAs
Triosephosphate isomerase	Dihydroxyacetone-P ⇌ D-Glyceraldehyde 3-P
Tripeptidase (Zn^{2+} or Co^{2+})	Hydrolysis of tripeptides

Note: IP_3, inositol 1,4,5-trisphosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate; * Indicates the presence of zinc finger structure.

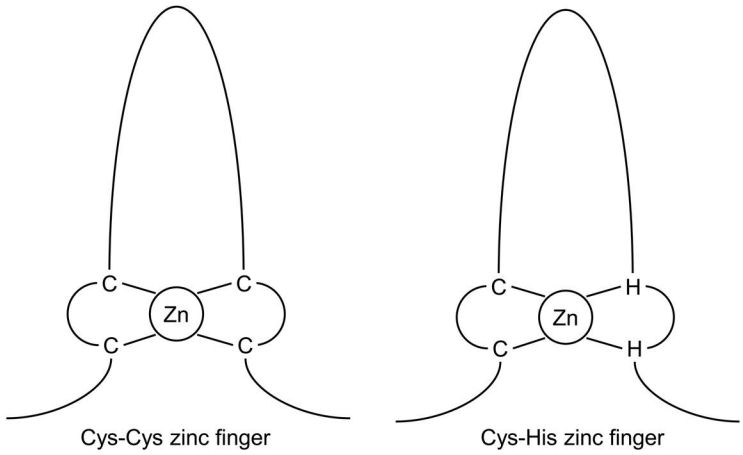


FIGURE 10.13 The zinc-finger structure in protein. This unique structure coordinates zinc ions with cysteine and histidine residues in proteins to stabilize them. Zinc-finger proteins help to stabilize proteins while binding DNA to exert their biological functions, such as the regulation of gene expression.

a polypeptide molecule) in proteins (Figure 10.13), Zn stabilizes the macromolecules (e.g., insulin and nucleic acids) and regulates gene expression in cells. Thus, Zn plays a significant role in genomic stability and antioxidative responses; the metabolism of protein (both protein synthesis and degradation), nucleic acids, carbohydrate, lipids, and energy; integrity and function of cells (including epithelial cells); transport of vitamins A and E in the blood; immune response; reproduction (e.g., male and female fertility); and collagen and keratin formation, as well as healthy development and repair of hair, skin, and nails. Furthermore, high dietary Zn (e.g., 2425–3000 ppm ZnO) can reduce the number of bacteria (e.g., *Enterobacteriaceae*, the *Escherichia* group, and *Lactobacillus* spp.) in the stomach, small intestine, and large intestine of weaned piglets (Starke et al. 2014). The inclusion of 0.3% ZnO (3000 ppm) in diets can prevent diarrhea while enhancing the growth and feed efficiency of weanling pigs. Of note, Zn (e.g., in calamine lotion consisting of 8% calamine and 8% ZnO) is now used topically in medicine to relieve the itching, pain, and discomfort of skin irritations caused by insect bites, chickenpox, shingles, poisonous plants, heat rash, acne, and hives, while reducing the weeping and oozing of the blisters (Mayo Clinic 2023). Indeed, the medical use of calamine dates back to 1500 BC.

Deficiency symptoms: Zn deficiency is widespread in farm animals if their diets are not supplemented with adequate Zn. Zn is essential for many metabolic pathways (including protein synthesis, cell division, and the removal of oxidants such as superoxide anion), as noted previously. Thus, in animals, Zn deficiency results in reduced food intake, growth restriction, and poor food utilization; gastrointestinal ulcerative colitis, diarrhea, and anorexia; hypogonadism, low sperm counts, fetal abnormalities, and infertility; skin abnormalities (e.g., hyper-keratinization of the skin, known as parakeratosis in pigs), dermatitis, and impaired wound healing; impaired growth and development of the cells that mediate innate immunity and of T- and B-lymphocytes, resulting in impaired immune function; impaired activation of the extracellular signal-regulated kinases (ERK1/2) in the brain (leading to neurological dysfunction); eye lesions and photophobia; and behavioral change (Harris 2014; Hojyo and Fukada 2016). Of note, bilateral cataracts occur in animals such as salmonids and rainbow trout due to a Zn deficiency caused by the low intake and reduced intestinal absorption of Zn (particularly when diets contain high levels of calcium, phosphorus, or phytate; Hardy 2025). This nutritional deficiency leads to oxidative stress and damage in the lens and can be prevented by increasing Zn levels in feed mineral premixes.

Excess: Zn toxicity rarely occurs in farm animals under practical feeding conditions but may result from excessive Zn supplementation to diets (Pond et al. 1995). Excessive intake of Zn results in oxidative stress and apoptosis in animal cells (Formigari et al. 2007). Because the absorption of Zn, Fe, Cu, and Ni by the apical membrane of the enterocyte shares the same transporter, DCT1 (Table 10.6), excessive Zn results in impaired absorption of Fe, Cu, and Ni by the small intestine. Many of the toxic effects of Zn result from Cu and Fe deficiencies. Symptoms of Zn toxicity include reduced feed intake and impaired growth; gastric irritation, abdominal pain, nausea, vomiting, and diarrhea; the impaired absorption of dietary Fe and Cu, the abnormal metabolism of Fe and Cu, and anemia; increased risk for infectious disease; cardiovascular and neurological dysfunction; and damage to reproductive tracts and impaired reproduction in both males and females (Cai et al. 2005; Georgievskii et al. 1982). Of note, Georgievskii et al. (1982) reported that concentrations of Fe (as $FeSO_4.7H_2O$) in the ruminal fluid ≥0.3 mg/ml are toxic to ruminal microbes as assessed by the rate of cellulose digestion.

HIGHLY TOXIC MINERALS

Al, As, Cd, Pb, and mercury have no biological or nutritional function but are highly toxic to animals at relatively low concentrations in food [e.g., >0.306 ppm in swine diets (Liao et al. 2020) or drinking water (e.g., 0.1 to 0.5 ppm in water for aquatic animals; Neville and Campbell 1988)].

TABLE 10.21
Total Content of Toxic Metals in Feeds for Animals

Feed	Cadmium (Cd)	Lead (Pb)	Arsenic (As)	Mercury (Hg)
		mg/kg dry matter		
Ingredients				
Barley grain	0.11	0.97	—	0.006
Citrus pulp meal	0.19	0.76	—	—
Corn gluten	0.12	2.07	0.13	—
Corn grain	0.06	0.56	0.26	—
Dicalcium phosphate	1.53	2.26	4.17	—
Fish meal	0.40–0.57	0.52–2.22	2.39–4.7	0.10
Limestone	2.56	9.79	0.46	—
Molasses	<0.10	2.24	0.20	—
Rapeseed, extracted	0.15	0.60	—	—
Soybean meal	0.07–0.14	0.93 to <1.00	<0.10	0.022
Sugar beet pulp	0.14–0.29	1.47–2.24	1.06	—
Sunflower meal	0.41	—	—	0.003
Sunflower seeds	0.16–0.65	0.37	—	—
Mineral premix, unspecified	0.58–1.79	3.38–5.50	3.03–6.8	0.02
Meat and bone meal	—	0.81	—	—
Wheat	0.19	0.26	0.06	0.003
Forages and roughages				
Grass/herbage (fresh)	0.62	4.93	—	0.02
Hay	0.11–0.73	3.64–3.89	0.05 to 0.10	0.005 (alfalfa)
Grass silage	0.09–0.10	<1.0–2.02	0.10–0.16	—
Corn silage	0.16–0.28	<1.0–2.19	0.05 to <0.10	—
Straw	0.10	<1.00	<0.10	—
Complete feeds				
Ruminants, unspecified	0.11	0.34	0.27	0.012
Poultry, unspecified	0.16	1.16	1.83	0.039
Poultry, layers	0.16	0.87	0.20	—
Poultry, broilers	0.19–0.23	0.52 to <1.00	0.18–0.34	—
Pigs, unspecified	0.09	1.03	0.62	0.032
Pigs, weaner	0.13	<1.00	0.43	—
Pigs, <17 weeks	<0.10–0.16	0.77 to <1.00	0.39–0.72	—
Pigs, >17 weeks	0.07 to <0.10	0.38 to <1.00	0.28–0.31	—
Pigs, sows	0.09	0.70–2.10	0.39–0.85	—

Source: López-Alonso (2012); Nicholson et al. (1999).
Note: —, data are not available.

They are potential environmental pollutants in the air, ground and drinking water, and soil and can readily enter the body through the mouth, lungs, skin, and eyes via feeding, inhalation, and contact. Trace amounts of these minerals are usually present in animal feeds (Table 10.21). Maximum tolerable levels of dietary minerals, which are defined as the dietary levels that, when fed for a defined period of time, will not impair animal health or performance (NRC 2005), are summarized in Table 10.22. The concentrations of Al, As, Cd, Pb, and mercury in most common feedstuffs, forages, pastures, and drinking water are usually less than the maximum tolerable levels and, therefore, do not impair the growth, development, lactation, or reproduction of grazing ruminants or monogastric animals. However, accidental exposure of animals to high doses of Al, As, Cd, Pb, and mercury is extremely harmful and can be fatal (Vázquez et al. 2015).

Aluminum (Al)

Al was discovered by Humphry Davy in 1808. It was subsequently isolated by Friedrich Wöhler in 1927 through reacting a small quantity of K with an excessive amount of $AlCl_3$. The name of Al is derived from the Latin word *alumen*, meaning "alum", a bitter mineral that has been used for antisepsis and dyeing since ancient times. Al is the third most abundant element in the earth's crust. Al is chemically reactive with more than 100 different minerals and has a strong affinity for oxygen to form oxides. Its most common oxidation state is +3, but this mineral also exists as Al^{1+} and

TABLE 10.22
Maximum Tolerable Levels of Dietary Minerals for Animals (on a Dry Matter Basis)

Mineral	Cattle	Sheep	Swine	Poultry	Horse	Rabbit	Rodents	Fish
Aluminum, ppm	1,000	1,000	200	200	200	200	200	—
Arsenic, ppm								
Inorganic	30	30	30	30	30	50	30	5
Organic	100	100	100	100	100	100	100	—
Boron, ppm	150	150	150	150	150	150	150	—
Bromine, ppm	200	200	200	2,500	200	200	300	—
Cadmium, ppm	10	10	10	10	10	10	10	10
Calcium, %	1.5	1.5	1	1.5[a], 5[g], 1.2[h]	2	2	2	0.9
Chromium, ppm								
Chloride form (Cr^{3+})	100	100	100	500	100	100	100	—
Oxide form (CrO)	3,000	3,000	3,000	3,000	3,000	3,000	30,000	3,000
Cobalt, ppm	25	25	100	25	25	10	25	—
Copper, ppm	40	15	250	250	250	800	500	100
Fluorine, ppm	40[a], 100[g], 50[c]	60[a], 150[b], 60[f]	150	150	40	40	150	—
Iodine, ppm	50	50	400	300	5	5	—	—
Iron, ppm	500	500	3,000	500	500	500	500	—
Lead, ppm	100	100	10	10	10	30	10	10
Magnesium, %	0.6	0.6	0.24	0.5[a], 0.75[g]	0.8	0.3	0.5	0.3
Manganese, ppm	2,000	2,000	1,000	2,000	400	400	2,000	—
Mercury, ppm								
Inorganic	—	—	0.2	0.2	0.2	—	0.2	—
Organic	2	2	2	1	1	2	1	1
Molybdenum, ppm	5	5	150	100	5	5	7	10
Nickel, ppm	100	100	250	250	50	50	50	50
Phosphorus, %	0.7	0.6	1	1[a], 0.8[g], 1[h]	1	1	0.6	1
Potassium, %	2	2	1	1	1	3	1	—
Selenium, ppm	5	5	4	3	5	2	5	2
Silver, ppm	—	—	100	100	—	—	—	>3
Silicon, %	0.2	0.2	—	—	—	—	—	—
Sodium chloride, %	4.5[a], 3.0[d]	4	3	1.7	6	3	4	4[i]
Sulfur, %	0.3–0.5[e]	0.3–0.5[h]	0.4	0.4	0.5	—	0.5	—
Tin, ppm	100	100	100	100	100	—	100	—
Vanadium, ppm	50	50	10	25[a], <5[g],10[h]	10	10	—	—
Zinc, ppm	500	300	1,000	500	500	500	500	250

Source: The data for rabbits are taken from Pond et al. (1995); and Georgievskii et al. (1982). The data for other animals are taken from NRC (2005).

Note: Production phases of animals, types of animals, or diets are: [a] young; [b] finishing; [c] adult; [d] lactating; [e] 0.3% in concentrate diets and 0.5% in high-forage diets; [f] breeding; [g] laying hens; [h] other production phases; and [i] most freshwater fish.

Al^{2+}. At a neutral pH, Al forms $Al(OH)_3$, which is barely soluble in water. In addition, Al can react with phosphates to yield Al phosphate ($AlPO_4$) precipitates, rendering P unavailable for animals.

$$Al^{3+} + 3OH^- \rightarrow Al(OH)_3 \downarrow$$

$$Al^{3+} + PO_4^{3-} \rightarrow AlPO_4 \downarrow$$

Sources: Water-soluble forms of Al include aluminum sulfate, aluminum chloride, aluminum nitrate, and aluminum acetate, whereas water-insoluble forms of Al include aluminum oxide and aluminum hydroxide. Al is present in the air, water, and many plant-sourced foods (CDC 2017). Concentrations of Al in drinking water range from <0.001 to 1.029 mg/L, depending on the region, whereas those in groundwater at a neutral pH generally fall below 0.1 mg/L. Forages contain 0.3 to 3.3 g Al/kg DM, and these levels of Al do not pose a risk to grazing ruminants (Niles 2017). The content of Al in cereal grains is generally <5 ppm. Under normal feeding conditions, this mineral does not accumulate in the products and tissues of healthy animals to any significant extent. However, toxic

levels of Al intake may occur in (a) ruminants foraging on plants that are contaminated with large amounts of Al in soil, and (b) nonruminants (e.g., swine and poultry) consuming diets that contain Al-contaminated feed ingredients or are supplemented with high levels of zeolites such as sodium zeolite A ($Na_{12}[(AlO_2)_{12}\ (SiO_2)_{12}]$-$27H_2O$) to provide silica.

Absorption and transport: In the small intestine, Al^+ is oxidized to Al^{2+} by membrane-bound oxidase. Al^{2+} is absorbed by the enterocyte via the apical membrane DCT1. Citric acid and other organic acids (e.g., ascorbic, oxalic, lactic, and tartaric acids) can enhance the intestinal absorption of Al possibly by increasing its solubility. Within the enterocyte, Al is transported by ferritin to the basolateral membrane. This mineral exits the enterocyte across its basolateral membrane into lamina propria via unknown transporters, which may include MRPs 3 and 4 for transporting GSH-conjugates. The rate of intestinal absorption of dietary Al is generally low (0.5% of ingested Al in food matrix; 0.3% of Al in drinking water), except when Al is complexed with organic ligands (e.g., citrate, tartarate, and glutamate) (Cunat et al. 2000; Krewski et al. 2007). Absorbed Al enters portal circulation. In the blood, about 80% and 20% of Al is bound to transferrin (via its Fe-binding sites) and albumin, respectively. Through receptor-mediated endocytosis, the Al-transferrin complex crosses (a) the blood-brain barrier into brain cells and (b) the plasma membrane of osteoblasts into the bones. The Al not absorbed by the intestine is excreted in the feces. Nearly all (>95%) of the blood-borne Al is excreted via the urine.

Toxicity: Symptoms of Al toxicity are consistent across species, including neurodegeneration, bone loss, anemia, and growth depression. Al inhibits Ca^{2+}-, Fe^{2+}-, and Mg^{2+}-dependent enzymes, and interacts with cell membrane proteins and lipids, thereby impairing cell signaling pathways, reducing intestinal nutrient transport, interfering with cell metabolism, inhibiting nucleic acid and protein synthesis, enhancing the production of reactive oxygen species, and impairing neurotransmission (Jaishankar et al. 2014). The latter causes oxidative stress and damages tissues. A dietary level of >500 ppm Al (on a DM basis) is toxic to animals (Jones and Hunt 1983). The greatest complications of Al toxicity occur in the brain (Niles 2017). In fish and crustaceans, high levels of Al also inhibit (a) gill enzymes that are essential for the uptake of ions from the surrounding water, and (b) the diffusion of O_2 and CO_2 through the gills, leading to asphyxiation (Rosseland et al. 1990). In all animals, excess Al results in digestive disorders, low appetite, growth restriction, low feed efficiency, P deficiency, and the demineralization of bone (Igbokwe et al. 2019). The latter results in hypercalcemia (elevated concentrations of Ca^{2+} in serum), which can induce the loss of Mg^{2+} in the urine of all animals and may contribute to the development of grass tetany in ruminants (Eppe et al. 2023). Of practical importance in nutrition, an elevated intake of Al reduces intestinal phosphate absorption and whole-body bioavailability, as well as feed intake, growth, and development in animals (Crowe et al. 1990). In humans, long-term, low-level exposures to Al may increase risk for Alzheimer's disease (Nayak 2002).

Arsenic (As)

As was discovered by Albertus Magnus in 1250 through extracting gold-like pigment from an As ore (As_2S_3). In 1649, Johann Schröder published methods for preparing As from As trioxides (a white solid). The name of As is derived from the Latin *arsenicum* or Greek *arsenikon*, meaning "a strong or muscular character". The most common oxidation states for As are −3 in the arsenides, which are alloy-like intermetallic compounds, +3 in the arsenites, and +5 in the arsenates and most organoarsenic compounds. As can occur as As_4O_6 (a gas) or as AsO_3 (a crystal) that gives rise to arsenic acid [H_3AsO_4 or $AsO(OH)_3$] in water. Arsenic acid is similar to phosphoric acid in chemical structure, such that arsenate and phosphate salts behave very similarly in biochemical reactions. In foods and animal tissues, As usually occurs in combination with S and metals. There is no evidence for As being a physiologically or nutritionally essential mineral in animals.

Sources: As is present in the air, water, and many plant-sourced foods (López-Alonso 2012; Welch et al. 2000). Concentrations of As in drinking water range from <5 to 150 μg/L, depending on the region, whereas those in groundwater range from <1 to 10 μg/L. Forages contain 0.25 to 3.5 mg As/kg DM, and these levels of As do not pose a risk to grazing ruminants (López-Alonso 2012). The content of As in cereal grains ranges from 3 to 285 μg/kg, depending on the plant species and region (Zhao et al. 2010). In cereal grains, As exists as 30% inorganic As (mainly arsenite) and 70% dimethylarsinic acid (Moore et al. 2010). At low levels (e.g., 0.306 ppm As in a diet containing 36.7% rice bran), As does not accumulate in the products and tissues (e.g., blood and skeletal muscle) of healthy animals to any significant extent (Liao et al. 2020).

Popular As-containing poultry and swine feed additives included roxarsone, arsanilic acid, carbarsone, and nitarsone. These substances have been used to prevent parasitic diseases (e.g., coccidiosis caused by coccidia) in poultry and swine since the 1940s. Animals receiving an appropriate level of supplemental As exhibited improvements in growth performance, feed efficiency, and intestinal health (Liao et al. 2020). However, there is concern over the accumulation of As in animal tissues due to its supplementation at elevated doses. For example, relatively high levels of As metabolites are present in chicken livers after the removal of roxarsone from the As-supplemented feed 5 days before slaughter (FDA 2018).

Absorption and transport: Dietary As from grain products (e.g., rice bran) is poorly absorbed by the GIT of animals (only ~25% of the dietary intake in swine) due to high fiber content, with most of the unabsorbed As being excreted in the feces; Liao et al. 2020). By contrast, animals (e.g., rats and humans) absorb dietary organic As (as in

shrimp) with a high rate of efficiency (~90%; NRC 1977). In the stomach and small intestine, dietary As^{5+} is reduced to As^{3+} by vitamin C. As^{3+} and As^{5+} are absorbed by the apical phosphate transporters into the enterocyte, where any As^{5+} is further reduced to As^{3+} by intracellular vitamin C and GSH. In the enterocyte, As^{3+} may bind to metallothionein and GSH. This mineral exits the enterocyte across its basolateral membrane via unknown transporters, which may include MRPs 3 and 4 for transporting GSH conjugates. Absorbed As^{3+} enters the portal circulation, where any As^{5+} is further reduced to As^{3+}. In the portal blood, As^{3+} binds to albumin and is subsequently taken up by the liver (hepatocytes), where As^{3+} is converted into arsenobetaine and dimethylarsenic by *S*-adenosylmethionine-dependent methyltransferase. The liver releases arsenobetaine and dimethylarsenic into the blood, where dimethylarsenic binds to haptoglobin and the hemoglobin α-chain (released from erythrocytes) to form a ternary dimethylarsinous-hemoglobin-haptoglobin complex (Naranmandura and Suzuki 2008). This complex and arsenobetaine (which does not bind to any protein) are transported via the blood circulation to the kidneys, which then excrete arsenobetaine and dimethylarsenic via the urine.

Very little As is stored in the tissues of healthy animals. For example, two days after the administration of As into rats, only 0.3% of the administered As is retained in the body (Harris 2014). Because of their roles in As metabolism and transport, S-containing amino acids (methionine and cysteine) and GSH play important roles in the detoxification of inorganic As to nontoxic or weakly toxic organoarsenics in animals (Figure 10.11).

Toxicity: As is a well-known poison. A diet containing 0.612 ppm As (e.g., due to the inclusion of 73.5% rice bran, which contains As) reduces feed intake and BW gain by 24.5% and 36.7%, respectively, in 26- to 72-kg growing pigs (Liao et al. 2020). The toxicity of As is one of the most common cases of heavy metal poisoning in cattle and other domestic animals, which results from dipping powder, herbicides, hide preservatives, insecticides, and timber preservatives (Moxham and Coup 1968). On farms, As poisoning is associated with trash dumps, old sheds where arsenicals may have been stored (and forgotten), and old containers of arsenicals discarded inappropriately. Inorganic As is a very toxic metal, because it binds with the sulfhydryl groups of about 200 enzymes, thereby inactivating them (Ratnaike 2003). These enzymes are involved in a wide array of reactions, including energy metabolism, protein synthesis, DNA synthesis and repair, immunity, antioxidative response, and neurotransmission. In addition, as an anion structurally analogous to phosphate anion, inorganic As is an inhibitor of Na^+-dependent phosphate transport (NaPi-2b-mediated transport) in the small intestine (Villa-Bellosta and Sorribas 2010) and possibly of phosphate-activated glutaminase. A dietary level of >5 ppm As (on a DM basis) is toxic to animals (Jones and Hunt 1983). Acute As poisoning is initially associated with nausea, vomiting, abdominal pain, severe diarrhea, and a burning sensation in the mouth. Chronic exposure to As results in multiorgan failure (including headaches, muscle weakness, chronic pain, and peripheral neuropathy), skin lesions, as well as skin, lung, and bladder cancers (Jaishankar et al. 2014). The International Agency for Research on Cancer (IARC 2014) indicates that the inorganic form of As is a class-I nonthreshold carcinogen to humans. Trivalent As compounds (arsenites; As^{3+}) are more toxic than the pentavalent forms (arsenates, As^{5+}).

Cadmium (Cd)

Cd was discovered by Friedrich Strohmeyer in 1817. Its name is derived from the Latin word *cadmia*, meaning "calamine" (zinc carbonate, $ZnCO_3$), or from the Greek word *kadmeia* with the same meaning. Cd occurs in the oxidation state +2 in most of its compounds. This mineral is chemically similar to Zn and forms complexes with Zn and Pb in sulfide ores. Cd was the first metal isolated from an ore byproduct of Zn. Cd and its compounds are highly water-soluble compared to other metals. Cd sulfide and selenide are commonly used as pigments in plastics.

Sources: Cd is present in the air, water, and many plant-sourced foods (WHO 2011a). Concentrations of Cd in unpolluted natural water are generally <1 μg/L, whereas those in drinking water range from <0.2 to 26 μg/L, depending on the region. Of note, in contaminated water, concentrations of Cd are generally >1 μg/L, such as the maximum value of 100 μg/L recorded in the Rio Rimao, Peru. Forages contain 2 to 12 mg Cd/kg DM, and these levels of Cd do not pose a risk to grazing ruminants (López-Alonso 2012). The content of Cd is usually <10 μg/kg DM in fruits and vegetables and about 25 μg/kg wet weight in cereals. Cd is accumulated in the tissues of animals at 10–100 and 100–1000 μg/kg wet weight in the liver and kidneys, respectively.

Absorption and transport: Dietary Cd is absorbed in the form of Cd^{2+} by the small intestine into the lamina propria via the apical transporter CDT1 (Ryu et al. 2004). In the enterocyte, Cd^{2+} is carried by metallothionein to the basolateral membrane. This mineral exits the enterocyte across its basolateral membrane via an exporter called metal transporter protein 1 (MTP1; Ryu et al. 2004). Absorbed Cd^{2+} enters the portal circulation, where it is transported bound to transferrin and albumin. In the blood, Cd^{2+} is taken up by extraintestinal tissues via Fe transporters and receptors. Inside the cells, this mineral binds to metallothionein for storage. The Cd not absorbed by the intestine is excreted in the feces. However, Cd can accumulate in the liver and kidneys, where it has multiple cytotoxic effects. Most Cd in the blood circulation is excreted via the urine.

The rate of intestinal absorption of dietary Cd is generally low (usually 0.5–3% of ingested Cd in animals and 5–7% in humans; Asagba 2013) and is negatively affected by dietary Fe content. Dietary deficiency of Fe results in an elevation of the intestinal absorption of dietary Cd. In addition, the inadequate intake of dietary protein or amino acids impairs the transport of Cd in the blood and the urinary excretion of Cd, thereby exacerbating Cd toxicity in animals.

Toxicity: Cd is a metabolic antagonist to Zn, Cu, and Ca in animals, therefore inhibiting enzymes involved in energy metabolism, protein synthesis, DNA synthesis and repair, immune response, antioxidative reactions, and neurotransmission (Jaishankar et al. 2014; Vázquez et al. 2015). A dietary level of 50 ppm Cd (on a DM basis), which is provided as $CdCl_2$, Cd succinate, or Cd propionate, can result in intoxication to animals, whereas a dietary level of 640 ppm (on a DM basis) causes rapid deterioration in their health status (Jones and Hunt 1983). Symptoms of acute Cd poisoning include stomach irritation, vomiting, and diarrhea. Inhaling higher levels of Cd can result in severe damage to the lungs and respiratory dysfunction. Chronic exposure to excess Cd causes (a) necrotizing enteritis and intestinal atrophy; (b) damage to endothelial cells of the microvasculature, resulting in hemorrhagic necrosis in some organs (e.g., testes and skin); (c) disturbances in Ca metabolism, osteoporosis (skeletal damage), and fragile bones; (d) formation of renal stones and renal dysfunction; and (e) damage to the liver and lungs (Jaishankar et al. 2014; Jones and Hunt 1983; Vázquez et al. 2015).

Lead (Pb)

It is unknown when humans first discovered Pb, but this mineral has been mined for over 6,000 years. Pb has two main oxidation states (+4 and +2) and does not react with oxygen. In the earth, Pb rarely occurs as an element and instead is present in ores [e.g., lead sulfide (PbS)]. Compounds of lead (PbF_2, $PbCl_2$, and $PbSO_4$, which are poorly soluble in water) are usually found in the +2 oxidation state.

Sources: Pb is present in the air, water, plant-sourced foods, and some animal products (WHO 2011b). Concentrations of Pb in drinking water range from 1 to 100 μg/L, whereas mean values in groundwater range from 1 to 70 μg/L, depending on the region. Forages contain 0.3 to 16 mg Pb/kg DM, depending on the region, and these levels of Pb do not pose a risk to grazing ruminants (Khan et al. 2012). The content of Pb in vegetables ranges from <0.2 to 2 mg/kg DM (McBride et al. 2014), and the content of Pb in cereal grains is generally <60 μg/kg DM (Zhang et al. 1998).

Absorption and transport: In the stomach and small intestine, dietary Pb^{4+} is reduced to Pb^{2+} by vitamin C. Pb^{2+} is absorbed by the small intestine into the lamina propria via the apical transporter CDT1 (Bressler et al. 2004). In the enterocyte, Pb^{2+} binds to metallothionein and possibly GSH for transport to the basolateral membrane. This mineral exits the enterocyte across its basolateral membrane via unknown transporters, which may include MRPs 3 and 4 for transporting GSH conjugates. Absorbed Pb enters the portal circulation. In the blood, Pb binds to hemoglobin in red blood cells for transport to extraintestinal tissues. This mineral can accumulate in tissues due to its low rate of clearance from the body. The half-life of Pb in the blood and soft tissues is about 40 days for adults, and this increases to about 20 years in the bones (Jaishankar et al. 2014).

The rate of intestinal absorption of dietary Pb is generally low (usually 1–2% of ingested Pb) and is negatively affected by the dietary intakes of Fe, Ca, and P (Bressler et al. 2004). Dietary deficiency of Fe, Ca, and P facilitates the intestinal absorption of dietary Pb and, therefore, exacerbates the toxicity of dietary Pb. The severity of lead poisoning can also be increased by an excess or deficiency of dietary protein intake (Levander 1979).

Toxicity: Pb binds to the sulfhydryl groups of many enzymes and displaces Ca, Fe, Zn, Mg, or Na ion as a cofactor for many enzymes (Vázquez et al. 2015). In addition, Pb inhibits key enzymes involved in heme synthesis from glycine and succinate. Thus, excess Pb inactivates a wide array of enzymes involved in energy metabolism, protein synthesis, DNA synthesis and repair, immunity, antioxidative response, and neurotransmission, while enhancing the production of reactive oxygen species and damaging lipids, proteins, and nucleic acids in cell membranes and organelles (Jaishankar et al. 2014). Dietary intake of 6 mg Pb/kg BW per day is toxic to postweaning mammals, and the dietary intake of 2.7 mg Pb/kg BW can be fatal to neonates (Jones and Hunt 1983). Acute Pb poisoning results in low appetite, headache, hypertension, abdominal pain, nausea, vomiting, renal dysfunction, and fatigue, as well as weakness in the fingers, wrists, or ankles (Assi et al. 2016). Chronic exposure to Pb can cause anemia, hypertension, infertility, birth defects, muscular weakness, brain damage and neurological dysfunction, renal failure, bone loss and abnormalities, and cancers (Assi et al. 2016; Jones and Hunt 1983).

Mercury (Hg)

It is unknown when humans first discovered mercury, but the use of this mineral by Egyptians dates back to about 1500 B.C. Mercury resists a loss of an electron from its outer orbital to form a cation, and behaves similarly to noble gases. However, mercury reacts with atmospheric hydrogen sulfide, whereas ionic mercury and organomercury have a high affinity for reduced sulfhydryl groups, including those of cysteine and GSH (Ballatori 2002). In nature, mercury occurs in +1 (e.g., HgCl) and +2 (e.g., $HgCl_2$ and HgS) oxidation states, with Hg^{2+} being the major species of mercury. Note that $HgCl_2$ is readily soluble in water, HgCl has a low solubility in water, and HgS (mercury sulfide) is barely soluble in water. The mercury element (Hg) is a liquid at room temperature (e.g., 20–25°C). Different forms of mercury have very different metabolic fates in animals (Figure 10.14).

Sources: Mercury is present in the air, water, and many plant-sourced foods mainly in three forms: metallic elements, inorganic salts (mostly Hg^{2+}), and organic compounds (Hg^{2+}; Clarkson 2002). They possess different toxicity and bioavailability to animals. Microbes can convert inorganic mercury into methyl mercury. Organomercury compounds, which include methylmercury (MeHg; CH_3HgX), are soluble in water and occur in the +2 oxidation state.

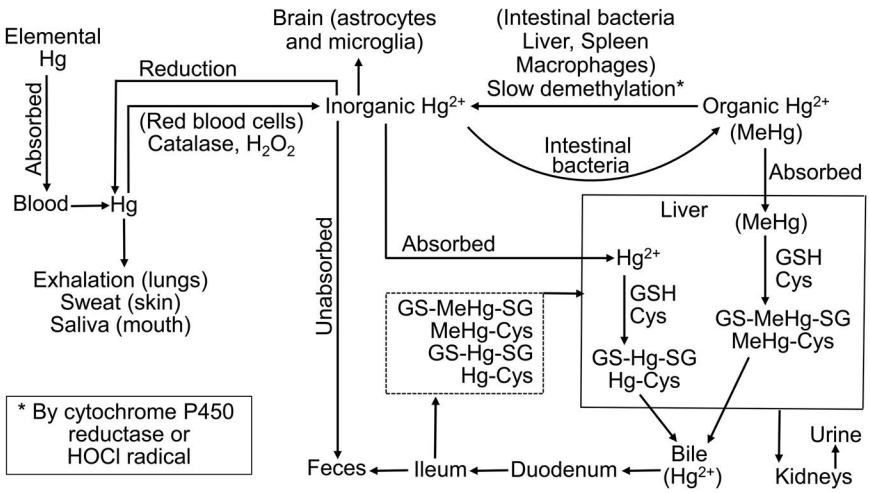


FIGURE 10.14 Metabolism of elemental, inorganic, and organic mercury in animals via different pathways. Intestinal bacteria, erythrocytes, liver, and kidneys play important roles in the detoxification of mercury through the formation of glutathione- and cysteine derivatives. These end products are released from the liver to the bile and then to the small intestine for excretion in the feces. Because of the enterohepatic circulation, most of mercury's glutathione- and cysteine derivatives are absorbed from the ileum into the liver, thereby prolonging the life of mercury in animals. Excess mercury is stored in the brain and can cause neurological toxicity. * Methylmercury is demethylated by organomercury lyase to form inorganic mercury, which is poorly absorbed by the intestine.

Concentrations of mercury in drinking water range from 5 to 100 ng/L (with a mean value of 25 ng/L), whereas those in ground and surface water are generally <0.5 μg/L and can reach >2 μg/L, depending on the region (WHO 2005). Forages generally contain <0.1 mg mercury/kg DM, depending on the region, and this low level of mercury does not pose a risk to grazing ruminants (EFSA 2008). The content of mercury in vegetables ranges from 5 to 75 μg/kg DM, and the content of mercury in cereal grains ranges from 4 to 30 μg/kg DM (Tkachuk and Kuzina 1972). Fish meal may contain <0.5 ppm Hg (EFSA 2008).

Absorption and transport

Elemental Mercury (a Liquid)

The epithelial cells of the GIT are barriers to elemental mercury in diets due to the absence of its transporter. The tight junctions of the gut are virtually impermeable to elementary mercury. Studies with rats indicate that <0.01% of the ingested elemental mercury is absorbed from the GIT (Clarkson 2002). In erythrocytes, elemental mercury is oxidized by catalase and H_2O_2 to form inorganic Hg^{2+}. The element is excreted from animals via exhalation, sweat, and saliva, respectively, through the lungs, sweat, and feces.

Inorganic Mercury

Ionic mercury (Hg^{2+}) binds to cysteine to form an Hg-cysteine complex, which is taken up by the enterocyte via the apical cysteine transporters (Ballatori 2002). Ionic mercury can also be transported by the small intestine via DCT1 (Vázquez et al. 2015). Inside the enterocyte, ionic mercury binds to metallothionein, GSH, and cysteine. This mineral exits the enterocyte across its basolateral membrane via cysteine transporters, MRPs, and possibly the organic anion transporters (Ballatori 2002; Nigam et al. 2015). Absorbed ionic mercury enters the portal circulation. In the blood, it binds to albumin, GSH, and cysteine and is carried to tissues for uptake by cysteine transporters. In rats, a small amount of ionic mercury is reduced to elementary Hg (Clarkson 2002). Ionic mercury is stored in the brain (mainly astrocytes and microglia), and most ionic mercury is conjugated with GSH and cysteine for export to the duodenum via the bile. The entero-hepatic circulation allows for the reabsorption of most inorganic mercury by the ileum into the liver. The GS-Hg^{2+}-SG and cysteine-Hg^{2+} complexes that are not absorbed by the ileum are then excreted in the feces.

In the lumen of the stomach and small intestine, some inorganic mercury is converted into methylmercury by bacteria (Ballatori 2002). The absorption of the resultant organomercury by the small intestine will be the same as the dietary methylmercury (see the sections below).

The rate of the absorption of dietary inorganic mercury depends on its solubility and ease of dissociation in the GIT. About 7–20% of ingested inorganic mercury is absorbed by the small intestine of animals (Clarkson 2002; Jaishankar et al. 2014; Suttle 2010). Dietary intake of divalent minerals negatively affects the intestinal absorption of inorganic mercury. Adequate dietary intake of amino acids facilitates the excretion of inorganic mercury from the body. The half-life of inorganic mercury in animals ranges from 2 to 3 months.

Organic Mercury

Monomethyl-mercury is the predominant form of organic mercury in plant and fish products. In the lumen of the small intestine, a small amount of organic mercury is demethylated by luminal bacteria to inorganic mercury (Clarkson 2002), which is absorbed by the small intestine,

as described previously. Organic mercury (Hg^{2+}) binds to cysteine to form a CH_3-Hg-cysteine complex, which is structurally similar to methionine. The CH_3-Hg-cysteine complex is taken up by the enterocyte via the apical methionine transporters (Ballatori 2002). Inside the enterocyte, the organic mercury binds mainly to metallothionein and, to a lesser extent, to GSH and cysteine. This mineral exits the enterocyte across its basolateral membrane via methionine transporters and MRPs (Vázquez et al. 2015). Absorbed organic mercury enters the portal circulation. In the blood, it binds to albumin, GSH, and cysteine and is transported to tissues, where the CH_3-Hg-cysteine complex is taken up by methionine transporters. In certain extraintestinal tissues and cells, such as the liver, spleen, and macrophages, methylmercury undergoes slow demethylation to form inorganic mercury (NRC 2000), which is excreted from the body, as described previously. In the liver, some organic mercury is conjugated with GSH and cysteine in the liver for excretion to the bile for export to the duodenum. Similar to inorganic mercury, the entero-hepatic circulation allows for the reabsorption of most organic mercury by the ileum into the liver. The GS-$MeHg^{2+}$-SG and cysteine-$MeHg^{2+}$ complexes that are not absorbed by the ileum are then excreted in the feces. About 90% of dietary MeHg absorbed by the small intestine is excreted from animals in the form of inorganic mercury (NRC 2000).

About 90–95% of ingested organic mercury is absorbed by the small intestine of animals (Jaishankar et al. 2014; NRC 2000; Suttle 2010). Through competitive interaction with the transmembrane methionine transporters, dietary methionine and large neutral amino acids negatively affect the intestinal absorption of organic mercury. In addition, an adequate intake of dietary amino acids facilitates the excretion of organic mercury from the body. The half-life of organic mercury in animals ranges from 2 to 3 months.

Dimethylmercury and diethylmercury, which are used for laboratory research, are volatile and lipid-soluble liquids. They are extremely poisonous and dangerous to animals. Dimethylmercury and diethylmercury readily diffuse into the enterocytes and, thereafter, enter the systemic circulation. These two compounds are transported in the blood as lipoprotein complexes. In the liver, spleen, and macrophages, dimethylmercury and diethylmercury are converted into monomethyl mercury, which is subsequently excreted from the body, as described previously.

Toxicity: Mercury has a high capacity for binding to the sulfhydryl groups of proteins, thereby inhibiting their enzymatic and other biological activities (Vázquez et al. 2015). In addition, mercury causes oxidative stress, DNA damage, microtubule destruction, mitochondrial defects, and lipid peroxidation (Jaishankar et al. 2014). Thus, mercury in any form is poisonous by severely affecting the neurological, digestive, and renal organ systems. A dietary level of >2 ppm Hg (DM basis) is toxic to animals (Jones and Hunt 1983). Symptoms of mercury intoxication include diarrhea, headache, abdominal pain, nausea, vomiting, muscle twitching and atrophy, tremors, weakness, impaired neurological function, loss of coordination, kidney malfunction, respiratory failure, vision impairment, and even death. Treatment of mercury toxicity includes (a) the use of Se, which binds Hg and improves antioxidative capacity, as an antidote (Bjørklund 2015); and (b) chelation drugs (e.g., edetate Ca disodium and penicillamine) to reduce mercury absorption and facilitate its excretion in the feces.

SUMMARY

Minerals are inorganic substances that are important for: (a) skeletal growth, development, and function (e.g., Ca, P, and F); (b) cellular metabolism by serving as cofactors of a plethora of enzymes [e.g., Na^+-K^+-ATPase, Ca^{2+}-dependent protein kinase, and arginase (Mn)]; and (c) physiological functions by regulating gene expression and biological activities of proteins (e.g., Zn in the Zn figure structure, Cu and Fe in the mitochondrial respiratory chain, and boron in the cross-linking of collagens). Based on their quantitative needs, nutritionally essential minerals are classified as macrominerals (Na, Cl, K, Ca, P, Mg, and S) or microminerals (Fe, Cu, Co, Mn, Zn, I, Se, Mo, Cr, F, Sn, V, Si, Ni, B, and Br). Common supplemental sources of minerals for animal diets are summarized in Table 10.23. Toxic

TABLE 10.23
Common Supplemental Sources of Minerals for Animal Diets[a]

Mineral	Common Supplemental Source
Calcium	Calcium carbonate (limestone), dicalcium phosphate, monocalcium phosphate, calcium sulfate, and defluorinated phosphate
Phosphorus	Dicalcium phosphate, monocalcium phosphate, defluorinated phosphate, monoammonium phosphate, and sodium phosphate
Magnesium	Magnesium oxide, magnesium sulfate, and magnesium carbonate
Potassium	Potassium chloride, and potassium sulfate
Sodium	Sodium chloride and sodium bicarbonate
Sulfur	Sodium sulfate, calcium sulfate, and elemental sulfur
Iron	Ferrous sulfate, ferrous carbonate, and organic forms of Fe
Copper	Copper sulfate, copper oxide (CuO), copper oxide (Cu_2O), tribasic copper chloride (Cu_20H_3Cl), copper carbonate, and organic forms of Cu
Chromium	Chromium picolinate (swine only) and chromium propionate (swine, cattle, broilers, turkeys, horses)
Manganese	Manganese sulfate, manganese oxide, manganese hydroxychloride (Mn_2OH_3Cl), and organic forms of Mn
Iodine	Calcium iodate, ethylenediamine dihydroiodide (EDDI), potassium iodate, and potassium iodide
Zinc	Zinc sulfate, zinc oxide, zinc hydroxychloride ($Zn_5Cl_2OH_8$), zinc carbonate, and organic forms of Zn
Selenium	Sodium selenate and selenium yeast

[a] Many organic forms of microminerals (Zn, Cu, Mn, and Fe) are commercially available.

minerals that have no physiological or nutritional function include Al, As, Cd, Pb, and Hg. Transition metals have multiple oxidation states and, therefore, participate in intracellular oxidation-reduction reactions. Because of their similar electron configurations, many minerals have similar chemical properties. Although requirements of animals for essential microminerals are quantitatively low, all of them, along with essential macrominerals, must be supplied in diets for mammals, birds, fish, and other animals. Compared with mature animals, dietary requirements of animals for all nutritionally essential minerals are much higher for growing, gestating, and lactating animals.

Minerals are absorbed primarily by the small intestine and transported in the blood through different mechanisms (Figure 10.15). Specifically, minerals are absorbed into the intestinal epithelial cell (primarily the jejunum of the small intestine for most minerals) through its apical membrane via specific transporters (e.g., ZIP4 for Zn, Na channels for Na^+, Cl^- channels for Cl^-, CTR1 for Cu^{2+}, and Ca transporter for Ca^{2+}), as well as transporters with a broad specificity for a group of ions (e.g., DCT1 for divalent metals; phosphate transporters for phosphate, V, and As; and NaP_i2b for Si and phosphate). For trafficking in enterocytes, all macrominerals but Ca^{2+} occur as free ions, whereas most microminerals bind cytosolic proteins (e.g., ferritin and metallothionein), GSH, or cysteine. Minerals exit the intestinal epithelial cell across its basolateral membrane into the lamina propria via specific transmembrane transporters (e.g., Na^+-K^+-ATPase for Na^+, ZnT1 for Zn^{2+}, and ferroportin for Fe^{2+}), vesicle discharge, receptor-mediated exocytosis, or MRPs 3 and 4 that transport GSH conjugates. Absorbed minerals enter the portal circulation. In the blood, all macrominerals but Ca^{2+} occur as free ions, and most microminerals bind proteins (e.g., ferritin and metallothionein) in plasma. Tissues take up minerals from the blood via specific transporters, transporters with a broad specificity for a group of ions, or receptor-mediated endocytosis.

It is important to choose suitable biological samples to assess the nutritional status of minerals in an animal. For example, concentrations of inorganic P and Mg in serum or plasma, but not in the liver, are good indicators of their dietary intake by an animal. In contrast, concentrations of Zn, Cu, and Mn in serum or plasma do not necessarily reflect their deficiency or excess in an animal, but their hepatic concentrations are useful indicators of their nutritional status. Concentrations of Ca in either plasma (serum) or the liver are not a good measure of Ca nutritional status in animals. Furthermore, whole blood Se (including Se in RBCs) is valuable to assess the nutritional status of Se in an animal, but concentrations of Se in serum or plasma are not. Finally, although elevated intakes of some minerals (e.g., Na, Cl, K, and S) are associated with increases in their urinary excretion, fecal excretion of other minerals (e.g., Mg, Ca, P, and Zn) is generally more than their urinary output because of relatively low rates of intestinal absorption.

Except for Se, nutritionally essential minerals are utilized in the form in which they are absorbed from the intestine. As electrolytes, minerals are essential for (a) the maintenance of extracellular and intracellular osmolarity, water balance, and blood pressure and flow; and (b) the conduction of electrical impulses that control the physiological activities of the heart, skeletal muscle, and brain. Furthermore, metals play structural roles in proteins, participate in oxidation-reduction reactions, transport and store O_2, and function in the active sites of

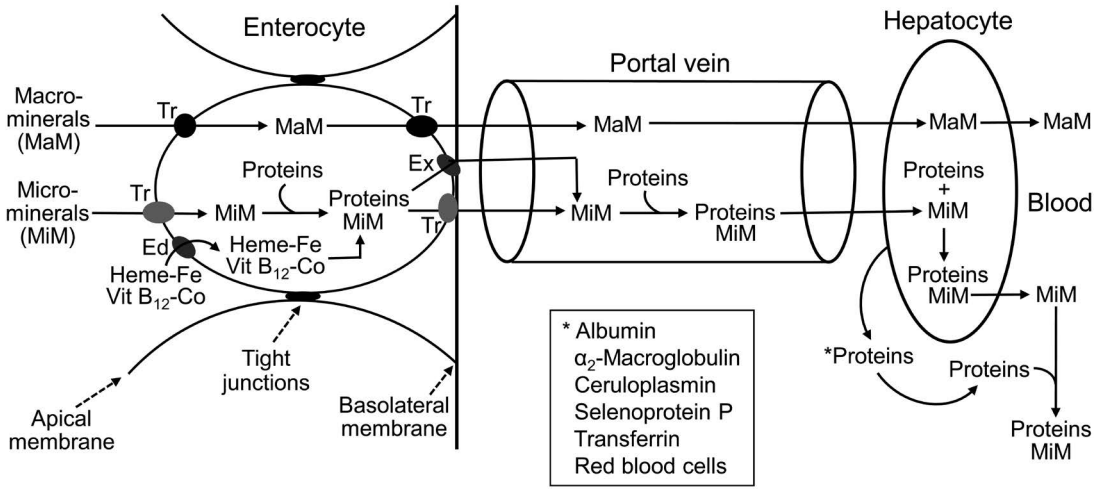


FIGURE 10.15 Intestinal absorption of dietary minerals and their transport in blood. In the lumen of the small intestine, dietary macrominerals (MaM) and microminerals (MiM) are absorbed by enterocytes through various apical membrane transporters (Tr). Heme-iron and vitamin B_{12}-complexed cobalt enter the cells via endocytosis (Ed). Inside the enterocyte, MaM exist as free ions, whereas MiM bind to specific proteins for transport to the basolateral membrane. MaM and MiM exit the enterocytes through the basolateral membrane, whereas exocytosis (Ex) may mediate the export of some MiM. Absorbed minerals enter the portal vein and are subsequently taken up by hepatocytes. The liver releases the minerals into the blood, where MaM are transported as free ions, and MiM bind to albumin and specific proteins (synthesized by hepatocytes) for transport to tissues. * The types of proteins are indicated in the insert box.

TABLE 10.24
Minerals-Related Metabolic Disorders (Including Metabolic Diseases) in Ruminants and Nonruminants

Metabolic Disorder	Cause	Major Symptoms
Anemia	Deficiency of iron	Fatigue, stunting, pale skin, dizziness, impaired immunity, and cold feet
Dental caries	Deficiency of fluorine	Tooth decay, dental caries, bone weakness, and possibly osteoporosis
Electrolyte imbalance	Imbalance of Na, K, and Cl	Low osmolarity, spasms, tetany, paralysis, cardiac dysfunction, and death
Goiter	Deficiency of iodine	Enlarged thyroid, stunting, obesity, and neurological dysfunction
Grass tetany	Mg ↓ (blood Mg^{2+} <0.4 mM)	Muscle cramps, seizures, paralysis, and death in (grazing ruminants
Keshan's disease	Deficiency of selenium	Oxidative stress, damage of tissues (particularly heart), and death
Menkes disease	Impaired absorption of Cu	Low Cu levels in serum, liver, and brain; easily broken and kinky hair
Milk fever (cows)	Ca ↓ (blood Ca^{2+} <1.25 mM)	Impaired calcium signaling, spasms, paralysis, and unconsciousness
P deficiency syndrome	Deficiency of phosphorus	Hemolysis, fatigue, infertility, muscle weakness, tremors, and ataxia
PEPM	Excess sulfur in diet	Abnormal structure and function of CNS; star-gazing
Wilson's disease	Impaired efflux of Cu from the liver to blood	Accumulation of copper in the liver and other tissues; deficiency of Cu in serum; a green or golden pigmented ring around the corner of eyes

Note: CNS, central nervous system; Cu, copper; P, phosphorus; PEPM, polioencephalomalacia.

enzymes involved in nutrient and energy metabolism. Dietary requirements of animals for these nutrients are affected by physiological states, environment, and disease. The common symptoms of their dietary deficiencies include poor appetite, growth restriction, and low feed efficiency; nausea and vomiting; compromised immune response; reduced fertility; the impairment of bone growth and development; oxidative stress; and, in severe cases, death (Table 10.24). Of note, these problems also occur in response to an excess intake of dietary minerals. In feeding practices, caution must be taken to (a) recognize mineral-mineral and mineral-organic constituent interactions that are important in animal nutrition (e.g., Mg-K, Ca-P, Mg-Ca, Zn-Cu, Zn-Fe, Cu-Mo-S, Cu-Fe, Zn-Cu-Fe, and Fe-vitamin C); and (b) minimize the entry of toxic metals without physiological or nutritional function into animals via feedstuffs, drinking water, and other environmental sources (e.g., volcanic eruptions, mining, and smelting).

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11 Nutritional Requirements for Maintenance, Production, and Health

Mammals (e.g., cattle, pigs, rats, and sheep) and birds (e.g., chickens, ducks, and geese) are endotherms (warm-blooded animals) and maintain a constant internal temperature through changes in internal heat production in response to a cold or a warm environment (Collier and Gebremedhin 2015; Mount 1978). Most mammals and birds have body temperatures of about 37°C and 40°C, respectively. In contrast, ectotherms (cold-blooded animals; e.g., fish, shrimp, amphibians, reptiles, insects, and worms) do not maintain a constant body temperature because their body temperature changes with alterations in the temperature of their living environment (van de Pol et al. 2017). Thus, per kilogram of body weight (BW), metabolic rates differ markedly between warm- and cold-blooded animals (Blaxter 1989). However, all animals have requirements for energy and nutrients to sustain their physiological needs for survival, growth, development, reproduction, and physical activities. In animals, growth is defined as an increase in cell number, cell size, or both, whereas development refers to progressive changes in the structure, shape, and function of cells, tissues, or organs. Nutritional requirements of animals generally refer to their requirements for nutrients in diets.

Dietary energy is contained in the three major classes of macronutrients: carbohydrates, lipids, and protein/amino acids. The requirement for energy or nutrients that keeps the animal at zero energy or nutrient balance is defined as the maintenance requirement for energy or nutrients. In other words, maintenance is a state in which there is no gain or loss of nutrients by the body. Different animal species, individuals of the same species, and the same animals at different physiological states have different maintenance requirements for energy, amino acids, glucose, fatty acids, vitamins, minerals, and water (Pond et al. 1995). When nutrient intake exceeds maintenance requirements, animals grow and produce products such as muscle, eggs, milk, and wool (Bergen 2021; Campbell 1988). Thus, the total requirement for energy or nutrients is the sum of the needs for maintenance plus production (including growth, lactation, gestation, and work), which is of practical importance in animal nutrition (NRC 2001, 2011, 2012). The same nutrients are needed for both maintenance and production. However, the amounts of energy or nutrients required for maintenance generally differ from those for production. Likewise, the efficiencies of energy or nutrient utilization for maintenance are different from those for production (McDonald et al. 2021). In practice, the requirements of animals for energy and nutrients [e.g., amino acids, fatty acids, calcium (Ca), and phosphorus (P)] can be determined through a combination of several techniques, including calorimetry, feeding trials, slaughter experiments, and isotope studies.

For healthy, physically active adult animals (e.g., mature dogs, cats, horses, and boars) that do not gain tissue protein or produce a product (e.g., eggs, milk, conceptus, or wool), requirements for energy and nutrients should be greater than their maintenance requirements. This is because muscular activity requires energy and is also associated with enhanced metabolism of nutrients in a tissue-specific manner (Newsholme and Leech 2009). Protein and fats are deposited in growing animals only when (a) the dietary intake of energy and amino acids exceeds maintenance requirements for energy and amino acids and (b) the requirements for all other nutrients are met. However, in gestating dams, their fetuses can grow despite inadequate maternal intake of energy, dry matter (DM), or macronutrients, because mothers mobilize nutrients from their tissues to provide an endogenous source of nutrients (Wu et al. 2006). Similarly, lactating cows produce a large amount of milk even though they exhibit a negative energy balance in the first month after calving (NRC 2001). This illustrates dynamic changes, or plasticity, in nutrient metabolism and requirements of animals in their life cycles. Inadequate intake of nutrients reduces the growth and productivity of the animal, whereas excesses in their intake have environmental consequences such as contributions to nitrogen (N) and P pollution, as well as global warming (Wu et al. 2014a).

Malnutrition is defined as deficiencies, excesses, or imbalances in the dietary intakes of energy and/or nutrients (amino acids, carbohydrates, fatty acids, vitamins, minerals, and water) and can be classified as either undernutrition or overnutrition. Starvation (a severe or total lack of food for a prolonged period) is the most extreme form of undernutrition. Malnutrition may occur in all members of the animal kingdom, depending on the availability of food, gastrointestinal function, and other environmental factors (such as disease, ambient temperatures, and air quality). As explained in Chapters 5–10, either undernutrition or overnutrition results in poor animal health and metabolic disorders, while increasing risks for infectious and chronic diseases. For example, in cattle, P deficiency results in the delayed onset of puberty, irregular or an absence of estrous cycles, lower conception rates, reduced fertility, longer intervals from calving to conception, high rates of embryonic/fetal

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deaths, stillborn, or weak offspring, and reduced calf crops (Stenhouse et al. 2022b).

The requirements of animals for nutrients are dynamic. Therefore, environmental factors (e.g., ambient temperatures) and the diseased state of animals must be considered in ration formulation. Diets must provide all nutrients in proper amounts and balances. Optimizing animal nutrition is essential to maximizing the production of high-quality animal products for human consumption, economic returns in animal agriculture, and the longevity of animals. The major objective of this chapter is to highlight the principles of nutritional requirements of animals for maintenance, production, and health.

NUTRITIONAL REQUIREMENTS FOR MAINTENANCE

Species Differences in Maintenance Requirements for Nutrients

As detailed in Chapters 5–9, there are both qualitative and quantitative differences in nutrient metabolism between ruminants and nonruminants, among mammalian species, or different developmental stages of the same species. Let's take domestic pigs, dogs, and cats as examples (Tables 11.1, 11.2, and 11.3). Pigs are omnivores, whereas

TABLE 11.1
De Novo Formation of Nutrients in Dogs, Cats, and Pigs

	Formation De Novo			
Nutrient and Energy	**Dog**	**Cat**	**Pig**	**Comment**
Water	Yes	Yes	Yes	Insufficient formation of H_2O from nutrient oxidation
Amino acids	Yes	Yes	Yes	Dependent on breeds, sex, age, physical activity, environmental factors, disease
Fatty acids	Yes	Yes	Yes	Elongation; introduction of new double bonds between Δ^1 and Δ^9 carbons
Glycogen	Yes	Yes	Yes	Synthesis primarily in the liver and skeletal muscle of all animals
Glucose	Yes	Yes	Yes	High rate of synthesis from amino acids in cats; Pigs < Dogs < Cats
Arginine	Ins	LM	AD, Ins	Species differences in expression of arginine-synthetic enzymes in enterocytes; insufficient synthesis in dogs possibly due to low intestinal activities of P5CS and POX; low intestinal activities of P5CS and OAT in cats; Pigs > Dogs > Cats
Taurine	Yes; Ins[a]	LM	Yes	Sufficient synthesis in most breeds of dogs; limited synthesis in cats due to low hepatic activities of CDO and CSD; Pigs > Dogs > Cats
Felinine	No	Yes	No	Synthesized from cysteine in cats; used for intra-species communication
Phe plus Tyr	No	No	No	Carbon skeletons of Phe and Tyr are not formed de novo; Phe → Tyr in animals
Palmitoleic acid	Yes	Yes	Yes	$C_{16:1}$; active synthesis from palmitic acid ($C_{16:0}$) by Δ^9 desaturase in all animals
Oleic acid	Yes	Yes	Yes	$C_{18:1}$; formed from palmitic acid ($C_{16:0}$) by elongation and then Δ^9 desaturase
α-Linolenic acid	No	No	No	$C_{18:3}$; no double bond can be formed beyond the Δ^9 carbon in animal cells
Linoleic acid	No	No	No	$C_{18:2}$; no double bond can be formed beyond the Δ^9 carbon in animal cells
Arachidonic acid from LA	Yes	LM	Yes	$C_{20:4}$; active synthesis from linoleic acid (LA) in dogs and pigs; very limited synthesis from linoleic acid in cats due to very low Δ^6 desaturase activity
EPA from α-LA	Yes; Ins[b]	LM	Yes	$C_{20:5}$; active synthesis from α-linolenic acid (α-LA) in dogs and pigs; very limited synthesis from α-linolenic acid in cats due to very low Δ^6 desaturase activity
DHA from α-LA	Yes; Ins[b]	LM	Yes	$C_{22:6}$; active synthesis from α-linolenic acid in dogs and pigs; very limited synthesis from α-linolenic acid in cats due to very low Δ^6 desaturase activity
Vitamin A from β-carotene	Yes	No	Yes	Active synthesis from β-carotene in dogs and pigs; no synthesis in cats due to the absence of β-carotene dioxygenase in the small intestine and other tissues
Vitamin D_3[c]	LM	LM	Yes	Limited synthesis from 7-dehydrocholesterol in dogs and cats even when exposed to sunlight; active synthesis in pigs when exposed to sunlight
Vitamin C	Yes	Yes	Yes	Active synthesis from glucose in dogs, cats, and pigs; required in heat stress
Niacin[d]	Yes; Ins	No	Yes; Ins	Active synthesis from L-tryptophan in dogs and pigs; no synthesis in cats

Source: Li and Wu (2024).

[a] In some breeds such as golden retrievers, American Cocker Spaniels, and a small population (1.3–2.5%) of the Newfoundlands.

[b] During gestation.

[c] In both dogs and cats, the skin has a low concentration of 7-dehydrocholesterol and a high activity of 7-dehydrocholesterol Δ^7 reductase. The latter rapidly converts 7-dehydrocholesterol into cholesterol, thereby limiting the formation of vitamin D_3.

[d] The liver of cats has all the enzymes for converting tryptophan, but also has a relatively high activity of picolinate decarboxylase. This results in the diversion of 2-amino-3-carboxymuconate semialdehyde (an intermediate of the niacin-synthetic pathway) towards the formation of α-ketoadipate rather than the production of nicotinate mononucleotide.

Note: AD, age-dependent (a high rate of citrulline synthesis from glutamine/glutamate and proline at birth; inadequate synthesis of citrulline at 7- to 21-days of age; and increased synthesis of citrulline after weaning); CDO, cysteine dioxygenase; CSD, cysteinesulfinate decarboxylase; DCM, dilated cardiomyopathy; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; Ins, insufficient; 4-LKs and 5-LKs, series 4 and 5 leukotrienes, respectively; 2- and 3-PGs, series 2 and 3 prostaglandins, respectively; LM, very limited; OAT, ornithine aminotransferase; P5CS, pyrroline-5-carboxylate synthase; POX, proline oxidase.

TABLE 11.2
Dietary Requirements of Nutrients by Dogs, Cats, and Pigs

	Dietary Requirements			
Nutrient and Energy	**Dog**	**Cat**	**Pig**	**Comments on Metabolism, Function, and Dietary Requirements**
Water	Yes	Yes	Yes	Essential for whole-body homeostasis and life ; high requirement in all animals
Energy	Yes	Yes	Yes	Required for survival, growth, development, and health; obesity occurs with excess deposition of white fats
Amino acids[a]	Yes	Yes	Yes	Building blocks of proteins and precursors of bioactive molecules; dietary requirements: Pigs < Dogs < Cats
Fatty acids[b]	Yes	Yes	Yes	Fatt acids serve as major metabolic fuels and other functions
Starch[c]	No	No	No	Major source of glucose in domestic dogs and pigs after weaning but not cats; excess causes gut dysfunction in cats
Glycogen[c]	No	No	No	Present in meat; a source of glucose in dogs and cats but insignificant in domestic pigs
Dietary fiber	No	No	No	Not required as an essential nutrient; plays important roles in intestinal health
Glucose[d]	No	No	No	Exclusive metabolic fuel for brain and red blood cells; high rate of synthesis in cats
Sweet substances[e]	No	No	No	Dogs and pigs select for, but cats avoid, sweet substances
Arginine[f]	Yes	Yes	Yes	Essential for the urea cycle to detoxify ammonia; dietary requirement: Pigs < Dogs < Cats
Taurine[g]	Yes	Yes	No	A deficiency of taurine causes DCM and retinal damage; dietary requirement: Dogs < Cats
Felinine	No	No	No	Synthesized from cysteine; used for communication
Phe plus Tyr	Yes	Yes	Yes	Required for eumelanin synthesis for black hair color and skin health
α-Linolenic acid	Yes	Yes	Yes	Precursor of EPA and DHA; nutritionally essential for all animals
Linoleic acid	Yes	Yes	Yes	Precursor of arachidonic acid; nutritionally essential for all animals
Arachidonic acid[h]	No	Yes	No	Essential for the synthesis of 2-PGs and 4-LKs; nutritionally essential for cats
EPA[i]	No	Yes	No	Essential for the synthesis of 3-PGs and 5-LKs; nutritionally essential for cats
DHA[i]	No	Yes	No	Essential for the growth and development of brain; nutritionally essential for cats
Vitamin A[j]	Yes	Yes	Yes	Essential for the integrity and function of retinal cells, reproduction, and health
Vitamin D_3	Yes	Yes	Yes	Effectively used in all animals; essential for the intestinal absorption of Ca and P
Vitamin D_2	No	No	No	Dogs and pigs, but not cats, can effectively utilize vitamin D_2 from sun-dried plants
Vitamin C	No	No	No	Essential for many enzyme-catalyzed reactions and integrity of connective tissue
Niacin	Yes	Yes	Yes	A component of NAD(P) for redox reactions and poly(ADP-ribose) polymerase

Source: Li and Wu (2024).

[a] Cats have greater endogenous losses of nitrogen and greater requirements for dietary protein than pigs and dogs. Cats select high-protein diets over low-protein diets, whereas dogs select medium-level protein diets over high-protein diets.

[b] Dogs select for high-fat diets over low-fat diets; whereas cats prefer animal (e.g., beef) fats to plant fats.

[c] Adequate intake of starch or glycogen by pigs and carnivores can beneficially reduce their use of amino acids for gluconeogenesis and ammonia production. Cats have a low ability to digest dietary starch and glycogen. In contrast to pigs, the saliva of dogs and cats has no or little α-amylase activity. Pancreatic α-amylase activity is high in the small intestine of pigs and dogs but is limited in cats.

[d] Cats have a greater ability to synthesize glucose from amino acids and other glucogenic substrates than pigs and dogs. The liver of cats lacks glucokinase.

[e] The tongue of cats lacks receptors for sweet substances.

[f] Pigs and dogs can synthesize arginine to maintain nitrogen balance but the endogenously synthesized arginine is inadequate to meet functional needs such as ammonia detoxification via the urea cycle and possibly nitric oxide synthesis. Cats have a very low rate of arginine synthesis via the intestinal-renal axis. Dogs and cats require dietary arginine for health and survival.

[g] Pigs and most breeds of dogs synthesize sufficient taurine but some dogs of certain breeds do not. Healthy pigs have no requirement for dietary taurine.

[h] Arachidonic acid is synthesized from linoleic acid via Δ^6 desaturase in pigs and dogs but the activity of this enzyme is very low in cats. Dogs and pigs have no requirement for dietary arachidonic acid if linoleic acid intake is sufficient.

[i] Eicosapentaenoic acid (EPA) and docosahexaenoic acid are synthesized from α-linolenic acid via Δ^6 desaturase in pigs and dogs but the activity of this enzyme is very low in cats. Pigs and dogs have no requirement for dietary eicosapentaenoic acid and docosahexaenoic acid if α-linolenic acid intake is sufficient.

[j] Pigs and dogs can synthesize vitamin A from β-carotene in the enterocytes of their small intestine via β-carotene 15,15-dioxygenase, but this enzyme is absent from cats. Diets for dogs can use β-carotene to replace vitamin A, and this vitamin must be included in diets for cats.

Note: AA, amino acid, AD, age-dependent (high at birth; inadequate at 7- to 21-days of age, as well as during pregnancy and lactation); DCM, dilated cardiomyopathy; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; 4-LKs and 5-LKs, series 4 and 5 leukotrienes, respectively; 2- and 3-PGs, series 2 and 3 prostaglandins, respectively.

TABLE 11.3
Daily Maintenance Requirements of Growing, Gestating, and Lactating Swine for Dietary Energy and Amino Acids

Maintenance Requirement for Energy and Nutrient	50-kg Growing Pig (FI = 2 kg DM/day)		200-kg Gestating Sow (FI = 2 kg DM/day)		200-kg Lactating Sow (FI = 5 kg DM/day)	
	Amount	Ratio to Lys	Amount	Ratio to Lys	Amount	Ratio to Lys
Metabolizable energy (ME)[a]	106	1.492	100	2.874	110	2.378
Amino acids with no *de novo* synthesis and crude protein (mg/kg $BW^{0.75}$/day for amount or g/g for ratio to Lys)[b]						
Histidine	25.00	0.352	12.09	0.348	19.74	0.427
Isoleucine	62.32	0.877	29.78	0.856	38.76	0.838
Leucine	88.78	1.249	36.03	1.036	53.41	1.154
Lysine	71.05	1.000	34.79	1.000	46.26	1.000
Methionine	19.68	0.277	9.17	0.264	10.16	0.220
Methionine + Cysteine	72.77	1.024	36.80	1.058	36.33	0.785
Phenylalanine	64.27	0.905	25.03	0.720	35.66	0.771
Phenylalanine + Tyr	104.96	1.477	45.49	1.308	62.14	1.343
Threonine	97.33	1.370	44.53	1.280	56.78	1.227
Tryptophan	26.98	0.380	10.94	0.314	13.97	0.302
Valine	83.89	1.181	37.82	1.087	65.81	1.423
Crude protein	2057.73	28.96	979.33	28.15	1320.51	28.55
Amino acids synthesizable in animal cells (AASAs; mg/kg $BW^{0.75}$/day for amount or g/g for ratio to Lys)[c]						
Alanine	385.50	5.426	190.65	5.480	255.80	5.530
Arginine	78.41	1.104	38.72	1.113	52.01	1.124
Asparagine	42.03	0.592	20.16	0.579	27.39	0.592
Aspartate	49.60	0.698	24.41	0.702	33.09	0.715
Glutamine	598.30	8.421	292.50	8.410	386.73	8.358
Glutamate	98.61	1.388	48.78	1.402	64.20	1.388
Glycine	149.05	2.098	88.24	2.536	114.49	2.475
Proline	160.75	2.262	96.92	2.786	124.46	2.691
Serine	51.49	0.725	24.96	0.717	33.16	0.717

[a] The amount of ME and the ratio of ME to Lys are expressed as kcal/kg $BW^{0.75}$/day and kcal/mg, respectively (NRC 2012).

[b] Standardized ileal digestible amino acids (NRC 2012).

[c] Minimum maintenance requirements. The values were estimated on the basis of (a) the composition of amino acids (AAs) in the carcass proteins of 50-kg pigs (barrows), 200-kg gestating sows, and 200-kg lactating sows, which was determined as previously described (Li and Wu 2020; Wu et al. 1999); (b) whole-body glucose synthesis from alanine and glutamine; and (c) the use of glutamine by the small intestine and kidneys. The molecular weights of intact AAs were used in the calculations. The composition (g/kg protein; mean ± SEM, n = 6) of AAs in the carcass proteins of 50-kg pigs was: Ala, 64.6 ± 0.5; Arg, 65.7 ± 0.6; Asn, 35.2 ± 0.5; Asp, 41.5 ± 0.4; Cys, 12.8 ± 0.4; Gln, 50.1 ± 0.5; Glu, 82.6 ± 0.8; Gly, 124.8 ± 1.8; His, 20.2 ± 0.5; Ile, 34.5 ± 0.6; Leu, 66.7 ± 0.5; Lys, 59.5 ± 0.4; Met, 18.2 ± 0.5; Phe, 33.4 ± 0.6; Pro + 4-hydroxyproline, 134.6 ± 1.5; Ser, 43.1 ± 0.9; Thr, 34.2 ± 0.6; Trp, 10.8 ± 0.3; Tyr, 26.4 ± 0.3; and Val, 41.3 ± 0.3. The composition (g/kg protein; mean ± SEM, n =6) of AAs in the carcass proteins of 200-kg gestating sows was: Ala, 61.6 ± 0.5; Arg, 62.6 ± 0.4; Asn, 32.6 ± 0.6; Asp, 39.4 ± 0.4; Cys, 12.1 ± 0.3; Gln, 47.3 ± 0.2; Glu, 78.8 ± 0.8; Gly, 142.6 ± 0.9; His, 19.0 ± 0.3; Ile, 32.8 ± 0.4; Leu, 63.3 ± 0.4; Lys, 56.2 ± 0.5; Met, 17.3 ± 0.5; Phe, 31.8 ± 0.4; Pro + 4-hydroxyproline, 156.6 ± 1.6; Ser, 40.3 ± 0.4; Thr, 32.1 ± 0.5; Trp, 10.1 ± 0.2; Tyr, 24.6 ± 0.3; and Val, 39.1 ± 0.4. The composition (g/kg protein; mean ± SEM, n =6) of AAs in the carcass proteins of 200-kg lactating sows was: Ala, 62.6 ± 0.3; Arg, 63.6 ± 0.4; Asn, 33.5 ± 0.4; Asp, 40.5 ± 0.4; Cys, 12.2 ± 0.4; Gln, 47.3 ± 0.3; Glu, 78.6 ± 0.6; Gly, 140.1 ± 1.0; His, 18.6 ± 0.3; Ile, 33.1 ± 0.4; Leu, 63.8 ± 0.4; Lys, 56.6 ± 0.6; Met, 17.1 ± 0.3; Phe, 32.1 ± 0.4; Pro + 4-hydroxyproline, 152.3 ± 0.9; Ser, 40.6 ± 0.7; Thr, 32.5 ± 0.4; Trp, 10.2 ± 0.2; Tyr, 24.9 ± 0.5; and Val, 39.8 ± 0.6.

Note: DM, dry matter; FI, feed intake.

dogs and cats are carnivores. Dogs have evolved to adapt to omnivorous diets (containing both meat and starch-rich plant ingredients) but still prefer to eat meat. By contrast, domestic cats must consume animal-sourced foods for survival, growth, and development. Pigs, dogs, and cats can synthesize glucose, vitamin C, and many amino acids (such as alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, and serine). Pigs can synthesize de novo arginine (albeit insufficiently), as well as vitamin D_3 from 7-dehydrocholesterol, but both dogs and cats have a limited ability to form de novo arginine and vitamin D_3. Compared with pigs and dogs, cats have greater endogenous N losses

and higher dietary requirements for amino acids (particularly arginine, taurine, and tyrosine), B-complex vitamins (niacin, thiamin, folate, and biotin), and choline; exhibit greater rates of gluconeogenesis; are less sensitive to amino acid imbalances and antagonism; are more capable of concentrating urine through renal reabsorption of water; and cannot tolerate high levels of dietary starch due to limited pancreatic α-amylase activity. Thus, the excess intake of starch and glycogen may be detrimental for the digestive tract and may increase the risk of metabolic disorders in cats (Li and Wu 2024). In addition, dogs (for most breeds) and pigs can form sufficient taurine from cysteine, arachidonic acid from linoleic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from α-linolenic acid, all-*trans*-retinol from β-carotene, and niacin from tryptophan. These synthetic pathways, however, are either absent or limited in all cats due to (a) no or low activities of key enzymes (including pyrroline-5-carboxylate synthase, cysteine dioxygenase, Δ^6-desaturase, β-carotene dioxygenase, and quinolinate phosphoribosyltransferase) and (b) diversion of intermediates to other metabolic pathways. Pigs and dogs can thrive on one large meal daily, select high-fat over low-fat diets, and consume sweet substances. By contrast, cats eat more frequently during light and dark periods, select high-protein over low-protein diets, refuse dry food, enjoy a consistent diet, and cannot taste sweetness.

Energy Requirements for Maintenance

The basal metabolic rate (BMR) of an animal is often used to estimate its maintenance requirement for energy (Baldwin 1995). The BMR is the energy expended in the fasting animal, which is also known as fasting catabolism (Chapter 8). When expressed as net energy (NE), the BMR represents the maintenance requirement for energy. However, when expressed as metabolizable energy (ME) for fed animals, the maintenance requirement includes the BMR plus the heat increment of the diet and can be measured in fed animals at zero energy balance (Chwalibog 1991). Energy requirements for the basal metabolism are explained by (a) intracellular protein turnover (protein synthesis and degradation); (b) maintenance of cellular ionic gradients, especially the Na and K gradients; (c) substrate cycles (e.g., substrate cycles in glucose and fat intermediary metabolism); (d) biosynthetic processes (e.g., gluconeogenesis, protein synthesis, ureagenesis); (e) active transport of organic nutrients (e.g., amino acid transport by cells, glucose reabsorption by renal epithelial cells, and urea excretion by the kidneys); and (f) contraction of skeletal, cardiac, and smooth muscles (Baldwin and Bywater 1984; Kelly et al. 1991; Milligan and Summers 1986). These variables are summarized in Table 11.4.

The BMR is determined using direct or indirect calorimetry. Any factors that influence fasting metabolism should be eliminated (Blaxter 1989). In addition, previous feeding levels, which can affect gene expression and substrate concentrations in tissues, influence steady-state heat production and BMR in fasted animals (de Lange et al. 2006). Therefore, it is important that the test animal be in the postabsorptive, resting, and healthy state; fed a balanced diet before testing; and housed in its thermoneutral zone. Specifically, the gastrointestinal tract of the animal must first be free of feed residues. The excretion of feed residues from the gastrointestinal tract can be monitored by observing the disappearance of a marker (e.g., dye) in feed particles, and it takes a longer time in ruminants than in nonruminants (Chapter 1). For example, in cows, about 80% of the feed particles ingested through the mouth are excreted within 80 h, and the excretion rate is reduced thereafter; therefore, the BMR in ruminants is often measured 10 days after the last meal (Chapter 1). Second, the metabolic rate of an animal is a function of its physiological state, in which the body temperature should be constant. However, under disease conditions (e.g., fever and infections) or exposure to toxins, substrate oxidation is altered, contributing to changes in basal energy requirements. Third, the test animal must be placed within its thermoneutral (comfort) zone (Table 11.5), within which heat production by the resting animal is minimal and is sufficient to offset heat loss. Thermoneutral zones, which differ among animal species and even among different breeds of the same species or different sizes of the same breed, may be reduced when the ambient humidity is high. At a high temperature, high humidity prevents cooling by reducing the rate of evaporation of sweat. Technically, the relative humidity inside an animal calorimeter should not exceed 50% (e.g., 37–45%

TABLE 11.4
Energy Requirements for Basal Metabolism in Animals

Physiological Activity	Maximum Contribution (%)
Intracellular protein turnover	25
Maintenance of cellular ionic gradients	25
Substrate cycles in metabolism	15
Biosynthetic processes in metabolism	15
Active transport of organic and inorganic nutrients	10
Contraction of skeletal, cardiac, and smooth muscles	10

Source: Baldwin (1995); Kelly et al. (1991); Milligan and Summers (1986).

TABLE 11.5
Thermoneutral Zones (TNZ) of Animals

Animal Species	TNZ (°C)	Animal Species	TNZ (°C)
Cat	30–38	Man	26–28
Cattle (calf)	15–20	Rat	26–28
Cattle (lactating cow)	15–20	Sheep	20–25
Dog (with long hair)	13–16	Swine (postweaning)	20–26
Fowl	15–26	Swine (neonatal)	33–35
Goat	15–20	Turkey poults	20–28

Source: Bondi (1987); Farmer and Edwards (2022).

for sheep; Pinares and Waghorn 2012). Thus, care should be taken to account for these variables when measuring the BMR of animals. The maintenance requirements of mammals, birds, and reptiles for energy can be estimated at twice the BMR (Kirkwood 1991).

Additional Factors Affecting the Basal Metabolic Rate

Metabolic Size of Animals

In mammals and birds, an important element in determining the BMR is to ensure that the animal maintains a constant body temperature, as noted previously. In fish and crustaceans, the BMR is also important to sustain life. Heat loss to the environment is proportional to (a) the difference between body temperature and ambient temperature and (b) body-surface area. Because the body temperatures of all farm mammals are nearly the same, their body surface area is the major factor determining heat loss (Blaxter 1989). This notion also applies to birds and fish, whose body temperatures are usually higher and lower, respectively, than those of mammals. The surface area of the animal, which is often difficult to measure, is generally proportional to the 2/3 power of the BW (White and Seymour 2002). However, much evidence from the studies of mammalian species ranging from the 20-g mouse to the 4-ton elephant indicates that heat loss is proportional to the 3/4 power of the BW (Brody 1945). Thus, the 3/4 power of the BW (i.e., $BW^{0.75}$) is generally referred to as the metabolic BW or metabolic size (Chapter 8). Some authors also used $BW^{0.73}$ to express the BMR in certain species. Of interest, variations of BMR per metabolic body size among animal species are much lower than values expressed per kilogram of BW (Table 11.6). In mammals, the maintenance requirement for energy supports its equilibrium over a long period of time and can be calculated as $70.5 \times BW\ (kg)^{0.73}$ kcal/day (Brody et al. 1945), which can be simplified as $70 \times BW\ (kg)^{0.75}$ kcal/day (Kleiber 1961).

Age and Sex of Animals

The age of an animal is another major factor affecting its BMR. The BMR usually decreases with postnatal age (Turner and Taylor 1983), which corresponds to a decrease in circulating concentrations of thyroid hormones (Segal et al. 1982). For example, the BMRs (MJ/kg $BW^{0.73}$) of cattle at different ages are 0.586 (1 month), 0.565 (3 months), 0.523 (6 months), 0.419 (18 months), 0.398 (24 months), 0.377 (36 months), and 0.356 (48 months; Bondi 1987). The BMR also decreases with a reduction in lean body mass. An increase in skeletal-muscle mass and activity has the effect of enhancing the BMR, but an increase in white adipose tissue (WAT) has the opposite effect (Blaxter 1989). Of interest, the metabolic rate of the fetus is relatively low, compared with that of newborns. For example, the rate of heat production per kilogram of tissue in fetal lambs is about the same as that of mature ewes at rest (Bondi 1987). However, within 24 h after birth, the BMR of the newborn lamb is two times greater than that of the fetus because of the need for heat production to maintain the body temperature in extrauterine life. Oxidation of fatty acids by brown adipose tissue contributes to nonshivering thermogenesis in neonatal lambs and many other animals such as cattle, rats, and humans (Satterfield and Wu 2011). Some species (e.g., pigs and chicks) lack brown adipose tissue before or after birth.

Hormonal profiles in plasma differ between males and females during the growing-finishing periods and adult life. For example, the concentrations of thyroid hormones in plasma are greater in males than in females (Segal et al. 1982). Thus, the BMR of males is generally higher than that of nonpregnant females of similar age and size. Similarly, intact males (e.g., boars) have a greater BMR and gain less body fat than castrated males (e.g., barrows; Knudson et al. 1985). Furthermore, factors (e.g., ambient temperatures, disease, and dietary intakes of tyrosine, phenylalanine, and iodine) that affect the synthesis and degradation of thyroid hormones can also greatly influence the BMR (Kim 2008).

Normal Living Conditions of Animals

The BMR is measured in fasting animals (Blaxter 1989). It is difficult to translate the values of the BMR into practical recommendations for maintenance requirements of normal living animals because of (a) the heat increment of feeding, (b) the muscular activity of the free-moving animal, and (c) changes in an environment that deviates from the thermoneutral zones for the animals (Bondi 1987). Thus, it is preferable to determine the NE requirement for maintenance using fed animals with an energy balance of zero. Alternatively, the rate of fasting heat production may be multiplied by a factor of greater than 1 (i.e., 1.15 for housed animals and 1.25–1.5 for outdoor grazing animals) to obtain the true BMR of animals under feeding conditions (Osuji 1974). The factors of 1.15 to 1.25–1.5 are recommended because (a) the act of eating, chewing, gut motility, standing and walking, and other physical activities in the housed animals produce some heat, which amounts to ~15% of the heat produced by fasting metabolism; and (b) grazing animals expend more energy by walking longer distances to forage foods than their housed counterparts and generate a large amount of heat, amounting to ~25–50% of the heat produced by fasting metabolism (Osuji 1974).

TABLE 11.6
Basal Metabolic Rates of Adult Animals

Animal Species	Body Weight (BW, kg)	Basal Metabolic Rate (MJ/day)		
		Per Animal	Per kg BW	Per kg $BW^{0.75}$
Cow	500	34.1	0.0682	0.323
Pig	70	7.50	0.107	0.310
Man	70	7.09	0.101	0.293
Sheep	50	4.31	0.0861	0.229
Dog	28	3.37	0.127	0.293
Cat	4	0.652	0.163	0.231
Fowl	2	0.60	0.300	0.357
Rat	0.3	0.12	0.400	0.296

Source: Bondi (1987); Nguyen et al. (2004).

Diurnal Changes

Some animal species exhibit diurnal changes in the BMR. For example, in beagle dogs living in a thermoneutral environment with a 12-h light/12-h dark cycle, their rate of heat production is the lowest in the morning (124 kcal/kg $BW^{0.75}$/day), gradually increases to a maximum in the evening (195 kcal/kg $BW^{0.75}$/day), and then declines through the night (Besch and Woods 1977). Such changes in heat production with a circadian rhythm are largely independent of food intake. The rates of heat production by dogs during the dark and light periods are similar (Woods and Besch 1971). In contrast, there is no circadian rhythm in heat production in cats (Riond et al. 2003).

Protein and Amino Acid Requirements for Maintenance

Amino acids are building blocks of proteins and regulators of protein kinases, including the mechanistic target of rapamycin (MTOR; the master activator of protein synthesis), as highlighted in Chapter 7. Animals degrade protein and amino acids continuously; therefore, there must be a maintenance requirement for amino acids to replace their daily losses (Campbell 1988). In addition, the small intestines of mammals, fish, and crustaceans require large amounts of glutamate, glutamine, and aspartate to fulfill metabolic needs for energy, nucleotide synthesis, and growth (Jia et al. 2017; Li et al. 2020. 2021b, 2021c; Wu 1998). Although avian enterocytes have a limited ability to degrade glutamine due to low glutaminase activity (Wu et al. 1995), those cells may extensively oxidize glutamate and aspartate for ATP production. The maintenance requirement for protein is the sum of the amounts of N excreted in the urine, feces, and lungs, skin, and hair when the animal is at N equilibrium (Reeds and Garlick 2003). In practice, the maintenance requirement for amino acids can be determined by feeding the test animal two or more levels of dietary protein or amino acids and then extrapolating the BW to zero amino acid intake (ARC 1981). Note that the energy requirement for maintenance must be met in N-balance studies to minimize the use of body protein as an energy source to reduce urinary N excretion. Under ideal conditions, urinary N excretion (so-called endogenous N, meaning that its immediate precursors are derived from tissues rather than feed) should be minimal. The minimum excretion of urinary N is proportional to metabolic body size (McDonald et al. 2021). Because different amino acids are utilized through different metabolic pathways and at different rates, and because many amino acids are synthesized *de novo* from common substrates in animals, it is technically challenging to determine the true maintenance requirements for amino acids. Animals have particularly high maintenance requirements for alanine and glutamine, as shown for growing, gestating, and lactating swine (Table 11.3). This is because (a) both alanine and glutamine are major substrates for the synthesis of glucose (the exclusive metabolic fuel for the brain in the postabsorptive state and for erythrocytes at all times), and (b) arterial blood glutamine provides most energy for the small intestine in the postabsorptive state and also plays a key role in regulating acid-base balance through renal ammoniagenesis (Wu 2022a).

Fecal N excretion derived from the gastrointestinal epithelium and bacteria, as well as other endogenous sources, is called metabolic fecal N (Chapter 7). In nonruminants, amounts of metabolic N are estimated by feeding an N-free diet to animals and measuring fecal N excretion. However, estimates obtained with N-free diets are lower than the true maintenance protein requirements of animals receiving normal amounts of dietary amino acids, due to lower rates of whole-body protein turnover and amino acid catabolism in animals fed amino acid-free diets. Furthermore, feeding an N-free diet inhibits fermentation in the rumen of ruminants (Chapter 5), and this method is therefore not applicable to ruminants. Rather, the regression technique, in which fecal N (g/100 g fecal DM; Y-axis) is plotted as a function of feed N (g/100 g fecal DM; X-axis) and extrapolated to zero N intake, is often used to estimate the amount of metabolic N in ruminants (Hironaka et al. 1970).

Ruminants produce more metabolic N than nonruminants (Cao et al. 2021; Zhang et al. 2021). For example, the amount of fecal N excreted per day is about 1–2 g/kg DM in nonruminants and about 4–6 g/kg DM intake in mature ruminants (e.g., 280 g metabolizable protein/day in a 600-kg cow). This reflects a much larger gastrointestinal tract and a much greater number of bacteria in the digestive tract of ruminants as compared to nonruminants. Depending on species and age, the maintenance requirements of various animals for true protein are 1–6 g/kg $BW^{0.75}$/day and are affected by the environment in which they live (ARC 1981; NRC 1995, 2001). This is equivalent to 1.8–11 g true dietary protein/kg $BW^{0.75}$/day, given that their true digestibility is 85% and the efficiency of utilization of truly digestible protein for maintenance is 65% (i.e., 1 or 6 ÷ 85% ÷ 65% = 1.8 or 11). Corresponding to changes in the rates of whole-body protein turnover and amino acid oxidation, the maintenance requirements for dietary protein or amino acids decrease with age but increase with cold or heat stress, or disease.

Fatty Acid Requirements for Maintenance

Like other organic nutrients, fatty acids constantly undergo turnover in an animal and are lost from the body via oxidation to CO_2 and H_2O. Although saturated and monounsaturated fatty acids are not dietarily essential, they supply a large portion of energy for carnivores and nonruminant animals, thereby sparing some amino acids and digestible carbohydrates in diets. As indicated in Chapter 6, arachidonic acid ($C_{20:4}$ ω6), EPA ($C_{20:5}$ ω3), and DHA ($C_{22:6}$ ω3) are all polyunsaturated fatty acids (PUFAs) that are important components of cell membranes and have many physiological functions. Of note, these three PUFAs are particularly abundant in the brain, retina, and erythrocytes. In most young adult animals (e.g., pigs, humans, dogs, poultry, and rodents) that have high Δ^6 desaturase activity, linoleic acid is readily converted into arachidonic acid, and α-linolenic acid into EPA and DHA. Although ruminal microbes can synthesize both ω3 and ω6 PUFAs from acetyl-CoA, ruminants have maintenance dietary requirements for these nutrients for normal metabolism and health. Thus, linoleic acid and α-linolenic acid are dietarily essential for both ruminants and nonruminants and

must be provided to those animals ultimately from diets to maintain membrane fluidity, basal metabolism, and survival of cells. However, during prenatal and neonatal periods, Δ^6 desaturase activity may not be well developed, and the fetuses and infants of those species benefit from adequate dietary intakes of EPA and DHA for optimal brain development and health. Likewise, Δ^6 desaturase expression may be reduced in elderly individuals of these species; therefore, a sufficient provision of EPA and DHA can improve cognitive function. Young or adult cats have very limited Δ^6 desaturase activity; therefore, linoleic acid, EPA, and DHA are dietarily essential for their maintenance, growth, and health.

Because long-chain fatty acids (including PUFAs) are stored for a prolonged period of time (at least days) in WAT, liver, and other tissues, maintenance requirements for these nutrients cannot be readily determined in a short-term experiment (Bondi 1987). A long-term study is required to achieve this goal, and at the same time, both energy and N balances of the animal must be maintained. The minimum requirements for PUFAs by animals can be determined through feeding different dietary levels of PUFAs and then observing growth performance and immune responses, as well as the appearance and health of skin and hair. A deficiency of PUFAs often results in dermatitis, bleeding gums, skin necrosis, and hair loss. The Agricultural Research Council (ARC, 1981) reported minimal (maintenance) requirements of 30–90 kg pigs for linoleic acid (0.31 g/kg BW/day) and arachidonic acid (0.21 g/kg BW/day). The maintenance requirements of adult dogs for linoleic acid, α-linolenic acid, and EPA + DHA were estimated to be 11, 0.44, and 0.44 g/kg dietary DM, respectively (Li and Wu 2024).

Carbohydrate Requirements for Maintenance

All animals are capable of gluconeogenesis and therefore do not have maintenance requirements for dietary glucose, fructose, or other sugars to maintain life. However, adequate intake of starch or glycogen is beneficial for optimum health by reducing the use of protein and amino acids to synthesize glucose, as well as preventing ammonia toxicity (due to amino acid oxidation) and acid-base imbalance (due to an excess production of sulfate and H^+; Chapter 7). Ruminants do have dietary requirements for plant-sourced nonstarch polysaccharides to maintain the normal populations and functions of the ruminal microbes (Chapter 5). Specifically, nonstarch polysaccharides (e.g., cellulose, hemicellulose, fructans, and β-glucans) are fermented in the rumen, generating large amounts of short-chain fatty acids (SCFAs), which help maintain ruminal fluid pH between 5.5 and 6.8 for optimum growth, activity, and survival of ruminal microbes. Additionally, in the fed state, propionate is the major glucogenic substrate, whereas both acetate and butyrate are major sources of energy in ruminants. Sources of dietary fiber for ruminants include forages (e.g., grasses), corn silage, corn, soybean hulls, alfalfa hay, straw, distiller's dried grains and solubles (DDGS), and wheat middlings (Knoell et al. 2024). In both ruminants and nonruminants, nonstarch polysaccharides are fermented in the hindgut to form large amounts of SCFAs (major metabolic fuels for epithelial cells of the large intestine). In addition, these complex carbohydrates stimulate intestinal motility, prevent constipation, decrease the absorption and plasma levels of triacylglycerols (TAGs), and reduce risks for colon cancers. Thus, dietary fiber is crucial for intestinal and metabolic health in both ruminants and nonruminants. A typical diet for lactating cows is shown in Table 11.7 to illustrate

TABLE 11.7
Composition and Nutrient Content of Feedstuff Ingredients in a Typical Diet for Lactating Cows

Ingredient	Percent (%)	Ingredient	Percent (%)	Nutrient Content[d] in a typical diet	Percent (%)
Corn silage	37.0	Blood meal	0.89	Crude protein	17.8
Ground corn	12.8	Calcium carbonate	1.63	Crude fat	4.5
DDGS (corn)	12.1	Sodium bicarbonate	0.56	ADF	18.5
Soybean meal	11.3	Calcium-LCFA soaps	1.63	NDF	29.3
Soypass®[,a]	3.06	Salt (NaCl)	0.33	fNDF[e]	20.7
Soybean hulls	1.43	Magnesium oxide	0.31	Lignin	3.5
Alfalfa hay	6.1	Calcium diphosphate	0.07	Starch	24.2
Straw	4.2	Rumen-protected Met	0.07	Sodium (Na)	0.39
Molasses, dried	2.55	Rumen-protected Lys	0.11	Potassium (K)	1.53
Lard	1.62	Vitamin premix[b]	0.05	Sulfur (S)	0.33
Whey	2.56	Mineral premix[c]	0.04	Ash (minerals)	7.9

Source: Knoell et al. (2024).

[a] Providing rumen-protected protein in soybean meal (LignoTech, Overland Park, KS).

[b] Providing 120,000 IU of vitamin A, 24,000 IU of vitamin D, and 800 IU of vitamin E per day.

[c] Containing 13.9% Ca, 0.03% P, 0.42% Mg, 0.20% K, 4.20% S, 0.08% Na, 0.03% Cl, 445 mg Fe//kg, 60,021 mg Zn/kg, 17,375 mg Cu/kg, 43,470 mg Mn/kg, 287 mg Se/kg, 527 mg Co/kg, and 870 mg I/kg.

[d] The content of dry matter in the diet = 60.1%. Dietary cation-anion gap (mEq/kg of DM) = [(Na + K) – (Cl + S)]/kg of DM = 254.

[e] Calculated as % DM – forage NDF fraction (as % of DM).

Note: ADF, acid detergent fiber; DDGS, dried distillers grains with solubles; LCFA, long-chain fatty acid; NDF, neutral detergent fiber.

the importance of plant-sourced feedstuffs in the nutrition and production of these animals.

Vitamin Requirements for Maintenance

Vitamins are excreted in the urine and feces. Thus, it is customary to determine maintenance requirements for water- and lipid-soluble vitamins by measuring their excretion (including metabolites) in urine and feces under conditions of dietary depletion (Combs and McClung 2017), analogous to methods used for estimating maintenance requirements for amino acids (Chapter 7). Because the digestion and absorption of lipid-soluble vitamins requires fats, dietary provision of lipids is a prerequisite for assessing the bioavailability of those vitamins. The ARC (1981) summarized the minimum (maintenance) vitamin requirements for pigs. For example, the minimum retinol requirements for 5–40 kg pigs, 40–90 kg pigs, and breeding sows (or gilts) are 22, 16, and 14 μg/kg BW/day, respectively. In addition, the minimum thiamin, riboflavin, pantothenic acid, pyridoxine, and vitamin B_{12} requirements for 90-kg pigs are 17, 28, 110, 28, and 0.10 μg/kg BW/day, respectively. Ruminants consuming plant-based diets with sufficient carbohydrates, proteins, and cobalt have no maintenance requirements for dietary vitamins because of their synthesis by ruminal bacteria (NRC 2011). It is noteworthy that a deficiency of most water-soluble vitamins can occur rapidly in animals, with such symptoms as skin lesions, neurological disorders, anemia, and fatigue (Chapter 9). Like the deficiencies of PUFAs, it takes a prolonged period of time for animals to exhibit symptoms due to deficiencies of lipid-soluble vitamins, such as blurry vision, failure of blood clotting after a cut, injury, anemia, and abnormal bone development (Combs and McClung 2017).

Mineral Requirements for Maintenance

In animals, minerals are essential for extracellular and intracellular osmolarity, as well as the growth and development of bones and teeth (Chapter 10). These nutrients are excreted primarily in the urine and, to a much lesser extent, in feces (Chapter 10). Because minerals are not degraded in animals, their maintenance requirements by the animal can be determined by measuring their obligatory losses in urine plus feces under conditions of dietary depletion (Harris 2014). Alternatively, radioactive or stable isotopes can be used to determine the excretion of endogenous minerals. Let us use Ca as an example. The excretion of endogenous Ca by an adult nonlactating cow weighing 500 kg is 8.1 g/day (Bondi 1987). Assuming that the true absorption rate of dietary Ca in cattle is 45%, the maintenance requirement for dietary Ca would be 36 mg/kg BW/day [i.e., (8.1 g/day ÷ 0.45)/500 kg]. The mineral requirement for maintenance is generally proportional to metabolic body size. For example, the maintenance requirement for Ca by the nonlactating cow is about 0.325 mg/kJ of fasting heat production. The ARC (1981) summarized the obligatory losses of minerals from growing pigs as follows (μg/kg BW/day): Ca, 32; P, 20; magnesium, 400; sodium, 1140; and potassium, 130. These values are to meet maintenance requirements for those minerals.

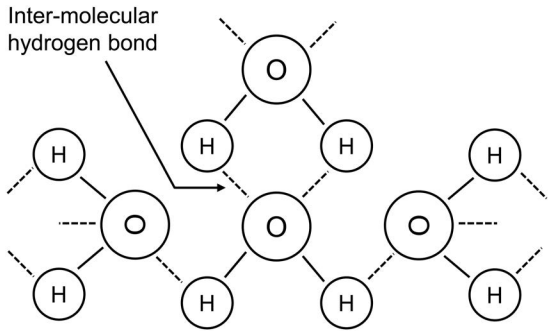


FIGURE 11.1 The structure of the water molecule. Water (H_2O) is a small bent molecule consisting of one oxygen and two hydrogen atoms. The oxygen atom is connected to each hydrogen atom by a covalent bond. Each water molecule can potentially form four intermolecular hydrogen bonds with surrounding water molecules.

Water Requirements for Maintenance

Water (H_2O) is the most abundant nutrient in animals. This polar molecule contains one oxygen and two hydrogen atoms (Figure 11.1). The oxygen atom is connected to each hydrogen atom by a covalent bond, with the oxygen atom carrying a partial negative charge and the hydrogen atoms each having a partial positive charge. Water exists in a liquid (between 0 and 100°C at the standard pressure of 1 atm), solid (ice, <0°C), or vaporous (>100°C) state. At physiological temperatures, water is a chemically stable liquid in animals. Because of its polarity, each water molecule can potentially form four intermolecular hydrogen bonds with surrounding water molecules.

The content of water in the fat-free body is 72–75% in various animal species (Table 11.8). Most water in the body

TABLE 11.8
Composition of Nutrients in the Fat-Free Bodies of Animals

Animal	Body Weight (kg)	Water (%)	Crude Protein (%)	Ash (%)	Carbohydrates (%)
Cat	4	73.9	20.9	4.57	0.65
Cattle	500	71.0	22.0	5.97	1.05
Hen	2.5	73.0	22.3	3.96	0.76
Horse	650	72.6	20.4	5.77	1.18
LMB	0.055	76.8	18.3	4.71	0.27
Man	65	72.5	19.3	7.76	0.47
Pig	125	75.3	19.5	4.68	0.50
Rat	0.35	73.2	22.0	4.17	0.68
Rabbit	2.6	72.5	23.1	3.98	0.42
Sheep	80	72.7	22.4	4.30	0.56

Source: The data for largemouth bass (LMB) are taken from Li et al. (2021d). The data for other animals are recalculated, after the inclusion of carbohydrates (glycogen plus glucose), from Blaxter (1989).

is present inside the cells (intracellular water), but a significant proportion of water is outside the cells (extracellular water), as noted in Chapter 1. Lean and obese animals may have 70% and 40% of their BW as water, respectively. Dietary water is absorbed primarily by the small intestine and the large intestine. It was assumed for years that water passes through biological membranes only by simple diffusion through lipid bilayers until the discovery of water channel proteins known as aquaporins (AQPs; Agre and Kozono 2003). Because water passes through cell membranes relatively slowly by simple (passive) diffusion, rapid water flow across biological membranes is primarily mediated by AQPs, the water-transporting protein channels. AQPs are a family of small (28–30 kD) integral membrane proteins that primarily transport water, but some APQs also transport glycerol, urea, and other solutes across the plasma membrane of cells (Zhu et al. 2015). The movement of water via AQPs is driven by osmotic gradients. To date, 13 AQP isoforms (AQPs 0–12) have been identified in mammals. Based on their structural and functional properties, AQPs are divided into three subgroups: classical aquaporins (AQPs 0, 1, 2, 4, 5, 6, and 8), aquaglyceroporins (AQPs 3, 7, 9, and 10), and superaquaporins (AQPs 11 and 12; Table 11.9). AQPs are widely distributed in reproductive (both male and female), respiratory, digestive, excretory, circulatory, muscular, and other systems of animals (Zhu et al. 2022).

Water is lost from the animal body periodically through urine and feces and constantly through evaporation from the skin and respired air via the lungs (McDonald et al. 2021). The loss of water through the skin and lungs is known as insensible perspiration. Water losses are positively related to body size and are affected by nutritional, physiological, and environmental factors. For example, water loss via feces increases as the proportion of undigested dietary material increases (Bondi 1987). In addition, as the concentrations of glucose, ketone bodies, and urea in plasma increase, water loss via urination increases. Furthermore, water loss through evaporation and respiration increases as the ambient temperature increases. For example, Maynard et al. (1979) reported that an average *Bos taurus* cow weighing 450 kg and consuming 10 kg of DM/day drank 28, 41, and 66 L of water when the ambient temperatures were 4, 21, and 32°C, respectively.

TABLE 11.9
Classification and Characteristics of Aquaporins (AQP) in Animals

Classification	Isoform	Substrates for AQP
Aquaporin	AQP0	Water
	AQP1	Water, CO_2, NO, and NH_3
	AQP2	Water
	AQP4	Water, CO_2, O_2, and NO
	AQP5	Water and CO_2
	AQP6	Water, anions, urea, and glycerol
	AQP8	Water, urea, and NH_3
Aquaglyceroporin	AQP3	Water, urea, glycerol, and NH_3
	AQP7	Water, urea, glycerol, and NH_3
	AQP9	Water, urea, glycerol, other solutes, and NH_3
	AQP10	Water, urea, and glycerol
Superaquaporin	AQP11	Uncertain
	AQP12	Uncertain

Source: Zhu et al. (2015). AQPs 3, 7, 8, 9, and 10 can transport boric acid.

Water is essential to life. This nutrient dissolves many salts and hydrophilic organic molecules (e.g., sugars, amino acids, nucleic acids, and enzymes) and is the solvent in all biochemical reactions. When acids (e.g., HCl, glutamic acid, aspartic acid) dissolve in water, they yield their corresponding anions. An adequate volume of water in the body is necessary to maintain the intracellular and extracellular concentrations of all nutrients (including amino acids, glucose, fatty acids, and electrolytes) within physiological ranges. Thus, water is required to maintain osmo-equilibrium in all animals. In addition, as a component of synovial fluid, water plays an important role in lubricating the joints to facilitate muscle locomotion. Also, as the major constituent of cerebrospinal fluid, water helps to provide cushion for protecting the nervous system. Furthermore, water absorbs heat produced from chemical reactions to minimize increases in body temperature. Therefore, animals must obtain sufficient water for health, survival, and production. This is particularly important for (a) lactating dams, (b) young mammals within the first week after weaning, (c) all animals living at high ambient temperatures, and (d) all sick animals with fever and an elevated body temperature. Thus, severe dehydration in animals results in their death.

The sources of water in animals are drinking water, water-containing food, and the endogenous provision of water through macronutrient metabolism. The complete oxidation of 100 g of protein, carbohydrate, and fat to CO_2 generates 41, 60, and 109 g of metabolic water, respectively (Chapters 5–7). The daily water intake of animals is usually 1.5 to 3 times their DM intake but increases in response to a plethora of factors, including the high protein and salt content of the diet, environmental temperature, lactation, gestation, egg production, disease, and exercise, as noted previously. Average requirements of animals for water in a temperate climate are summarized in Table 11.10.

There are species differences in tolerance to water deficiency among animals. For example, cats have a lower physiological thirst drive than dogs and concentrate their urine (through the reabsorption of water in the lumen of kidney tubules into the blood) to a greater degree than dogs, which helps to conserve water (Li and Wu 2024). In addition, the dense hair of cats can minimize the evaporative loss of water through the skin. Thus, cats may have a lower requirement for exogenous water than dogs under similar feeding conditions and can tolerate mild dehydration better than dogs. However, a loss of 20% water from cats can be fatal (Anderson 1982). In all animals, drinking too much

TABLE 11.10
Average Daily Requirements of Animals for Water in a Temperate Climate

	Daily Water Requirements	
Animal Species	**L**	**L/kg body weight**
Beef cow (lactating, 500 kg)	60	0.12
Beef cattle (450 kg)	40	0.09
Cat (4 kg)	0.2	0.05
Dairy cow (lactating, 500 kg)	90	0.18
Dairy cow (maintenance, 500 kg)	60	0.12
Dog (30 kg)	1.65	0.055
Horse (medium work, 450 kg)	40	0.09
Horse (lactating, 450 kg)	50	0.11
Men (70 kg)	3	0.043
Poultry, hen (2 kg)	0.5	0.25
Rat (0.3 kg)	0.075	0.25
Swine (growing, 30 kg)	6	0.20
Swine (growing, 60–100 kg)	8	0.10
Swine (lactating sow, 150 kg)	18	0.12
Sheep (lactating ewe, 50 kg)	6	0.12
Sheep (fattening lamb, 50 kg)	4	0.08

Source: Maynard et al. (1979); Wu et al. (2023).

water can result in adverse effects. Animals drinking an excessive amount of water (e.g., >100 ml/kg BW/day for cats) may appear thirsty but actually have potential health problems (e.g., diabetes mellitus, chronic kidney disease, and hyperthyroidism).

Use of Energy and Its Substrates for Maintenance

Different nutrients are utilized in animals through different pathways (Chapters 5–10). For example, the rumen ferments carbohydrates to produce SCFAs, as highlighted in Chapter 6. As discussed in Chapter 8, the energetic efficiency of ATP production from the oxidation of protein in mammals (which synthesize urea from ammonia) is higher than that in birds (which synthesize uric acid from ammonia). In both mammals and birds, but not fish or crustaceans, the energetic efficiency of ATP production from the oxidation of fats or glucose is greater than that for proteins or amino acids (Chapter 8). Thus, protein is generally not used as the major metabolic fuel in the whole body of mammals and birds. However, this is not the case with fish because certain amino acids (e.g., glutamate, glutamine, and aspartate) are important energy substrates for their metabolically active tissues, including not only the small intestine but also the skeletal muscle, liver, and kidneys (Jia et al. 2017; Li et al. 2020; Wu 2022a). If there are differences in nutrient digestibility and content, different feedstuffs, such as corn grain and soybean meal (SBM), may contribute differentially to the maintenance of animals.

Even at maintenance requirements, animals synthesize proteins and fats. However, both proteins and fats undergo continuous degradation (Chapters 6 and 7). In growing or producing animals, the maintenance requirement is coupled to the production requirement. Therefore, the total requirement is generally used to formulate diets for farm animals (NRC 2001, 2011, 2012). Because the energetic efficiencies of synthetic processes vary with precursors and products (Chapter 8), energy substrates have different efficiencies with which they are used for maintenance and production. For example, in pigs, the efficiencies of amino acids and fatty acids for maintenance are 78% and 95% of those for glucose, respectively, whereas the efficiencies of amino acids and glucose for fat deposition are 61% and 83% of those for fatty acids (ARC 1981). In growing beef cattle, the efficiencies of ME used for both maintenance and BW gain increase with increasing ME in the diet within a physiological range (2.0–3.2 Mcal/kg diet; Blaxter 1989). This is because dietary intake of energy (primarily in the forms of water-soluble and insoluble polysaccharides) by ruminants above the maintenance level can enhance microbial protein yields and SCFA production in the rumen (de Faria and Huber 1983; Hackmann and Firkins 2015). This results in (a) reduced production of gases (e.g., CH_4) by the gastrointestinal tract, (b) increased hepatic gluconeogenesis from propionate, (c) increased lipogenesis from acetate and butyrate in WAT, (d) increased ATP production from acetate and butyrate in diverse tissues, and (e) increased availability of amino acids for protein synthesis in skeletal muscle and other tissues, leading to reduced excretion of nitrogenous wastes in the urine and feces. When a quantity of dietary energy is not used by animals for maintenance and production, an energetic inefficiency in feed utilization occurs (van Milgen et al. 2001). This concept helps us to better understand the nutritional requirements of animals for production.

NUTRITIONAL REQUIREMENTS FOR PRODUCTION

Diets for productive animals must meet the requirements for energy and specific nutrients (amino acids, fatty acids, carbohydrates, minerals, vitamins, and water) for (a) the formation of milk (mammals), eggs (poultry), and wool (e.g., sheep); (b) the growth of tissues, such as skeletal muscle; and (c) work (horses and cattle; Figure 11.2). Because amino acids cannot be synthesized solely from fatty acids and carbohydrates, animals must consume protein or amino acids from diets (Wu et al. 2014b). A deficiency of dietary protein results in low appetite, poor growth (fetuses and young animals), impaired production of body proteins [including immunoglobulins (Igs, also known as antibodies)], depressed reproductive performance, reduced milk production, and impaired egg laying. Providing adequate protein or amino acids in ruminant (e.g., beef cattle and cows) and nonruminant (e.g., pigs and poultry) diets is essential for the optimal health and productivity of animals [e.g., the ability of an animal to grow, reproduce, and

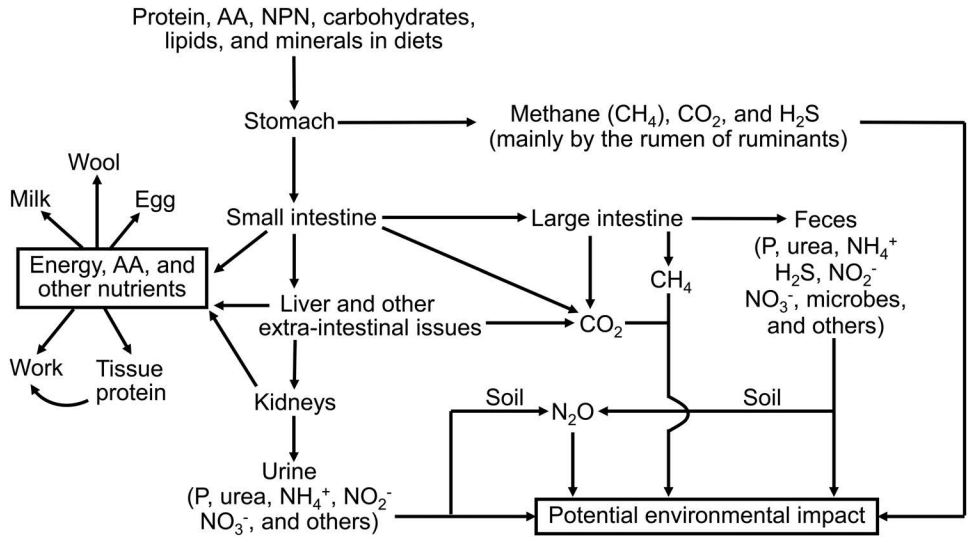


FIGURE 11.2 Metabolism of nutrients to methane (CH_4), CO_2, urea, ammonia, hydrogen sulfide (H_2S), nitrous oxide (N_2O), and other products in animals. Dietary nutrients are terminally digested and absorbed in the small intestine. The kidneys and liver contribute amino acids and glucose for utilization. Metabolism of protein, carbohydrates, and lipids in the gastrointestinal tract (particularly the rumen of ruminants) generates methane and CO_2, whereas metabolism of protein also produces ammonia and H_2S. Nitrogenous substances in urine and feces are fermented by bacteria in the soil to form N_2O. Optimizing the formulation of diets is an attractive means to mitigate the potential impact of animal production on greenhouse gas emissions and the environment. AAs, amino acids; NPN, nonprotein and non-amino acid nitrogen.

produce useful outputs (e.g., milk, eggs, wool, and muscular work)], as well as farm profitability.

Nutritional requirements of animals for production are often determined from factorial analysis of their products (e.g., tissue protein accretion, milk, wool, and eggs) and must be considered along with their nutritional requirements for maintenance. The efficiency of utilization of dietary energy and nutrients for production depends on animal species, tissue, and nutritional adequacy. Although all nutrients are essential for animal production, the dietary ratios of protein/energy, arginine/lysine, Ca/P, and ω3 PUFAs/ω6 PUFAs are of particular interest to producers when practical rations are formulated. Enhancing the efficiency of nutrient utilization can help sustain the global animal industry while mitigating its adverse impact on the environment. Because there are large variations in dietary energy and nutrient requirements among animals, the values recommended for group feeding may not be used for individual precision feeding (Remus et al. 2019).

There are biological interactions in absorption, interorgan transport, and metabolism between and among nutrients (Chapters 5–10). Some interactions are beneficial to maximize their effects on health, growth, development, and production in animals (e.g., vitamin B_6, B_{12}, and folate), but some can result in adverse responses (e.g., arginine and lysine). Thus, the toxic effect of an excessive nutrient can also be the cause of a secondary deficiency of another nutrient. Examples for nutrient cooperation and antagonism are given in Table 11.11. Relationships between and among nutrients affect their biological functions and dietary requirements. Available evidence shows that there may be species- and age-specific differences in nutrient interactions (Baker 2001; Persia et al. 2003; Wu 2022a). Findings from studies of farm and laboratory animals can be applied to the prevention and treatment of diseases in humans.

Suboptimal Efficiencies of Animal Protein Production in Current Agricultural Systems

Natural resources for growing feedstuffs and pasture plants are becoming increasingly limited worldwide. A major goal of animal nutrition is to fully realize the genetic potential of livestock, poultry, fish, and crustaceans for reproduction, growth (including accretion of protein in skeletal muscle), and resistance to disease, while preventing an excessive accumulation of WAT (Wu et al. 2014a). In animals (e.g., mammals, birds, fish, and invertebrates), mitochondria play an important role in ATP production; mitochondrial function greatly influences energetic efficiency and therefore feed efficiency (Bottje and Carstens 2009). Biological efficiency of animal production, which refers to how effectively feed is converted into tissues (e.g., skeletal muscle and hair), milk, or eggs in agricultural operations, is a major determinant of economic efficiency to minimize costs for the production of livestock, poultry, fish, and crustaceans (Campbell 1988; He et al. 2021; Li et al. 2021b,c; Zhang et al. 2021). Levels of economic efficiency are usually set by practitioners, and there is not a gold standard or a universally established minimum above which the biological

TABLE 11.11
Physiological and Metabolic Interactions between and among Nutrients in Animals

Nutrient Interactions	Comments
Arginine × Citrulline	Citrulline can replace part of dietary arginine requirement in terrestrial animals
Arginine × Lysine	Antagonism in intestinal absorption and uptake by extraintestinal cells
Arginine × Proline	Inter-conversion in most mammals (including pigs, ruminants, and rodents)
Bromine × Iodine	Bromine prevents iodine toxicity
Calcium × Phosphorus	Intestinal absorption; food intake; whole-body homeostasis
Calcium × Zinc	Calcium inhibits the intestinal absorption of Zn; interaction affects food intake
Calcium × Vitamin D	Intestinal absorption of calcium; bone health and growth
Choline × Betaine	Choline is converted into betaine via choline oxidase (a mitochondrial enzyme)
Co^{3+} (inorganic) × Fe^{2+}	Have identical electronic configurations and interfere with each other's absorption
Copper (Cu) × Arsenic	Arsenic reduces Cu absorption
Copper × Cysteine	Cysteine can chelate Cu and, therefore, reduces the absorption and toxicity of excess Cu
Copper × Iron (Fe)	Interfere with each other's absorption. Reduction of Fe^{3+} to Fe^{2+} in Mit depends on Cu
Copper × Heme	Cu deficiency reduces the conversion of Fe^{3+} into Fe^{2+} in mitochondrial electron system
Copper × Molybdenum (Mo)	Mo interferes with Cu absorption and prevent the toxicity of excess dietary Cu
Copper × Mo × Sulfur	Interfere with each other's absorption[a]
Copper × Sulfur	Form the insoluble CuS that is not absorbed by the gastrointestinal tract of ruminants.
Copper × Vitamin C	Vitamin C reduces Cu absorption
Copper × Zinc	Interfere with each other's absorption by the intestine; regulation of food intake
Cysteine × Potassium sulfate (SO_4^{2-})	Increase food intake by chicks; causes acid-base imbalance in excess
Cysteine × Glutathione	Cysteine is used for glutathione synthesis; antioxidant reactions
Cysteine × Lanthionine	Intestinal cysteine absorption and whole-body cysteine metabolism
Cysteine × Taurine	Cysteine is catabolized to generate taurine, but not vice versa.
Cysteine (Cys) × Zn	Promotes each other's absorption. Intestinal absorption of Zn requires Cys-rich proteins.
Glutamine × Fructose (or Glucose)	Glucosamine essential for conceptus growth but results in insulin resistance in adults
Glycine × Folate	Excess glycine disturbs one-carbon unit metabolism and is toxic to the brain.
Glycine × 4-Hydroxyproline	4-Hydroxyproline is converted into glycine in animal tissues.
Glycine (Gly) × Serine (Ser)	Interconversion in animals; the rate is greater for Ser to Gly than Gly to Ser.
Glycine × Taurine × LCFA	Taurine and/or glycine (components of bile acid) are required for LCFA absorption
Glycine × Threonine	Glycine is formed from threonine, but glycine cannot be converted into threonine
Histidine × Carnosine (His-β-alanine)	Dietary histidine stimulates carnosine synthesis; carnosine affects His requirement
Iron × Manganese	Interfere with each other's absorption
Iron × Tryptophan	Iron is required for the conversion of tryptophan to niacin
Iron × Zinc	Interfere with each other's absorption
Leucine × Isoleucine × Valine	Imbalance and antagonism among these amino acids
Lysine × Lysinoalanine	Intestinal lysine absorption and whole-body Lys metabolism
Methionine × Choline	*S*-Adenosyl-methionine is used to synthesize choline; this pathway is limited in chicks.
Methionine × Creatine	Methionine is utilized for creatine synthesis.
Methionine (Met) × Cysteine	Met is catabolized to generate cysteine. Cysteine can replace part of dietary Met.
Phenylalanine (Phe) × Tyrosine	Phe is catabolized to generate tyrosine; Tyrosine partially spares dietary Phe.
Phosphorus × Vitamin D	Intestinal absorption of phosphorus; bone health and growth
Phosphorus × Manganese (Mn)	Dietary phosphate reduces the intestinal absorption of Mn.
Riboflavin × Vitamin B_6	Affect amino acid metabolism
Selenium × Arsenic	Arsenic ameliorates selenium toxicity.
Selenium × Methionine	Methionine increases the intestinal absorption of selenium; antioxidant reactions
Selenium (from selenite) × Cysteine	Antioxidant reactions; cysteine increases Se absorption and affects Se requirements.
Selenium × Sulfur	Selenium spares dietary sulfur possibly due to antioxidative effects.
Tryptophan × Niacin	Tryptophan is a precursor for the synthesis of niacin in most animals except for cats.
Tryptophan × Lysine	Shares the same transporter at intestinal basolateral membrane and extraintestinal cells
Vitamin A × β-Carotene[b]	Vitamin A is formed from β-carotene in most animals except for cats.
Vitamin B_{12} × Vitamin B_6 × Folate	Metabolism of Met, Cys, and homocysteine; recycling of homocysteine into methionine
Vitamin B_{12} × Cobalt	Required for glucose synthesis in ruminants
Vitamin C × Zn	Vitamin C increases Zn absorption; Zn can reduce the oxidation of vitamin C.

[a] In the rumen, dietary sulfur is reduced to sulfide. The latter interacts with molybdenum to form thiomolybdates, which bind Cu and prevent Cu absorption by the small intestine. In addition, absorbed thiomolybdates increases the excretion of Cu by the liver into the bile, impairs the transport of Cu in the blood, and removes Cu from metalloenzymes in cells.

Note: LCFA, long-chain fatty acid.

efficiency of animal production would be considered adequate (Wu et al. 2014b).

Nitrogen utilization efficiency refers to the percentage of dietary N (proteins, amino acids, and non-amino acid N such as ammonia and urea) being converted into proteins, peptides, and amino acids in body tissues, milk, eggs, wool, and other useful products in animals. About 97% of N in the body exists as proteins (Wu 2022a). Because of physiological and biochemical constraints, the digestion of feeds and the conversion of dietary proteins to tissue proteins in animals remain suboptimal (Table 11.12). As indicated in Chapter 7, dietary protein has no nutritive value until it is digested by the gastrointestinal tract, and digestion is a key factor affecting the efficiency of N utilization by animals. Due to differences in digestive systems and nutrient metabolism, there are species differences in the efficiency of dietary N utilization for protein gain (Table 11.12). Because both insulin sensitivity in skeletal muscle and the rate of whole-body protein synthesis decrease with age, young animals have a greater N utilization efficiency than older ones. Nutritional strategies to improve the retention of dietary N as protein in animals include (a) supplementing functional amino acids [e.g., arginine, branched-chain amino acids (BCAAs), glutamine, glutamate, glycine, and tryptophan) to plant protein-based diets to reduce intestinal amino acid catabolism, increase the entry of dietary amino acids into the portal circulation, and activate cell signaling pathways (including the MTOR); (b) optimizing the proportion and amounts of all proteinogenic amino acids in diets through the use of synthetic amino acids, including those that can be synthesized in the body; (c) creating and augmenting pathways for the endogenous synthesis of amino acids (e.g., arginine, glutamine, proline, and threonine) through genetic or metabolic engineering; and (d) alleviating adverse impacts of the environment (e.g., heat stress and air pollution) on animal metabolism and health. In strict carnivores, dietary taurine is essential as a nonproteinogenic amino acid for the optimum efficiency of N utilization (Wu et al. 2014b).

Catabolism of amino acids generates CO_2, ammonia, hydrogen sulfide, methane, urea, and uric acid, further reducing the efficiencies of growth, lactation, and reproduction in animals (Chapter 7). Although bacteria in the rumen have a high capacity for converting nonprotein N into amino acids, this process generates a large amount of ammonia, and its use for the synthesis of amino acids and protein occurs at suboptimal rates (Gilbreath et al. 2021). Thus, the efficiency

TABLE 11.12
Suboptimal Efficiencies of Production of Animal Protein in Current Agricultural Systems[a]

Animal	Weeks of Production	Efficiency for Protein Gain (%) (Based on Whole Body Protein)[b]	Efficiency for Protein Gain (%) (Based on Edible-Protein)[c]
Broiler chickens	6	39.9	33.3
Beef cattle (feedlot, cereal-fed)	54	18.9	12.1
Beef cattle (grazing)	76	10.5	6.7
Cows (milk production)	44	17.9	17.9
Hybrid striped bass[d]	26	34.2	15.4
Laying hens (eggs)[e]	55	34.0	31.3
Pigs (growing)	25	29.6	23.3
Sheep (cereal-fed)	13	28.6	18.3
Sheep (grazing)	13	11.8	7.6
Sheep (Merino; wool production)	52	15.8	22.5
White-leg shrimp	8	24	15.8

Source: Li et al. (2021a.b); Wu (2023); Wu et al. (2014c).

[a] Animals were fed their conventional diets. Protein is expressed as crude protein (CP).

[b] Calculated as whole-body protein deposited in animal/protein intake from diet × 100%. These data are physiologically meaningful.

[c] Calculated as edible protein deposited in product (e.g., tissue, eggs, or milk)/protein intake from diet × 100%. The ratio of live body weight to edible meat (kg/kg) is 515:288 for grazing beef cattle, 540:302 for feedlot ("cereal") beef cattle, 109:78.1 for pigs, 2.54:2.0 for poultry, 0.50:0.24 for fish, and 0.015:0.0099 for white-leg shrimp. In edible meat, the weight of bone is deducted from the total weight of carcass. The content of CP (g/100 g fresh weight) in cow's milk, beef meat, pig meat, poultry meat, and eggs is 3.62, 19.7, 20.5, 19.1, and 12.7, respectively. The content of CP in the nonedible part of beef, pork, or poultry is 14%.

[d] Hybrid-striped bass (HSB) grows from 120 g to 610 g over a 6-month period. The efficiency for protein gain was calculated as edible protein based on the assumption that fish-processing industries generate 45% edible protein from HSB. The content of CP in HSB is 18.3%.

[e] A laying hen produces 17.7 kg edible eggs (295 eggs × 60 g/egg) in 55 weeks. The edible egg does not include the eggshell.

of utilizing dietary protein to produce whole-body protein is less than 40% for nonruminants and less than 25% for ruminants (Wu et al. 2014b). In addition to species differences, the efficiency of protein synthesis from dietary amino acids in skeletal muscle and other tissues decreases with increasing age. For example, approximately 70%, 55%, 50%, and 45% of dietary protein is converted into tissue protein in 14-day-old sow-reared pigs, 30-day-old pigs (weaned at 21 days of age to a corn- and SBM-based diet), 110-day-old pigs, and 180-day-old pigs, respectively (Wu et al. 2014b). Similarly, approximately 52%, 48%, 45%, and 41% of dietary protein is converted into tissue protein in 1-, 2-, 4-, and 6-week-old broiler chickens fed corn- and SBM-based diets, respectively. Furthermore, the sensitivity of skeletal muscle to insulin decreases as the age of the animal increases (Chapter 7). Thus, we are facing enormous challenges to sustain the production of high-quality protein by farm animals because feedstuff resources are becoming increasingly limited. Optimizing dietary formulations is an attractive means to mitigate the potential impact of animal production on greenhouse gas emissions and the environment.

Nutritional Requirements for the Reproduction of Females

Improving the efficiency of reproduction is of great economic importance in animal production (Stenhouse et al. 2022a; Wu et al. 2006). In both males and females, fertility depends on genetic traits and environmental factors. Adequate nutrition is essential for the normal reproductive functions of all animals, including sperm production, ovulation [controlled by gonadotropin-releasing hormone (GnRH) and luteinizing hormone (LH)] (Figure 11.3), conception, and parturition (Evans and Anderson 2017). Conversely, undernutrition delays the maturation of the reproductive system and impairs its function after puberty. Physiological levels of nitric oxide (NO) and polyamines (metabolites of arginine and proline) play important roles in reproduction by stimulating the release of GnRH from the hypothalamus (Dhandapani and Brann 2000) and polyamine synthesis (Bazer et al. 2014; Wu et al. 1998). In addition, through cell signaling and nutrient utilization, metabolic hormones (e.g., leptin, insulin, and ghrelin) are key mediators of fertility in males and females. Furthermore, progesterone, released by

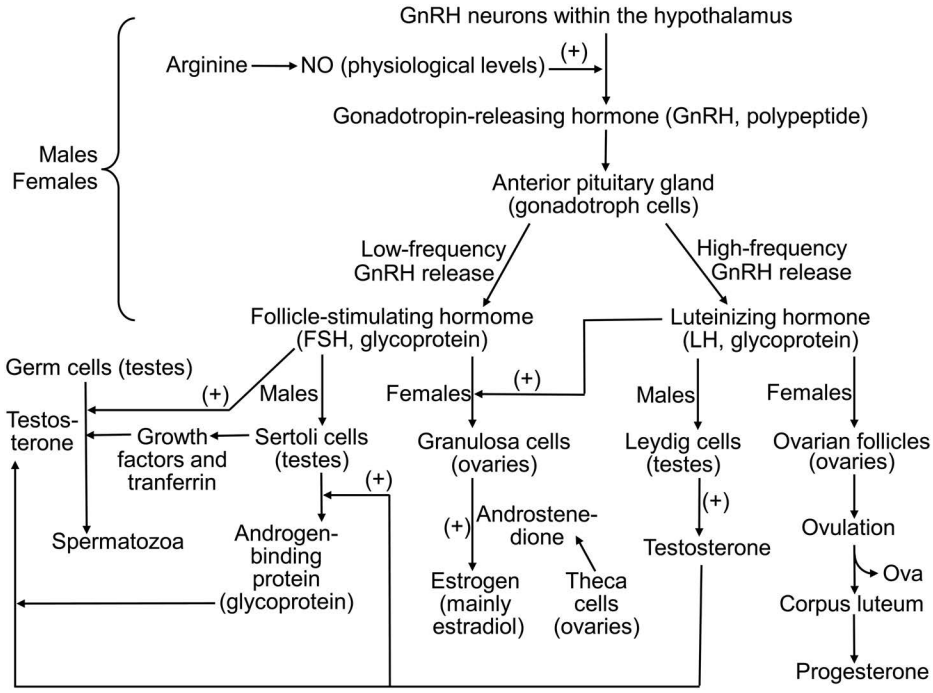


FIGURE 11.3 The hypothalamic-pituitary-gonadal axis and hormones for animal reproduction. The frequency of release (pulses) of gonadotropin-releasing hormone (GnRH) from the hypothalamus is critical to stimulate the production of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) by the anterior pituitary gland. In males, GnRH is secreted in pulses at a low constant frequency. In females, the frequency of GnRH pulses varies from low during most of the estrous cycle to high to induce a surge in LH required for ovulation of ova from ovarian follicles and the formation of corpora lutea that produce progesterone. Both FSH and LH act synergistically to enhance (a) the release of estrogen and progesterone from the ovaries of females and (b) the generation of testosterone and spermatogenesis in the testes of males. Each ovarian follicle contains an oocyte (ovum) with surrounding granulosa cells and theca cells in the ovaries, whereas spermatogonia, Sertoli cells, and Leydig cells are present in the testes. Estrogen, progesterone, and testosterone have negative feedback effects on GnRH release. These reproductive hormones are synthesized from cholesterol, a metabolite of fatty acids. Physiological levels of nitric oxide (NO, a metabolite of arginine) and adequate white adipose tissue for leptin production, for example, play important roles in regulating reproduction in both males and females by stimulating the cGMP-dependent NO signaling pathway.

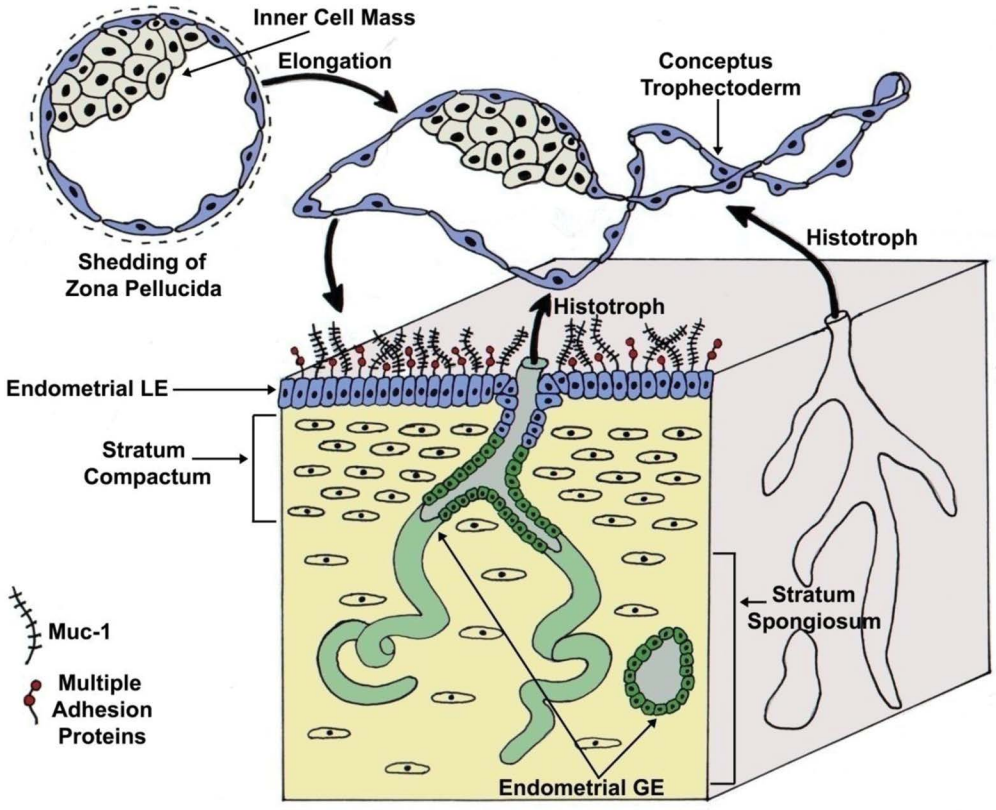


FIGURE 11.4 Uterine secretions for embryonic survival, growth, and development in mammals. During pregnancy, progesterone, estrogen, and other factors stimulate the endometrial luminal and glandular epithelia to secrete proteins, hormones, cytokines, nutrients, and other substances, which are collectively called histotroph. These molecules support the growth and development of the conceptus (embryo/fetus and associated placental membranes). (Reproduced from Bazer et al. 2014, with permission from Elsevier.)

the ovaries and placenta, is an essential hormone for establishing and maintaining pregnancy in mammals (Bazer et al. 2008). For example, in pigs and sheep, progesterone, along with other factors including estrogen, acts on the endometrial luminal and glandular epithelia to regulate the synthesis and secretion of certain components of histotroph (Figure 11.4). The latter is a mixture of molecules including proteins [e.g., secreted phosphoprotein 1 (osteopontin)], growth factors, hormones, cytokines, amino acids, and other molecules that support the growth and development of the conceptus (embryo/fetus and associated placental membranes; Johnson et al. 2014b, 2023; Wu et al. 2023b).

Embryonic Developmental in Mammals

Stages of Embryonic Development

Development of conceptuses in different mammalian species differs in (a) the duration of the preimplantation period and (b) the type of implantation (noninvasive versus invasive) and placentation [epitheliochorial in pigs, horses, whales, and dolphins; synepitheliochorial in ruminants (e.g., cattle, sheep, and goats); endotheliochorial in terrestrial carnivores (e.g., cats, dogs, and tigers); and hemochorial in primates (e.g., humans), rabbits, and rodents (e.g., rats and mice)]; (Bazer et al. 2014). However, the early stages of embryonic development and the phases of blastocyst implantation are similar among mammalian species (Figure 11.5). For early development of the zygote (a fertilized ovum), successive cleavage events occur at Stage 1, resulting in the formation of a 32- to 64-cell morula. At Stage 2 of development, the morula forms a blastocyst characterized by two distinct cell populations: (a) the inner cell mass, which develops into the embryo; and (b) the trophectoderm, which gives rise to the chorion of the placenta. Within the uterine lumen, the events of blastocyst development prior to implantation are divided into five phases: (a) shedding of the zona pellucida, (b) precontact and blastocyst orientation, (c) apposition of the trophectoderm and uterine luminal epithelium, (d) adhesion of trophectoderm to uterine luminal epithelium, and (e) invasion of the blastocyst into the uterine endometrium, which is unique to species (e.g., rodents and primates) that have invasive implantation (Bazer et al. 2008). Interferon tau is the pregnancy recognition signal produced and secreted by trophectoderm cells of ruminant conceptuses (Days 10–21 in sheep and 14–24 in cattle; Bazer et al. 2020). In contrast, estrogens are the pregnancy recognition signals in pigs (Days 11–12 of gestation), whereas prolactin and placental lactogen are pregnancy recognition signals in rodents, and chorionic gonadotrophin is the pregnancy recognition signal in primates (Bazer 2015). In mammals, the priority of organ development at the embryonic stage (from greatest to least) is neural tubes (that later become

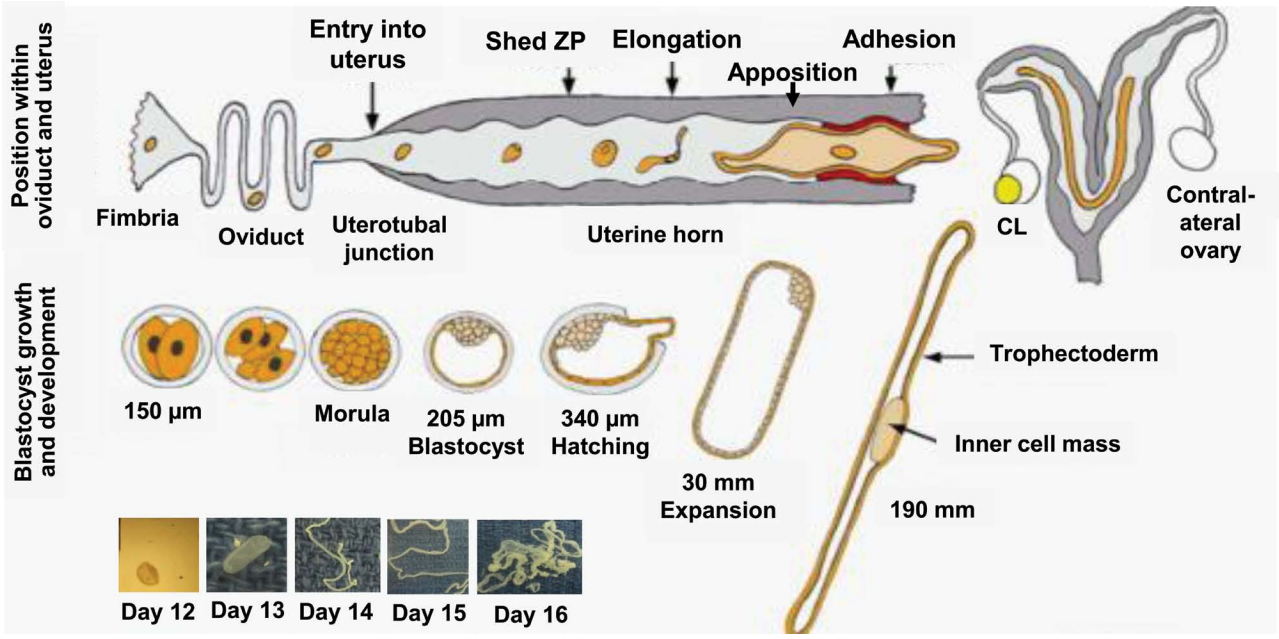


FIGURE 11.5 Growth and development of ovine embryos/conceptuses within the oviduct and uterus. Fertilization between sperm and eggs (ova) inside the oviduct results in the formation of embryos. The embryos enter the uterus, where they undergo rapid elongation from spherical to tubular and filamentous forms as conceptuses that require proliferation, migration, and cytoskeleton reorganization of trophectoderm cells. Apposition and attachment of the conceptus trophectoderm to the uterine luminal and glandular epithelia are intricately orchestrated during implantation. (Reproduced from Bazer et al. 2014, with permission from Elsevier.)

the brain, spinal cord, and other neural tissues of the central nervous system), head, and heart; ears, eyes, limb buds (that later become arms and legs), skin, nose, blood vessels, spleen, thymus, lungs, the organs of the digestive system, and the organs of the urinary system; bones and the organs of the reproductive system; and skeletal muscles and adipose tissue. In the following sections, examples of conceptus development are provided for sheep (ruminants) and pigs (nonruminants).

In sheep, embryos enter the uterus on day 3 after breeding, develop to spherical blastocysts, and then transform to large spherical (day 10, 0.4 mm), tubular, and filamentous forms between days 12 (1×33 mm), 14 (1×68 mm), and 15 (1×150–1×190 mm) of pregnancy (Bazer et al. 2012a,b). Meanwhile, extraembryonic membranes extend into the contralateral uterine horn between days 16 and 20 of pregnancy. Elongation of ovine conceptuses is a prerequisite for central implantation involving apposition and adhesion between the trophectoderm of the conceptus and uterine luminal and superficial glandular epithelia. Later, there is loss of the luminal epithelium, which allows intimate contact between the trophectoderm and the uterine basal lamina proximal to uterine stromal cells between days 18 and 50–60 of gestation. These events in the development of the ovine conceptus are illustrated in Figure 11.5.

In swine, following fertilization, zygotes develop and cleave into 2- and 4-cell stage embryos in the oviduct (Bazer et al. 2010). After entering the uterus around day 3 of gestation, embryos continue to cleave, develop to blastocysts by days 7–8 of gestation, and then hatch from the zona pellucida. Thereafter, blastocysts migrate within the uterine lumen to achieve equal spacing among themselves and then undergo dramatic changes in morphology from an expanded spherical shape to tubular and filamentous forms between days 10 and 15 of pregnancy. The diameter of spherical blastocysts is only 5–10 mm by day 10 of gestation. However, when reaching a spherical diameter of 10 mm on about day 11 of gestation, it takes only 3 or 4 h for blastocysts to elongate to tubular and then filamentous conceptuses that are 150–200 mm in length, and by day 15 they approach approximately 1,000 mm in length (Geisert and Yelich 1997). This dramatic morphological change occurs initially through cellular remodeling rather than cellular hyperplasia, but the final phase of elongation between days 12 and 15 involves both cellular hyperplasia and cellular remodeling (Geisert et al. 1982). Porcine conceptuses initiate attachment of trophectoderm to uterine luminal epithelia on day 13 of pregnancy, and implantation is accomplished by about day 18 of gestation in advance of placentation and formation of a true epitheliochorial placenta. The chorioallantoic placenta is evident by day 20 of gestation and grows rapidly thereafter. Of note, the uterine-placental bilayer develops progressively complex folds to augment the surface area of contact between the chorionic membrane and luminal epithelial cells. The placental and uterine microvasculature lies immediately beneath these epithelia, and therefore the development of placental folds is important for efficient transport of nutrients from

TABLE 11.13
Stages of Fetal and Postnatal Development of Skeletal Muscle in Pigs

Stage	Days of Gestation	Major Events
1	From conception to 25 days of gestation	Embryonic myogenesis from a common mesenchymal precursor
2	From 25 to 50 days of gestation	Formation of primary muscle fibers (rapid fusion of primary myoblasts)
3	From 50 to 90 days of gestation	Formation of secondary muscle fibers (formed on the surface of primary fibers)
4	From 90 to 95 days of gestation	Establishment of muscle fiber numbers
5	Day 114 of gestation (term birth)	Total numbers of muscle fibers are fixed at birth
6	After birth	Growth of skeletal muscle by increasing the size of its fibers (hypertrophy)[a]; and the maturation of skeletal muscle

Source: Ji et al. (2017).

[a] Hypertrophy is defined as an increase in the size of the skeletal-muscle cell (also known as muscle fiber), whereas hyperplasia refers to an increase in the number of cells.

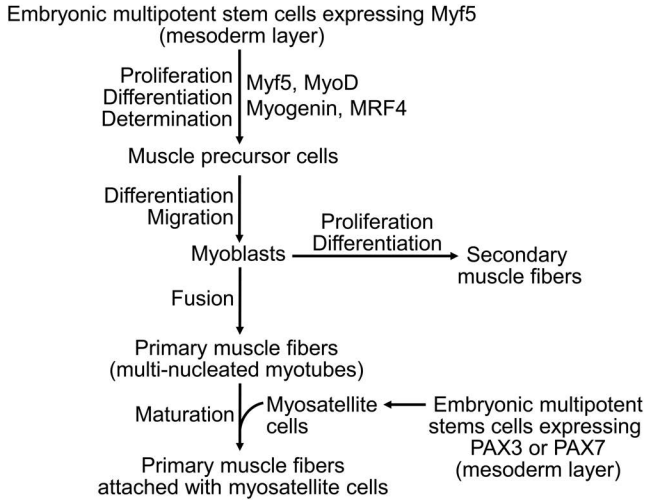


FIGURE 11.6 Embryonic lineage for development of skeletal muscle in animals. MRF4, muscle regulatory factor 4; PAX3, paired box protein 3; PAX7, paired box protein 7.

the mother to the developing conceptus. Before day 35 of gestation, porcine conceptuses are uniformly distributed within each uterine horn. After this time in gestation, uterine capacity becomes a limiting factor for fetal growth even though the fetuses are distributed relatively uniformly (Ford et al. 2002). In fetal pigs, primary muscle fibers form from the rapid fusion of primary myoblasts between days 25 and 50 of gestation, whereas secondary muscle fibers form on the surface of primary fibers between days 50 and 90 of gestation (Table 11.13).

Embryonic Origins of Fetal Tissues

The embryo differentiates to form three germ layers: endoderm (the innermost layer), mesoderm (the middle layer), and ectoderm (the outer layer; Senger 1997). The endoderm is the origin of the digestive tract, liver, lungs, pancreas, thyroid gland, and other glands. The mesoderm is the origin of the circulatory system, skeletal muscle, bones, reproductive tracts, kidneys, and urinary ducts (Pownall et al. 2002). The lineage of skeletal muscle development is shown in Figure 11.6. The ectoderm is the origin of the central nervous system, sense organs, mammary glands, sweat glands, skin, hair, and hooves. Before placentation, the embryo receives nutrients from uterine secretions and oxygen from its surrounding environment. The timelines of conceptus development differ among mammals, such as swine and sheep (Bazer et al. 2010). However, maternal under- or overnutrition during early gestation negatively affects conceptus development in all species (Hoffman et al. 2016; Wu et al. 2006).

Functions of the Placenta

As noted previously, the placenta starts to develop during early gestation in mammals (e.g., as early as day 18 of gestation in pigs), and then placental blood vessels are clearly visible (e.g., day 25 of gestation in pigs). In all species, a functional placenta transports nutrients, respiratory gases, and products of their metabolism between the maternal and fetal circulations, which is crucial for fetal survival, growth, and development (Wang et al. 2012). Rates of utero-placental blood flow depend on placental vascular growth (a result of angiogenesis and vasodilation of blood vessels) and placental vascularization, which are greatly enhanced by physiological concentrations of NO and polyamines (Reynolds et al. 2006). To support increased uterine and placental blood flows, placental angiogenesis increases markedly from the first to the second and third trimesters of gestation (e.g., term = 114 days in pigs and 147 days in sheep) and continues to increase during the last days of gestation. Uptake of nutrients by the uterus or the fetus is determined by both rate of blood flow and concentrations of nutrients in the arterial and venous blood. Thus, uptakes of both macro- and micro-nutrients by the uterus are greater in pregnant women than in nonpregnant women (Reynolds et al. 2006). Conversely, impaired placental blood flow contributes to intrauterine growth restriction (IUGR) in mammalian pregnancies (Wu et al. 2004).

Embryonic Developmental in Poultry

Embryonic development and hatching in poultry have been described by Moran (2007). Cell division inside a fertilized egg starts approximately 5 h after fertilization and continues as the egg passes along the oviduct and is laid. It generally takes 21 days of favorable incubation conditions for the egg to fully develop and hatch as a chick that can breathe air with its lungs. These physiological processes are outlined as follows.

The Zygote

After a sperm fertilizes an egg in the infundibulum of a hen, a zygote (a single cell with a set of half chromosomes

from each parent) is formed. About 5 h after fertilization, the zygote enters the isthmus and develops via cell division into the blastoderm of 8 cells on the egg yolk surface. After a 75-min passage through the isthmus, the blastoderm enters the uterus and grows into 256 cells within a 4-h passage through the uterus. The blastoderm continues to develop and has ~20,000 cells. Thereafter, the egg is laid when most of the cells of the area pellucida remain at the egg yolk surface to form the epiblast and the other area pellucida cells migrate into the subgerminal cavity (the space between the blastoderm and the yolk) to form the polyinvagination islands (primary hypoblast). The fully developed blastoderm encloses a fluid-filled cavity called the blastocoel.

Formation of Ectoderm, Endoderm, and Mesoderm

At the early stage of incubation, the blastoderm within the laid egg grows via cell division to form one layer of cells over the yolk and then two layers of cells, namely the ectoderm layer (uppermost) and the endoderm (underneath) layer. At this time, the central cells of the blastoderm separate from the egg yolk, creating a cavity where the embryo further develops a third layer of cells called the mesoderm. The ectoderm is the origin of the nervous system, parts of the eyes, the feathers, beak, claws, and skin. The endoderm leads to the respiratory system, the digestive system, and secretory organs. The mesoderm generates the skeleton, muscles, circulatory, reproductive, and excretory systems.

Extraembryonic Membranes

The avian embryo develops extraembryonic membranes to obtain nutrients within the incubated egg. These special membranes are the amnion (a sac that is filled with fluid in which the embryo floats), allantois (with an extensive circulatory system connected to that of the embryo), chorion (whose inner shell membrane fuses with the allantois), and yolk sac (enclosing the yolk to nourish the embryo). A fully developed allantois surrounds the embryo to fulfill the respiratory, excretory, and some digestive functions. Blood vessels appear on day 2 of embryonic development. The yolk sac utilizes nutrients within the yolk by synthesizing and secreting digestive and catalytic enzymes as well as releasing metabolites into the yolk. Any remaining, unused yolk material in the yolk sac at hatching is used by the chick during the first 2–3 days after hatching.

Effects of Nutrients and Related Factors on the Reproductive Performance of Females

Overall Undernutrition or Overnutrition

Undernutrition not only affects the onset of puberty and sexual development but also impairs ovulation and fertilization. Maternal undernutrition, particularly protein deficiency, reduces the weight of the dam more than that of her offspring, because maternal tissues are mobilized to provide nutrients to the fetus, which has a high priority for nutrients (Ashworth 1991; Pond et al. 1991). The protection of the fetus may not occur when the mother is deficient in nutrients, particularly arginine, folic acid, vitamin A, and iron (Wu et al. 2012). The early gestation period is critical for placental growth, differentiation, angiogenesis, vascularization, and fetal organogenesis, whereas most of the fetal growth occurs in the last trimester of pregnancy (Reynolds et al. 2006). Before birth, the fetus is nurtured in the sterile, protected, moist, and warm environment of the uterus and receives nutrients via the umbilical vein (parenteral nutrition) and also by swallowing amniotic fluid (enteral nutrition).

Either under- or overnutrition increases the incidence of early embryonic mortality (Ji et al. 2017). In animal production, it is desirable for females to conceive soon after parturition to maximize economic returns. As a result, breeding and conception must occur in ruminants when cows are lactating or within the first week after the termination of lactation in nonruminants. For example, cows are often bred shortly after the peak of lactation, and pigs are bred within 5 days following a 21-day period of lactation. The challenge is that milk production often induces temporary negative energy and protein balances in most females, which is not conducive to successful reproduction (Wu et al. 2006). Indeed, in dairy breeds of cattle, sheep, and goats, high milk output interferes with reproduction because of negative energy and protein balances in the dams. For this reason, in dairy cattle, the conception rates of heifers are usually much higher than those for lactating cows, although fertility rates are low for both beef and dairy cows under stress conditions (e.g., heat stress and malnutrition; Thatcher et al. 2010; Santos et al. 2016). Furthermore, either under- or overnutrition increases risk for preterm births in mammals (Wu et al. 2012) due to underlying complex mechanisms explained in Figure 11.7.

Another example of production-imposed underfeeding is a reduction in rations for gestating swine. Specifically, gilts or sows are fed a restricted amount of feed (i.e., only about 2 kg of diet/day, compared with a normal feed intake of about 4–6 kg/day if they are fed ad libitum) during pregnancy. The goal of this restricted feeding is to reduce embryonic mortality and excessive maternal fat accumulation (Wu et al. 2010, 2011). The latter is a major factor impairing feed intake and milk production by lactating sows during the first week postpartum. A disadvantage of restricted feeding is an inadequate provision of L-arginine, an amino acid that is necessary for optimum conceptus growth and survival.

Nutrition affects the early resumption of the ovarian cycle during the postpartum period after females give birth, as well as the success of conception (Chen et al. 2012). For example, adequate body condition and positive energy balance are important for the onset of estrus, fertilization of the oocyte, and high conception rates in animals. Note that suckling delays the resumption of the ovarian cycle in both ruminants and nonruminants through its effect on the central nervous system to disrupt the pattern of pulsatile release of GnRH from the hypothalamus and hence LH from the pituitary (McNeilly et al. 1994). However, overfeeding dams in late lactation or during the dry period may

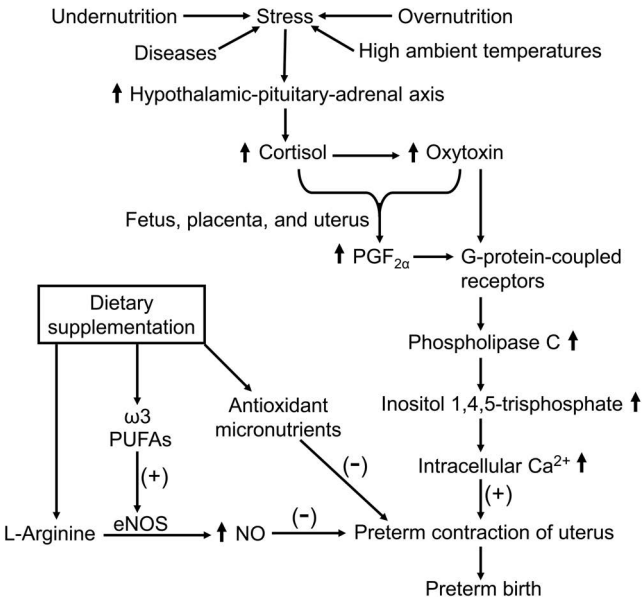


FIGURE 11.7 Biochemical mechanisms responsible for malnutrition-induced preterm birth and its amelioration by dietary supplementation with nutrients in mammals (including pigs, cattle, and humans). Maternal undernutrition or overnutrition represents a stress factor that can stimulate the release of cortisol and oxytocin from the adrenal gland and the posterior pituitary gland, respectively. These hormones enhance the production of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) by the fetus, placenta, and uterus. Oxytocin and $PGF_{2\alpha}$ bind to their respective G-protein-coupled receptors, leading to an increase in intracellular concentrations of Ca^{2+} via the phospholipase C and inositol 1,4,5-trisphosphate pathway. High levels of Ca^{2+} activate the preterm contraction of the uterus, thereby stimulating preterm birth. In contrast, physiological levels of nitric oxide (NO), which is synthesized from arginine by endothelial NO synthase (eNOS), inhibit the preterm contraction of the uterus. The generation of NO in the vasculature is up-regulated by ω3 PUFAs. Dietary supplementation with antioxidant micronutrients, ω3 PUFAs, and arginine can be beneficial in reducing the risk of preterm delivery in pregnant mammals. The signs (+) and (–) denote activation and inhibition, respectively. (Courtesy of Wu et al. 2012, an open-access article.)

result in the so-called metabolic syndrome (e.g., dyslipidemia) associated with impaired reproductive function. In litter-bearing species, such as pigs and prolific ewes, the level of nutrition greatly affects ovulation rate and, therefore, fertility and fecundity. Feeding sows and ewes with low body condition scores a high-level energy diet for a few weeks before mating can enhance ovulation rates and the number of fertilized embryos. However, this practice, which is known as "flushing", increases oxidative stress in the conceptus and the rate of embryonic mortality and is no longer recommended to producers.

Maternal Protein and Arginine Intake

Low intake of protein delays puberty in females (Muñoz-Calvo and Argente 2016). A deficiency in dietary protein reduces the fertility of females after puberty. In addition, a deficiency in dietary protein decreases food intake and is, therefore, associated with deficiencies in intake of other nutrients. Producers must pay attention to adverse effects of a high-level intake of protein by gestating dams because high levels of ammonia are particularly toxic to embryos and cause large metabolic burdens on maternal organs (e.g., liver and kidneys). For example, in gestating gilts, increasing dietary content of crude protein (CP) from 12 to 16% progressively reduces the number of live-born piglets per litter (Ji et al. 2017). In addition, an excess of dietary protein, which is supplemented in the form of SBM, is associated with reduced conception rates and increased embryonic losses in dairy cows (Butler 1998; Laven and Drew 1999). The underlying mechanisms may include (a) increased production of ammonia, which induces oxidative stress (Haussinger and Görg 2010), the depletion of α-ketoglutarate (an intermediate of the Krebs cycle for ATP production) via glutamate dehydrogenase, and elevated pH in cells (Wu 2022a); (b) increased generation of H_2S and homocysteine to cause further oxidative stress (Herring et al. 2018); (c) reduced secretion of histotroph from the uterus; (d) the impairment of blood flow (including utero-placental blood flow) and nutrient transport due to decreased NO availability; and (e) low production of progesterone from ovaries (Butler 1998). Dietary supplementation with arginine (e.g., 0.4–0.8% in swine diets) after day 14 of gestation can improve embryonic and fetal survival through enhancing placental angiogenesis and growth, utero-placental blood flow, folding at the uterine-placental interface and its structural development, MTOR cell signaling in the conceptus, and removal of excess ammonia (Wu et al. 2017).

Deficiencies of Minerals and Vitamins

Diets that are deficient in minerals and vitamins, particularly P, Ca, manganese, copper, zinc, cobalt, vitamin A, and vitamin E, impair fertility in females (Clagett-Dame and Knutson 2011). Dietary supplementation with a deficient mineral or vitamin can improve reproductive performance. A deficiency of P, which is common in ruminants grazing on forages or herbage grown in P-deficient soil, delays puberty, inhibits the expression of estrus, and reduces the numbers and weights of calves or lambs. This effect of P deficiency is often associated with low food intake and inadequate provision of other nutrients. In both ruminants and nonruminants, retention of the placenta following parturition, which delays the next conception by causing uterine infection, may also result from nutritional deficiencies in selenium, copper, vitamin A, and vitamin E.

Diseases, Toxins, Stress, and Excess Minerals

Diseases (e.g., infections by bacteria, viruses, or parasites), toxins, air pollution, stress (physical or psychological), goitrogens (causing enlargement of the thyroid), as well as high levels of nitrates and plant estrogens (e.g., isoflavones) can reduce conception rates in both ruminants and nonruminants (Bazer et al. 2014; Jefferson and Williams 2011). These negative factors impair the metabolic activities

and functions of the uterus, placenta, and ovaries. Of note, SBM, the most common protein supplement for all farm animals, contains genistein (an isoflavone), as well as weak goitrogens (possibly 1-methyl-2-mercaptoimidazole and potassium thiocyanate) that inhibit thyroid peroxidase (an enzyme required for the synthesis of thyroid hormones). Thus, genistein may contribute to infertility when high levels of dietary SBM are fed to females of reproductive age. The goitrogens in soybeans can be partially inactivated by heat. Just like the adverse effects of their deficiencies, excessive intakes of sodium, P, Ca, and fluorine can also reduce conception rates in animals.

Prenatal Growth and Development of Skeletal Muscle

There are remarkable developmental changes in myofiber types in prenatal skeletal muscle. In mammalian fetuses, there are two types of developing muscle fibers during pregnancy: primary and secondary. During the first third period of pregnancy (e.g., between days 25 and 50 of gestation in pigs), primary myoblasts (derived from mesenchymal stem cells) fuse and differentiate to form primary myotubes and then become a small number of primary myofibers. The formation of new myofibers is termed myogenesis, in which multipotent stem cells are converted into committed muscle cells (called myogenic progenitor cells) via differentiation. The primary myofibers serve as templates for the formation of secondary myofibers during the second third period of pregnancy (e.g., between days 50 and 90 of gestation in pigs), when secondary myoblasts (also derived from mesenchymal stem cells) line up along the primary myotubes, fuse, and differentiate to form secondary myotubes and then secondary myofibers. The latter are the majority of myofibers in fetal skeletal muscles. During fetal development, both myogenic precursor cells and myoblasts proliferate to increase their numbers through a net increase in protein synthesis. Satellite cells (mononucleated cells), which appear underneath the newly formed basal lamina around each myofiber, participate in the formation of primary and secondary myotubes during fetal development. In mammals, myogenesis continues for muscle hypertrophic growth through the addition of myonuclei to existing secondary myofibers during late gestation. At birth, the total number of myofibers is fixed, type I myofibers account for only a small proportion of total myofibers, and type II myofibers make up most myofibers. Thus, the prenatal development of myofibers is a major factor regulating the postnatal growth of offspring. Interestingly, type IIB myofibers are completely absent at birth in some muscles (e.g., serratus ventralis thoracis muscle) of sheep.

Prenatal myogenesis is under the control of regulatory proteins in mesenchymal stem cells. These proteins include (a) Wingless and Int (Wnt) for initiating myogenesis and (b) paired box gene (Pax) 3 and Pax 7 for stimulating the expression of myogenic regulatory factors (MRFs) that promote myogenic differentiation. Among MRFs, myogenin (a muscle-specific transcription factor) induces myogenesis through activating the fusion of myoblasts into myotubes, MRF-4 (expressed at the very early stage of myogenesis) plays a dual role in both determination and differentiation of the muscle lineage, and Myo-D and Myf-5 stimulate the differentiation of multipotent myogenic precursor cells into myoblasts.

Intrauterine Growth Restriction

Definition and Occurrence of IUGR

IUGR is defined as impaired growth and development of the mammalian embryo/fetus or its organs during pregnancy (Wu et al. 2006). In practice, IUGR is identified as fetal or birth weights less than two standard deviations of the mean BW for gestational age. For example, for crossbred sows (Yorkshire × Landrace dams and Duroc × Hampshire boars), the mean birth weight is 1.4 kg, and a piglet with a birth weight of less than 1.1 kg is considered to have IUGR (Ji et al. 2017). Multiple genetic and environmental factors contribute to IUGR. Although the fetal genome plays an important role in growth potential *in utero*, convincing evidence shows that the intrauterine environment is a major determinant of fetal growth (Wu et al. 2006). For example, results of embryo-transfer studies clearly indicate that the recipient mother, rather than the donor mother, strongly influences fetal growth (Brooks et al. 1995). Among intrauterine environmental factors, under- or overnutrition plays the most critical role in influencing placental and fetal growth. Among livestock species, pigs exhibit the highest rates of embryonic mortality, IUGR, and neonatal deaths because of their unique biological characteristics (Figure 11.8). These problems are further exacerbated by (a) restricting the feed intake of gestating swine (e.g., by 50–60% compared with ad libitum intake) to prevent excess maternal fat accretion and (b) a variety of factors encountered in the different phases of swine production, including extreme ranges in environmental temperatures, feed hygiene and safety, suboptimal nutrition, and disease (Ji et al. 2017). Both qualitative and quantitative requirements for dietary nutrients may differ between normal and IUGR offspring. Thus, it is nutritionally beneficial to house IUGR offspring separately and adopt different feeding programs than those for normal offspring.

Consequences of IUGR

IUGR has permanent negative impacts on organ structure, neonatal adjustments, preweaning survival, postnatal growth, feed efficiency, lifetime health, skeletal-muscle composition, physical strengths, excessive accumulation of WAT, meat quality, reproductive performance, and the onset of adult diseases (Oksbjerg et al. 2013; Wu et al. 2006). Altered organ mass and structure, such as reduced numbers of pancreatic islets, reduced numbers of kidney glomeruli, or reduced numbers of secondary (but not primary) muscle fibers, are consequences of IUGR and equally important to functional consequences (Foxcroft et al. 2006). How IUGR negatively affects embryonic and fetal development is

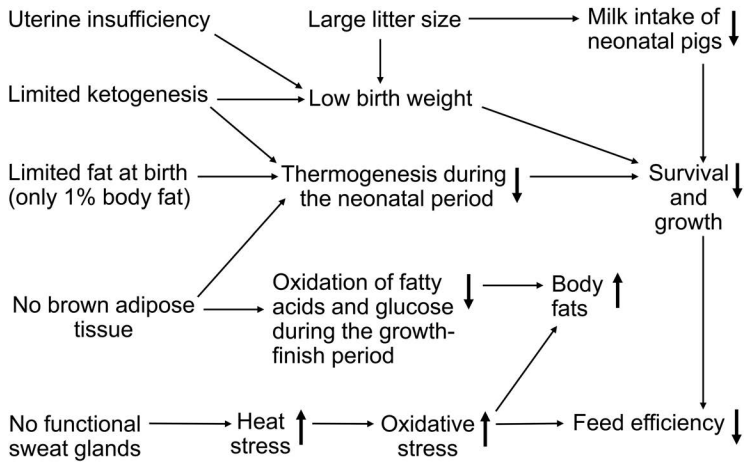


FIGURE 11.8 Unique biological characteristics of swine that contribute to high rates of neonatal mortality and morbidity. Pigs have no brown adipose tissue (BAT) and little ketogenesis before or after birth and a limited amount of fats at birth. These factors collectively result in a low rate of thermogenesis during the neonatal period. Because of uterine insufficiency, restricted feeding during gestation for the prevention of excessive maternal fat gain, and a large litter size (particularly an increased litter size due to improved genetic selection), high rates of intrauterine growth restriction (IUGR) occur in swine. Furthermore, because lactating sows that are commonly fed plant protein-based diets do not produce sufficient milk, the intake of nutrients by the suckling piglets is inadequate for their maximum survival and growth. Failure to maintain body temperature or receive adequate nutritional support results in the high rates of morbidity and mortality in neonatal pigs. Additionally, postweaning pigs accumulate a large amount of body fat and are highly susceptible to heat stress due to their lack of functional sweat glands, therefore leading to the excessive production of reactive oxygen species under conditions of high ambient temperatures. Provision of adequate nutrients (particularly amino acids) is crucial in reducing neonatal mortality and fat deposition while improving lean tissue gain and feed efficiency in pigs, particularly those with IUGR. The symbols "↓" and "↑" denote decrease and increase, respectively.

unknown, but this problem likely involves an impairment of protein synthesis in skeletal muscle. Interestingly, reduced concentrations of glycine in the skeletal muscle of neonatal IUGR pigs last for 6 months later at a market weight (He et al. 2023a). Although IUGR may be a natural mechanism to protect the dam in case of maternal undernutrition, it adversely affects the survival and growth performance of the progeny and the efficiency of animal production. Effective solutions must be developed to prevent and treat IUGR in mammals.

Determination of Nutrient Requirements by Gestating Dams

Nutrient requirements during pregnancy can be estimated by combining the maternal and fetal requirements for maintenance and growth (i.e., the factorial method). Estimation of dietary needs for energy and nutrients during gestation can be based on (a) the rates of accretion of placental, fetal, and uterine tissues and (b) the efficiency of utilization of energy and nutrients for gains in the growth of those tissues. The additional needs for the growth of maternal tissues must be met for females that are bred before reaching mature size. Over the past 25 years, much attention has been directed to the studies of dietary requirements of gestating dams (e.g., pigs, cattle, and sheep) for functional amino acids (e.g., arginine, glutamine, glutamate, and glycine; Gilbreath et al. 2021; Mateo et al. 2007, 2008; Wu et al. 2010, 2011, 2018, 2022a, 2024). Of particular note, glutamine reacts with fructose-6-P (a metabolite of fructose or glucose) to generate glucosamine that is essential for placental and embryonic growth and development via activating the MTOR cell signaling and the synthesis of glycoproteins (Figure 11.9). However, these metabolic pathways also favor insulin resistance and cancer growth in diseased states (Bazer et al. 2024).

Recently, there has been a growing interest in dietary requirements of P by gestating dams because such information is limited (Stenhouse et al. 2022b). The NRC (2007) estimated that the requirements of sheep for P varied between 0.15% and 0.31% of dietary DM, with a convenient value being set at 0.25% of dietary DM intake. In pregnant sheep consuming 22 g dietary DM (with 0.31% P)/kg BW/day, daily P intake is 69 mg/kg BW/day (Satterfield et al. 2013). At this level of P intake, pregnant sheep exhibit a large negative P balance (Braithwaite 1983). Thus, 0.25–0.31% P in the diet (on a DM basis) is inadequate and likely a significant nutritional deficiency for pregnant sheep. However, this issue has received little attention from sheep researchers and producers. The birth weights of lambs from pregnant sheep consuming 86 mg P/kg BW/day during weeks 16 to 21 of gestation are greater than those of lambs from pregnant sheep consuming 25, 38, or 122 mg P/kg BW/day (Sykes and Dingwall 1976). Phosphorus intake by pregnant sheep fed a diet with 0.4% P (on a DM basis) is 88 mg/kg BW/day, which may be an optimal P intake to meet the requirements of these animals. It is unlikely that a dietary

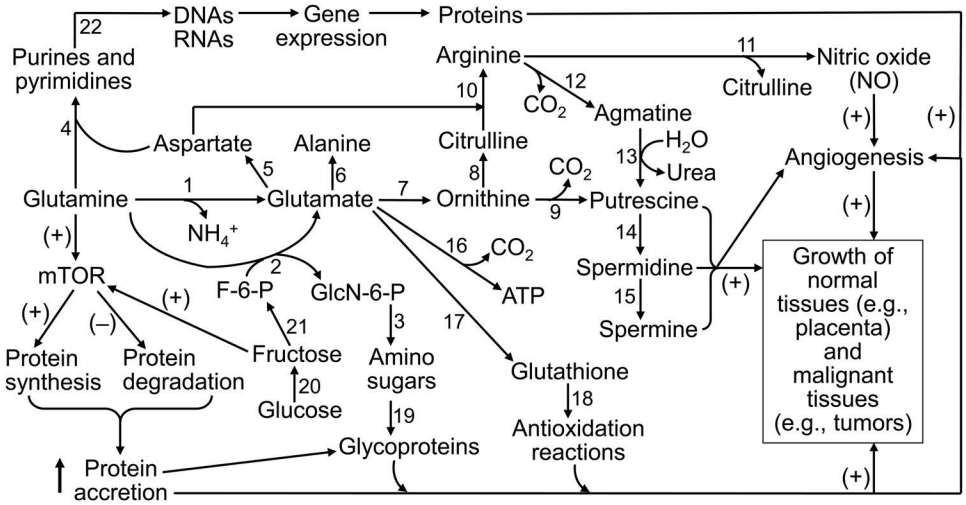


FIGURE 11.9 Mechanisms responsible for glutamine, glucose, and fructose metabolism to stimulate the growth of both normal and malignant tissues in humans and animals. Glutamine is degraded via multiple pathways to generate glutamate, aspartate, alanine, ornithine, citrulline, arginine, glucosamine, and CO_2, purines, and pyrimidines. Ornithine and arginine are substrates for the synthesis of polyamines (putrescine, spermidine, and spermine), whereas nitric oxide (NO) is formed from arginine oxidation. Glutamate is required for the production of glutathione as the most abundant low-molecular-weight antioxidant. Amino sugars (which derive the carbohydrate moiety from fructose-6-phosphate, a product of fructose) are required for the generation of glycoproteins as essential components of membranes, cytoplasm, and the extracellular matrix. Purine and pyrimidine nucleotides are precursors of DNAs and RNAs that are necessary for protein synthesis. Furthermore, both glutamine and fructose activate the mTOR cell signaling pathway to stimulate protein synthesis and inhibit protein degradation, leading to protein accretion in cells. NO (a major vasodilator to promote blood flow and nutrient supply), polyamines, protein accretion, and redox balance are crucial for angiogenesis and the growth of both normal and malignant tissues in humans and animals. Abbreviations: F-6-P, fructose-6-phosphate; GlcN-6-P, *N*-acetylglucosamine-6-phosphate; mTOR, mechanistic target of rapamycin. The enzymes that catalyze the indicated reactions are (1) phosphate-activated glutaminase; (2) glutamine:fructose-6-phosphate transaminase; (3) glucosamine-phosphate *N*-acetyltransferase, phosphoacetylglucosamine mutase, UDP-GlcNAc pyrophosphorylase, and UDP-GlcNAc 4-epimerase; (4) a series of enzymes for purine and pyrimidine syntheses; (5) glutamate-oxaloacetate transaminase; (6) glutamate-pyruvate transaminase; (7) pyrroline-5-carboxylate synthetase and ornithine aminotransferase; (8) ornithine carbamoyltransferase; (9) ornithine decarboxylase; (10) argininosuccinate synthase and argininosuccinate lyase; (11) nitric oxide synthase; (12) arginine decarboxylase; (13) agmatinase; (14) spermidine synthase; (15) spermine synthase; (16) a series of enzymes for glutamate oxidation (including glutamate transaminases and glutamate dehydrogenase); (17) γ-glutamyl-cysteine synthetase and glutathione synthetase; (18) glutathione-dependent antioxidative enzymes (including glutathione peroxidase, glutathione *S*-transferase, and thioltransferase); (19) a series of enzymes for incorporation of amino sugars into proteins; (20) aldose reductase and sorbitol dehydrogenase; (21) fructokinase and hexokinase; and (22) a series of enzymes for DNA and RNA syntheses. The sign (+) and (–) denote activation and inhibition, respectively. (Courtesy of Bazer et al. 2024, an open-access article.)

level of 0.4–0.5% P (on a DM basis; 88–110 mg P/kg BW/day) affects feed intake by pregnant sheep, because P intake at 110 or 193 mg/kg BW/day does not affect either the feed consumption or the health of pregnant sheep (Braithwaite 1983; Ternouth and Budhi 1996). The NRC (2007) indicated that the maximum P level in the diet of pregnant sheep could be 0.6% (on a DM basis) without any adverse effects. Future experiments are warranted to define the impact of dietary P intake on fetal growth and development in pregnant mammals (including sheep, cattle, and pigs).

Nutritional Requirements for the Reproduction of Males

Overall Undernutrition or Overnutrition

After a prolonged period of overall undernutrition (e.g., global reduction in food or energy intake), semen quality in males (including bulls, rams, and boars) is reduced. For example, the concentrations of fructose (the source of energy for sperm motility) and citrate are reduced in the seminal fluid of undernourished males. Undernutrition impairs the development of the testes and the synthesis of testosterone in males. At the other end of the nutrition spectrum, overnutrition induces oxidative stress and decreases semen quality, thereby contributing to male infertility.

Protein and Arginine Intake

Physiological levels of NO stimulate GnRH release from the hypothalamus and, therefore, testosterone production and spermatogenesis in the testes (Figure 11.2). Similar to the problems seen in females, low intake of protein and energy also delays puberty in males (Dance et al. 2015). In addition, a deficiency in dietary protein reduces food intake, leading to deficiencies in other nutrients that are necessary for spermatogenesis (Ghorbankhani et al. 2015). The maintenance requirement of males for energy

and protein is greater than that for females, because males have a greater proportion of lean tissue than females due to testosterone-induced muscular hypertrophy (Herbst and Bhasin 2004). Furthermore, as reported for mammals, including boars (Louis et al. 1994), low protein intakes reduce libido and semen volume. Of note, although the average ejaculate of an adult male (e.g., bull and boar), including spermatozoa and accessory secretions, contains only a relatively small amount of DM, the seminal fluid contains relatively high levels of polyamines and arginine (Wu et al. 2009). For example, concentrations of polyamines in porcine seminal fluid are 90 μM compared with those in plasma (3–5 μM), and dietary supplementation with 1% arginine-HCl in sexually active boars for 30 days enhanced concentrations of polyamines in seminal fluid by 63% compared with the control group (Wu et al. 2009). Concentrations of polyamines and arginine may be reduced in the seminal fluid of malnourished males. In humans, low levels of polyamines and arginine in semen are associated with infertility (Wu et al. 2021). For example, feeding an arginine-deficient diet to adult men for 9 days decreased sperm counts and the percentage of motile sperm by 90% (Holt and Albanese 1944). Remarkably, oral administration of L-arginine (e.g., 0.5 g/day for 6–8 weeks) to infertile men increased the number and motility of sperm, resulting in successful pregnancies for their female partners (Tanimura 1967). Furthermore, dietary supplementation with L-arginine or L-citrulline (e.g., 5 g/day over 6 weeks) to impotent men with impaired endogenous synthesis of NO can enhance sexual function (Cormio et al. 2011; Wu et al. 2021).

Deficiencies of Minerals and Vitamins

A deficiency of vitamin A causes degeneration of the germinal epithelium of the testes, thereby reducing or eliminating spermatogenesis (Clagett-Dame and Knutson 2011). As in females, a severe and prolonged deficiency of vitamin A results in the complete failure of reproduction in males (Chapter 9). The testicular tissue and spermatozoa contain high levels of zinc, and thus the zinc requirement for testicular growth and normal sperm production is greater than that for animal growth. Inadequate intake of zinc impairs Leydig cell function and reduces sperm counts in males (Abbasi et al. 1980). In addition, deficiencies in P, selenium, and copper (Chapter 10), as well as in vitamin C, folate, and vitamin E (Chapter 9), also contribute to infertility in males.

Diseases, Toxins, Stress, and Excess Minerals

Diseases, toxins, air pollution, stress (physical or psychological), and goitrogens reduce reproductive performance in males, as in females (Meldrum et al. 2016). All of these factors impair the metabolic activities and functions of testes and other parts of the reproductive tract. In addition, excessive intakes of iron and copper are highly toxic in males and females (Chapter 10). Large amounts of those two minerals damage the structure of male gonads and spermatozoa, cause defective spermatogenesis, reduce libido, and induce oxidative injury in multiple cell types, thereby impairing fertility (Tvrda et al. 2015).

Fetal and Neonatal Programming

Alterations in maternal and fetal nutrition and endocrine status during gestation result in developmental adaptations that may permanently change the structure, physiology, and metabolism of offspring, therefore predisposing the individuals to metabolic defects and reduced food efficiency for lean tissue growth, as well as cardiovascular, metabolic, and endocrine diseases in adult life (Wu et al. 2004). These adverse effects may be carried over to the next generation or beyond. This phenomenon of transgenerational impacts is known as fetal programming, which is mediated by stable and heritable alterations in gene expression through covalent modifications of DNA and histones without changes in DNA sequences (namely, epigenetics). The mechanisms responsible for the epigenetic regulation of protein expression and functions include chromatin remodeling, DNA methylation (occurring at the 5'-position of cytosine residues within CpG dinucleotides), and histone modifications (acetylation, methylation, phosphorylation, and ubiquitination; Ji et al. 2016). Like maternal malnutrition, undernutrition during the neonatal period also reduces growth performance and feed efficiency (e.g., by 5–10% in postweaning pigs), increasing the days necessary to reach a market BW (Ji et al. 2017). Supplementing functional amino acids (e.g., arginine and glutamine) and vitamins (e.g., folate) plays a key role in activating the mammalian target of rapamycin signaling and regulating the provision of methyl donors for DNA and protein methylation. For example, dietary supplementation with arginine reduces the incidence of IUGR and within-litter variations in birthweights of piglets (Wu et al. 2017). Therefore, these nutrients are beneficial for the dietary treatment of metabolic disorders in offspring with IUGR or neonatal malnutrition. These mechanism-based strategies hold great promise for the improvement of the efficiency of animal production.

Nutritional Requirements for the Postnatal Growth of Animals

Rapid growth of skeletal muscle, mainly protein accretion, is economically important for livestock, poultry, and aquaculture industries worldwide (Wu et al. 2014a). As noted in Chapter 7, the balance between the rates of protein synthesis and degradation is the determinant of tissue growth. Therefore, over the past 50 years, extensive research has focused on the regulation of intracellular protein turnover in skeletal muscle (Hernandez-García et al. 2016; Reeds and Mersmann 1991). The maximal growth rate of animal tissues depends on both genetic and environmental factors. An animal can fully express its genetic potential only when its requirements for energy and all nutrients (e.g., amino acids, fatty acids, carbohydrates, minerals, and vitamins) are met, as noted previously.

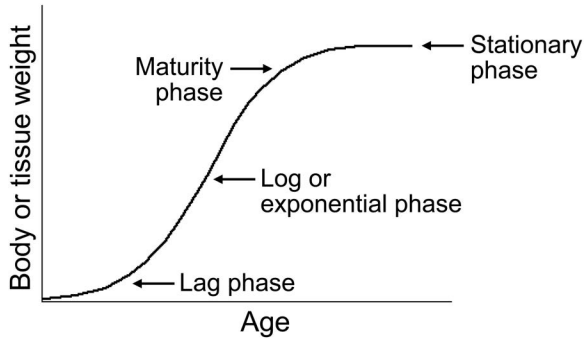


FIGURE 11.10 The general curve describing animal growth over time (age). This sigmoid curve consists of lag, log or exponential, maturity, and stationary phases.

Components of Animal Growth

The general sigmoid curve of animal growth, expressed as increases in absolute body or tissue weight with age, is shown in Figure 11.10. This curve consists of four phases: lag, log or exponential, maturity, and stationary. In farm animals, the head and extremities develop early, and the hindquarters and loin region develop very late during the growing-finishing period (Wagner et al. 1999). The patterns of accretion of protein and fat in animals vary with species and developmental stages (Gerrard and Grant 2002). However, as age advances, weight gain generally consists of an increasing percentage of fat and a decreasing percentage of water (ARC 1981; NRC 2001, 2011, 2012). In contrast, the percentage of protein in the body generally increases gradually after birth until animals reach sexual maturity (Susenbeth and Keitel 1988). In fully mature animals, excess energy is stored almost entirely as fats, and only a relatively small amount of proteins may be deposited as a cellular component of white adipocytes, muscle fibers, hair, and keratinized cells of skin.

An increase in BW is the most common measure of growth due to its simplicity. However, the growth of any animal is characterized by increases in the weight of its muscles, WAT, bones, and internal organs that are associated with the enhanced accretion of protein, minerals, water, and fats (van Es 1976). Because the deposition of 1 g of protein in the body is associated with the retention of 3 g of water (Wu et al. 2014b), a net synthesis of 1 g of protein in tissues (primarily skeletal muscle, which is 40–45% of the BW) results in a weight gain of 4 g. Rapid muscle-protein accretion leads to fast animal growth. For example, young animals have a higher rate of net protein synthesis and, therefore, a higher rate of growth than older ones (Davis et al. 1998). However, the opposite may not always hold true. This is because weight gain may not necessarily result from lean tissue growth but may simply be caused primarily by fat accumulation in the body.

As a hydrophobic molecule, triacylglycerol (TAG, fat) is deposited in the body of animals without the retention of water. Although fat may be deposited in various tissues, it is stored mainly in subcutaneous WAT, the abdominal cavity, and connective tissue (Smith and Smith 1995). A certain amount of fat among muscle fibers within skeletal muscle (called marbling fat) confers a good taste to meats eaten by consumers. As more fat is accumulated in animals than protein, water content in the body decreases. Thus, in ruminants and pigs, water content is generally 75–80% at birth but decreases to 45–50% at their market weights (ARC 1981, NRC 2001). The expansion of fats during the finishing period results primarily from an increase in the size of white adipocytes. Results from growth studies involving cattle and pigs indicate that the deposition of 1.4 g of fat in the animal body is associated with the replacement of 0.4 g of water and that the actual gain in the whole body is only 1 g (van Es 1976; Pond et al. 1995). Thus, weight gain increases to a lesser extent when fat is accreted in the body compared to the accretion of the same amount of protein.

Despite concerns over the adverse effects of overweight or obesity on health, animals require fats for normal physiological functions (Chapter 6). For example, although obesity increases the risk for infertility in both males and females (Norman 2010), an adequate amount of body fat is necessary for reproduction, such as the pulsatile GnRH release (Sam and Dhillo 2010). This can be explained by the facts that (a) leptin secretion from WAT is necessary for attainment of puberty (Cardoso et al. 2014) and (b) testosterone, estradiol, progesterone, and all other steroid hormones are synthesized from cholesterol, which is a lipid synthesized from fatty acids (Kiess et al. 2000).

Postnatal Growth and Development of Skeletal Muscle

In early postnatal life, muscle hypertrophic growth occurs through the addition of myonuclei to existing secondary myofibers. During all phases of postnatal growth, there is no net formation of new muscle fibers, but the size of myofibers and the total number of nuclei in skeletal muscle increase, with satellite cell hyperplasia exceeding myofiber hypertrophy. In addition, satellite cells (residing near myofibers) contribute to the postnatal growth of skeletal muscle. Furthermore, when skeletal muscle is damaged, mitogenic factors released by the damaged muscle stimulate the migration of satellite cells to the wounded site, where they fuse and differentiate to form new myotubes. Thus, in cooperation with myogenin, satellite cells play a key role in postnatal muscle gain, as well as the repair and regeneration of damaged muscle fibers in adults.

During the postnatal development of skeletal muscle, some type II myofibers are converted into type I myofibers. Thus, as animals grow, the proportion of type I myofibers increases, but the proportion of type II myofibers decreases. In adult animals, the numbers of type I and type II myofibers are about 30% and 70%, respectively. Aging is associated with a decrease in type IIB myofibers and an increase in type IIA myofibers.

Recent studies have shown that heavy-weight fast-growing meat-type broiler chickens develop breast degenerative myopathies known as spaghetti (pale-soft-exudative; PSE)

meat, hard or woody breast, and white striping, therefore resulting in product downgrades or condemnation (Velleman 2023). These problems occur due to the modern genetic selection of chickens for high growth rates that leads to changes in the morphological structure of breast muscle in broilers: increases in muscle mass and fiber diameters but decreases in blood supply, spacing between individual fibers, and fiber bundles. Such broilers may have metabolic defects in antioxidative responses, the repairment of muscle fibers with necrosis and fibrosis, and the ability of satellite cells to regenerate healthy myofibers. Breast muscle myopathies may be prevented or alleviated by dietary supplementation with (a) antioxidative amino acids, which may also promote protein synthesis and inhibit proteolysis in skeletal muscle (e.g., arginine, glutamine, glycine, 4-hydroxyproline, and proline); (b) vitamins (e.g., vitamins A, C, and E); and (c) minerals (e.g., selenium, copper, and zinc), and ω3 PUFAs (e.g., α-linolenic acid, EPA, and DHA). Studies are warranted to test this novel hypothesis.

Absolute versus Relative Rate of Animal Growth

The absolute growth rate refers to the amount of BW gain per unit of time (e.g., g/day). The relative growth rate of animals is commonly expressed in terms of changes in BW within a given period of time (e.g., %/day). The absolute rates of whole-body growth in animals increase, but the relative rates of their whole-body growth decrease as they approach sexual maturity. However, the absolute or relative rates of fat and protein depositions in the body may not necessarily follow the pattern of growth for the whole body (Table 11.14). A combination of feed efficiency, animal price, and meat yield determines the market weight of farm animals (Thornton 2010). Thus, animals are sold to the market at a heavier BW than usual when their numbers

TABLE 11.14
Important Roles of Dietary Protein and Amino Acids in Animal Nutrition

1. Metabolic and hormonal profiles
(a) Maintaining optimal concentrations of amino acids and proteins (including albumin) in plasma
(b) Maintaining endocrine balance, as well as optimal concentrations of insulin, growth hormone, IGF-I, and thyroid hormones in plasma
(c) Maintaining optimal antioxidative reactions; reducing oxidative stress
(d) Maintaining optimal synthesis of neurotransmitters
(e) Reducing excess deposition of white-adipose tissue
(f) Maintaining optimal whole-body energy expenditure

2. Nutrient absorption and transport
(a) Promoting intestinal absorption of nutrients, including vitamins, minerals, amino acids, glucose, and fatty acids
(b) Promoting the transport of vitamins, minerals, and long-chain fatty acids in blood and among various tissues
(c) Helping to store vitamins and minerals in cells

3. Protein synthesis and growth
(a) Promoting protein synthesis and increasing proteolysis in skeletal muscle and whole body
(b) Preventing growth stunting of the young; improving development (including cognitive development) of the young
(c) Preventing intrauterine growth restriction and its life-long negative consequences on postnatal growth, metabolism and health (e.g., increasing risk for obesity, infection, and cardiovascular abnormalities)
(d) Increasing skeletal muscle mass; maintaining physical strengths
(e) Enhancing feed efficiency

4. Organ structure
(a) Preventing cardiac structural abnormalities
(b) Preventing the loss of calcium and bones, and dental abnormalities
(c) Preventing hair breakage and loss; maintaining optimal production of pigment; maintaining normal hair structure and appearance
(d) Preventing pale skin, dry or flaking skin, and skin atrophy
(e) Maintaining optimal immune responses; reducing risk for, and mortality of, infectious diseases

5. Health and reproduction
(a) Improving cardiovascular function; preventing hypertension or hypotension; reducing risks for headache and fainting
(b) Preventing excess fluid retention in tissues; preventing peripheral and periorbital edema (particularly swelling in the abdomen, leg, hands, and feet)
(c) Preventing emotional disorders (e.g., moodiness, severe depression, and anxiety), irritability, and insomnia
(d) Preventing a loss of libido; improving fertility (including spermatogenesis in males and conception in females); reducing embryonic loss; enhancing pregnancy outcomes

are lower due to disease outbreak or other causes, so that more meat can be produced per animal at the expense of a reduced efficiency of nutrient utilization.

Regulation of Animal Growth by Anabolic Agents

As noted in Chapter 7, many hormones play an important role in animal growth, primarily by regulating skeletal-muscle protein synthesis and catabolism. A net increase in tissue protein synthesis (i.e., the rate of protein synthesis is greater than the rate of protein degradation) results in weight gain. Insulin, growth hormone, and insulin-like growth factor-I (IGF-I) are anabolic hormones (Etherton and Bauman 1998). Interestingly, tissue sensitivity to hormones decreases with advancing age in animals and humans. Thus, the frequency of feeding can affect the pulsatile release of these hormones and other anabolic nutrients in plasma (El-Kadi et al. 2020). This concept can have important implications for improving the efficiency of feed utilization and reducing the costs of animal production. The higher the rate of gain in BW, the shorter the time required for the animal to reach a slaughter weight or full productive performance, and the smaller the proportion of feed used for maintenance (Ji et al. 2017). Insulin resistance often occurs in aging animals and in gestating dams during the last trimester of gestation, which leads to their mobilization of fats and a reduction in protein synthesis.

Growth-promoting agents can improve the efficiency of meat production and result in leaner meat. There are two major types of these agents in ruminants and nonruminants: (a) hormone-like substances [e.g., β-agonists (Figure 11.11)]; and (b) antibiotic-like substances (Reeds and Mersmann 1991). These agents also reduce WAT in the body and enhance the growth of the animals through different mechanisms (Gerrard and Grant 2002).

β-Agonists

Ractopamine (known as Paylean for swine and Optaflexx for cattle) is a feed additive that promotes lean-tissue growth in swine and cattle (Bohrer et al. 2013; Lean et al. 2014). This agent acts as a trace amine-associated receptor 1 (TAAR1) agonist and also as a β-adrenoreceptor agonist that stimulates both β_1 and β_2 adrenergic receptors to enhance lipolysis in skeletal muscle (Johnson et al. 2014a). Ractopamine has been banned as a feed additive in many countries, including the European Union, China, and Russia because of concern over adverse effects of its residue in meat on human health. However, ractopamine has been approved for use in some countries, such as the United States, Japan, and Canada to improve meat production by livestock fed an adequate-protein diet. Other growth promoters available for cattle and sheep include zeranol and trienbolone (Figure 11.5) that may be administered as feed

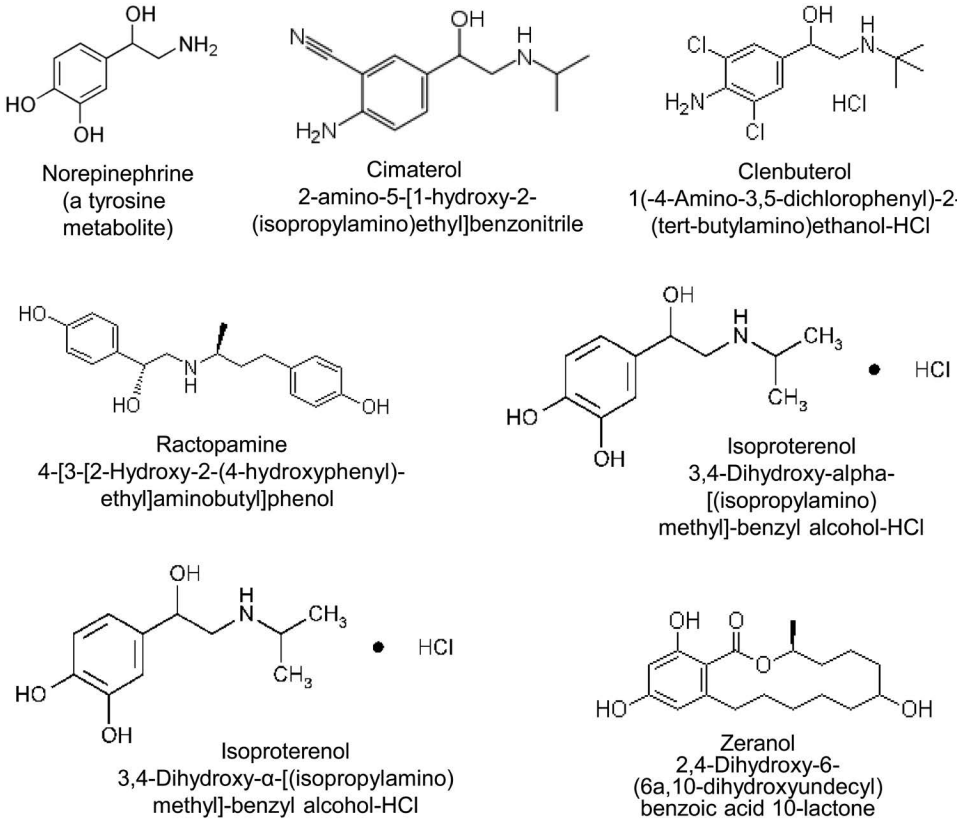


FIGURE 11.11 The chemical structures of β-agonists used in animal production. These are analogues of norepinephrine (a metabolite of tyrosine) but are not steroids.

additives, oil-based injections, or subcutaneous implants to animals. In recent years, in-feed lubabegron fumarate (Experior; a β_3-agonist) has been used at doses of 1.5, 3.5, or 5.5 mg/kg of dietary DM to increase weight gain and feed efficiency, while reducing ammonia production in feedlot beef cattle (steers and heifers; Kube et al. 2021).

All β-agonists have a chemical structure similar to that of norepinephrine, which is a metabolite of tyrosine and a catecholamine (Reeds and Mersmann 1991). The modes of action of these agents involve β-adrenoceptors and the Gβγ subunits of G-protein, both of which are localized on the cell membrane. β-Agonists bind to the membrane-bound β-adrenoceptors, thereby activating adenylate cyclase to produce cyclic adenosine monophosphate (cAMP) from ATP and then promote cAMP-dependent lipolysis in cells (e.g., white adipocytes) via protein kinase A signaling (Figure 11.12). In addition, β-agonists promote protein synthesis and reduce protein degradation in skeletal muscle via the stimulation of the Gβγ subunits of the cell membrane-bound G-protein, which leads to activation of the phosphoinositol 3-kinase (PI3K)-protein kinase B (Akt) signaling pathway (Koopman et al. 2010). The growth signal is integrated via the MTOR pathway, which is a master regulator of protein synthesis and an inhibitor of autophagy (a pathway of lysosomal proteolysis) (Chapter 7). β-Agonists (including catecholamines) inhibit calpain (Ca-dependent proteases) in skeletal muscle through β2-adrenoceptors and cAMP signaling (Navegantes et al. 2001). Because of their anabolic effects on enhancing net protein accretion, β-agonists could provide an effective treatment for ameliorating skeletal muscle wasting in a catabolic state and promoting muscle hypertrophy in healthy animals.

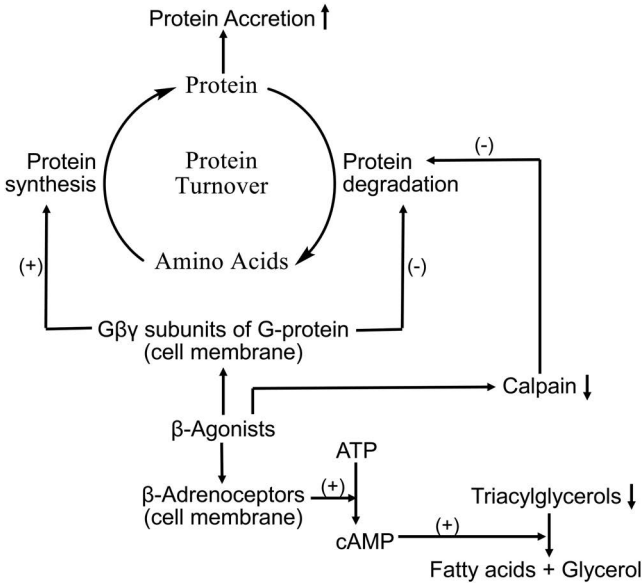


FIGURE 11.12 Mechanisms for β-agonists to enhance lean tissue growth and reduce fat deposition in animals. β-Agonists act on (a) β-adrenoceptors on the cell membrane to activate adenylate (adenylyl) cyclase, which converts ATP into cAMP in cells (e.g., adipocytes); and (b) the Gβγ subunits of the cell membrane-bound G-protein, resulting in the activation of the phosphoinositol 3-kinase (PI3K)–protein kinase B (Akt) signaling pathway to increase protein synthesis and inhibit protein degradation. In addition, the β-agonists reduce calpain activity in skeletal muscle via unknown mechanisms.

Specific Growth-Promoting Agents for Ruminants

In ruminant nutrition, antibiotic-like substances have been used as growth-promoting agents because of their actions on ruminal and/or intestinal microflora to modify the quantity and quality of the fermentation products (Cameron and McAllister 2016). Furthermore, monensin, an antibiotic produced by certain bacteria, has gained wide acceptance as a feed additive for ruminants because it improves feed utilization efficiency by altering ruminal fermentation in favor of propionate production (Chapter 13).

Compensatory Growth

Compensatory growth (the ability of an animal to recover growth after periods of underfeeding) is an interesting area of investigation in animal biology. Animals that have been subjected to a period of undernutrition and restricted growth exhibit compensatory growth during a subsequent period of realimentation (Hornick et al. 2000). Expressed as a percent increase in body or tissue weight, such animals grow faster than other animals of similar age when an adequate feed supply becomes available. This biological phenomenon has been observed in mammals, birds, fish, and reptiles (Boersma and Wit 1997). Over time, the BW of animals with prior nutritional restriction may become similar to those of animals that did not experience such stress (Gerrard and Grant 2002). However, high rates of compensatory growth often result in excessive fat deposition in WAT and, therefore, negatively affect animal health (Gerrard and Grant 2002). The major reasons for the compensatory growth may result from increases in feed intake upon realimentation, the absorptive capacity of the gastrointestinal tract, insulin secretion, the hepatic production of IGF-I, and feed efficiency (partly due to an increase in the fractional rate of protein synthesis and a decrease in the fractional rate of protein degradation in skeletal muscle), in association with concomitant decreases in intestinal microbial activity and the rates of whole-body catabolism of amino acids (Boersma and Wit 1997; Heyer and Lebret 2007; Ishida et al. 2011; Won and Borski 2013; Zhu et al. 2017).

Whether IUGR offspring can fully catch up with those with a normal birth weight in terms of weight gains will depend on the severity of fetal growth restriction, as well as weight gains (particularly protein accretion) during the suckling period and the first weeks postweaning (Ji et al. 2017). The earlier in life nutritional deficiencies occur and the longer the period of food deprivation, the less compensatory growth will be realized. Because the metabolic status (particularly hepatic or gastrointestinal function) of IUGR offspring differs from that of offspring with a

TABLE 11.15
Growth Performance of Barrows and Gilts between 18 and 125 kg Body Weights

Variable	Changes between 18 and 125 kg Body Weights (BWs)
Daily weight gain	Increases progressively between 18 and 82 kg BW for barrows and gilts
	Decreases gradually between 82 and 125 BW for barrows and gilts
	Barrows > gilts between 18 and 125 BW
Daily protein gain	Increases progressively between 18 and 73 kg BW for barrows and gilts
	Decreases gradually between 73 and 125 BW for barrows and gilts
	Barrows > gilts before 90 kg BW; barrows = gilts between 90 and 125 BW
Daily fat gain	Increases progressively between 18 and 90 kg BW for barrows and gilts
	Small change between 90 and 125 BW for barrows and gilts
	Barrows > gilts between 18 and 125 BW
Feed:Gain (g/g)	Increases progressively between 18 and 125 kg BW for barrows and gilts
	Barrows = gilts between 18 and 50 kg BW; Barrows > gilts between 50 and 15 kg BW

Source: Wagner et al. (1999).

normal birth weight, different dietary formulations must be developed for each phenotype of animals. In addition, because myocytes and adipocytes are derived from the common mesenchymal precursor (Sordella et al. 2003), increased amounts of the WAT develop at the expense of skeletal muscle when embryonic myogenesis is impaired. Thus, maternal protein nutrition during gestation decreases postnatal muscle growth but increases intramuscular fat content in IUGR pigs (Rehfeldt et al. 2004). In addition, the numbers of secondary myofibers, but not primary myofibers, are affected by the uterine environment (Dwyer et al. 1994). This further helps to explain why differences in prenatal and postnatal growth rates between IUGR pigs and normal littermates positively correlate with a smaller ratio of secondary myofibers to primary myofibers and with a smaller size of myofibers in IUGR pigs (Handel and Stickland 1987).

Critical Role of Dietary Amino Acid Intake in Animal Growth

Amino acids are the building blocks of protein. As indicated previously, changes in the abundance of skeletal muscle account for most animal growth. Because protein is the most abundant component of DM in muscle, and because amino acids are constituents of protein, dietary amino acid intake plays a critical role in influencing the growth, feed efficiency, and health of all animals (Table 11.14). Thus, an active area of research in nutrition is dietary protein and amino acid requirements for growing animals (Escobar et al. 2006; Humphrey et al. 2008; Liao et al. 2018), particularly ruminants, which exhibit a low feed efficiency (Brake et al. 2014; Wilkinson 2011). Dietary protein or amino acid intake is of great importance for animal producers based on both biological and economic considerations. The efficiency of utilization of dietary energy, fat, and carbohydrates depends on the quantity and quality of dietary protein (Campbell 1988; Reeds and Mersmann 1991). Beef cattle are known for their low rate of conversion of dietary protein into body protein, and the efficiency of grazing cattle is only 1/2 that of high-producing dairy cows (Table 11.15). Depending on species, the efficiency of dietary protein used for tissue protein deposition is about 70–75% in milk-fed neonates and 40–45% in animals approaching a market weight and consuming corn- and SBM-based diets (Wu 2022a; Wu et al. 2014b). Thus, sufficient knowledge of protein metabolism and the requirements of growing animals (especially ruminants) will increase the efficiency of feed utilization and prevent the waste of energy and amino acids.

As discussed in Chapter 7, classic feeding trials involving several levels of dietary protein or amino acids are required to determine total protein requirements for animal growth. The measured endpoints are usually N retention and weight gain. When energy is insufficient, N balance may become negative even if sufficient protein is fed (McDonald et al. 2021). This necessitates adequate provision of dietary lipids, carbohydrates, vitamins, and minerals, as well as drinking water, when studies of N balance or growth trials are conducted. On the other hand, an excess of dietary protein or amino acid intake is wasteful, and feeding a surplus of protein or amino acids may not increase the protein content of the tissue accounting for live-weight gain and may result in negative impacts on animal health and the environment (Wu et al. 2014c).

Recommendations of nutritionally nonessential amino acid (NEAA) requirements for animals should depend on expected rates of growth, optimal reproduction, optimal health, and in the case of farm animals (e.g., livestock, poultry, fish, and crustaceans), also production performance including feed efficiency (Wu et al. 2014c). Recent advances in the analysis of all proteinogenic amino acids

TABLE 11.16
Texas A&M University's Optimal Ratios of True Digestible Amino Acids for Swine Diets[a]

	Growing Pigs (kg)[b]				Gestating Pigs[b,c]		
Amino Acid	**5–10**	**10–20**	**20–50**	**50–110**	**d 0–90**	**d 90–114**	**Lactating Sows[b]**
	% of diet (as-fed basis)						
Alanine	1.14	0.97	0.80	0.64	0.69	0.69	0.83
Arginine	1.19	1.01	0.83	0.66	1.03	1.03	1.37
Asparagine	0.80	0.68	0.56	0.45	0.50	0.50	0.66
Aspartate	1.14	0.97	0.80	0.64	0.61	0.61	0.94
Cysteine	0.32	0.28	0.24	0.20	0.19	0.19	0.26
Glutamate	2.00	1.70	1.39	1.12	0.89	0.89	1.81
Glutamine	1.80	1.53	1.25	1.00	1.00	1.60	1.38
Glycine	1.27	1.08	0.89	0.71	0.48	0.48	0.75
Histidine	0.46	0.39	0.32	0.26	0.29	0.29	0.39
Isoleucine	0.78	0.66	0.54	0.43	0.45	0.45	0.66
Leucine	1.57	1.33	1.09	0.87	1.03	1.03	1.41
Lysine	1.19	1.01	0.83	0.66	0.51	0.51	0.80
Methionine	0.32	0.28	0.24	0.20	0.16	0.16	0.25
Phenylalanine	0.86	0.73	0.60	0.48	0.54	0.54	0.77
Proline	1.36	1.16	0.95	0.76	0.89	0.89	1.24
Serine	0.70	0.60	0.49	0.39	0.45	0.45	0.74
Threonine	0.74	0.65	0.55	0.46	0.41	0.41	0.56
Tryptophan	0.22	0.19	0.17	0.14	0.11	0.11	0.18
Tyrosine	0.67	0.57	0.46	0.37	0.40	0.40	0.62
Valine	0.85	0.72	0.59	0.47	0.55	0.55	0.72

Source: Wu (2014).

[a] Except for glycine, all amino acids are L-isomers. Values are based on true ileal digestible amino acids. Crystalline amino acids (e.g., feed-grade arginine, glutamate, glutamine and glycine), whose true ileal digestibility is 100%, can be added to a diet to obtain their optimal ratios. The molecular weights of intact amino acids were used for all the calculations. The content of dry matter in all the diets is 90%. The content of metabolizable energy in the diets of growing pigs, gestating pigs, and lactating pigs is 3330, 3122, and 3310 Kcal/kg diet, respectively.

[b] Fed ad libitum (90% dry matter).

[c] Fed a 2-kg diet per day on Days 0–90, and a 2.3-kg diet per day on Days 90–114.

in food and animal-sourced proteins have made it possible to determine dietary intakes of these amino acids by animals. Based on results of published studies, Wu (2014) proposed Texas A&M University's optimal ratios of amino acids in typical corn- and SBM-based diets for pigs (Table 11.16) and chickens (Table 11.17) at various phases of growth and production. These recommended values are based on true ileal digestibilities of dietary amino acids and can be readily converted to percentages of total amino acids in the diet (g/100 g diet). Animal nutritionists should move beyond the "ideal protein" concept in dietary formulation to maximize the efficiency of utilization of amino acids for protein deposition in skeletal muscle and other tissues (e.g., wool and skin/hide) of economic importance, as well as the production of milk by mammals and of eggs from laying hens (Wu and Li 2022).

Phase Feeding of Animals

Animals at different developmental ages or production stages require different amounts of nutrients for maintenance, growth, and productive performance (e.g., gestation, lactation, and muscular work). Thus, it is beneficial to adopt phase feeding for animals on farms. For example, in the United States, most breeding pigs (gilts and sows) are housed in gestation and farrowing facilities, weaned pigs are moved to nursery barns, and eventually to finishing barns. The housing of animals at the same production stage in the same facilities can offer the following advantages: facilitating the provision of specialized rations and management to animals, improving the efficiency in the use of land and diets, preventing or mitigating disease outbreaks on farms, and aiding in the sustainability of animal agriculture.

TABLE 11.17
Texas A&M University's Optimal Ratios of True Digestible Amino Acids in Diets for Chickens[a]

Amino Acid	Age of Chickens			
	0 to 21 days[b]	21 to 42 days[c]	42 to 56 days[d]	Laying Hens
	% of lysine in diet			
Alanine	102	102	102	110
Arginine	105	108	108	126
Asparagine	56	56	56	88
Aspartate	66	66	66	126
Cysteine	32	33	33	35
Glutamate	178	178	178	177
Glutamine	128	128	128	193
Glycine	176	176	176	120
Histidine	35	35	35	50
Isoleucine	67	69	69	85
Leucine	109	109	109	185
Lysine	100	100	100	100
Methionine	40	42	42	46
Phenylalanine	60	60	60	65
Proline	184	184	184	160
Serine	69	69	69	98
Threonine	67	70	70	74
Tryptophan	16	17	17	23
Tyrosine	45	45	45	50
Valine	77	80	80	95

Source: Wu (2014).

[a] Except for glycine, all amino acids are L-isomers. Values are based on true ileal digestible amino acids.

[b] Patterns of amino acid composition in the ideal protein are the same for male and female chickens. The amounts of digestible lysine in diet (as-fed basis; 90% dry matter) are 1.12% and 1.02% for male and female chickens, respectively.

[c] Patterns of amino acid composition in the ideal protein are the same for male and female chickens. The amounts of digestible lysine in diets (as-fed basis; 90% dry matter) are 0.89% and 0.84% for male and female chickens, respectively.

[d] Patterns of amino acid composition in the ideal protein are the same for male and female chickens. The amounts of digestible lysine in diets (as-fed basis; 90% dry matter) are 0.76% and 0.73% for male and female chickens, respectively.

Nutritional Requirements of Mammals during Weaning

All mammalian neonates undergo weaning, which refers to the full transition of the puppy's diet from its mother's liquid milk to the solid growth diet. In current production systems, early weaning is adopted to increase the productivity of dams, reduce risks for the transmission of infectious diseases from mother to her young, and facilitate management on farms. For example, pigs, lambs, and calves are weaned at approximately 3 weeks, 3 months, and 7 months of age, respectively (Wu et al. 2024). In dogs and cats, weaning can begin at 4 weeks of age through separation from their mothers for a few hours at a time each day and is completed at ~ 8 weeks of age (Case et al. 2011). A small amount of creep feed (for livestock) or canned food (for dogs and cats) can be gradually introduced to all mammalian neonates before weaning so that they can adapt more easily to solid foods (Kim et al. 2013; Li and Wu 2024). The palatable food usually consists of ingredients of animal origin, such as meat, poultry, fish, and animal by-products that are mixed with water at a 1:1 ratio (g/g; Case et al. 2011).

Weaning is a stressful period for young mammals. Without adequate transition, they often refuse food intake or eat only a small amount of food, have abnormal alterations in the intestinal microbiota, experience atrophy and damage in the small intestine, exhibit diarrhea growth restriction, and even die during the first several days (e.g., mainly 1–7 days in piglets) post weaning (Figure 11.13). Maintaining a healthy gut is key to successful weaning in all mammals (Kim and Duarte 2021). An effective nutritional method is to supplement weaning diets with animal-sourced protein (e.g., fishmeal and blood meal), functional amino acids (e.g., arginine, methionine/cysteine, glutamate, glutamine, glycine, proline, and tryptophan), organic acids, probiotics, or prebiotics (Fan and Kim 2024; Li and Wu 2021; Liao 2021). This will be discussed in more detail in Chapter 13. Likewise, care must be taken to protect the intestinal microflora and prevent intestinal disorders in postweaning dogs and cats, such as gastritis, abnormal stool, gut atrophy, diarrhea, enteritis, leaky gut syndrome, inflammatory bowel disease, and vomiting (Case et al. 2011).

Nutritional Requirements for Milk Production

Newborn mammals depend on milk for survival and growth; therefore, lactation is critical for their survival, growth, and development. The composition of nutrients in the milk of mammals varies among species (Table 11.18) or breeds within a species, the stage of lactation, and different milking intervals. Data on the content of select amino acids, fatty acids, minerals, and vitamins in the

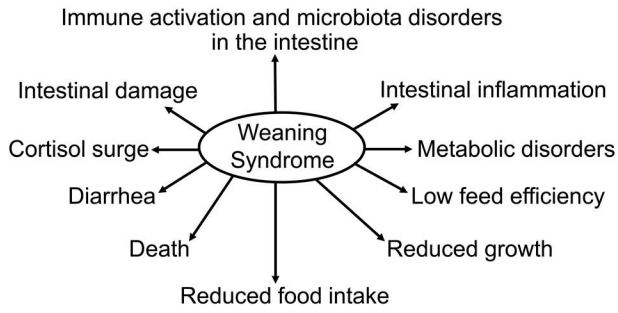


FIGURE 11.13 Weaning wasting syndromes in young mammals. Because of endocrine, behavioral, food, and environmental changes, weaning, particularly early weaning, is a traumatic event for young mammals (e.g., piglets and calves). This results in digestive and immunological dysfunction as well as malnutrition and infection, leading to diarrhea, dehydration, growth depression, and even death.

TABLE 11.18
Composition of Mature Milk of Domesticated and Wild Mammals[1]

Animal Species	Fat	Casein	Whey Protein	Total Protein	NPN Subs	Lactose	Total Carb	Ca	Ash	DM[a]
Antelope[b]	72	48	14	62	18	42	47	2.6	13	212
Baboon	46	4.7	7.3	12	3.0	60	77	0.44	3.0	141
Bat	133	---	---	80	5.0	34	40	2.2	6.8	265
Bear (black)	220	88	57	145	7.5	3.0	27	3.6	19	419
Bear (grizzly)	185	68	67	135	7.0	4.0	32	3.4	13	372
Bear (polar)	331	71	38	109	5.6	4.0	30	3.0	12	488
Beaver	182	85	23	108	6.3	17	22	2.4	20	338
Bison	35	37	8.0	45	3.0	51	57	1.2	9.6	150
Buffalo	77	38	7.0	45	5.2	40	47	1.9	8.0	192
Blue whale	423	73	36	109	6.6	10	13	3.4	16	568
Camel	45	29	10	39	5.6	49	56	1.4	7.0	153
Cat (domestic)	108	31	60	91	10	42	49	1.8	6.2	264
Chimpanzee	37	4.8	7.2	12	2.0	70	82	0.36	11	144
Cow (domestic)[c]	37	28	6.0	34	2.2	49	56	1.2	7.1	136
Coyote	107	---	---	99	---	30	32	2.4	9.0	247
Deer	197	94	10	104	14	26	30	2.6	14	359
Dog (domestic)	95	51	23	74	23	33	38	2.0	12	242
Dolphin	330	39	29	68	3.0	10	11	1.5	7.5	420
Donkey (Ass)	14	11	9.0	20	3.2	61	68	0.91	4.5	110
Elephant	116	19	30	49	4.1	51	60	0.80	7.6	237
Ferret	80	32	28	58	6.7	38	44	2.3	8.0	197
Fin whale	286	82	38	120	6.2	2.0	26	3.0	16	454
Fox	63	---	---	63	4.0	47	50	3.4	10	190
Giant panda	104	50	21	71	10	12	15	1.3	9.4	209
Giraffe	125	48	8.0	56	2.2	34	40	1.5	8.7	232
Goat (domestic)	45	29	5.0	34	5.8	43	47	1.4	7.9	139
Goat (mountain)	57	24	7.0	31	5.3	28	32	1.3	12	136
Gorilla	19	13	9.0	22	1.9	62	73	3.2	6.0	122
Guinea pig	39	66	15	81	12	30	36	1.6	8.2	176
Hamster	126	58	32	90	11	32	38	2.1	14	279
Horse (domestic)	19	14	8.3	22	3.6	62	69	0.95	5.1	119
Human[d]	42	4.4	6.6	11	2.8	70	80	0.32	2.2	138
Kangaroo	21	23	23	46	4	<0.01	47	1.6	12	130
Lion	189	57	36	93	6.6	27	34	0.82	14	337
Llama	42	62	11	73	9.6	60	66	1.7	7.5	198
Mink	80	---	---	74	12	69	76	1.3	10	252
Moose	105	---	---	135	18	33	38	3.6	16	312
Mouse (Lab)	121	70	20	90	11	30	36	2.5	15	273
Mule[e]	18	---	---	20	3.0	55	62	0.76	4.8	108
Musk ox	110	35	18	53	7.0	27	33	3.0	18	221
Opossum	61	48	44	92	4.6	16	20	4.2	16	194
Peccary	36	40	15	55	5.7	66	71	1.2	6.4	174
Pig (domestic)	80	28	20	48	5.4	52	58	3.1	9.2	201
Pronghorn	130	---	---	69	7.2	40	43	2.5	13	262
Rabbit	183	104	32	136	11	18	21	6.3	20	371
Rat (Lab)	126	64	20	84	6.3	30	38	3.2	15	269
Reindeer	203	86	15	101	14	28	35	3.1	14	367
Rhesus monkey	40	11	5.0	16	1.6	70	82	0.40	26	166
Rhinoceros	4.0	11	3.0	14	2.3	66	72	0.56	3.7	96
Sea lion	349	86	50	136	4.1	0.0	6.0	0.76	6.4	502
Seal (fur)	251	46	43	89	6.9	1.0	24	0.70	5.0	376
Seal (gray)	532	50	52	102	10	1.0	26	2.0	7.0	677
Seal (harp)	502	38	21	59	2.4	8.9	23	1.2	3.9	590

(*Continued*)

TABLE 11.18 (*Continued*)
Composition of Mature Milk of Domesticated and Wild Mammals[1]

Animal Species	Fat	Casein	Whey Protein	Total Protein	NPN Subs	Lactose	Total Carb	Ca	Ash	DM[a]
Seal (hooded)	404	---	---	67	5.0	0.0	10	1.2	8.6	496
Sheep (domestic)	74	46	10	56	2.7	48	55	1.9	9.2	195
Sperm whale[f]	153	32	50	82	6.3	20	22	1.5	8.0	270
Squirrel (gray)	121	50	24	74	16	30	34	3.6	12	257
Tree shrew	170	---	---	85	19	15	20	1.3	8.0	302
Water buffalo	74	32	6.0	38	5.8	48	55	1.9	7.8	181
Water shrew	200	---	---	100	---	1.0	30	---	20	350
White whale	220	82	38	120	3.7	2.0	18	3.6	16	378
Wolf	96	---	---	92	4.8	32	35	4.0	25	253
Yak	68	36	7.0	43	2.5	50	54	1.3	8.0	176
Zebra	21	12	11	23	2.6	74	82	0.8	3.5	132

Source: Rezaei et al. (2016).

[1] Values are g/kg whole milk. Nonprotein nitrogen = total nitrogen – protein nitrogen. Nitrogen content in milk protein is 15.67%. The amount of nonprotein nitrogenous substances (g/kg whole milk) is calculated as the amount of nonprotein nitrogen (g/kg whole milk) × 6.25. Caseins include α_{S1}-casein, α_{S2}-casein, β-casein, γ-casein, and *k*-casein. Whey proteins include α-lactoalbumin, β-lactoglobulin, serum albumin, immunoglobulins, lactoferrins, lysozymes, amino acid oxidases, xanthine oxidase, and other enzymes.

[a] Including fat, protein, NPN, lactose plus other carbohydrates, and minerals (total ash). When data on total carbohydrates have not been reported, ratios of lactose to other carbohydrates in milk are estimated to be 15:1 (g/g).

[b] Gemsbok antelope;

[c] Concentrations of urea, creatinine, and amino sugars are 317, 127, and 392 mg/L whole mike, respectively.

[d] Concentrations of urea, creatinine, and amino sugars are 274, 209, and 1111 mg/L whole mike, respectively.

[e] *Mule* is a domesticated, hybrid animal produced by crossing a female horse with a male *donkey*.

[f] Pygmy sperm whale.

Note: AA, amino acid; Ca, calcium; Carb, carbohydrates; DM, dry matter; NPN Subs, nonprotein nitrogenous substances (including free amino acids, small peptides, urea, ammonia, uric acid, creatine, creatinine, and other low-molecular weight nitrogenous substances); ---, the lack of data in the literature.

milk of some mammals are provided in Table 11.19. Colostrum is the first secretion from the mammary gland immediately and within the first few days after parturition. Colostrum is characterized by high concentrations of Igs (predominantly IgG and small amounts of IgA and IgM) that are efficiently absorbed by enterocytes via the neonatal Fc receptor (FcRn) and necessary for conferring passive immunity due to the underdeveloped immune system of the newborn (Table 11.20). These antibodies are produced by plasma cells (a type of differentiated B-lymphocytes) residing in various tissues (e.g., the bone marrow, lymph nodes, spleen, mammary glands, connective tissue, intestine, and blood). For mammals (e.g., pigs, horses, whales, and ruminants) that lack the placental transfer of Igs from mother to fetus and for terrestrial carnivores (e.g., cats and dogs) that have a limited placental transfer of Igs, colostrum is essential for protecting their neonates from infections. Even for mammals (e.g., humans, rabbits, and rodents) whose placentae transfer significant amounts of Igs from mother to fetus, colostrum plays a crucial role in building the immune system of their infants. In addition to Igs and oligosaccharides, colostrum and mature milk also contain nonnutrient substances (e.g., osteopontin, IGF-I, and other bioactive factors). Of note, N-containing substances (primarily β-casein, α-lactalbumin, other proteins, and free amino acids) are highly abundant organic nutrients in the milk of farm animals (Table 11.21), but the content of fat and lactose in milk is also high on a DM basis. Adequate production of milk is essential for high efficiencies in livestock production enterprises. However, in livestock species (e.g., sows and cows), milk production is often a limiting factor for maximum preweaning growth of offspring (Dunshea et al. 2005; Rezaei et al. 2016). To meet maximum milk production, lactating dams need to increase their feed intake per kilogram of BW by 50–80%, compared with their nonlactating counterparts, depending on the stage of lactation (Bell 1995; Kim et al. 2013). This is particularly important for cows that normally exhibit insulin resistance and metabolic syndrome during the critical transition period from ~21 days prepartum to ~21 days postpartum (Santos et al. 2016). Therefore, an important goal of animal nutrition research is to understand lactation biology. Available evidence indicates that the dietary intake of nutrients (particularly amino acids, including arginine, BCAAs, glutamate, and glutamine through activating MTOR and serving as the building blocks of proteins) and energy, endocrine status, and blood flow to the mammary glands play major roles in regulating milk production (Farmer 2019; Kim and Wu 2009; Rezaei et al. 2022a,b; Zhang et al. 2019).

TABLE 11.19
Concentrations of Select Amino Acids, Fatty Acids, and Minerals in the Milk of Mammals

Nutrient	Cat	Dog	Cow	Goat	Pig
Proximate analysis (g/100 g milk)					
Water	79	77.3	87.7	87.0	79.9
Dry matter	21	22.7	12.3	13.0	20.1
Crude protein	7.5	7.5	3.3	3.6	4.8
Crude fat	8.5	9.5	3.6	4.1	8.0
Lactose	4.0	3.3	4.7	4.0	5.2
Energy					
Metabolizable energy (kcal/100 g milk)	121	146	64	69	110
Metabolizable energy (kJ/100 g milk)	506	610	268	288	462
Amino acids (mg/100 g milk)					
Arginine	347	420	119	119	186
Methionine	188	194	82	80	91.8
Taurine	27	33	0.13	9.8	19.0
Fatty acids (g/100 g milk)					
Arachidonic acid (C20:4; ω6; $\Delta^{5,8,11,14}$)	0.11	0.04	0.09	0.17	0.04
Linoleic acid (C18:2; ω6; $\Delta^{9,12}$)	1.3	1.1	0.10	0.11	1.1
α-Linolenic acid (C18:3; ω3; $\Delta^{9,12,15}$)	0.16	0.03	0.05	0.04	0.09
Oleic acid (C18:1; ω9; Δ^{9})	2.3	3.8	1.0	0.98	2.2
Palmitic acid (C16:0)	1.3	2.5	0.94	0.91	2.0
Macrominerals (mg/100 g milk)					
Calcium	180	240	119	126–133	200
Chloride	---	---	85–110	110	100–110
Magnesium	9	11	14	11–14	11
Phosphorus	162	180	93	97–111	142
Potassium	103	120	150	190–204	89
Sodium	90	80	53	38–56	42
Microminerals (mg/100 g milk)					
Copper	0.11	0.33	0.05	0.022–0.07	0.09
Iodine	---	0.009	0.015–0.049	0.007	0.035–0.058
Iron	0.35	0.70	0.05	0.046	0.20
Manganese	0.039	0.011	0.003	0.006	0.007
Selenium	---	0.011	0.0024	0.002	0.0042
Zinc	0.66	0.95	0.44	0.15–0.38	0.68
Vitamins					
Vitamin A (IU/100 g milk)	651	867	156	207	160
Vitamin D (IU/L milk)	92	30 + 218[a]	40	122	360

Source: Borucki Castro et al. (2011); Corbee et al. (2012); Csapó et al. (1996); Iwarsson et al. (1973); Li and Wu (2024); Meschy (2000); Quesnel et al. (2008); Zhang et al. (2022).

[a] 30 IU of vitamin D/L milk and 218 IU of 25-hydroxy-vitamin D/L milk.

Note: ---, data are not available.

Mammary Gland

The lactating mammary gland is metabolically active and requires considerably more nutrients than the whole body on a per-kilogram basis, although this organ represents only 5–7% of the BW (Akers 2002). The lactating gland is a highly organized organ with a compound, tubulo-alveolar structure. Proteins, some amino acids, fats, and lactose are synthesized and secreted by mammary epithelial cells (MECs) in response to hormones (e.g., prolactin, insulin, and glucocorticoids). The alveoli are connected to a duct system through which secreted milk flows into the teat canal. The milk can be removed from the teat canal by suckling or milking (Figure 11.14). The lactating mammary gland is composed of connective tissue surrounding the secretory and ductal epithelial cells. The cellular components of the connective tissue consist of fibroblasts, blood vessels, and leukocytes, whereas noncellular components include collagen and other proteins in connective tissue (Inman et al. 2015). In addition, extensive WAT exists within the stroma of the mammary gland. This fat pad (an extraparenchymal

TABLE 11.20
Composition of Immunoglobulins (Ig G) in Colostrum and Mature Milk (g/L)

Animal	Type of milk	Ig A	Ig G	Ig M
Cow	Colostrum	3.9	50.5	4.2
	Mature milk	0.20	0.80	0.05
Goat	Colostrum	0.9–2.4	50–60	1.6–5.2
	Mature milk	0.03–0.08	0.1–0.4	0.01–0.04
Sheep	Colostrum	2.0	61	4.1
	Mature milk	0.06	0.3	0.03
Sow	Colostrum	10–26	62–94	3–10
	Mature milk	3.4–5.6	1.0–1.9	1.2–1.4
Human	Colostrum	17.4	0.43	1.6
	Mature milk	1.0	0.04	0.1

Source: Rezaei et al. (2016).

TABLE 11.21
Composition of Proteins in the Mature Milk of Mammals

Protein	Cow	Pig	Sheep	Goat	Horse	Human
	g/L milk					
Caseins	28	28	46	29	13.6	4.4
α-S1	10.6	20	16.6	4.3	2.4	0.52
α-S2	3.4	2.4	6.4	5.8	0.2	0.0
β	10.1	2.3	18.3	11.2	10.7	2.8
κ	3.9	3.3	4.5	7.7	0.24	1.0
Whey proteins	6.0	20	10	5.0	8.3	6.6
α-Lactalbumin	1.2	3.0	2.4	0.85	2.4	2.8
β-Lactoglobulin	3.2	9.5	6.4	2.3	2.6	0.0
Lactoferrin	0.02–0.2	0.1–0.25	0.1	0.02–0.2	0.58	3–4
Transferrin	0.02–0.2	0.02–0.2	0.1	0.02–0.2	0.1	0.02–0.03
Immuno-globulins	1.1	6.6	0.4	0.4	1.6	1.2
Serum albumin	0.4	0.5	0.5	0.6	0.4	0.5

Source: Rezaei et al. (2016).

tissue) is enlarged during the early phases of fetal and postnatal development but decreases progressively in mass during the periods of puberty, pregnancy, and lactation.

Structural development of the mammary gland occurs in utero during fetal growth and the prepubertal and postpubertal periods, pregnancy, and lactation (Figure 11.15). This process is known as mammogenesis. The mammary gland develops rapidly during sexual maturation under the influence of hormones such as estrogen, progesterone, adrenal corticoids, prolactin, growth hormone, and thyroxine (Musumeci et al. 2015). Most of the mammogenesis occurs during gestation under the control of various hormones (particularly estrogen, growth hormone, IGF-I, progesterone, placental lactogen, and prolactin) in a species- and stage-dependent manner. Thus, progesterone and prolactin promote lobuloalveolar development to form alveolar buds. Following parturition, the cessation of progesterone secretion is necessary for the initiation of milk production, because progesterone inhibits the secretion of hormones from the anterior lobe of the pituitary gland (Girmus and Wise 1992). Thus, at the onset of lactation, mature alveoli

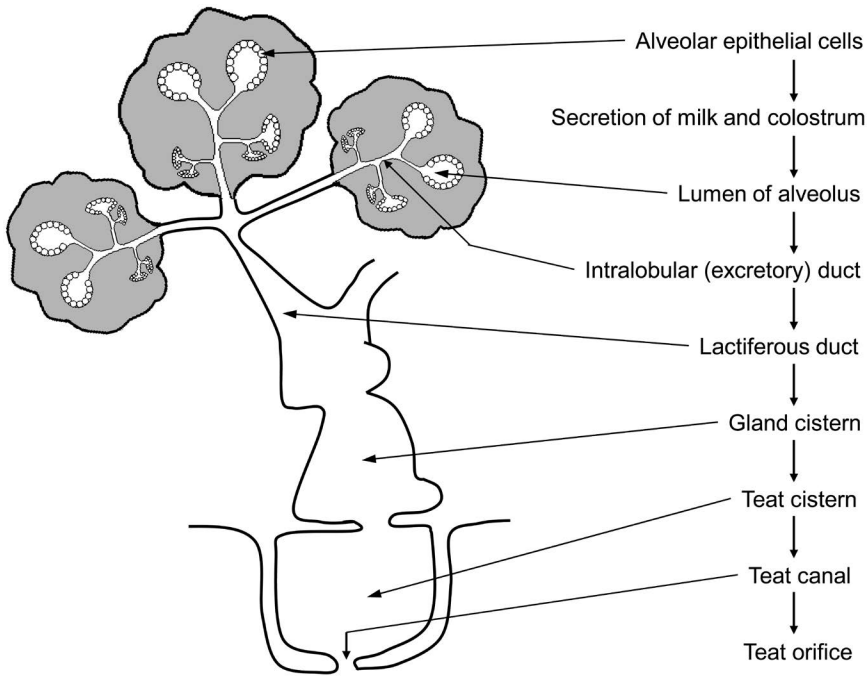


FIGURE 11.14 The basic structure of the mammary gland. Mammary epithelial cells are responsible for the synthesis and release of milk by lactating mammals. The alveoli are connected to a duct system through which the secreted milk flows into the teat canal, from which it can be removed by suckling or milking. (Courtesy of Rezaei et al. 2016, an open-access article.)

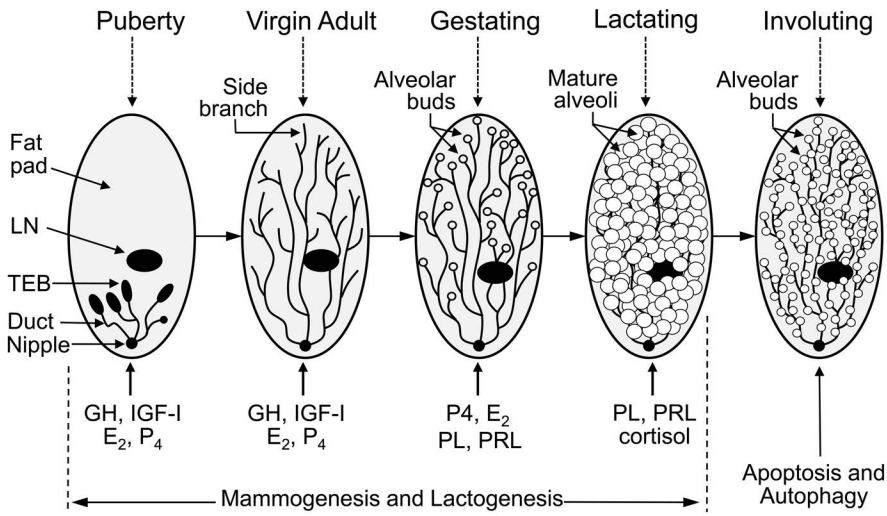


FIGURE 11.15 Development of the mammary gland in mammals. Mammogenesis begins during fetal development. After birth, mammary ducts elongate through cell proliferation. At the onset of puberty, high concentrations of growth hormone and insulin-like growth factor-I in plasma stimulate mammary duct proliferation to form terminal end buds (TEBs) at the tips of the ducts. Under the influence of estrogen, TEBs actively proliferate to form ductal branches, which fill the mammary fat pad. After this stage, the TEBs regress. During pregnancy, progesterone and prolactin promote lobuloalveolar development to form alveolar buds. At the onset of lactation, mature alveoli capable of producing and secreting milk are formed. Suckling of the nipple by the neonate results in contractions of the myoepithelial cells around the alveoli, causing the milk to be ejected through the ducts into the nipple. Upon weaning, lactation stops, and the mammary gland undergoes involution through apoptosis and autophagy to its nonlactating state. E2 = estrogen; GH = growth hormone; IGF-I = insulin-like growth factor-I; LN = lymph node; P4 = progesterone; PL = placental lactogen; PRL = prolactin. (Courtesy of Rezaei et al. 2016, an open-access article.)

capable of producing and secreting milk are formed. Milk secretion is maintained by suckling and/or milking, which stimulates the secretion of prolactin and other hormones active in the mechanism of milk secretion. Suckling of the nipple by the neonate also causes the oxytocin-induced contraction of the myoepithelial cells around the alveoli to eject the milk through the ducts into the teat canal. Upon weaning, lactation stops, and the mammary gland undergoes involution through apoptosis and autophagy to its nonlactating state (Rezaei et al. 2016).

Milk Synthesis by MECs

The milk is produced in MECs arranged in alveoli (or acini) and is drained through a system of arborizing ducts toward the body surface. The natural length of lactation differs among mammals. For example, cows lactate for about 40 weeks, with the peak of lactation at 6–8 weeks after calving; ewes lactate for about 12–20 weeks, with the peak of lactation at 2–3 weeks after lambing; and sows lactate for about 6–8 weeks, with the peak of lactation at 3–4 weeks after farrowing (Akers 2002; Bell 1995; Manjarin et al. 2014). In all mammals, the synthetic capacity of the mammary gland depends largely on the number and efficiency of functional MECs, and milk synthesis is regulated at both transcriptional and post-transcriptional levels (Osorio et al. 2016). As part of the systemic circulation, a pair of major arteries provide blood to the mammary gland. Uptake of nutrients from a large volume of blood flow is required for milk production. For example, in lactating cows, the production of 1 L of milk requires the passage of approximately 500 L of blood through the mammary gland (Braun and Forster 2012). During lactation, blood flow through the mammary gland is very rapid, and nutrients are actively taken up from the blood by cells of the mammary gland.

Different methods have been developed to study the biosynthesis of milk constituents in the mammary gland. Determination of arteriovenous (A–V) differences in a substance entering and leaving the mammary gland is the simplest method (Trottier et al. 1997). Based on A–V differences in a substance, the rate of its extraction can be calculated as $(A - V)/A \times 100\%$. If the rate of blood flow (ml/min) is known, it is multiplied by the rate of extraction to obtain the absolute rate of uptake of molecules by the mammary gland. The mammary glands of lactating goats take up, from the arterial blood, a large amount of acetate, glucose, lactate, amino acids, β-hydroxybutyrate, and TAG, but only a small quantity of glycerol, long-chain fatty acids, and phospholipids (Table 11.22).

1. Proteins and amino acids in milk
   True protein in milk. Approximately 80–95% of the N in milk is present in true protein (casein, lactalbumin, and lactoglobulin) and free amino acids, with the remaining N primarily as urea, ammonia, creatine, and creatinine (Rezaei et al. 2016). Albumin, κ-casein, and Igs in colostrum and milk are derived from their direct passage from maternal blood into the lumen of the alveoli. Protein concentrations are greater in colostrum than in mature milk. In colostrum, concentrations

TABLE 11.22
Concentrations of Milk Precursors in the Mammary Arteries and the Rates of Extraction of Nutrients by the Mammary Glands of Lactating Goats

Substance	Arterial Concentration (mg/L)	Extraction Rate (%)
Blood		
Acetate	89	63
Glucose	445	33
Lactate	67	30
Oxygen (volume as liter)	119	45
Plasma		
Amino acids	2.7–68.5	15–72
Glycerol	3.4	7
β-Hydroxybutyrate	58	57
Free long-chain fatty acids	87	3
Phospholipids	1600	4
Sterols	1040	0
Triglycerides	219	40

Source: Mepham and Linzell (1966).

of albumin and globulins are greater than those for casein (Tables 11.13 and 11.14). In milk, about 25% of DM and a similar proportion of energy are in the protein fraction (Table 11.23). A high-producing cow produces 1–1.5 kg of protein per day, and a rapidly growing calf or steer deposits about 250 g of protein per day (Tucker 2000). Thus, milk production requires a large amount of dietary protein.

Milk contains whey proteins and caseins. After milk has been coagulated or curdled by rennin (chymosin) or any edible acidic substance (e.g., lemon juice or vinegar), the liquid portion (whey) consists of water-soluble globular proteins (called whey proteins), and the solid masses (curds) are caseins. Whey proteins include a mixture of α-lactalbumin, β-lactoglobulin, serum albumin, Igs, transferrin, and lactoferrin. The proportions of whey proteins and caseins in milk vary among animal species: cows, 18% and 82%, respectively; humans, 60% and 40%, respectively; and pigs, 42% and 58%, respectively. Caseins are relatively hydrophobic and therefore poorly soluble in water

TABLE 11.23
Percentage of Total Calories Represented by Fat, Protein, and Carbohydrates in the Milks of Mammals[a]

Animal	Fat (kcal/kg milk)	Protein + AAs (kcal/kg milk)	Carb (kcal/kg milk)	Total Energy (kcal/kg milk)	% Calories as Fat	% Calories as protein + AAs	% Calories as Carb
Cat	1014	518	192	1725	58.8	30.1	11.1
Cow	347	189	220	756	46.0	25.0	29.0
Deer	1850	599	118	2567	72.1	23.4	4.6
Dog	892	464	149	1505	59.3	30.8	9.9
Dolphin	3099	378	43	3520	88.0	10.7	1.2
Donkey	131	119	267	517	25.4	23.0	51.6
Elephant	1089	275	235	1600	68.1	17.2	14.7
Goat	423	200	184	807	52.4	24.8	22.8
Guinea pig	366	470	141	977	37.5	48.1	14.4
Hamster	1183	518	149	1851	63.9	28.0	8.0
Horse	178	130	270	578	30.8	22.4	46.8
Human	394	65	314	773	51.0	8.4	40.6
Kangaroo	197	259	184	641	30.8	40.5	28.8
Mouse	1136	518	141	1796	63.3	28.9	7.9
Pig	751	275	227	1254	59.9	22.0	18.1
Rabbit	1718	767	82	2567	66.9	29.9	3.2
Rat	1183	470	149	1802	65.7	26.1	8.3
Reindeer	1906	583	137	2627	72.6	22.2	5.2
Rhesus monkey	376	92	321	789	47.6	11.6	40.7
Sea lion	3277	745	24	4046	81.0	18.4	0.6
Sheep	695	313	216	1224	56.8	25.6	17.6
Squirrel (gray)	1136	443	133	1712	66.4	25.9	7.8
Wolf	901	238	212	1351	66.7	17.6	15.7
Yak	639	238	212	1088	58.7	21.8	19.5

[a] The energy values of fat, protein + amino acids (AAs), and total carbohydrates were calculated on the basis of their combustion energy of 9.39, 5.40, and 3.92 kcal/g, respectively.

Note: Carb, carbohydrates.

but are present in milk as a suspension of particles called Ca-casein micelles. Although both whey protein and casein contain relatively low amounts of arginine and glycine, there are two major differences in nutrition between these two types of protein. First, compared with caseins, whey proteins contain more cysteine, glutamate, leucine, and tryptophan but less arginine, glutamine, serine, and threonine (Wu 2022a). Second, individual amino acids are released more rapidly from whey proteins ("fast-release" proteins) than caseins ("slow-release" proteins) in the small intestine. Thus, the consumption of whey protein may stimulate postprandial muscle protein synthesis and accretion more effectively than casein or casein hydrolysates in animals.

Processing of milk protein in MECs. Proteins that are synthesized in the rough endoplasmic reticulum (RER) are either secreted proteins (e.g., casein, β-lactoglobulin, and α-lactalbumin), membrane-bound proteins (e.g., extracellular matrix proteins involved in cell-cell contacts and membrane-bound enzymes), or intracellular proteins [e.g., enzymes, structural proteins (e.g., keratin), and fatty acid-binding proteins] (Ollivier-Bousquet 2002). Within MECs, the newly synthesized proteins are transferred from the RER to the Golgi apparatus, where they are processed for transport out of the cells. Casein is secreted as a micelle, which is formed in the Golgi from casein, Ca, and P. Casein, which exists in several forms, represents the bulk of the protein in the mature milk of all nonhuman species: 82–86% in ruminants, 52–80% in nonruminants, and 40% in humans (Table 11.21). Casein contains phosphorylated amino acid residues (e.g., serine, threonine, and tyrosine residues) and Ca. Although milk protein is generally rich in most proteinogenic amino acids, the content of arginine, glycine, and methionine in casein is relatively low (Wu 2022a).

Free amino acids in milk. Some amino acids, including BCAAs and arginine, are extensively catabolized by the lactating mammary gland (Wu 2022a). The nitrogenous products of BCAA catabolism are mainly glutamine and alanine. The nitrogenous products of arginine catabolism are ornithine, proline, glutamate, glutamine, NO, citrulline, and polyamines. As a result of extensive arginine degradation, arginine content in the milk of all species studied, except for the horse and cat, is remarkably low (Manjarin et al. 2014). Milk-borne polyamines are important for promoting the maturation and growth of the neonatal intestine. Interestingly, there is little catabolism of proline and glutamine in mammary tissue due to the lack of proline oxidase and glutaminase, and this helps explain the abundance of those two amino acids in milk proteins (Rezaei et al. 2016).

2. Lactose in milk
   Glucose is transported from blood into MECs across their basolateral membrane via specific transporters (predominantly GLUT1; Zhao and Keating 2007). Approximately 60% or more of the glucose extracted by the lactating mammary gland is used for lactose synthesis, with the remaining portion of the glucose being used for fat synthesis, oxidation, and perhaps alanine synthesis. Studies with ^{14}C-glucose have shown that blood glucose is the major precursor for both moieties (i.e., glucose and galactose) of the lactose molecule (Baxter et al. 1956). Glycerol, a hydrolytic product of triglycerides, is a precursor for glucose synthesis in the liver of the lactating dam, and thus plays a role in galactose and lactose production by MECs. In these cells, both glucose and galactose enter the Golgi to form lactose via a series of enzyme-catalyzed reactions (Chapter 5). The formation of lactose in the Golgi results in the movement of water from the extracellular space (and thus the blood) into the cytoplasm and then into the Golgi, where lactose ultimately becomes a component of milk. Lactose contributes to most of the osmotic pressure in milk (Table 11.17) and is therefore of nutritional and physiological importance for suckling neonates.
3. Lipids in milk
   In the milk of most mammals (including cows and swine), except for a few (including horses), 98% of the lipids are TAG (Palmquist 2006). The lipids of equine milk contain 80% TAG (Stoneham et al. 2017). Sterols, including cholesterol (the major milk sterol), represent less than 0.5% of the total milk lipid fraction, and the concentrations of phospholipids are low in milk (Jensen 2002). However, phospholipids are essential components of the milk fat globule. Fats are not water-soluble, do not contribute to the osmotic pressure of milk, and are present in milk as an emulsion. Concentrations of fat in the milk of different species vary greatly. For example, the milk of ruminants has relatively high concentrations of butyrate, other SCFAs, odd-numbered fatty acids, and branched-chain fatty acids (Park and Haenlein 2006). A unique component of the milk of ruminants is conjugated linoleic acid, which has many important roles in nutrition (Chapter 6). $C_{18:1}$, $C_{16:0}$, and $C_{18:0}$ are the most abundant fatty acids in bovine milk, as are $C_{18:1}$ and $C_{16:0}$ in sow's milk (Palmquist 2006). The fatty acids of milk have two major sources: blood triglycerides as lipoproteins and/or de novo synthesis.

   Fatty acids from lipoproteins in MECs. During the passage of triglycerides from blood to the

mammary tissue, fatty acids and glycerol are released by lipoprotein lipase bound to the endothelial surface of the capillary wall (Chapter 6). Following their passage through the capillary wall into MECs, fatty acids and glycerol are taken up by these cells. The circulating low-density lipoproteins (LDLs) also enter the mammary tissue via the process of endocytosis mediated by the membrane-bound LDL receptor (Osorio et al. 2016). Approximately 50% of fatty acids in milk are derived from blood lipoproteins (Palmquist 2006).

De novo synthesis of fatty acids in the MECs. In the MECs of lactating animals, fatty acids are synthesized from glucose, acetate, butyrate, acetoacetate, and β-hydroxybutyrate in a species-dependent manner (Chapter 6). De novo synthesis by MECs contributes about 50% of the fatty acids in milk (Palmquist 2006). Glucose metabolism through the pentose cycle generates the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) for fatty acid synthesis. The ketone bodies are important precursors for the synthesis of fatty acids in the milk of ruminants, rodents, and humans, but not pigs. This is because ketogenesis is limited in swine (including lactating sows) due to a deficiency of 3-hydroxy-3-methyl-glutaryl-CoA synthase in the liver (Chapter 5). Fatty acids (up to C_{18}) in sow's milk can be synthesized from blood glucose and acetate (Linzell and Mepham 1969). In contrast, there is little conversion of glucose into fatty acids in the mammary gland of ruminants (Chapter 6).

Synthesis of phospholipids and cholesterol by MECs. The phospholipids in milk are synthesized from diacylglycerol and phosphatidic acid by MECs (Chapter 6). Phospholipids are transported among organelles of MECs via spontaneous membrane diffusion, cytosolic phospholipid transfer proteins, vesicles, and membrane contact sites (Vance 2015). The cholesterol in milk lipids originates predominantly from blood and, to a lesser extent, de novo synthesis in MECs (Ontsouka and Albrecht 2014).

Synthesis of fats by the MECs. Fatty acids, glycerol, and monoacylglycerides are taken up by MEC for the synthesis of triglycerides in the smooth endoplasmic reticulum (Chapter 6), leading to the formation of small lipid droplets. Numerous small lipid droplets fuse to yield larger droplets, which move toward the apical membrane. The large lipid droplets exit the MECs through their apical membranes into the lumen of alveoli and the duct system of the mammary gland. Except for horses, fat is the major energy substrate for the suckling neonates of mammals (Table 11.23). The concentration of lipids in the milk of horses is only about 50% of that in the cow's milk.

4. Minerals in milk
   Minerals in blood pass into the mammary gland to become components of milk (Shennan and Peaker 2000). For farm mammals, colostrum may contain about twice the amount of most minerals in mature milk, and the concentration of iron in colostrum may exceed that in milk by 10- to 17-fold. However, concentrations of potassium are lower in colostrum than in milk (Park and Haenlein 2006). Concentrations of Ca and P in milk are much greater than in blood (13 and 10 times greater for Ca and P, respectively). Milk differs from plasma in concentrations of minerals and is more like value for intracellular fluid. Dietary intake of most trace minerals greatly influences their concentrations in milk (Suttle 2010). However, concentrations of copper, iron, and zinc in milk may not be altered significantly in response to dietary supplements.
5. Milk's organic substances not synthesized in MECs
   A number of organic substances in maternal plasma are transported through MECs into the mammary duct system essentially unchanged, including vitamins and Igs as found in blood or mucosal secretions (Hurley and Theil 2011). Vitamins in the blood enter MECs through its basolateral membrane via specific transporters or receptor-mediated endocytosis (Chapter 9). Igs bind to specific receptors on the basolateral surface of MECs, enter the cells, and are then exported into the lumen of the alveolus through the apical side of the cell via endocytic vesicles (or transport vesicles; Hurley and Theil 2011). In this process, the membrane of transport vesicles fuses with the inner surface of the apical membrane of MECs and releases Igs into the lumen of the alveolus. As the transport vesicles traverse the cell, they do not seem to interact with the Golgi, secretory vesicles, or lipid droplets. Serum albumin may also be transported through MECs via this clathrin-mediated mechanism (Monks and Neville 2004). Because there is no serum albumin receptor, serum albumin molecules are probably internalized into MECs along with Igs in the transport vesicles.

Release of Milk Proteins, Lactose and Fats from MECs to the Lumen of the Alveoli

In MECs, the Golgi apparatus participates in the processing of milk proteins as glycosylated proteins, the synthesis of lactose and fats, and osmotic gradients for the transport of water, and therefore plays an important role in the synthesis of milk components (Rezaei et al. 2016). Milk proteins and lactose are transported to the apical area of MECs via secretory vesicles, which make their way to the apical membrane

via a mechanism involving microtubules made of polymerized tubulin (Shennan and Peaker 2000). Tubulin is one of several cytoskeletal proteins that form the cellular scaffolding that provides structure to the cell. Keratin is another cytoskeletal protein. The apical membrane of MECs and the membrane of the secretory vesicle fuse, resulting in an opening through which the vesicle contents are discharged into the lumen of the alveoli.

Because fats and long-chain fatty acids are insoluble in water, the lipid droplets in milk are covered by a thin membrane consisting of protein and phospholipids to form milk fat globules on the endoplasmic reticulum membrane (Palmquist 2006). Approximately 13% of protein binds to lipids in milk (Wu and Knabe 1994). These lipid droplets are released from the endoplasmic reticulum into the cytosol and transported to the apical membrane of MECs for secretion into the lumen of the alveoli via exocytosis.

Efficiency of Energy Utilization for Milk Production

Energy (primarily ATP) is required for milk synthesis. In MECs, about 90% of the ATP is generated via the mitochondrial electron transport system (Rezaei et al. 2016). When there are no changes in body reserves of lactating animals, the gross efficiency of milk production is the ratio of the energy in milk (output) to the energy in the feed consumed (input) during a given period. The net efficiency of milk production is the ratio of energy in milk to the energy in feed, excluding maintenance requirements. Energy requirements for lactation are calculated factorially by summing those for maintenance, milk production, and changes in BW. In essence, milk yield and its energy content [e.g., bovine and sow's milk (Table 11.23)] determine energy requirements for lactation. In milk, fat content varies the most, protein content varies less, and the content of minerals and lactose varies the least (Table 11.18).

There is a negative energy balance in lactating cows, goats, ewes, and sows during early lactation (Baldwin and Bywater 1984). In cows, the efficiency of utilization of energy during early lactation is as follows: feed ME to milk = 0.644; body fats to milk = 0.824; feed ME to body fats = 0.747; and feed ME to body fats during the dry period = 0.587 (Bondi 1987). Thus, fat deposition during lactation is 27% more efficient than that in the dry period. The efficiency in the conversion of feed into milk in high-producing cows is increased by 10% through intramuscular administration of recombinant bovine growth hormone to high-producing dairy cows (Etherton and Bauman 1998). A possible alternative solution is dietary supplementation with rumen-protected arginine because this technique is highly effective in enhancing milk production by sows (Mateo et al. 2008). In addition to DM intake, water consumption by lactating mothers greatly affects the volume of milk produced daily. Overall, the efficiency of milk production decreases with advanced lactation because of the decreasing metabolic activity in MECs (Gorewit 1988).

Nutritional Requirements for the Production of Muscular Work

In underdeveloped nations, cattle are still used to plow the soil (Pearson et al. 2003). In developed countries, increasing numbers of horses are used for racing and other recreational purposes. In many regions of the world, horses, donkeys, mules (male donkeys × female horses), yaks, and camels are used for transport. Physical performance is the major output of the working cattle, horses, and riding animals in agriculture and transport (Pearson et al. 2003). As noted in Chapter 1, cattle are ruminants, whereas horses are monogastric herbivores with a relatively small stomach and a voluminous cecum and colon. The large intestine of horses has digestive functions similar to those of ruminants (Chapter 1). However, horses do not ruminate, and the gases produced by fermentation in the cecum and colon escape from the gastrointestinal tract through the rectum (Chapter 1). Because of the posterior compartments of fiber digestion in horses, ingested intact fiber enters the large intestine. Consequently, the rate of passage of digesta from the stomach to the distal end of the small intestine is greater in horses than in cattle (McDonald et al. 2021). Although the microflora and its biochemical activity in the cecum and colon of horses are similar to those in ruminants, fiber digestion in horses is not as efficient as in ruminants (Bondi 1987). Diets containing <15% fiber are well digested by horses, which utilize dietary fiber more efficiently than rabbits (Chapter 6). Donkeys, mules (offspring of male donkeys × female horses), yaks, and camels are all herbivores, which consume plants (e.g., grass, vegetables) as their diets.

Energy Conversion in Skeletal Muscle

Skeletal muscle converts chemical energy (e.g., ATP) directly into mechanical (dynamic) energy (Lieber and Bodine-Fowler 1993). Thus, muscle differs from the steam engine and the internal combustion engine because these engines convert chemical energy first into thermal energy (heat) and the latter into dynamic energy. Skeletal muscle consists of fibers made of myofibrils. There are two major important proteins in myofibrils: actin and myosin. During contraction, bundles of actin and myosin filaments slide into each other to generate force. The energy required for contractions of skeletal muscle is supplied directly by the enzymatic hydrolysis of ATP to ADP plus phosphate in the presence of Ca and magnesium (Newsholme and Leech 2009). Interestingly, myosin, in association with actin, has ATPase activity.

$$\text{ATP} \rightarrow \text{ADP} + \text{Pi} + \text{Mechanical Energy} + \text{Heat}$$

Under normal feeding conditions, fatty acids and glucose are the major sources of energy for skeletal muscle in mammals and birds (Jobgen et al. 2006). Glycogen, ketone bodies, and free amino acids (e.g., glutamine and BCAAs) are also sources of energy for the muscles of these animals. In the contracting muscle, the amount of glycogen is gradually reduced. Under predominantly anaerobic conditions,

ATP is produced in skeletal muscle through glycolysis, an inefficient pathway for ATP production (Chapter 5). By contrast, the oxidation of amino acids provides most ATP in the skeletal muscles of fish and crustaceans for locomotion and metabolic processes (Li et al. 2021b,c).

Although contractions of skeletal muscle specifically require ATP and its concentration in the muscle is greater than that in the liver, phosphocreatine is the major high-energy phosphate stored in the muscle and brain. Indeed, the concentration of phosphocreatine in skeletal muscle (19 mmol/kg or 25 mM) is much greater than that of ATP (4.5 mmol/kg or 6 mM). Creatine is synthesized from arginine, glycine, and methionine through an interorgan cooperation (mainly the liver, kidney, and muscle) in terrestrial animals (Wu 2022a), but teleost fish form creatine primarily in their skeletal muscle (He et al. 2024). Thus, adequate protein nutrition is crucial for maintaining muscular work.

$$\text{Creatine} + \text{ATP}^{4-} \rightleftharpoons \text{Phosphocreatine}^{2-} + \text{ADP}^{3-} + \text{H}^{+} \left(\text{Creatine kinase}\right)$$

When ATP is used by skeletal muscle for contraction, this reaction (catalyzed by creatine kinase) proceeds from the right to the left (Newsholme and Leech 2009). With the depletion of ATP, fatigue sets in, and after prolonged muscular activity, the accumulation of lactic acid results in muscle cramps (Allen et al. 2008). At rest, the energy in ATP is transferred to creatine and is then stored in phosphocreatine; namely, the reaction proceeds from the left to the right. In tissues (e.g., skeletal muscle and brain), creatine kinase exists in both the mitochondria and the cytosol.

High Requirements for Dietary Energy, Protein, and Minerals for Muscular Work

Muscular Work-Induced Increases in Nutrient Metabolism

A working animal expends more energy than a resting one, and therefore requires higher intakes of dietary energy substrates (fats, glucose, and amino acids; Jobgen et al. 2006). In addition, rates of muscle protein turnover are enhanced in response to muscular work, with outcomes depending on its intensity and dietary intake of energy and protein (Allen et al. 2008). Thus, additional dietary amino acids for protein synthesis to offset the loss of body protein must be supplied in diets. There are suggestions that dietary intake of protein and energy by subjects be increased by 30% and 60%, respectively, for moderate and intense exercise (Wu 2016). Furthermore, the rates of production of oxidants are greater in the working animal compared with the resting one. Thus, requirements for dietary antioxidant nutrients (e.g., vitamins E, A, and C) must be augmented accordingly for the working animal compared with the resting one (Allen et al. 2008). Note that vitamin E is particularly important for the maintenance of the structural integrity of skeletal muscle.

Intensive work is associated with sweating and thus the loss of large amounts of water, sodium, and chloride, as well as small amounts of potassium, magnesium, Ca, and P (Keen 1993). This requires supplementation of water, sodium chloride, and other minerals in a continuous and balanced manner. Dehydration is the major danger under conditions of hard work or intensive heat. For example, a horse with intense exercise may need up to 60 L of water and 100 g of sodium chloride per day to avoid dehydration (Zeyner et al. 2017). A consequence of severe dehydration is collapse and death.

Energetic Efficiency of Muscular Work

The upper limit of the efficiency of conversion of dietary energy into work is set by the maximum efficiency for the conversion of fats, glucose, and amino acids into ATP (e.g., 55% for fatty acids and glucose; 37% for glutamine; 46% for BCAAs) (Wu 2022a). In fish and shrimp, which lack the urea cycle and directly excrete ammonia into the surrounding water, fat, glucose, and proteins have similar energetic efficiencies (Chapter 8). The working animal must also walk, and fish must swim regularly, and these activities require energy expenditure. Unlike an engine, the working animal or swimming fish must spend energy on maintenance regardless of working, walking, other physical activities, or resting. Thus, dietary nutrients are required by exercising animals for both maintenance and muscular power production.

In mammals and birds, the efficiency of the conversion of dietary fat or glucose into work is greater than that for protein or amino acids, as noted previously. For a 500-kg horse that works 8 h/day to pull a 67-kg plow at the speed of 4000 m/h, the efficiency in the use of ME for plowing (the useful work done, muscular power production) is 31%, and the efficiency in the use of ME beyond maintenance for the useful work done is 24% (Table 11.24). The latter value is

TABLE 11.24
Efficiency in the Use of Metabolic Fuels for Muscular Power Production in Horses

1. A 500-kg horse worked 8 h/day to pull a 67-kg plough at the speed of 4 km/h (32 km/day).
2. Total requirement for metabolizable energy (ME) = 135,208 kJ/day.
3. Maintenance requirement for ME = 47,301 kJ/day.
4. ME requirement for walking = 19,968 kJ/day (624 kJ/km × 32 km/day = 19,968 kJ/day)[a].
5. ME requirement for muscular power production = 67,939 kJ/day (135,208 – 47,301 – 19,968 kJ/day = 67,939 kJ/day).
6. Energy value of the useful work done (ploughing) = 21,026 kJ/day [67 kg × 32 km/day = 2,144 (kg•km)/day = 21,026 kJ/day][b]
7. Efficiency of ME for the useful work done (ploughing) = 31% (21,026 kJ/day ÷ 67,939 kJ/day × 100% = 31%)
8. Efficiency of ME beyond maintenance for the useful work done (ploughing) = 24% [21,026 kJ/day/(19,968 + 67,939 kJ/day) × 100% = 24%]

Source: Adapted from Bondi (1987).

[a] ME requirement for walking by a 500-kg horse is 624 kJ/km. Thus, 32 km/day × 624 kJ/km = 19,968 kJ/day.

[b] Calculated on the basis of 1 kg•km = 9.807 kJ. Thus, 2,144 (kg•km)/day × 9.807 kJ/(kg•km) = 21,026 kJ/day.

similar to the efficiency of converting dietary protein into milk protein in lactating cows (Table 11.14).

Benefits of Moderate Muscular Activity in Enhancing Animal Growth and Feed Efficiency

Moderate muscular work (e.g., walking or swimming) increases the rates of whole-body oxidation of fatty acids and glucose to minimize fat accretion in the body while enhancing tissue insulin sensitivity and activating the MTOR signaling pathway to promote protein synthesis and deposition in skeletal muscle (Jobgen et al. 2006). As noted previously, fat reduction in tissues is not associated with a loss of water, but the accretion of 1 g of protein is accompanied by the retention of 3 g of water in the body. Thus, in response to moderate exercise, animals with adequate intakes of energy and nutrients (including amino acids) exhibit a greater growth rate and a higher feed efficiency compared with animals with minimal physical activity. For example, Murray et al. (1974) reported that female pigs (12–60 kg) with a 6×10 min treadmill exercise regimen three afternoons a week (Monday, Wednesday, and Friday) for 9 weeks exhibited a 15% increase in both weight gain and feed efficiency (weight gain/feed intake) during weeks 7 to 9 of the experiment, compared to the control group. Furthermore, regular swimming enhances growth rates and feed efficiencies in several fish species, including rainbow trout, Masu salmon, striped bass, and zebrafish (Davison and Herbert 2013). These findings indicate an important role for moderate muscular activity in improving the efficiency of feed utilization for lean-tissue growth in animals.

Nutritional Requirements for the Production of Wool and Feathers

Wool Production in Sheep and Goats

The term wool is usually restricted to describing the fibrous protein derived from the specialized skin cells (follicles) in animals. Sheep (e.g., Merino sheep) and goats (e.g., Cashmere and Angora goats) produce wool for making high-quality clothes. The wool consists of wool fiber (almost entirely protein and wool α-keratins produced by the epidermal cells), wool wax (mainly esters of cholesterol and other alcohols produced by the sebaceous glands), and water (Reis and Gillespie 1985). Keratin has a high content of cysteine, which is synthesized from methionine in the liver (Chapter 7). Of note, the content of the sulfur (S)-containing amino acids in keratin is about 15% (14% cysteine plus 0.4% methionine), which is 12 and 10 times that in SBM and microbial proteins, respectively (Bradbury 1973). Thus, wool production requires a large amount of both cysteine and methionine. To produce a fleece containing 3 kg of protein in one year, the sheep would need to deposit 8.2 g of protein/day in the wool.

In sheep and goats, wool growth depends on the quality and quantity of dietary protein digested, as well as the profile of amino acids absorbed in the small intestine. The efficiency of truly digestible protein for wool/hair growth (including water retention) is about 60%, and the protein deposited in wool accounts for ~ 20% of total protein synthesis in the skin (Liu et al. 1998). A 50-kg Merino ewe must absorb ~ 135 g of protein/day to achieve her maximum rate of wool growth (Qi and Lupton 1994). If such a ewe is provided with a diet containing the ME of 12 MJ/day (i.e., twice its maintenance requirement), 101 g of microbial protein would be synthesized per day to yield only 69 g of amino acids absorbed by the small intestine (i.e., $101 \times 0.8 \times 0.85 = 69$ g). To achieve maximum growth of wool, the diet of the sheep must be supplemented with a good source of undegraded protein and an additive rich in S-amino acids. Thus, wool growth in sheep responds well to dietary intakes of protein, energy, minerals, and vitamins, and the response is affected by the genotype and reproductive status of the animal (Reis and Sahlu 1994). Similar findings have been reported for Cashmere and Angora goats (Galbraith 2000).

Feather Growth and Color of Birds

In birds, feathers are formed in the follicles of the epidermis, which produce β-keratin proteins and wax, and have distinctive colors (Haake et al. 1984). These complex integumentary structures cover ~ 75% of the bird's skin and aid in flight, thermal insulation, and waterproofing. The wax consists of short-, medium-, and long-chain fatty acids (Chapter 3). The colors of feathers are produced by pigments, which are tyrosine metabolites [melanins (brown and beige pheomelanins, as well as black and gray eumelanins)], plant substances known as carotenoids (red, yellow, or orange), or copper-containing porphyrins (Bennett and Thery 2007). Tetrahydrobiopterin, oxygen, copper, iron, and zinc are cofactors in the synthesis of melanins from tyrosine (Wu 2022a). In animals, vitamin C stabilizes tetrahydrobiopterin and promotes the intestinal absorption of iron, and is therefore required for feather growth. The pigments refract, reflect, or scatter the selected wavelengths of light to produce various colors.

Feathers are composed of 85–90% protein, 1.5% lipids, 8–10% water, and 1–2.5% minerals (Murphy et al. 1990). The most abundant amino acids in the β-keratin proteins of feathers are glycine, proline, serine, cystine, and BCAAs, as reported for the rachis (central shafts) and barbs (serial paired branches) of penguin feathers (Table 11.25). Similar results were reported for feathers of fowl (Akahane et al. 1977). Notably, glycine accounts for 13.7% of total amino acids (mol/mol) in chicken feather keratin (Gregg and Rogers 1986). The three most abundant amino acids are those that can be synthesized de novo, but it is unknown whether their formation is sufficient for feather development in birds. Because cysteine, tyrosine, and BCAAs are not synthesized de novo in animals, avian diets must contain not only high levels of small neutral amino acids but also aromatic, S-containing, and large neutral amino acids to ensure the optimal growth of feathers. There is evidence that the dietary intake of protein affects the development of feather colors in birds (Meadows et al. 2012).

TABLE 11.25
Composition of Amino Acids (AAs) in Feathers of Birds within the Genus *Pygoscelis*

AA	Rachis of Three Species of Penguins			Barbs of Three Species of Penguins			Chicken Feather Keratin	Whole Feather of Fowl
	P. adeliae	*P. antarctica*	*P. papua*	*P. adeliae*	*P. antarctica*	*P. papua*		
	μmol/g dry mass			μmol/g dry mass			(%, mol/mol)	% of AAs residues
Ala	722	634	641	499	503	506	8.7	5.6
Arg	328	321	326	337	335	339	3.8	4.7
Asx	464	496	525	628	630	615	5.6	6.3
Cystine	717	707	776	782	878	786	7.8	8.4
Glx	498	557	570	685	709	684	6.9	8.6
Gly	1477	1386	1405	1155	1096	1109	13.7	11.5
His	81	73	72	78	33	30	0.2	0.3
Ile	374	389	405	424	436	431	3.2	4.3
Leu	986	923	924	727	729	741	8.3	7.4
Lys	44	45	40	78	80	75	0.6	1.2
Met	73	68	73	103	112	101	0.1	0.3
Phe	216	203	235	203	178	199	3.1	3.6
Pro	1035	986	1002	1044	1074	1046	9.8	11.7
Ser	933	785	792	703	716	727	14.1	15.7
Thr	392	409	436	437	452	452	4.1	5.3
Tyr	222	254	230	243	257	215	1.4	1.6
Val	724	737	737	839	860	845	7.9	7.7
Trp	---	---	---	---	---	---	0.7	---

Source: Adapted, for the three species of penguins, from Murphy et al. (1990); for the whole feather of fowl, from Akahane et al. (1977); and, for chicken feather keratin, from Gregg and Rogers (1986).

Note: Asx, aspartate plus asparagine; Glx, glutamate plus glutamine; rachis, the central shaft of a feather; ---, data are not available. Penguins do not have teeth, but have barbs (papillae consisting of connective tissue) on their tongues and the roof of their mouths to catch and swallow their food or prey.

Nutritional Requirements for the Production of Eggs in Poultry

Egg production is of great economic importance in the poultry industry. Egg production provides a unique opportunity to augment the supply of some nutrients (e.g., ω3 PUFAs, taurine, and arginine) for human consumption. The egg within its shell contains all nutrients necessary to produce a viable embryo and is the sole reproductive product of birds.

Composition of Eggs

The egg consists of three basic parts: yolk, egg white, and shell (membranes; Table 11.26). The white portion is the major part of the egg. Ca carbonate, protein, and lipids are the main DM components of the egg's shell, white, and yolk, respectively (Gilbert 1971a). Egg white contains about 40 different proteins, such as ovalbumin (54%); ovotransferrin (12–13%, an antimicrobial agent); ovomucoid (11%, an inhibitor of trypsin); globulins (8%); lysozymes (3.4–3.5%, which split β-(1,4)-D-glucosaminides and lyse bacteria); ovomucin (1.5–2.9%, antiviral hemagglutination); flavoprotein (0.8%, which binds riboflavin); ovomacroglobulin (0.5%); ovoglycoprotein (0.5–1%); ovoinhibitor (0.1–1.5%, an inhibitor of trypsin, chymotrypsin, and other proteases); avidin (0.05%, which binds biotin); and papain inhibitor (0.1%, an inhibitor of papain and other proteases) (Gilbert 1971b). Egg white contains only a trace amount of lipids (0.02%), which includes triglycerides, diglycerides, free fatty acids, cholesterol ester, cholesterol, phosphatidylcholine, lysophosphatidylcholine, phosphatidylethanolamine, and sphingomyelin (Sato et al. 1973).

In egg yolk, the ratio of protein to lipids is 1:2, with the lipids being present as lipoproteins, including LDLs

TABLE 11.26
Chemical Composition of Eggs from Hens[a]

Substance	Shell (%)	White (%)	Yolk Shell (%)	Egg without Shell (%)	Egg with Shell (%)
Water	1.5	88.5	49.0	73.6	67.1
Protein	4.2	10.5	16.7	12.8	11.8
Lipid	trace	trace	31.6	11.8	9.8
Other OM	trace	0.50	1.1	1.0	0.64
Minerals[†]	94.3	0.50	1.6	0.80	10.6

Source: Gilbert (1971a).

[a] A 58-g egg consists of shell, 6.1 g (10.5%); yolk, 18 g (31.0%); and white, 33.9 g (58.5%).

[†] Mainly as calcium carbonate.

Note: OM, organic matter (including carbohydrates).

(containing 90% lipids) and high-density lipoproteins (McIndoe 1971). Egg yolk proteins consist of lipovitellin a (58%), lipovitellin b (11%), livetin a (4%), livetin b (5%), livetin g (2%), phosvitin (7%; a principal phosphoprotein that can bind iron and Ca), and apo (apolipoprotein) LDL (12%; McIndoe 1971). Egg yolk lipids include neutral lipids (65%), phospholipids (30%), cholesterol (5%), and carotenoids (8–20 mg/g yolk; Anton 2013; Blesso 2015). The phospholipids comprise phosphatidylcholine (83%), phosphatidylethanolamine (14%), sphingomyelin (0.8%), and phosphatidylinositol (0.15%; Anton 2013; Blesso 2015).

Formation of Eggs

Eggs are produced in the female reproductive tract through interorgan metabolism of nutrients (Figure 11.16). The formation of one egg by a laying hen requires ~24 h (Cunningham et al. 1984). Specifically, proteins and lipids in yolk are synthesized in the liver of a laying hen under the influence of estrogen and then transported to her ovary. The rates of hepatic protein synthesis and lipogenesis in mature laying hens may be 5 to 10 times those in the liver of immature females, and the concentrations of free fatty acids in plasma increase approximately 10-fold immediately prior to the laying of an egg. The composition of fatty acids in the liver and plasma is substantially altered in laying hens with respect to the composition of fatty acids in the yolk, with the proportions of palmitic and oleic acids being increased, whereas those of stearic and linoleic acids are decreased (Anton 2013; Gilbert 1971a).

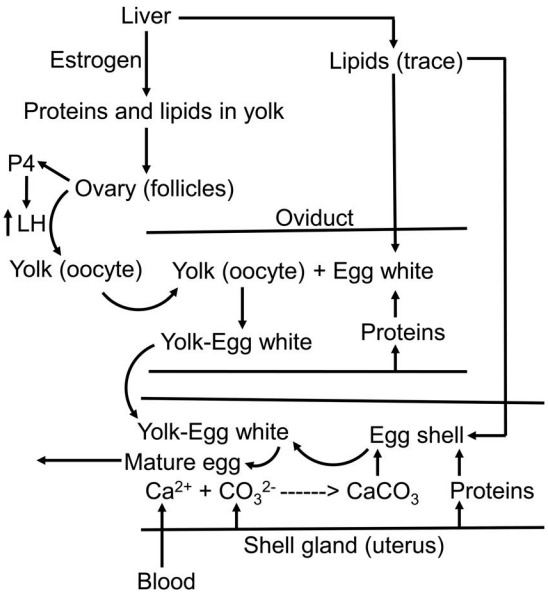


FIGURE 11.16 Formation of eggs by laying poultry. Under the influence of estrogen, the liver of a laying hen synthesizes and releases proteins and lipids (components of the yolk), which are transported to the follicles of the ovary for deposition and formation of yolk. Through the action of luteinizing hormone (LH), which peaks in response to a progesterone surge, the mature follicle releases the yolk into the oviduct, in which both estrogen and progesterone stimulate and maintain the synthesis of egg-white proteins. In the oviduct, the egg white is formed from albumen proteins, a trace amount of liver-derived lipids, as well as water, vitamins, and minerals. After the yolk is coated with the egg white, this complex enters the shell gland (or uterus), where the eggshell is generated from the deposition of calcium carbonate (main component) and other minerals. In domestic chickens, the formation of one egg requires about 24 h.

The yolk proteins and lipids from the liver are deposited in the ovary, along with water, amino acids, carbohydrates, vitamins, and minerals that are all ultimately derived from maternal blood (Anton 2013). As in mammals, under the influence of high concentrations of LH, a mature follicle of the ovary ovulates to release a yolk into the oviduct. Ovulation of the mature follicle requires not only LH but also progesterone (Cunningham et al. 1984). In chickens, 4 to 6 h prior to the LH surge, the circulating concentrations of progesterone (released from the largest and most mature ovarian follicle) are the greatest during a laying cycle. This peak in plasma concentrations of progesterone is necessary for the subsequent surge of LH to induce ovulation.

The next stage of egg formation involves the synthesis of egg-white proteins from blood-derived amino acids in the oviduct, which is stimulated and maintained by steroid hormones (e.g., estrogen and progesterone; Palmiter 1972). The egg white (also called albumen) is then formed from the oviduct-derived proteins, a trace amount of liver-derived lipids, as well as water, minerals, and vitamins that are all ultimately derived from maternal blood. The egg white is added to the surface of the yolk to form a complex. The coated yolk then enters the shell gland (or uterus).

In the shell gland, the eggshell is formed from the deposition of Ca carbonate (the main component) and other minerals, and this process requires about 17–20 h in hens (Burley and Vadehra 1989). Vitamin D and parathyroid hormone stimulate the intestinal absorption of Ca and its transport by the shell gland for eggshell formation. The protein in the eggshell is synthesized and secreted by the shell gland. Because the shell gland in the uterus does not store significant amounts of Ca, the shell gland derives Ca (2–2.5 g/egg) and other minerals from blood. The deposition of Ca carbonate ($CaCO_3$; containing 40% Ca) in the developing eggshell is maintained through continuous secretion of bicarbonate from the shell gland to produce a mature egg (Burley and Vadehra 1989). Note that CO_2 exists in the blood as dissolved gas (CO_2), carbonic acid (H_2CO_3), and bicarbonate (HCO_3^-). Eggshell color results from pigment deposition and, therefore, is affected by the diets of hens.

$$CO_2 + H_2O \rightarrow H_2CO_3 \quad \text{(Carbonic anhydrase or spontaneously)}$$

$$H_2CO_3 \rightarrow H^+ + HCO_3^- \quad \text{(Spontaneously)}$$

$$HCO_3^- \rightarrow H^+ + CO_3^{2-} \quad \text{(Spontaneously)}$$

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \quad \text{(Spontaneously)}$$

High Requirements for Dietary Energy, Protein, and Calcium for Egg Production

Requirements of energy, protein, and Ca by laying hens are particularly high (Burley and Vadehra 1989). Syntheses of egg yolk protein and lipids by the liver and of egg-white proteins by the oviduct require a large amount of ATP. In addition, vitamins (particularly vitamin D) and other minerals must be supplied in adequate amounts to ensure high production of eggs and high rates of hatchability and survival. The following calculations are examples of the minimum requirements of a 2-kg laying hen for dietary energy, protein, and Ca.

1. *Dietary energy requirement*
   A 58-g egg contains 91.8 kcal of gross energy (Pond et al. 1995). Based on the production of a 58-g egg/day and the digestibility of dietary energy (90%), at least 102 kcal of energy in the diet are used for egg production by a 2.0-kg hen. The dietary energy requirement for maintenance is 79.4 (i.e., 71.5/0.90) kcal/kg BW/day (159 kcal/day for a 2-kg hen) in a laying hen that does not gain BW (Table 11.6). Thus, the requirement of dietary energy is at least 261 kcal/day (102 + 159; production + maintenance) for the 2-kg laying hen. If the laying hen produces 250 eggs/year, at least 65.25×10^3 kcal of dietary energy (equivalent to 6.94 kg of fat) are used for this purpose.
2. *Dietary protein requirement*
   Based on the production of a 58-g egg/day and the digestibility of dietary protein (85%), at least 8.1 g of protein in the diet are used for egg production by a 2.0-kg hen (Pond et al. 1995). The dietary protein requirement for maintenance is 1.95 (= 1.66/0.85) g/kg BW/day (3.9 g/day for a 2-kg hen) in a laying hen that does not gain BW (Pond et al. 1995). Thus, the requirement of dietary protein is at least 12 g/day (8.1 + 3.9; production + maintenance) for the 2-kg laying hen. If the laying hen produces 250 eggs/year, at least 3.0 kg of dietary protein are used for this purpose.
3. *Dietary calcium requirement*
   The eggshell consists of about 95% Ca carbonate and 5% organic material (Burley and Vadehra 1989). Based on the production of a 58-g egg/day and the digestibility of dietary Ca (50%), at least 4.64 g Ca in the diet are used for egg production by a 2.0-kg hen. The dietary Ca requirement for maintenance is estimated to be 0.02 g/kg BW/day (0.04 g/day for a 2-kg hen) in a laying hen that does not gain BW (Pond et al. 1995). Thus, the requirement of dietary Ca is at least 4.68 g/day (4.64 + 0.04; production + maintenance) for the 2-kg laying hen. If a laying hen produces 250 eggs/year, at least 1.17 kg of Ca in the diet are used for this purpose.

Nutritional Requirements for the Optimum Growth and Health of Bone, Skin, and Hair

Skin and hair are specialized connective tissues with important biological and social significance. In mammals, hair is mostly made up of protein, and skin contains protein as the most abundant DM. The keratinization of skin and the normal growth of hair may account for 25–30% of the daily protein requirements of cats and dogs (Connolly and Wu 2024). In some mammals, such as adult dogs and cats, up to 35% of daily protein intake is used to maintain their skin and hair. Keratin is the major protein in both the skin's epidermis and the hair. This protein is rich in cysteine, serine, and glycine (Table 11.27). Filaggrin, which aids in the aggregation and compaction of keratin intermediate filaments, is a physiologically important protein in the epidermis of skin and hair. Filaggrin is produced by the granular cells of the skin's epidermis and by the inner root sheath cells of the hair follicles and contains high amounts of serine, glutamate, glutamine, glycine, arginine, and histidine. Collagen and elastin (highly abundant in glycine and proline plus 4-hydroxyproline) are the major proteins in both the dermis and hypodermis (Table 11.28). Glycine, proline, and 4-hydroxyproline are the most abundant amino acids in skin proteins. Taurine is an abundant free amino acid in the epidermis (5–18 mM; mainly in keratinocytes) and the dermis (3–17 mM), whereas 4-hydroxyproline is present in high amounts in all layers of the skin (0.4–0.8 mM;

TABLE 11.27
Abundant Amino Acids in Major Proteins of the Skin and Hair of Mammals

Protein	Constituent in Skin and Hair	Major Amino Acids (%, mol/mol)
Collagen	About 70% of dry matter in the dermis (this layer is 90% of the skin's weight); 90% of total proteins in the skin; 75% of the skin's dry matter; the most abundant protein in the skin and the whole body	Gly, 33; and Pro + Hydroxyproline, 23
Elastin	A physiologically important protein in the skin and hair; 10% of total proteins in the skin, 5% of dry matter in the skin's dermis, and 2–4% of the skin's dry matter; 5% of total proteins in the hair; conferring elasticity in tissue	Gly, 36; and Pro + Hydroxyproline, 15
Keratins	About 90% of dry matter in the hair; 95% of epidermal proteins in the skin; 80–90% of dry matter in the epidermis; the most abundant protein in the hair	Cys, 16; Ser, 11; and Gly 9
Filaggrin	4–5% of dry matter in the epidermis of the skin; 3–4% of dry matter in the hair	Ser, 20; Glu + Gln, 20; Gly, 17; Arg 12; and His, 8

Source: Connolly and Wu (2024).

TABLE 11.28
Composition of Amino Acids (mol/100 mol of Residues) in the Hair of Mammals

Amino Acid	Cat	Dog	Human	Horse	Sheep
Alanine	5.1	5.1	4.6	5.5	5.2
Arginine	6.1	6.3	5.8	7.9	6.2
Asparagine	3.6	3.4	2.2	3.9	3.8
Aspartate	2.0	1.9	1.7	2.1	2.1
Cysteine	15.8	16.7	17.8	14.4	13.1
Glutamate	6.5	6.3	6.5	6.4	6.3
Glutamine	4.9	4.8	4.9	4.9	4.8
Glycine	9.5	7.8	6.4	6.4	8.6
Histidine	1.2	0.9	0.9	1.1	0.8
Isoleucine	2.5	2.5	2.6	3.6	3.0
Leucine	6.7	6.1	5.8	7.5	7.2
Lysine	2.9	3.9	2.7	2.9	2.7
Methionine	0.9	0.9	0.6	0.2	0.5
Phenylalanine	2.3	1.7	1.6	2.5	2.5
Proline	4.9	7.3	8.4	7.8	6.6
Serine	10.6	10.5	11.7	9.6	10.8
Threonine	6.4	6.2	6.8	5.5	6.5
Tyrosine	3.0	2.7	2.0	1.9	3.8
Valine	4.9	4.9	5.8	5.9	5.7

Source: Hendriks et al. (1998); and Connolly and Wu (2024).

Connolly and Wu 2024). The epidermis of the skin synthesizes melanin (the pigment in the skin and hair) from tyrosine and produces *trans*-urocanate from histidine.

Bone, which is another connective tissue providing the skeleton of animals, is composed of bone matrix and bone marrow and is required for locomotion and the protection of internal organs, as well as the maintenance of Ca and P homeostasis in the body. Chemically, there is 30% organic matter, 60% inorganic matter, and 10% water in bone, with collagen and elastin representing 90% and 1% of the organic component in the bone matrix, respectively (Lopez et al. 2024). On the other hand, joints consist of cartilage, tendons, ligaments, synovial joint capsules and membranes, and a synovial joint cavity filled with synovial fluid. Joints are responsible for resisting mechanical stress/trauma, aiding in locomotion, and supporting the overall musculoskeletal system. Notably, collagen accounts for about 70% of the DM in the cartilage. Sufficient intake of dietary nutrients (e.g., amino acids, vitamin C, and minerals) is essential for maintaining healthy bones and joints while preventing disorders of bone (e.g., fractures and nutritional osteopathies) and joints (e.g., fractures and inflammation), which cause pain and reduce the quality of life (Connolly and Wu 2024).

Cysteine, glutamate, glutamine, glycine, proline, serine, and tyrosine have traditionally been considered NEAAs in animal nutrition, but these amino acids are physiologically essential for maintaining the growth, development, structure, integrity, and health of the skin and hair (Cao et al. 2021; Connolly and Wu 2024), as well as bone and joints (Lopez et al. 2024). In addition, taurine has also been considered an NEAA in omnivores and most breeds of dogs (Li and Wu 2023) but is nutritionally essential in human infants, cats, and possibly carnivorous fishes and crustaceans (Wu 2022a). Animal-sourced foodstuffs are useful for balancing amino acids and provide functional organic nutrients (e.g., taurine, creatine, and 4-hydroxyproline) as well as essential minerals (e.g., Ca, P, copper, and zinc) in the diets of animals to maintain the structure, function, and health of their specialized connective tissues (Li and Wu 2018, 2022). Note that plant-sourced foods contain relatively low amounts of cysteine, glycine, proline, and serine and lack taurine.

Nutritional Requirements for Optimum Brain Development and Cognitive Function

The brain is the central commander of all physical activities and the expression of emotions in mammals (e.g., dogs and cats; Wu 2024a). With postnatal development, animals acquire information from the environment and learn about conceptual skills, as well as memorize, analyze, retrieve, and use the knowledge; this process is defined as cognition. Brain development, cognitive health, and mood (an emotional state of mind or feeling in individuals) critically depend on the neural network, including neurons, glial cells (namely, nonneuronal cells), and neurotransmitters (communicators between neurons). The latter include L-glutamate, L-aspartate, glycine, γ-aminobutyrate, D-aspartate, D-serine, NO, CO, H_2S, dopamine, norepinephrine, epinephrine, and serotonin (He and Wu 2020). In addition, some non-neurotransmitter metabolites of amino acids, such as taurine, creatine, and carnosine, also play important roles in brain development, cognitive health, behavior, and mood of mammals and birds. Furthermore, animals require both ω3 (α-linolenic acid, EPA, and DHA) and ω6 (linoleic acid and arachidonic acid) PUFAs for the development of the central nervous system. All of these fatty acids must be provided in the diets of some animals (e.g., cats), whereas ω3 PUFAs and linoleic acid must be included in the diets of all animals. As an essential component of membranes of neurons and glial cells, cholesterol is also crucial for cognitive development and function. Thus, inadequate protein or lipid nutrition will lead to mood disorders. Some amino acids (e.g., arginine, glycine, methionine, serine, taurine, tryptophan, and tyrosine) can help to alleviate behavioral and mood disorders (e.g., depression, anxiety, and aggression). As abundant providers of all these functional amino acids and lipids, animal-sourced foods [e.g., liver (Table 11.29)], intestinal mucosa, and meat] may play important roles in the brain development, cognitive function, and mood of animals, particularly carnivores (e.g., dogs and cats). This may explain, in part, why dogs and cats prefer to eat visceral organs of their prey (Li and Wu 2024). In addition, dietary tryptophan (the precursor of serotonin and melatonin) confers mood-stabilizing and

TABLE 11.29
Concentrations of Taurine and Lipids in the Livers of Animals

	Concentrations in Liver (mg/100 g of Fresh Liver)			
Animal	**Taurine[e]**	**Cholesterol[e]**	**EPA**	**DHA**
Beef (grass-fed)	104	402	151	83
Chicken	115	558	16	43
Lamb	101	491	155	171
Pig	136	360	33	90
Rat	118	364	38	612

Source: Wu (2024).
Note: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

calming effects and prevents aggressive behavior in animals such as dogs, cats, and weanling pigs (Li et al. 2006; Wu 2024a). Collectively, adequate provision of all nutrients in all phases of the life cycle (pregnancy, lactation, postnatal growth, and adulthood) is essential for optimizing neurological health, while preventing cognitive dysfunction and abnormal behavior.

Cognitive function declines with aging in animals, including dogs and cats. Clinical symptoms in affected individuals include disorientation, social distancing, an abnormal sleep–wake cycle, wandering, alterations in activity levels and patterns, as well as increased anxiety, irritability, and aggression (Stockman 2024). For example, 28–30% of 11- to 14-year-old dogs and 48–68% of dogs over 15 years exhibit cognitive dysfunction syndrome (Azkona et al. 2009; Milgram et al. 1994). Similarly, 28% of 11- to 14-year-old cats and 50% of cats over 15 years show cognitive dysfunction symptoms (Gunn-Moore et al. 2007; Landsberg et al. 2010). The cognitive dysfunction syndrome appears to be irreversible after it is established and is associated with severe brain atrophy (including the loss of neurons). One of the pathogenic factors is the accumulation of a protein called β-amyloid, which creates toxic conditions for neurons. The development of this brain disorder may be alleviated or prevented by improving nutrition [e.g., adequate intakes of antioxidative amino acids (e.g., arginine, taurine, and tryptophan), minerals, vitamins (e.g., vitamins E and C) and related foods/nutrients (e.g., a mixture of fruits and vegetables, α-lipoic acid, and L-carnitine), as well as PUFAs (e.g., EPA and DHA) in dogs and cats (Cotman et al. 2002; Pan 2021; Pan et al. 2013, 2018a,b).

Nutritional Requirements for Optimal Immunity in Animals

Animals (particularly aquatic animals) are constantly exposed to various pathogens (including bacteria, viruses, parasites, and fungi) in the environment, particularly under intensive production systems aimed at improving the efficiency of use of resources (Dominguez 2020; Smith 2019; Washburn 2020; Zhou et al. 2020). Infectious diseases can result in poor appetite, impaired digestion and absorption of dietary nutrients, enhanced catabolism of nutrients, reductions in growth and production performance (e.g., weight gain, feed efficiency, egg yield, hatchability, male and female infertility, embryonic and fetal deaths), mortality, and carcass condemnation, leading to substantial economic loss. Some of the major pathogens infecting farm animals and causing disease outbreaks are summarized in Table 11.30. Examples are African swine fever virus, the porcine reproductive and respiratory syndrome (PRRS) virus (causing the blue ear disease), and porcine epidemic diarrhea virus in swine; bovine respiratory syncytial virus, the *Brucella bovis* bacterium, and the bovine viral diarrhea virus in cattle; and the highly pathogenic avian influenza, the Newcastle disease virus, and *Eimeria* spp. (a protozoan parasite causing coccidiosis) in poultry.

The function of immune systems and the health of animals critically depend on dietary intakes of energy and nutrients, including amino acids, ω3- and ω6-PUFAs, minerals, and vitamins (Field et al. 2002; Li and Wu 2022). Mammals, birds, and fish are protected from infections by both the innate (natural, nonspecific) and the acquired (adaptive, specific) immune systems (Chapter 1). Innate immunity is conferred by anatomic, physiological, phagocytic, and inflammatory barriers. The skin and mucosal barriers (major components of the innate system) consist of collagen that is rich in glycine, proline, and 4-hydroxyproline. NO, a product of arginine catabolism in macrophages (a component of the innate system), is the major killer of pathogens, including bacteria, parasites, fungi, and viruses. Adaptive immunity relies on both cellular immunity (cell-mediated immunity) and humoral immunity (antibody-mediated immunity). Cellular immunity requires T-cytotoxic cells ($CD8^+$ T-lymphocytes) and T helper cells ($CD4^+$ T-lymphocytes), with the latter synthesizing and secreting various cytokines. The humoral immunity is mediated by antigen-specific antibodies synthesized and released by memory B-lymphocytes and plasma B-cells. Note that the adaptive system mainly depends on proteins synthesized from amino acids. Many micronutrients (including vitamins and minerals) are also crucial for the production of antibodies. In contrast to vertebrates (mammals, birds, and fish), crustaceans (e.g., crabs and shrimp), like other invertebrates, lack an adaptive immune system and depend solely on the innate immune system to defend against infectious pathogens (Little et al. 2005). However, as indicated in Chapter 1, animals (including fish and crustaceans) can develop innate immune memory or trained immunity.

The innate and adaptive immune systems are highly interrelated through various kinds of T- and B-lymphocytes (also called T- and B-lymphocytes), as well as cytokines (proteins) and signaling molecules. The proliferation of these cells and the production of the immune-mediating large- and small-molecular-weight substances require amino acids, as noted previously. In addition, glutamine and glucose are major metabolic fuels for lymphocytes,

TABLE 11.30
Common Infectious Pathogens Affecting Farm Animals

Animal	Pathogen	Major Symptoms
Swine	African swine fever virus	High fever, red and blotchy skin, skin lesions, diarrhea, and cough
	H1N1 "swine flu" virus	Fever (not always), running or stuffy noses, red eyes, and cough
	Porcine circovirus type 2	Fever, skin rashes, stomach ulcers, diarrhea, and difficulty breathing
	Porcine epidemic diarrhea virus (PEDV)	Severe watery diarrhea, severe vomiting, and dehydration
	PRRS virus (a member of genus *Arterivirus*)	Blue ear disease (reproductive failure, pneumonia, and blue ear)
	Brachyspira hyodysenteriae (bacterium)	Swine dysentery disease (mucohemorrhagic diarrhea)
	Actinobacillus pleuropneumoniae (bacterium)	Respiratory disorders (e.g., fever, cough, and difficulty breathing)
	Hemophilus parasuis (bacterium)	Upper respiratory disorders (e.g., fever, cough, and difficulty breathing)
	Mycoplasma hyopneumoniae (bacterium)	Respiratory disorders (e.g., fever, cough, and difficulty breathing)
	Erysipelothrix rhusiopathiae (bacterium)	Erysipelas (bright red skin), low fever, fluid-filled blisters
	Ascaris (parasite)	Found in the small intestine, resulting in irritation and malnutrition.
	Metastrongylus (lungworm, parasite)	Found in the respiratory tract, resulting in breathing difficulty
Cattle	Bovine parainfluenza virus	Usually mild, fever, nasal discharge, dry cough, and difficulty breathing
	Bovine respiratory syncytial virus	High fever, cough, nasal discharge, and difficulty breathing
	Bovine viral diarrhea virus types 1 and 2 (PI)	Fever, nasal discharge, oral lesions, diarrhea, pneumonia, and abortion
	Bovine herpes virus 1 (BoHV-1)	Fever, red nose, salivation, red and watery eyes, and difficulty breathing
	Bovine campylobacter (bacterium)	Uterine infection, infertility, abortion, and long calving interval
	Bovine leptospira (bacterium)	High fever in calves, blood infection, anemia, red urine, and abortion
	Brucella bovis (bacterium)	Infertility, abortion, vaginal discharge, and birth of weak calves
	Clostridium chauvoeia (bacterium)	High fever, rapid breathing, lameness, and swelling of legs
	Clostridium haemolyticum (bacterium)	Hemolysis, hemoglobinuria (red urine), and necrosis in tissues
	Clostridium novyia (bacterium)	Pale mucous membranes, difficulty breathing, ascites, and weakness
	Clostridium perfringens (bacterium)	Enterotoxemia (or purple gut), severe diarrhea, and hemorrhage
	Clostridium septicum (bacterium)	High fever, malignant edema, and swelling of legs around the wound
	Clostridium sordellii (bacterium)	Severe diarrhea, blacklegs, subcutaneous fascia, and fatal toxemia
	Clostridium tetani (bacterium)	Spasms, stiffness of skeletal muscle, and inability to breathe
Poultry	Avian encephalomyelitis virus	Ataxia, leg weakness, paralysis, and tremors of the head and neck
	Avian influenza (bird flu) virus	Fever, diarrhea, nasal discharge, coughing, sneezing, and weakness
	Avian leukosis virus (a very rare virus)	Diarrhea, dehydration, lesions in internal organs, and weakness
	Avian metapneumovirus	Swollen head, nasal discharge, torticollis, and opisthotonos
	Duck adenovirus 1	Anorexia, diarrhea, and cloacal hemorrhage
	Infectious bursal disease virus (IBDV)	Trembling, watery diarrhea, ruffled feathers, dehydration, vent pecking
	Marek's disease virus	Paralysis, anemia, dehydration, and sometimes diarrhea
	Newcastle disease virus	Muscular tremors, droopy wings, opisthotonus, circling, and paralysis
	Chlamydophila psittaci (bacterium)	Fever, nasal and ocular discharge, ruffled feathers, and weakness
	Mycobacterium avium (bacterium)	Emaciation, diarrhea, and atrophy of breast muscle; lung lesions are rare
	Mycoplasma gallisepticum (bacterium)	Runny nose, cough, swollen eyelids, pale comb, and blotchy skin
	Pasteurella multocida (bacterium)	Cholera (lameness, swollen wattles in chickens, pneumonia in turkeys)
	Salmonella (bacterium)	Dehydration, diarrhea, poor growth, and weakness
	Ascaridia galli (parasite)	Anorexia, diarrhea, dehydration, stunted growth, and ruffled feathers
	Eimeria spp. (a parasite causing coccidiosis)	Diarrhea, poor growth and egg production, and ruffled feathers
	Leucocytozoon spp. (parasite)	Anemia, anorexia, diarrhea with green droppings, and tachypnea
FW fish	*Aeromonas salmonicida* (bacterium)	Furuncles, hemorrhage, pale gills, skin ulceration, and erratic swimming
	Anchor worms (parasites)	Visible worms on scales, skin patches and red lesions, and hemorrhage
	Edwardsiella ictalurid (bacterium)	Swelling and ulceration of the head, erratic swimming, and hemorrhage
	Edwardsiella tarda (bacterium)	Loss of pigmentation, opacity of eyes, exophthalmia, and swelling
	Flavobacterium columnare (bacterium)	Skin lesions, fin erosion, gill necrosis, and white patches or spots in head
	Ichthyophonus hoferi (fungus)	Light gray, cottony growths on the skin, fins, gills, and eyes; spiralling
	Ichthyobodo (a parasitic protozoa)	Anorexia, flashing behavior, skin in steel-grey color, and blue mucus
	Fish lice (external parasites)	Hemorrhages, anemia, fin and scale loss, and erratic swimming
	Gill mites (common parasite)	Red and distended gills, gasping for breath, staying near water surface
	Hexamita (protozoa)	Lesions on the head and flanks, hole in the head, pale gills, hemorrhage
	Mycobacterium marinum (bacterium)	Skin lesions or ulcers, possibly bulging eyes and enlarged abdomen
	Oödinium limneticum (parasite)	Scratching, color loss, prickly scales, and clamped fins (velvet disease)
	Piscine novirhabdovirus (virus)	Hemorrhagic septicemia, anemia, pale gills, and erratic swimming
	Vibrio anguillarum (bacterium)	Red spots in the skin, swollen and dark skin lesions, and hemorrhage

Note: FW, freshwater; PI, persistently infected; PRRS, porcine reproductive and respiratory syndrome.

macrophages, and other cells of the immune system to support their integrity and function. Thus, adequate intakes of amino acids (particularly arginine, cysteine, glutamine, glycine, taurine, and tryptophan), fatty acids (particularly ω3 and ω6 PUFAs), minerals (particularly copper, iron, manganese, selenium, and zinc), and vitamins (particularly lipid-soluble vitamins) are essential for the development and function of the immune systems.

There are anatomic differences in the immune systems between terrestrial and aquatic animals (Hissen et al. 2023). In humans and other terrestrial mammals, the primary lymphoid organs include the thymus and the bone marrow, whereas the secondary lymphoid organs consist of the lymph nodes, spleen, and certain tissues in various mucous membrane layers [such as gut-associated lymphoid tissue (GALT) and mucosa-associated lymphoid tissue (MALT)] in the body. The thymus produces and matures T-lymphocytes so that they express specific T-cell markers, whereas the bone marrow (and the liver in fetus) generates B-lymphocytes that subsequently migrate to the thymus for maturation. Thymosin, a peptide hormone synthesized by the thymus gland, is necessary for the production and development of T cells within this organ. The secondary lymphoid organs house mature naive lymphocytes and initiate an adaptive Immune response to infectious pathogens. In birds, the primary lymphoid organs include not only the thymus and bone marrow but also the Bursa of Fabricius (the major site for the differentiation of B lymphocytes), whereas the secondary lymphoid organs consist of the spleen, GALT, MALT, and diffuse lymphoid tissues. In fish, which lack bone marrow and lymph nodes, the thymus and the head kidney are the primary lymphoid organs, whereas the spleen, GALT, and MALT are secondary lymphoid organs. In addition, teleost fish (e.g., hybrid striped bass and largemouth bass) have plasma cells (from B cells) that produce Igs of three isotypes: IgM, IgD, and IgT/IgZ. IgM are the primary Ig in serum, whereas IgT and IgZ are mucosa-specific Ig analogous to mammalian IgA and are present primarily in the intestine and skin mucosal tissues (Hissen et al. 2023).

Methods for Estimating Dietary Requirements of Animals for Nutrients

Feeding trials are necessary to determine dietary requirements of animals for nutrients. A dietary requirement can be defined as the amount or content of a dietary nutrient that yields the maximum response in production variables (e.g., BW gain, feed efficiency, milk production, egg laying, or litter size) in the animals. Reliable methods are needed to assess the dose-dependent response of animals to graded levels of a test nutrient in diets (Pesti et al. 2009). Such methods include (a) multiple comparison tests, (b) broken-line linear ascending (BLLA), (c) broken-line quadratic ascending (BLQA), (d) quadratic polynomials, and (e) saturation kinetics (Figure 11.17), depending on experimental design and data characteristics. The experimental animals may show a linear (first-order) or quadratic (second-order) increase in response for variables (e.g., BW gain and feed efficiency) to the increased dietary intakes of a test nutrient until a plateau (maximum response) is reached. At the plateau (namely, zero-order response), there are no additional changes for variables in response to increased dietary intakes of the test nutrient. Thereafter, the animals may exhibit a decrease in production variables in response to the excess intakes of the test nutrient due to its adverse or toxic effects.

A multiple comparison test [e.g., Tukey, Student-Newman-Keuls, or Duncan] is the post hoc test in the analysis of

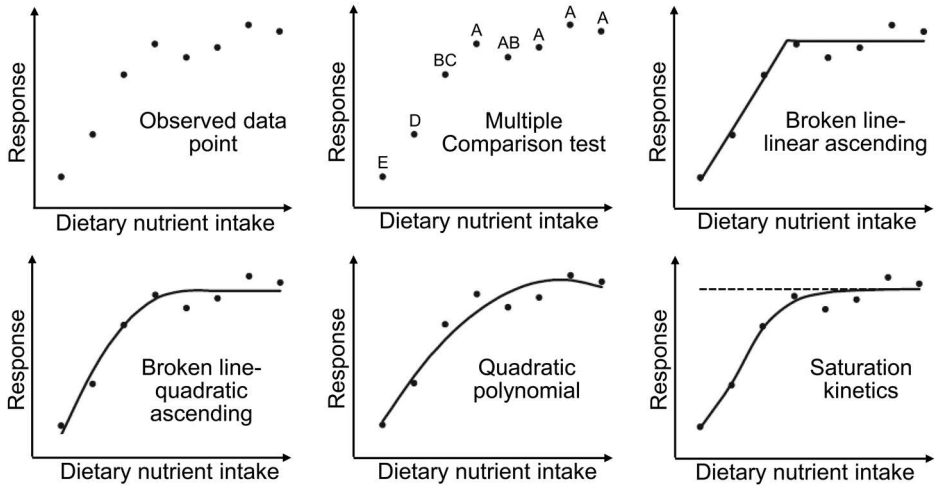


FIGURE 11.17 Methods for estimating dietary requirements of animals for nutrients. Animal feeding trials are conducted to measure dose-dependent responses for variables to graded levels of one or more nutrients in diets. Results can be used to estimate the dietary requirements of animals for nutrients based on the multiple comparison test, broken-line linear ascending (BLLA), broken-line quadratic ascending, quadratic polynomials, or saturation kinetics, depending on experimental design and data characteristics. In the multiple comparison test (e.g., the Student-Newman-Keuls, Duncan, or Tukey test), means not sharing the same letter differ statistically. Each method has its advantages and disadvantages. The BLLA model is commonly applied to assess dietary nutrient requirements. (Adapted from Pesti et al. 2009.)

variance (ANOVA), such as 1-way ANOVA for a single-factorial experimental design, to identify differences among the means of various treatment groups. The highest mean value for one group or a set of several groups is considered the dietary requirement for experimental animals. The Student-Newman-Keuls test is more stringent than the Duncan test and more powerful than the Tukey method in detecting significant differences among the means of treatment groups. Multiple comparisons are easily executed using software packages and conclusive, but yield a requirement value corresponding to only one level of dietary nutrient intake and lack precision.

The BLLA model is most commonly applied to assess dietary nutrient requirements. The slopes of the ascending and maximum (plateau) lines are 1 and 0, respectively (or statistically not different from 1 and 0, respectively). The inflection (break point) in the curve represents the dietary requirement for the test nutrient. This method closely reflects the classical concept for identifying nutrient requirements by animals and gives a requirement value that is more precise than the multiple comparisons test. In addition, the BLLA model fits very well the ascending linear (first order) and plateau portions of the response curve. However, more graded levels of a nutrient in diets are needed to better estimate the ascending and maximum (plateau) responses in the BLLA model. In addition, this model does not have a feature to fit any decreasing response data. Such a disadvantage is not an issue because responses to toxic levels of dietary nutrients are normally not target variables in experiments for determining optimal nutritional requirements.

The BLQA model offers the same advantages and disadvantages as the BLLA model. For example, the BLQA model satisfactorily fits the ascending quadratic (second-order) and plateau portions of the response curve. However, it is mathematically more complicated for the BLQA model to fit data in the ascending quadratic portion of the broken line than the BLLA model for the ascending linear portion of the response curve. Nonetheless, the BLQA model is more appropriate than the BLLA model to statistically analyze data with ascending quadratic characteristics.

Quadratic polynomials are useful to statistically analyze data with second-order characteristics that show increases in response for production variables with increasing dietary nutrient intakes within a certain range and then exhibit decreases in response to additional increases in dietary nutrient intakes. The GLM Procedure of the SAS uses the ordinary least squares method to fit second-order polynomial models. Quadratic polynomials are simple to use, fit the "increase" and "decrease" portions of the response curve well, and include features or parameters to identify toxic levels of excess nutrients in diets. However, this method cannot be applied to the statistical analysis of data in the plateau portion of the response curve.

Saturation kinetics are applied in the analysis of response data having a sigmoidal relationship between a measured variable (e.g., BW gain or feed efficiency) and dietary nutrient intake. This is analogous to the Michaelis-Menten kinetics in enzymatic reactions showing a sigmoidal relationship between velocity and substrate concentration (Chapter 1). For such data, a double-reciprocal plot, with 1/[Dietary nutrient intake] as the X-axis and 1/[Response] as the Y-axis, can reveal an intersection point (1/[Maximum response]) in the Y-axis. For fitting data that have the kinetic order of response not being equal to 1, the saturation kinetics model can include features such as an intercept and kinetic orders (e.g., 2 or 3). The NLIN Procedure of SAS can be used to fit nonlinear (e.g., sigmoidal) models. The saturation kinetics model can more accurately describe complex sigmoidal responses of test animals to dietary nutrient intakes than linear models based on putatively straight lines. However, this method calls for more levels of the test nutrient in diets to better estimate the ascending and maximum responses than with polynomials. In addition, it is more difficult to fit complex nonlinear models than simple polynomials. Furthermore, the saturation kinetics model cannot identify toxic levels of excess nutrients in diets.

METHODS FOR RATION FORMULATION

The feeding of animals often requires ration formulation, which involves the selection and combination of feed ingredients to meet the nutritional requirements of animals. The goal is to manufacture nutritive and palatable diets at the lowest possible cost that can support the maintenance, as well as optimum growth, development, productivity, feed efficiency, and health of animals. This requires sufficient knowledge of (a) the availability, price, and nutrient composition of feed ingredients; (b) nutritional requirements, physiological or pathological states, feed intake, and the desirable production level of animals; (c) priority for the balancing of dietary nutrients; and (d) biosecurity requirements on farms and housing facilities. Depending on the number of feed ingredients, skills, and feasibility, three methods have been used widely in ration formulation: the Pearson square, substitution, and computer-assisted linear programming (Gadberry 2019; Luce 2016). Feed intake can be expressed on a DM or as-fed basis, but the unit must be consistent in all calculations.

Pearson Square Method

This method, also called the box method, is useful for formulating a diet composed of a few ingredients. We can use, as an example, a corn- and SBM (44% CP)-based diet (providing sufficient nutrients, including 0.71% lysine and 0.46% Ca; as-fed basis) for the 100- to 135-kg finishing pig consuming a 2.93-kg diet (as-fed basis) per day. Corn grain and SBM contain 0.26% and 2.83% lysine, respectively.

Because lysine is a limiting amino acid for finishing pigs, we first consider balancing the content of lysine in a ration. In the formulation procedure, the ration consists of fixed and variable portions. The fixed portion (e.g., 8%) of the ration generally includes nonenergy and nonprotein feed ingredients, such as alfalfa, rice bran, wheat middlings, salt, vitamin premix, mineral premix, dicalcium phosphate, and ground limestone (Ca carbonate). The variable portion

(e.g., 92%) of the ration generally includes energy and protein feed ingredients, e.g., corn grain and SBM.

Lysine needed in 100 g of the ration = 0.71 g

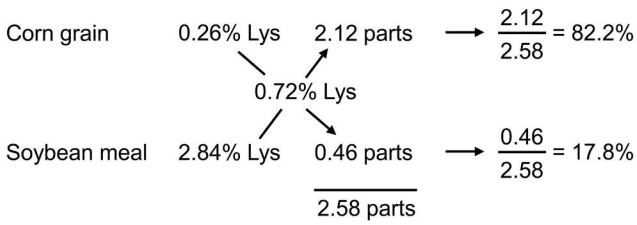
Lysine provided from 8 g of the fixed portion of the ration = 0.05 g

Lysine provided from 92 g of the variable portion of the ration = 0.66 g (i.e., 0.71 g − 0.05 g)

Lysine content in the variable portion of the ration = (0.66 g / 92 g) × 100 = 0.72%

Take the following steps in the calculation:

a. Place the value of lysine content (0.72%) in the variable portion of the ration in the center of the square.
b. Place the values of lysine content in corn grain (0.26%) and SBM (2.84%) on the left side of the square.
c. Subtract, on the diagonal, the smaller number from the larger number.
d. Place the absolute values of the across-diagonal differences (2.12 parts and 0.46 parts) on the right side of the square, and place the value of their sum (2.58 parts; i.e., 2.12 + 0.46) below the square.
e. Divide the two numbers on the right side of the square by their sum (i.e., 2.58) to yield the percentages of corn grain (82.2%) and SBM (17.8%) in the variable portion of the ration.



Thus, as calculated below, the final 100-g ration contains 75.62 g of corn grain and 16.38 g of SBM.

Content of corn grain in the final ration: 82.2% × 0.92 = 75.62%

Content of SBM in the final ration: 17.8% × 0.92 = 16.38%

Because plant-sourced feeds are often deficient in Ca, we next consider balancing the content of this macro-mineral in the diet by using Ca ingredients [dicalcium phosphate ($CaHPO_4$; containing 29.5% Ca by weight) and Ca carbonate ($CaCO_3$; containing 40.0% Ca)] and the Pearson square method. The total amount of Ca ingredients in the fixed portion of the ration is 1.75 g.

Ca needed in 100 g of the ration = 0.46 g

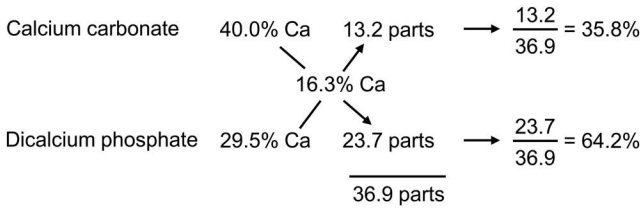
Ca provided from 92 g of the variable portion of the ration = 0.075 g

Ca provided from 8 g of the fixed portion of the ration except for Ca-rich ingredients = 0.10 g

Ca needed from Ca-rich ingredients in the fixed portion of the ration = 0.285 g (i.e., 0.46 − 0.075 − 0.10)

Ca content in 1.75 g of Ca-rich ingredients = (0.285 g / 1.75 g) × 100 = 16.3%

We can do Pearson square calculations as described previously for lysine, yielding 35.8% Ca carbonate and 64.2% dicalcium phosphate in 1.75 g of the fixed portion of the ration. This corresponds to 0.63 g of Ca carbonate and 1.12 g of dicalcium phosphate in the 8-g fixed portion of the 100-g ration.



Amount of Ca carbonate in the 8-g fixed portion of the ration: 35.8% × 1.75 g = 0.63 g

Amount of dicalcium phosphate in the 8-g fixed portion of the ration: 64.2% × 1.75 g = 1.12 g

Content of Ca carbonate in the final ration: (0.63 g / 100 g) × 100 = 0.63%

Content of dicalcium phosphate in the final ration: (1.12 g / 100 g) × 100 = 1.12%

Substitution Method

This method is based on the substitution of a portion of an estimated ration for another ingredient to correct nutrient deficiencies. Let's use again, as an example, a corn- and SBM-based diet for the 100- to 135-kg finishing pig consuming a 2.93-kg diet (as-fed basis) per day. The recommended dietary content of ME is 3300 kcal/kg diet (as-fed basis). Because corn grain is the major source of energy for growing swine, we first consider this ingredient (containing 3420 kcal ME/kg; as-fed basis) and assume that it provides all the needed ME in the diet. The following calculation indicates that corn grain alone exceeds the requirement of finishing pigs for energy by 3.6%.

Dietary energy requirement: 3300 kcal ME/kg diet × 2.93 kg diet/pig/day = 9669 kcal ME/pig/day

Energy from corn grain: 3420 kcal ME/kg diet × 2.93 kg diet/pig/day = 10021 kcal ME/pig/day

Next, we consider dietary lysine intake by the finishing pigs, because corn grain is severely deficient in this limiting amino acid. As illustrated below, a comparison with dietary lysine requirements reveals a deficit of 13.2 g of lysine/pig/day. Thus, adding a protein ingredient is necessary to balance dietary lysine.

Total lysine from corn grain: 2.6 g/kg × 2.93 kg = 7.62 g/pig/day

Dietary lysine requirement: 7.1 g/kg diet × 2.93 kg diet = 20.8 g/pig/day

Deficit of lysine intake = 20.8 g/pig/day − 7.62 g/pig/day = 13.2 g/pig/day

SBM is a good source of amino acids and is therefore substituted for a portion of corn grain. Because 1 kg of corn grain or SBM provides 2.6 or 28.4 g of lysine, respectively, replacing 1 kg of corn grain with 1 kg of SBM results in a net gain of 25.8 g of lysine (i.e., 28.4 – 2.6 = 25.8).

13.2 g of lysine needed ÷ 25.8 g of lysine gained from 1 kg of SBM = 0.512 kg SBM

Alternatively, the amount of SBM needed to provide sufficient lysine: 13.2 g ÷ (28.4 g − 2.6 g) /kg = 0.512 kg

Corn grain in the ration = 2.93 kg − 0.512 kg = 2.418 kg

Furthermore, we must balance Ca in the ration because a corn- and SBM-based diet is deficient in Ca. As illustrated below, a comparison with dietary Ca requirement reveals a deficit of 11.1 g of Ca/pig/day. Thus, adding a Ca ingredient is necessary to balance dietary Ca.

Total Ca from 2.418 kg of corn grain plus 0.512 kg of SBM: 0.3 × 2.418 + 3.2 × 0.512 = 2.36 g/pig/day

[Ca content in this mix = 2.36/(2418 + 512) = 0.08% (i.e., 0.8 g Ca/kg mix)

Dietary Ca requirement: 4.6 g/kg diet × 2.93 kg diet = 13.5 g/pig/day

Deficit of Ca intake = 13.5 g/pig/day − 2.36 g/pig/day = 11.1 g/pig/day

Let's assume that only one Ca-sourced ingredient (i.e., dicalcium phosphate) is used to provide the needed Ca. Thus, dicalcium phosphate is substituted for a portion of the corn grain plus SBM mix. As noted above, 1 kg of dicalcium phosphate provides 295 g of Ca. As explained previously for lysine, the amount of dicalcium phosphate needed to offset a dietary Ca deficit: 11.1 g ÷ (295 g – 0.8 g)/kg = 0.038 kg. This value accounts for 1.3% [i.e., (0.038/2.93 × 100) of the corn grain plus SBM mix. For this reason, the amounts of corn grain and SBM in the final ration are summarized as follows.

Corn grain in the ration: 2.418 kg × (100 − 1.3)/100 = 2.387 kg/day or 81.5% [i.e., (2.387/2.93) × 100]

SBM in the ration: 0.512 kg × (100 − 1.3)/100 = 0.505 kg/day or 17.2% [i.e., (0.505/2.93) × 100]

Dicalcium phosphate in the ration = 0.038 kg or 1.3% [i.e., (0.038/2.93) × 100]

Computer-Assisted Program

The Pearson square or substitution method is neither simple nor precise for formulating a ration with multiple feed ingredients. The composition of feed ingredients in the final ration should be adjusted according to the possible inclusion of additional components (e.g., NaCl, vitamin premix, and mineral premix). This process requires complex and tedious procedures. In such a case, the initial estimated values from the Pearson square or substitution method can provide a useful basis for precise ration formulation using a computer-assisted program such as an Excel spreadsheet. Essential information such as feed ingredients, their nutrient composition and digestibilities, their energy content (e.g., total or ME), and the nutritional requirements of animals is carefully entered into an Excel spreadsheet. Formulas for calculations can be built into the program to eliminate mathematical mistakes and human errors. A program operator should know the meanings of special symbols, such as "=" for equal to; "+" for addition; "–" for subtraction; "*" for multiplication; "/" for division; "%" for percentage; "^" for exponential power; "sqrt" for square root; "sum(A1:A6)" for the sum of values from rows 1 to 6 of column A; "sum(A1,A3,A6)" for the sum of values in rows 1, 3, and 6 of column A; "average(A1:A6)" for the mean of values in rows 1 to 6 of column A; and "average(A1:A3:A6)" for the mean of values in rows 1, 3, and 6 of column A.

The Excel spreadsheet can simultaneously show the balances of all nutrients in the ration. This computer-assisted program is simple, accurate, and robust for ration formulation and can be readily automated with reliable outputs. Based on new expected values for animal growth performance (e.g., daily weight gain and feed efficiency) and productivity (e.g., egg, milk, wool, and semen production, as well as meat quality), input parameters in the computation formulas can be easily revised to obtain desirable diets.

METHODS FOR ANIMAL FEEDING

Proper feeding (e.g., meal size and frequency, as well as nutritional, chemical, and physical properties of food) is essential for animals to obtain sufficient and balanced amounts of all nutrients for survival, health, immunity, growth, development, and reproduction. This is the scientific foundation for profitable and sustainable animal agriculture worldwide. Livestock, birds, fish, and crustaceans can be fed once, twice, three times, or more frequently daily through one or a combination of five methods: scheduled, full, restricted, free-access, and supplemental feeding, depending on animal species and production stage, as well as the availability of labor and farm facilities. For example, gestating swine can be fed a restricted amount of food (e.g., 2 kg/day) once or twice daily at approximately the same time(s) each day (Wu et al. 2010). Second, lactating sows are fed a full amount of their diets twice daily (Rezaei et al. 2022a,b). Third, preweaning piglets are naturally nursed by their mothers every 1 or 1.5 h. Interestingly, results of recent research indicate that intermittent (pulse) feeding is more effective than continuous feeding to increase muscle protein synthesis and whole-body growth in neonatal pigs maintained on total parenteral nutrition (El-Kadi et al. 2020). This is due to a sufficient accumulation of amino acids (including leucine, arginine, glutamine, and glycine) to activate the MTOR cell signaling (Suryawan et al. 2021). Fourth, postweaning pigs have free access to their diets between weaning (e.g., 5.5 kg) and a market weight (e.g., 130 kg; He et al. 2023a).

There is growing interest in properly feeding companion animals due to their increasing incidence of obesity. Dogs can consume a large meal within 10 min that is sufficient to meet daily nutrient requirements, but they usually eat 4 to 8 meals per day (Bradshaw 2006). Growing puppies should have free access to food or be fed 2–3 times daily. Young and adult dogs generally ingest food and drink water during the light period and may eat at night if they are hungry or dehydrated, but some breeds of dogs also eat and drink during the dark period (Mugford and Thorne 1980). Although adult dogs may do well on a regimen of eating one meal per day with possible health benefits, these animals should be offered foods at least twice daily to ensure adequate nutrition and welfare (Cline et al. 2021). Multiple meals per day may help dogs alleviate boredom while reducing the risk for the problem of gastric dilatation-volvulus in susceptible breeds of dogs. If overweight or obese, they should be fed either less or lower energy-dense diets. Caloric restriction may reduce oxidative stress and delay aging in dogs (Lawler et al. 2008). Of particular note, intermittent fasting on a ketogenic diet containing medium-chain triglycerides may confer both metabolic and immunological effects in healthy dogs (Leung et al. 2020). Dogs with special health issues or dietary needs may require individualized feeding programs.

In contrast to dogs, cats do not have clearcut circadian rhythms. Some cats are nocturnal (e.g., resting during the daytime and going out or walking at night), and their existing rhythms can be altered by minor disturbances such as human noises (Macdonald and Apps 1978). Cats generally eat frequent, small meals and drink water throughout the day and night and can voluntarily eat 12–20 meals/day, but fresh food should be offered daily (Kane et al. 1981). After they adapt to a new diet for 1 week, the size and number of meals are not affected by the type of diet. When cats eat a big portion of food at one time, they may immediately vomit it due to gastric irritation. Therefore, appropriate and frequent meals are critical for feline health. Pet owners can decide meal size and frequency for their companion animals based on breed, age, health condition, ambient environment, and practicality rather than relying simply on food availability.

Camara et al. (2020) reported that compared with the feeding of the same canned food four times/day, adult cats fed only once daily had greater plasma concentrations of glucagon-like protein-1, gastric inhibitory protein, and some amino acids within the initial postprandial hours (as expected because of a greater intake of nutrients at a single time), but a lower value for the respiratory quotient (RQ) in the fasting state. These authors interpreted the data to indicate that a reduced RQ value may result from a greater rate of whole-body fatty acid oxidation and that feeding once daily may be a beneficial management strategy for indoor cats to promote satiation and lean body mass. However, a low RQ value in the fasting state may also result from a greater rate of the conversion of amino acids (released from the breakdown of muscle protein; possibly an adverse effect) into glucose, because this process has an RQ value of 0.333 (Chapter 8).

MOST COMMON MISCONCEPTIONS REGARDING ANIMAL NUTRITION

Due to an inadequate understanding of nutrition and metabolism, there are several common misconceptions about animal nutrition among producers. First, animals (e.g., poultry and swine) do not have dietary requirements for NEAAs. As noted previously, NEAAs must be sufficient in diets to optimize the health, growth and production performance, and feed efficiency of animals. Second, diets for animals (e.g., poultry and swine) must be formulated according to the "ideal protein" concept. This nutritional concept might have been ideal in the 1960s–1990s when our knowledge of cell- and tissue-specific metabolism of amino acids in animals was limited (Kim et al. 2005), but is now recognized to be flawed in livestock, poultry, fish, and crustacean nutrition (Wu and Li 2022).

Third, energy is a nutrient, and glucose plus fatty acids provide most energy for every tissue and every cell type in all animals. Energy is not a nutrient but is contained

in macronutrients, primarily protein, amino acids, lipids, and starch. Amino acids, fatty acids, and glucose have different metabolic fates and different physiological roles in a tissue- and cell-specific manner. The proportions of energy from these three types of macronutrients can affect the metabolic profiles and health (e.g., obesity development) of animals, such as poultry and swine. The sources of energy substrates vary with cell types. For example, sufficient glutamine, glutamate, and aspartate (primary sources of energy in mammalian fish, shrimp, and crab enterocytes) must be provided in diets to ensure the health of the small intestine (He and Wu 2022). In the case of poultry, adequate glutamate and aspartate are also important as the primary sources of energy in their enterocytes, whereas dietary glutamine undergoes only limited degradation in the small intestine and is effectively available to skeletal muscle for ATP production (He et al. 2021, 2022).

Fourth, recommendations for nutrient requirements must be strictly followed in the formulation of diets. This is not true because these values only serve as guidelines for practice. As noted previously, animals have dynamic requirements for nutrients, particularly amino acids, under various physiological, pathological, and environmental conditions. Animals (e.g., poultry and swine) of different breeds or genetic backgrounds may have different capacities for growth (e.g., rate of muscle gain) and production (litter size, as well as egg size and quality) and, therefore, different requirements for dietary nutrients. Fifth, monogastric animals (e.g., poultry and swine) only need grains or vegetables to thrive. These animals are omnivores rather than herbivores and can consume both plant-sourced feedstuffs and animal byproducts. Without any supplements, grains or vegetables alone cannot support the maximum growth or production performance of poultry and swine. Their diets must provide all nutrients in proper amounts and balance, such as amino acids (including lysine, methionine, glutamine, glycine, and Pro), essential fatty acids (e.g., ω3 and ω6 PUFAs), vitamins (e.g., vitamins A, D, E, and K, as well as biotin, pyridoxine, and folic acid), and minerals (e.g., Ca, P, iron, and zinc). As a reference, NRC (2012)-recommended nutrient requirements of swine at different stages of production are summarized in Table 11.31.

TABLE 11.31
Total Requirements (Maintenance Plus Production) of Swine for Nutrients at Different Stages of Production

	Growing Pigs at Different Body-Weight Range (kg)							G Sows (P1)[a]		G Sows (P4+)[b]		La sows[c]	
Variable	**5–7**	**7–11**	**11–25**	**25–50**	**50–75**	**75–100**	**100–135**	**<d90**	**>d90**	**<d90**	**>d90**	**P1**	**P2+**
Dietary energy content, kcal/kg diet (as-fed basis, 90% DM)													
NE	2448	2448	2412	2475	2475	2475	2475	2518	2518	2518	2518	2518	2518
DE	3542	3542	3490	3402	3402	3402	3402	3388	3388	3388	3388	3388	3388
ME	3400	3400	3350	3300	3300	3300	3300	3300	3300	3300	3300	3300	3300
Feed intake (FI, as-fed basis), body weight (BW) gain, and body protein (BP) deposition, g/day													
FI + wastage	280	493	953	1582	2229	2636	2933	2130	2530	2200	2600	5.95	6.61
BW gain	210	335	585	758	900	917	867	578	543	410	340	71	176
BP deposition	---	---	---	128	147	141	122	---	---	---	---	---	---
Dietary content of total amino acid and total crude protein (CP), % (as-fed basis)													
Arginine	0.75	0.68	0.62	0.50	0.44	0.38	0.32	0.31	0.40	0.20	0.27	0.48	0.47
Histidine	0.58	0.53	0.48	0.39	0.34	0.30	0.25	0.21	0.25	0.13	0.17	0.35	0.34
Isoleucine	0.88	0.79	0.73	0.59	0.52	0.45	0.39	0.34	0.41	0.22	0.28	0.49	0.47
Leucine	1.75	1.54	1.41	1.13	0.98	0.85	0.71	0.52	0.71	0.34	0.50	0.96	0.92
Lysine	1.70	1.53	1.40	1.12	0.97	0.84	0.71	0.58	0.76	0.37	0.52	0.86	0.83
Methionine	0.49	0.44	0.40	0.32	0.28	0.25	0.21	0.17	0.22	0.11	0.14	0.23	0.23
Met + Cys	0.96	0.87	0.79	0.65	0.57	0.50	0.43	0.39	0.51	0.27	0.38	0.47	0.46
Phenylalanine	1.01	0.91	0.83	0.68	0.59	0.51	0.43	0.32	0.42	0.21	0.30	0.47	0.46
Phe + Tyr	1.60	1.44	1.32	1.08	0.94	0.82	0.70	0.58	0.75	0.39	0.53	0.98	0.94
Threonine	1.05	0.95	0.87	0.72	0.64	0.56	0.49	0.44	0.55	0.32	0.42	0.58	0.56
Tryptophan	0.28	0.25	0.23	0.19	0.17	0.15	0.13	0.10	0.14	0.07	0.11	0.16	0.15
Valine	1.10	1.00	0.91	0.75	0.65	0.57	0.49	0.42	0.55	0.29	0.40	0.75	0.72
CP (N x 6.25)	22.7	20.6	18.9	15.7	13.8	12.1	10.4	9.6	12.8	6.8	9.6	20.5	17.9

(*Continued*)

TABLE 11.31 (*Continued*)
Total Requirements (Maintenance Plus Production) of Swine for Nutrients at Different Stages of Production

	Growing Pigs at Different Body-Weight Range (kg)							G Sows (P1)[a]		G Sows (P4+)[b]		La sows[c]	
Variable	**5–7**	**7–11**	**11–25**	**25–50**	**50–75**	**75–100**	**100–135**	**<d90**	**>d90**	**<d90**	**>d90**	**P1**	**P2+**
Dietary content of total minerals, % or amount/kg diet (as-fed basis)													
Calcium, %	0.85	0.80	0.70	0.66	0.59	0.52	0.46	0.58	0.79	0.41	0.64	0.59	0.57
Phosphorus, %	0.70	0.65	0.60	0.56	0.52	0.47	0.43	0.47	0.58	0.36	0.49	0.53	0.52
Sodium, %	0.40	0.35	0.28	0.10	0.10	0.10	0.10	0.15	---	---	---	0.20	---
Chloride, %	0.50	0.45	0.32	0.08	0.08	0.08	0.08	0.12	---	---	---	0.16	---
Magnesium, %	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.06	---	---	---	0.06	---
Potassium, %	0.30	0.28	0.26	0.23	0.19	0.17	0.17	0.20	---	---	---	0.20	---
Copper, mg/kg	6.00	6.00	5.00	4.00	3.50	3.00	3.00	10	---	---	---	20	---
Iodine, mg/kg	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	---	---	---	0.14	---
Iron, mg/kg	100	100	100	60	50	40	40	80	---	---	---	80	---
Mn, mg/kg	4.00	4.00	3.00	2.00	2.00	2.00	2.00	25	---	---	---	25	---
Se, mg/kg	0.30	0.30	0.25	0.20	0.15	0.15	0.15	0.15	---	---	---	0.15	---
Zinc, mg/kg	100	100	80	60	50	50	50	100	---	---	---	100	---
Dietary content of vitamins, IU or amount/kg diet (as-fed basis)													
Vitamin A, IU	2200	2200	1750	1300	1300	1300	1300	4000	---	---	---	2000	---
Vitamin D, IU	220	220	200	150	150	150	150	800	---	---	---	800	---
Vitamin E, IU	16	16	11	11	11	11	11	44	---	---	---	44	---
Menadione, mg	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	---	---	---	0.50	---
Biotin, mg	0.08	0.05	0.05	0.05	0.05	0.05	0.05	0.20	---	---	---	0.20	---
Choline, mg	600	500	400	300	300	300	300	1250	---	---	---	1000	---
Folacin, mg	0.30	0.30	0.30	0.30	0.30	0.30	0.30	1.3	---	---	---	1.3	---
Niacin, mg	30	30	30	30	30	30	30	10	---	---	---	10	---
PA, mg	12	10	9.0	8.0	7.0	7.0	7.0	12	---	---	---	12	---
Riboflavin, mg	4.0	3.5	3.0	2.5	2.0	2.0	2.0	3.75	---	---	---	3.75	---
Thiamin, mg	1.5	1.0	1.0	1.0	1.0	1.0	1.0	1.0	---	---	---	1.00	---
Vitamin B_6, mg	7.0	7.0	3.0	1.0	1.0	1.0	1.0	1.0	---	---	---	1.00	---
Vitamin B_{12}, μg	20	17.5	15	10	5.0	5.0	5.0	15	---	---	---	15	---

Source: National Research Council (NRC, 2012).

[a] Anticipated litter size of 12.5 and anticipated mean birth weight of 1.40 kg. For minerals other than calcium and phosphorus, as well as vitamins, the values for >day 90 gestating sows may be 100–125% of those for <day 90 gestating sows. Dietary NE content is 2518 kcal/kg diet (ME = 3300 kcal/kg diet), and feed intake + wastage is 2.21 kg/day.

[b] Anticipated litter size of 13.5 and anticipated mean birth weight of 1.40 kg.

[c] Mean daily weight gain of nursing pigs = 190 g/day during a 21-day lactation period. For minerals other than calcium and phosphorus, as well as vitamins, values for P2+ lactating sows may be 100–125% of those for P1 lactating sows. Dietary NE content is 2518 kcal/kg diet (ME = 3300 kcal/kg diet), and feed intake + wastage is 6.28 kg/day.

Note: G, gestating; La, lactating; P, parity; PA, pantothenic acid; ---, data are not available.

Sixth, diets for animals (e.g., poultry and swine) must include antibiotics for rapid growth. Although in-feed antibiotics can reduce the incidence of diseases such as diarrhea and coccidiosis, they can be replaced effectively by alternatives such as essential oils, functional amino acids, and organic acids. Overuse of in-feed antibiotics in animal diets can result in antibiotic resistance in both humans (through the consumption of products such as meat, milk, and eggs) and farm animals, thereby posing a threat to public health. Seventh, fish and crustaceans cannot effectively use crystalline amino acids. This is not true because dietary supplementation with free amino acids can improve the growth and health of fish and crustaceans (Li et al. 2021b,c). Indeed, juvenile hybrid striped bass can grow faster when fed a complete purified diet than a 60%-fish-meal-based ration that provides the same amounts of all nutrients (He et al. 2023b). Finally, dogs are omnivores because they consume both meat and plant-sourced food. As indicated in Chapter 1, dogs are carnivores in anatomy and physiology but have evolved to effectively use high-starch rations (e.g., 30% starch, on a DM basis; Wu 2024b). If given food choices, dogs prefer to eat meat rather than grains or vegetables. An animal that can consume a formulated diet containing large amounts of starch-rich foods

may not necessarily be classified as an omnivore in biology. For example, the rainbow trout, which can use high-starch rations (e.g., 36% starch, on a DM basis), is a carnivore, but not an omnivore, in taxonomy (Li et al. 2021b). Adequate knowledge of the principles of animal nutrition is necessary to dispel these common false myths in animal nutrition.

USE OF ANIMAL MODELS FOR STUDYING HUMAN NUTRITION AND METABOLIC DISEASES

Domestic and laboratory animals are useful for the study of human nutrition because the nature of its research often involves invasive tissue collections and surgical procedures and may result in potential harmful effects on fetuses, infants, and adults (Bazer et al. 2021; Bergen 2022; Reynolds et al. 2022; Smith and Govoni 2022; Stenhouse et al. 2022a,b). Table 11.32 summarizes the similarities, major differences, advantages, and disadvantages of porcine, ovine, bovine, avian, and fish models for biomedical research. Examples are highlighted in Table 11.33 for uncovering mechanisms responsible for (a) IUGR and the prevention of IUGR in underfed dams by increasing the availability of amino acids to the fetus through either the dietary realimentation or modulation of the uterine arginine-NO pathway; (b) the unusual high abundance of the arginine-family amino acids and serine in fetal fluids; (c) arginine deficiency and hyperammonemia in preterm infants; (d) abundance of polyamines as well as glutamine and proline in milk; (e) the obligatory role of dietary amino acids in intestinal integrity; (f) hyperglycemia-induced endothelial dysfunction; (g) ammonia toxicity in patients with *N*-acetylglutamate deficiency; (h) extrahepatic urea synthesis in the small intestine of postweaning mammals; (i) the susceptibility of neonates to intestinal disease; (j) biomarkers for intestinal adaptation in preterm neonates; (k) gout, retinal degeneration and detachment, diabetes, and ovarian cancer, as well as cardiovascular development and angiogenesis (chicken models); and (l) the utilization of high amounts of dietary protein, glycogenic hepatopathy, hepatic steatosis, black skin syndrome, and intestinal carcinogenesis (fish models) (Wu 2022b). In addition, over the past two decades, transgenic cattle have been generated that possess desirable production traits (including resistance to diseases and improved growth and lactational performance) and produce recombinant proteins with nutritional and therapeutic values (Hay et al. 2022; Monzani et al. 2022). Those proteins include tissue-type plasminogen activator, recombinant human growth hormone (nutrient metabolism), recombinant human albumin, recombinant anti-CD20 monoclonal antibody, human lactoferrin (antimicrobial in the small intestine), α-lactalbumin (milk protein), myelin

TABLE 11.32
Use of Animal Models for Studying Human Nutrition and Metabolic Diseases

Animal	Nutrition and Biomedical Research
Pig	Fetal and postnatal development of intestine and other tissues, effects of maternal nutrition on placental and fetal growth, fetal composition of AAs and other nutrients in the body, tissue-specific and whole-body metabolism of amino acids, syntheses of bioactive products by mammary glands, nutrient requirements of neonates and adults, development of infant formulas and TPN solutions, regulation of vasodilator production by endothelial cells, cardiovascular diseases and responses to exercise and diets, novel biochemical pathways and metabolic defects, development of immune systems[a], prevention and treatment of IUGR in mammals, production of recombinant proteins and antimicrobials, and microbial development in the small and large intestines
Sheep	Composition of AAs and PAs in fetal fluids and placentae, expression of AA and sugar transporters in the conceptus, syntheses of NO and PAs in placentae, prevention of IUGR in underfed dams through Arg supplementation, growth and lactation performance, skeletal muscle growth, development, and adaptation
Cow	Develop transgenic cattle with desirable production traits[b] and as bioreactors to produce recombinant proteins[c], and develop therapeutic treatment of citrullinemia[d]
Chicken	Elucidate mechanisms responsible for gout, retinal degeneration and detachment, diabetes, and ovarian cancer, as well as their prevention and treatment; study cardiovascular development and angiogenesis
Fish	Study mechanisms for the utilization of high dietary protein, glycogenic hepatopathy, hepatic steatosis, black skin syndrome, the absence of cancer in the intestine, and Arg deficiency in growth and survival
Rodents	Digestion, absorption, metabolism (synthesis and catabolism), and excretion of dietary nutrients; adaptations to nutritional, physiological, and environmental alterations; endocrine and nonendocrine disorders

Source: Wu (2022b).

[a] Including intestinal intraepithelial lymphocytes and Peyer's patches.

[b] Including resistance to diseases and improved growth and lactation performance.

[c] Including tissue-type plasminogen activator, recombinant human growth hormone, recombinant human albumin, recombinant anti-CD20 monoclonal antibody, human lactoferrin, α-lactalbumin, myelin basic protein, and human bile salt-stimulating.

[d] A rare Holstein and Holstein-Friesian-specific metabolic genetic disorder of cattle.

Note: AA, amino acid; Arg, arginine; IUGR, intrauterine growth restriction; NO, nitric oxide; Pas, polyamines (putrescine, spermidine, and spermine); TPN, total parenteral nutrition

TABLE 11.33
Similarities, Major Differences, as well as Advantages and Disadvantages of Farm and Rodents for Biomedical Research in Human Nutrition, Metabolism, and Health

Animal	Similarities to Humans	Major Differences from Humans	Advantages	Disadvantages
Pig	Anatomy, physiology, digestion, food intake, and the metabolism of nutrients (including AAs, glucose, and minerals); monogastric omnivores; mechanisms for nutrient transport; the regulation of blood flow	*Pregnancy:* Time of implantation, type of placentation, fetal fluid compartments, gestational length, and number of offspring; *Nutrition:* Amounts of nutrients (fat and iron) stored in the body at birth; *Metabolism:* Synthesis of ascorbic acid and the site of FA synthesis	Provides adequate tissue samples for various biochemical assays; convenient to work with both fetal and postnatal pigs; sensitive to dietary intakes of AAs and other nutrients; large databases in the literature on pigs	The growth of fetal pigs is not highly sensitive to maternal protein deficiency; high risk for abortion in pregnant pigs after surgical catheterization of blood vessels; no BAT; litter-bearing
Sheep	Metabolic pathways for nutrient utilization, the regulation of blood flow and thermogenesis, mechanisms for nutrient transport, the number of offspring, maternal size, embryogenesis, BAT and thermogenesis, and birth weight	*Pregnancy:* Time of implantation, type of placentation, fetal fluid compartments, pregnancy recognition signal, and gestational length; *Digestion:* Fermentation of nutrients in the rumen; *Metabolism:* Intestinal catabolism of BCAAs, the metabolism of glucose, ammonia, and SCFAs; gestational length; the site and substrates for FA synthesis	Well-established animal model for studying human pregnancy, Convenient to work with sheep, pregnant ewes are well adaptable to surgical procedures of placing catheters into maternal and fetal blood vessels, and a large database in the literature on sheep nutrition and physiology	Extensive fermentation of dietary nutrients in the rumen, most dietary nutrients must be protected from rumen degradation, sensitive to ketosis and copper toxicity, a high rate of mortality during late pregnancy for ewes with ≥ 3 fetuses
Cattle	Metabolic pathways for nutrient utilization, the regulation of blood flow and thermogenesis, mechanisms for nutrient transport, number of offspring, embryogenesis, gestational length, BAT and thermogenesis	*Pregnancy:* Time of implantation, type of placentation, fetal fluid compartments, and pregnancy recognition signal; *Digestion:* Fermentation of nutrients in the rumen; *Metabolism:* Intestinal catabolism of BCAAs, metabolism of glucose, ammonia, and SCFAs; the site and substrates for FA synthesis	Well-established animal model for studying human citrullinemia[a], easiness for in vitro and in vivo gene transfer studies, used historically for research on infectious diseases (e.g., tuberculosis, cowpox[b]), and the production of interferon γ	Extensive fermentation of dietary nutrients in the rumen, most dietary nutrients must be protected from rumen degradation, sensitive to ketosis and rumen, rumen bloat, high costs, and inconvenience
Chicken	Digestion and absorption of nutrients in the small intestine, the metabolism of most nutrients, monogastric omnivores, the regulation of blood flow, angiogenesis, mechanisms for nutrient transport, the site and substrates for FA synthesis, and BAT[c]	*Embryo:* Hatching of eggs in birds vs. mammalian embryos in the uterus, different length of embryonic development; *Digestion:* Proventriculus and gizzard, the expression of digestive enzymes in the small intestine, and lipid absorption via the portal vein *Metabolism:* Uric acid synthesis, the lack of hepatic gluconeogenesis from AAs, and high metabolic rate	Provides adequate tissue samples for various biochemical assays; convenient to work with pre- and post-hatching birds, sensitive to dietary intakes of AAs and other nutrients; unique to study gout, retinal degeneration[d], and angiogenesis, naturally "diabetic", spontaneously develops ovarian cancer	Different mechanisms for ammonia detoxification, no placenta for embryonic growth and development, different paths for the absorption of lipids and lipid-soluble vitamins, the lack of muscle GLUT4, mammalian antibodies are generally not applicable to birds
Rodent[e]	Anatomy, physiology, digestion, and nutrition; metabolic pathways for nutrient utilization, monogastric omnivores; mechanisms for nutrient transport; the regulation of blood flow; BAT and thermogenesis	*Pregnancy:* Time of implantation, type of placentation[f], fetal fluid compartments, gestational length, and number of offspring; *Digestion:* Fast gastric emptying and GI transit time (reaching post-absorptive state at 6 h after feeding vs 12 h in humans *Metabolism:* High metabolic rate (6 to 10 times that in humans)	Provides adequate tissue samples for various biochemical assays; convenient to work with both fetal and postnatal rodents; sensitive to dietary intakes of AAs and other nutrients; large databases in the literature on rodents; widely available; low cost	A short gestation period (21 days), ability to synthesize vitamin C; high rates of food intake and metabolism, rapid aging processing, small size for surgery, litter-bearing, and high variation among breeds

(*Continued*)

TABLE 11.33 (*Continued*)
Similarities, Major Differences, as well as Advantages and Disadvantages of Farm and Rodents for Biomedical Research in Human Nutrition, Metabolism, and Health

Animal	Similarities to Humans	Major Differences from Humans	Advantages	Disadvantages
Fish	Digestion and absorption of nutrients in the small intestine, metabolic pathways for nutrient utilization, mechanisms for nutrient transport and its nutritional regulation, detoxification of toxins, intestinal AA metabolism, the secretion of digestive enzymes from the gut and and pancreas, and the control of food intake	*Embryo:* Hatching of eggs in fish vs. mammalian embryos in the uterus, different length of embryonic development; *Digestion:* Some fish lack a stomach; a short gut; requires a long time for nutrient digestion and absorption; *Metabolism:* Most fish lack the urea cycle and have high dietary requirements for AAs; intolerable to dietary starch; limited oxidation of glucose and lipids in muscle; unique roles of the head kidneys[g]	Big fish provide adequate tissue samples for various biochemical assays; convenient to work with pre- and post-hatching fish, sensitive to dietary intakes of starch and other nutrients; unique to study starch-intolerable disease in the liver; useful for studying the usual absence of tumors in the small intestine; zebrafish is widely used in research	Different mechanisms for ammonia removal, no placenta for embryonic growth and development, different metabolic patterns for glucose and fatty acids in fish than in mammals, requires intensive labor, mammalian antibodies are generally not applicable to fish, and difficult to measure food intake, small size

Source: Wu (2022b).

[a] A disease due to the deficiency of argininosuccinate synthase (a urea-cycle enzyme).

[b] The first vaccine against the cowpox virus.

[c] Development of brown adipose tissue in response to cold challenge.

[d] Mutation in the photoreceptor guanylate cyclase.

[e] Rats and mice.

[f] Labyrinthine-type in rats (an intricate structure of interconnecting passages) vs. the villous-type in humans.

[g] A tissue that contains cytokine-producing lymphoid cells of the immune system; endocrine cells that secret cortisol, catecholamines, and thyroid hormones; and hematopoietic stem cells that are capable of hematopoiesis (the production of new blood cells).

Note: AA, amino acid; BAT, brown adipose tissue; BCAA, branched-chain amino acid; FA, fatty acid; GLUT4, glucose transporter 4; SCFA, short-chain fatty acids.

basic protein (neurological development), and human bile salt-stimulating lipase (lipid digestion). Furthermore, transgenic pigs have been generated to produce porcine growth hormone, carbohydratases, phytase, antimicrobials, and antiviral antibodies (Wu and Bazer 2019).

USE OF ARTIFICIAL INTELLIGENCE IN ANIMAL NUTRITION

Artificial Intelligence (AI) is a field of technology that enables machines (including computers, as well as any associated hardware and software) to autonomously simulate human learning, pattern identification, adaptation, problem solving, and decision making (Rosati 2024). AI relies on machine learning (e.g., neural networks and deep learning), which requires the input of large datasets and algorithms (comprising a set of rules, mathematical computations, and statistical analyses). Neural networks consist of interconnected nodes (also known as neurons) arranged in layers to process information, whereas deep learning refers to neural networks with multiple layers for the analysis of more complex data structures. The datasets (including numbers, figures, images, and texts) include those that are available in the public domain or to users and collected in a real-time manner from the Internet of Things (IoT). The IoT is a network of physical devices embedded with sensors, software, and connectivity that allow them to collect and exchange data. These data are often deposited in databases, which may be local, hosted in a private network, or stored in the cloud (i.e., computing services over the internet). It is important to distinguish between datasets (that are used to train and evaluate AI models) and databases (that are designed to store, manage, and retrieve those datasets). The quality of datasets is essential: the more accurate the data, the more effective the machine learning, and the more reliable the AI's outputs. Thus, AI encompasses both computer science and big data analytics to build systems capable of mimicking human intelligence by analyzing vast datasets to recognize patterns, generate insights, and make informed predictions or decisions.

AI can be readily applied to animal nutrition (Rosati 2024; Tedeschi 2022). In this area, datasets may include nutrient composition in feedstuffs, drinking water, plasma, tissues, and animal bodies; rates of tissue and BW metabolism and gains at different stages of the life cycle; feed, nutrient, and energy intakes, as well as meal size and frequency; sources of feed ingredients and their costs; carcass dressing percentage and quality; embryonic survival during

pregnancy, litter size, and postnatal survival; information on farm location, climate, waste excretion, labor cost, and management. Sensors fitted in animals may include ear tags, nose bands, tail bands, and ankle monitors, whereas monitoring cameras can be mounted on farm facilities and vehicles. Based on a variety of factors [e.g., animal's physiological states and physical activity levels; types of feeds and daily nutrient intake; anticipated costs of farm operations and expected levels of productivity (e.g., weight gain, milk yield, weaning weight, and egg production); and economic returns], AI may recommend a cost-effective dietary formulation, efficient supply chains, and rapid responses to changes in market demands. This can improve animal productivity and farm competitiveness, as well as enterprise and environmental sustainability. In addition, through the analysis of data from sensors (either directly or remotely connected to animals) and IoT devices, AI enables the real-time monitoring of animal and environmental conditions. This can result in individualized precision nutrition (i.e., tailored nutritional recommendations based on an individual's characteristics), automated and optimized feeding systems, and improved well-being. Furthermore, the AI's early detection of abnormal changes in animals' behavior, feeding patterns, respiration rates, and body temperatures may be indicative of health or nutrition issues, therefore allowing for timely intervention and preventive measures. Thus, AI is expected to transform animal nutrition and feeding systems in the future. However, farmers should consider AI-associated issues such as technical expertise, cost, data security, and ethics.

SUMMARY

The daily requirements of gestating, lactating, growing, egg-producing, or working animals for energy or nutrients consist of maintenance and production requirements. The energy requirement for maintenance is the amount of energy needed to keep the animal in energy equilibrium. Likewise, the nutrient requirement for maintenance refers to the amount of a specific nutrient needed to keep a zero balance of the nutrient in the animal. Animals grow or produce only after their maintenance requirements are met. The requirements of animals for dietary energy and nutrients vary with their genetic backgrounds and physiological states (e.g., gestation, lactation, and growth), as well as the environments in which they are living. The maintenance and production requirements (total requirements) are usually determined through experiments (e.g., calorimetry, feeding trials, or isotope approaches). However, the recommended values should be used only as references because dynamic changes in nutrient metabolism occur in animals under various conditions that are different from the settings of scientific experiments. In all cases, the physiology and metabolism of animals (e.g., milk production in mammals, work of cattle and horses, and feather formation and color in birds) must be borne in mind when assessing their energy and nutrient requirements during their life cycle.

Because a new life starts from a fertilized egg, optimizing maternal nutrition not only improves pregnancy outcomes but may also be beneficial for the health, growth, and development of several generations of offspring. Also, maximal milk production by dams will ensure the successful nurturing and survival of their newborns so they will efficiently gain tissue protein during the growing-finishing period. Likewise, sustaining egg production by hens and promoting muscle growth are critical for the competitiveness of the poultry industry. Achieving these goals critically depends on adequate nutritional support to the animals and can be facilitated by metabolic regulators (e.g., functional amino acids, β-agonists, and anabolic hormones). In the life cycle, compensatory growth of animals commonly occurs, which refers to their accelerated growth following a fetal or postnatal period of slowed growth and development, particularly as a result of nutrient deprivation. Fetal (embryonic) or neonatal programming plays a key role in regulating postnatal growth and survival of mammals, birds, and possibly fish and crustaceans, as well as their feed efficiencies for lean-tissue growth. Finally, the usefulness of any recommendations for dietary energy and nutrient requirements must be evaluated on a large scale under practical production conditions. Collectively, the science- and AI-based formulation of nutrient-balanced and adequate diets will not only improve the growth and productivity of animals but will also mitigate the impact of livestock, poultry, and aquaculture industries on the quality of the environment worldwide. This knowledge can be translated into effective means for optimizing human nutrition and health while preventing chronic metabolic disorders (including diseases).

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12 Regulation of Food Intake by Animals

Food provides an animal with energy, nutrients, and other bioactive molecules. Food intake is essential to the maintenance, growth, development, reproduction, and health of all animals and, therefore, must be regulated in an exquisite manner. Enteral nutrients are provided to animals through either food intake via the mouth in normal feeding or direct infusion to the gastrointestinal tract (GIT) in special circumstances. In modern intensive or extensive farming systems, livestock, poultry, and aquatic animals have free access to food under most production conditions (e.g., for growth, lactation, and fattening for mammals; growth for birds and fish). The brain, which is a highly complex organ (Figure 12.1), collaborates with the GIT (known as the brain-gut axis) and white adipose tissue (WAT) to control the appetite (defined as the desire for food) and satiety (defined as the feelings of fullness between meals) of animals via neuronal and hormonal signals (Buchanan and Johnson 2007; Daniel et al. 2013; Li et al. 2023a). Other factors that influence appetite include sensory responses to the appearance, texture, smells, and tastes of food, as well as behavioral, social, and environmental issues. Likewise, both physiological (e.g., gender, age, concentrations of metabolites and neurohormones in plasma, gastrointestinal volume, and energy status) and environmental (e.g., ambient temperatures, humidity, and noise levels; dietary nutrient composition; and daily meal schedules) factors also affect satiety in animals (Table 12.1).

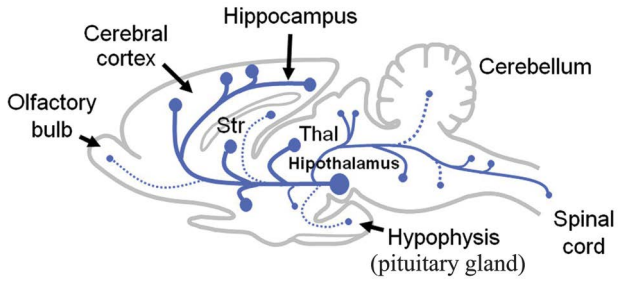


FIGURE 12.1 Structure of the central nervous system in animals. The brain is a highly complex organ. It consists of various integrated parts in the forebrain, midbrain, and hindbrain that produce different neurotransmitters for communication between neurons. GABAergic neurons are located in the hippocampus, thalamus, basal ganglia (in the forebrain), hypothalamus, and brainstem. Cholinergic neurons are found in the striatum (as part of the basal ganglia). Dopaminergic neurons extend from the midbrain (e.g., the substantia nigra pars compacta, the ventral tegmental area, the arcuate nucleus, and the retrorubral area) through the forebrain (e.g., the olfactory bulb). Histaminergic neurons are confined to the tuberomammillary nucleus of the posterior hypothalamus. Serotonergic neurons are predominantly located in the brainstem raphe complex, a midline complex closely associated with the reticular formation within the midbrain, pons, and medulla. Thal, thalamus; Str, striatum. (Reproduced from a figure freely available in the public domain from Nieto-Alamilla et al. 2016.)

TABLE 12.1
Factors Affecting Food Intake of Animals

Factor	Examples
Nutritional status	Hunger, starvation, fasting, obesity, and thirst
Diet	Chemical characteristics (e.g., amount and balance of amino acids; energy content; composition of carbohydrates, lipids, vitamins and minerals; antinutritional factors; lipid peroxides; color, smell, and taste/flavor; rancidity and staleness; toxic substances); physical characteristics of diets (e.g., temperature, particle size, viscosity, hardness, and softness); ingredients, the methods of food processing, and the form of diet (liquid, pellet, or powder); palatability; and the quality of drinking water
Gene	Species (e.g., cattle, fish, horse, humans, pigs, poultry, and sheep), breeds (leghorn vs. broiler chickens; Meishan vs. offspring of Landrace x Yorkshire gilts and Duroc x Hampshire boars), and sex (males and females; boars vs. sows); and gene mutations
Physiological and metabolic factors in plasma	Age, pregnancy, and lactation; light, circadian clock, and melatonin; release of hormones and satiety signals from the gut and brain; concentrations of amino, acids, glucose, fatty acids and their metabolites (e.g., ammonia, lactate and ketone bodies) in plasma and brain; salivary production; digestibility; fill and motility of the gastrointestinal tract; possibly gut microbes; and cellular factors such as ATP/AMP ratio, endoplasmic reticulum stress, and oxidants
Physical state	Sleep deprivation, fatigue, bending, stretch, and long-distance transportation
Psychological state	Mixing of animals from different parents and different species
Pathology	Infection, inflammation, trauma, neoplasia, diabetes, obesity, cardiovascular disease, fetal growth restriction, nausea, vomiting, and the accumulation of damaged molecules in tissues
Environment	Ambient temperature (e.g., heat stress[a], cold, local heating); ambient humidity and noise; air pollution (e.g., $PM_{2.5}$, ammonium sulfate, ammonia, H_2S, CO, and CO_2); sanitation; and social interaction
Management	Housing (space, stocking density, and mixing), frequency of meals, weaning, individual and group hygiene, handling and treatment of animals, physical activity, dietary habits, social behavior, care, and transportation

[a] This is a particularly severe problem for animals without sweat glands, such as dogs, sheep, and swine.

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Overall, the amount of feed [or dry matter (DM)] intake by an animal is a function of its body weight (BW) or the size and frequency of meals (Baile and Forbes 1974). In growing animals, food intake is generally proportional to their weight gain. Thus, food intake is closely related to two important issues in nutrition: the growth, productivity, and health of animals as well as their development of obesity. Animal producers strive to increase the voluntary food intake of farm animals at some phases of production, such as lactation, weaning, postweaning, post-hatching, or post-larval growth, or when they are sick, while maximizing the efficiency of nutrient utilization and minimizing fat accretion in the body, as well as reducing the time needed for their raising to market weights (Li et al. 2021a,b; Sartin et al. 2011). In contrast, owners of companion animals are increasingly concerned about the food consumption of their dogs, cats, and other pets to reduce overweight and obesity (Zomer and Cooke 2024). Also, in all dams, maternal obesity before and after pregnancy impairs embryonic survival and increases the risk for fetal mortality and perinatal complications (Wu et al. 2006). The main objective of this chapter is to highlight the mechanisms responsible for the control of food intake by animals for meeting their nutrient requirements.

REGULATION OF FOOD INTAKE BY NONRUMINANTS

The central nervous system and peripheral tissues play important roles in the control of food intake by animals (Figure 12.2). Thus, their voluntary food consumption is influenced by a plethora of factors (Buchanan and Johnson 2007; Daniel et al. 2003; Forbes 2007; Obese et al. 2007). They are: (a) dietary physical and chemical characteristics [including particle size and texture (e.g., temperatures, color, viscosity, taste/flavor, hardness, softness, rancidity, and staleness); composition and content of nutrients [particularly nutritionally essential amino acids (EAAs)]; palatability (a property of both foods and animals); and presence of antinutritional factors and toxins]; (b) food availability and energy supply; (c) blood metabolites [e.g., amino acids, glucose, and short-chain fatty acids (SCFAs)]; (d) salivary production, digestive tract capacity, and gut-fill (food digestibility); (e) feedback signals from the GIT (e.g., ghrelin), WAT (leptin), pancreas (e.g., insulin), liver [glucose and insulin-like growth factor-I (IGF-I)], and brain [e.g., growth hormone (GH), neuropeptides, proteins, as well as cytokines [e.g., interleukins (ILs) and tumor necrosis factor-α (TNF-α)], in response to dietary intakes of nutrients and alterations in the circulating levels of hormones; (f) physiological status (e.g., gestation, lactation, and growth) and pathological conditions (e.g., fever, inflammation, infection, and cancer); and (g) environmental factors such as ambient temperatures, noise, air pollution, and social interaction.

In addition to the aforementioned physiological, food, and environmental factors, animals learn about the metabolic consequences of consuming foods with certain physical or sensory properties, including appearance, flavor, and texture, and choose preferentially or avoid foods that they have previously experienced (Catanese et al. 2016; Wadhera and Capaldi-Phillips 2014). Recent studies have identified receptors for food flavors in the tongue (Niot and Besnard 2017) and the GIT (Breer et al. 2012), which provide a biochemical basis for food selection by animals. Thus, animals can reject, adapt to, or maintain food intake according to their nutritional and physiological needs. Interestingly, some mammalian hibernators completely cease food intake

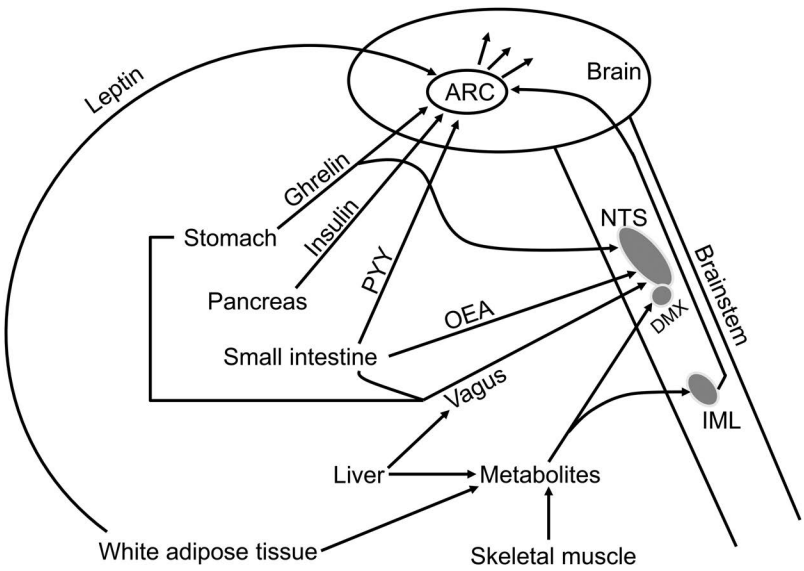


FIGURE 12.2 Regulation of voluntary food intake by animals at the gastrointestinal tract, liver, and brain levels. DMX, dorsal motor nucleus of the vagus nerve; IML, intermediolateral cell column; NTS, nucleus tractus solitaries; OEA, oleoylethanolamide; PYY, peptide YY.

for nearly 7 months regardless of ambient temperature and food availability (Florant and Healy 2012). Effects of chemical, physiological, and biochemical factors on food intake by animals are summarized in Table 12.1 and discussed in the following sections.

Control Centers in the Central Nervous System

Hypothalamus, Neurotransmitters, and Neuropeptides

The brain collects information from the special sensors and receptors in the GIT and other organs (Marx 2003). It was originally proposed that there were centers (the feeding center and satiety center) in the hypothalamus, which is located beneath the cerebrum of the brain (Auffray 1969). The hypothalamus is now known to be the main part of the brain that controls food intake by animals and to act with other brain regions to regulate hunger and satiety. The hypothalamus is made up of some nuclei, including the arcuate nucleus (ARC), paraventricular nucleus (PVN), ventromedial nucleus (VMN), dorsomedial hypothalamic nucleus (DMH), and dorsomedial nucleus (DMN). The feeding center [also known as the lateral hypothalamic area (LHA)] causes the animal to eat unless inhibited by the satiety center [also known as the ventromedial hypothalamus (VMH)] which receives signals from the body following food consumption (Figure 12.3). Thus, lesions (i.e., localized destructions of neurons) in the LHA result in underfeeding, whereas lesions in the VMH or the PVN cause overfeeding (Abdalla 2017). However, the LHA and the VMH are not the only parts of the brain that control food intake. The paraventricular nuclei of the hypothalamus, just ventral to the ventromedial nuclei, are particularly sensitive to the effects of neurotransmitters, including noradrenaline, neuropeptide Y (NPY), α-melanocyte-stimulating hormone (α-MSH, a melanocortin), and melanin-concentrating hormone (Kirouac 2015). Neurons within the central and peripheral nervous systems communicate with each other through these neurotransmitters. Most of them are metabolites of amino acids that either inhibit or stimulate appetite (Table 12.2).

The ARC contains two major types of neurons with opposing actions: agouti-related peptide and neuropeptide Y (AgRP)/NPY neurons and pro-opiomelanocortin (POMC)/cocaine-amphetamine regulated transcript (CART) neurons (Sutton et al. 2016). POMC is cleaved by prohormone convertase-1 into adrenocorticotropin hormone (ACTH), which is further cleaved by prohormone convertase-2 to form α-MSH. Activation of the AgRP/NPY neurons stimulates appetite. For example, fasting induces a large, leptin-dependent increase in the intrinsic action potential frequency of orexigenic AgRP/NPY neurons in the ARC, thereby signaling a strong appetite for eating food (Takahashi and Cone 2005). In contrast, activation of the POMC/CART neurons reduces appetite by causing the release of α-MSH (an anorexigenic neuropeptide) from the presynaptic terminals of the POMC neurons. By binding to the melanocortin-3 and -4 receptors (MC3R, MC4R) on the second-order neurons, α-MSH reduces food intake

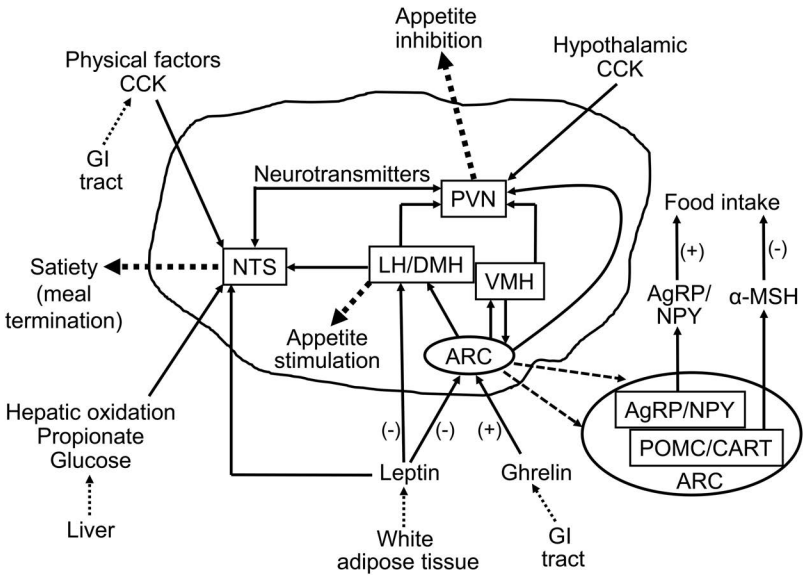


FIGURE 12.3 The centers of food intake control in the brain. The arcuate is located in the hypothalamus and contains two major types of neurons with opposing actions: agouti-related peptide/neuropeptide Y neurons (stimulating appetite) and pro-opiomelanocortin/cocaine-amphetamine regulated transcript neurons (inhibiting appetite). Ghrelin stimulates, but leptin inhibits, food intake by animals. AgRP, agouti-related peptide; ARC, arcuate; CART, cocaine-amphetamine regulated transcript; CCK, Cholecystokinin; DMH, Dorsomedial hypothalamus; LH, lateral hypothalamus [also known as lateral hypothalamic area (LHA)]; α-MSH, α-melanocyte-stimulating hormone; NPY, neuropeptide Y; POMC pro-opiomelanocortin; PVN, paraventricular nucleus; NTS, nucleus tractus solitarius; VMN, ventromedial nucleus. (+), stimulation; (–) inhibition. (Adapted from Sartin et al. 2011.)

TABLE 12.2
Hormones, Neuropeptides, and Neurotransmitters That Affect Food Intake (FI) by Animals

Substance	Major Source	Effect on FI
	Hypothalamus	
Acetylcholine and galanin (a neuropeptide)	Dorsomedial hypothalamic nucleus (DMH)	Increase
Agouti-related protein (AgRP)	Arcuate nucleus	Increase
γ-Aminobutyrate (GABA)	Various types of neurons	Increase
Dopamine	Neurons in arcuate nucleus (reward)	Increase
Neuropeptide Y (NPY)	Arcuate nucleus	Increase
Melanin-concentrating hormone (MCH)	Lateral hypothalamic area (LHA) and other regions (e.g., DMH) of the brain	Increase
Opioid peptides[a]	LHA, PE, PVN, and VMN	Increase
Orexin (also known as hypocretin)	LHA and DMN	Increase
Cocaine and amphetamine-related transcript (CART)	POMC neurons in arcuate nucleus, PVN, VMN, LHA, and median eminence	Decrease
Corticotrophin-releasing hormone (CRH)	Paraventricular nucleus (PVN)	Decrease
Kisspeptin (intracerebroventricular injection)	Arcuate nucleus and preoptic area	Decrease
Leukemia inhibitory factor	Arcuate nucleus	Decrease
Melanocortin	Ventromedial nucleus (VMN)	Decrease
α-Melanocyte-stimulating hormone (α-MSH)	POMC neurons in arcuate nucleus	Decrease
Neurotransmitters (dopamine and glucagon-like peptide-2)	DMH	Decrease
Neurotransmitters (dopamine and serotonin)	Dorsomedial nucleus (DMN)	Decrease
Neurotransmitters (dopamine, norepinephrine, and PACAP)	VMN	Decrease
Norepinephrine	PVN and VMH	Decrease
Oxytocin, thyrotropin-releasing hormone, and Urocortin	PVN	Decrease
Pro-opiomelanocortin (PMOC)	POMC neurons in arcuate nucleus	Decrease
Serotonin	PVN, LHA and VMN	Decrease
	Anterior pituitary gland	
Adrenocorticotropin hormone (ACTH)	Corticotropic cells	Increase
Growth hormone	Somatotropic cells	Decrease
	Brainstem	
Thyrotropin-releasing hormone (stimulating ghrelin release)	Caudal raphe nuclei	Increase
Neurotransmitters (GLP-1, norepinephrine, and oxytocin)	Nucleus tractus solitarius (NTS)[b]	Decrease
	Spinal cord	
Galanin	Sensory neurons located in the dorsal horn	Increase
Neurotransmitters (acetylcholine and substance P)	Intermediolateral cell column (IML)	Decrease
	The digestive system	
Ghrelin (acting on ARC neurons to release AgRP and NPY)	Enteroendocrine cells of the stomach (mainly) and the small intestine	Increase
Gastrin	Enteroendocrine cells	Increase
Motilin	Endocrine Mo cells of the small intestine	Increase
Nitric oxide	Enteroendocrine cells of the intestine	Increase
Serotonin	Enteroendocrine cells of the intestine	Increase
Cholecystokinin (CCK)	The small intestine	Decrease
Glucagon-like peptide-1 (GLP-1)	The small intestine	Decrease
Insulin-like growth factor-I (IGF-I)	Liver	Decrease
Insulin	Pancreatic β-cells	Decrease
Peptide YY (36-AA peptide)	Enteroendocrine cells of the intestine	Decrease
Oleoylethanolamide (OEA, FA derivative)	Enterocytes	Decrease
α-1-Acid glycoprotein (an acute-phase protein)	Primarily liver	Decrease

(*Continued*)

TABLE 12.2 (*Continued*)
Hormones, Neuropeptides, and Neurotransmitters That Affect Food Intake (FI) by Animals

Substance	Major Source	Effect on FI
	Reproductive systems	
Progesterone	Ovaries	Increase
Estradiol	Ovaries	Decrease
Testosterone	Testes	Increase
	White adipose tissue (WAT)	
Leptin	White adipocytes	Decrease
Adiponectin	White adipocytes	Decrease
Resistin	Lymphocytes and stromal cells	Decrease
Visfatin (intracerebroventricular injection)	White adipocytes in visceral WAT	Increase

[a] Examples are endorphins and enkephalins in the hypothalamus. They are derived from the cleavage of precursor proteins such as proenkephalin (PENK), prodynorphin (PDYN), and proopiomelanocortin (POMC).
[b] The communication from tissues in the digestive system to the NTS is transmitted along afferent fibers of the vagus nerve or sympathetic nerves.

Note: PACAP, pituitary adenylate cyclase-activating polypeptide (widely distributed in the hypothalamus); PE, periventricular nucleus.

and enhances energy expenditure (Figure 12.4). Thus, the POMC neurons connect with second-order neurons in other brain centers, which transmit the signals through the nucleus tractus solitarii (NTS) to the body. Many peptide-regulating hormones work through the ARC, but some may also have direct effects on the NTS and other brain centers. The roles of these neuropeptides as satiety signals are summarized in Table 12.2.

Ghrelin

Ghrelin is a peptide hormone (28 amino acids) produced by the enteroendocrine X/A cells in the stomach and the small intestine (Steinert et al. 2017). In the central nervous system, ghrelin acts on a G-protein receptor to increase the intracellular calcium (Ca) concentration and protein kinase C activity through the inositol 1,4,5-triphosphate/phospholipase C and diacylglycerol signaling pathway. At the ARC, ghrelin activates the AgRP/NPY neurons and inactivates the POMC/CART neurons, thereby stimulating appetite (Kojima and Kangawa 2005). Thus, ghrelin is the "hunger hormone", which opposes the actions of leptin and plays an important role in the short-term regulation of food intake (Chen et al. 2007; Steinert et al. 2017).

Leptin, Insulin, GH, IGF-I, and Reproductive Hormones

Leptin (from Greek *leptos*, meaning "thin"), insulin, GH, and IGF-I are synthesized and secreted by WAT, β-cells of the pancreas, somatotropic cells of the anterior pituitary gland, and the liver, respectively (Thon et al. 2016). Leptin (the "satiety hormone") and insulin act on the receptors in

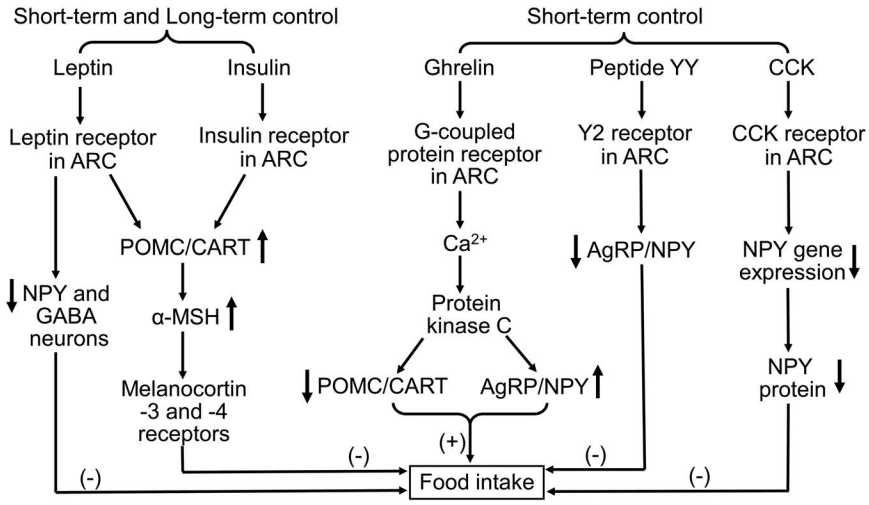


FIGURE 12.4 Regulation of food intake by leptin, insulin, and gastrointestinal peptides in animals. AgRP, agouti-related peptide; ARC, Arcuate; CART, cocaine-amphetamine regulated transcript; CCK, Cholecystokinin; α-MSH, α-melanocyte-stimulating hormone; NPY, neuropeptide Y; POMC pro-opiomelanocortin. ↑, increase; ↓, decrease; (+), stimulation; (–), inhibition.

the ARC of the hypothalamus to stimulate the production of POMC/CART, while inhibiting the release of AgRP/NPY, thereby suppressing food intake by animals (Figure 12.3). For example, the administration of leptin into either the ARC (15–150 ng/side) or the ventral tegmental area (VTA; 15–500 ng/side) of female rats decreased food intake for 72 h, inducing weight loss during the first 24 h (ARC) or 48 h (VTA) after the infusions (Bruijnzeel et al. 2011). Results from mouse studies indicate that leptin activates anorexigenic POMC neurons and increases the frequency of their action potentials in the ARC through two mechanisms: (a) membrane depolarization and (b) reduced inhibition by local orexigenic NPY/GABA neurons (Cowley et al. 2001).

There is evidence that insulin in plasma can cross the blood–brain barrier (BBB) into the brain via a process called receptor-mediated endocytosis (Pemberton et al. 2022). Through its receptors and downstream cell signaling in the brain, insulin can affect brain circuits involved in feeding behavior, mood, and cognition through neuronal mechanisms largely independent of glucose utilization (Chen et al. 2022). Thus, the administration of insulin (0.005–5 mU per site) into the VTA or ARC decreased food intake for 24 h but did not affect BW in rats (Bruijnzeel et al. 2011). Neither insulin nor leptin in the ARC affected brain reward thresholds. Likewise, 4-day infusions of IGF-I reduced feed intake by growing pigs (Dunaiski et al. 1997). Furthermore, GH increases the circulating levels of insulin and IGF-I while reducing those of ghrelin in humans (Engström et al. 2003). Therefore, when fat stores or plasma concentrations of leptin, insulin, GH, and IGF-I are elevated, the feed intake of animals is reduced as a compensatory response to reduce TAG deposition. However, when fat stores or plasma levels of leptin, insulin, GH, and IGF-I are reduced, AgRP/NPY neurons are activated and the POMC/CART neurons are inhibited, leading to increased food intake and weight gain (Figure 12.4).

Progesterone or testosterone increases food consumption and weight gain, but estrogens interact with hypothalamic neurons via estrogen receptors to decrease food intake, increase energy expenditure, and attenuate fat accumulation (Asarian and Geary 2006; Haddad-Tóvolli and Claret 2023). Conversely, the secretion of these hormones is affected by maternal and paternal nutrition (Chapter 11). In addition, the appetite-regulating neurotransmitters also have an intricate role in the control of metabolism and reproduction. For example, NPY promotes feed intake, and its gene expression in the ARC is increased with fasting (Brady et al. 1990). When food availability is low, NPY suppresses the pulsatile secretion of luteinizing hormone (LH) from the anterior pituitary gland and the release of insulin by the pancreatic β-cells but increases the secretion of GH from the anterior pituitary gland (Daniel et al. 2013; Ho et al. 1988). LH is primarily responsible for stimulating the production of testosterone by the testes in males or triggering ovulation by signaling the ovaries to release a mature egg during estrus, whereas insulin inhibits but GH promotes lipolysis in WAT (Daniel et al. 2013). The results are (a) a reduction in reproductive performance and (b) an increase in the circulating levels of free fatty acids in blood as metabolic fuels for survival.

Under the conditions of overnutrition, obesity, and type 2 diabetes mellitus, insulin signaling in the central nervous system is impaired, leading to insulin resistance and augmenting disease progression (Vogt and Brüning 2013). In addition, the circulating levels of leptin are increased in obese mammals such as rodents and humans, but they are largely unresponsive to leptin (a phenomenon called leptin resistance; Friedman 2019). This results in their continued overconsumption of food and further weight gain, although leptin would be expected to signal satiety and regulate energy balance. Therefore, as acute and long-term satiety signals, leptin, insulin, GH, IGF-I, and reproductive hormones play important roles in the control of energy homeostasis of the whole body.

Peptide YY

Peptide YY (PYY; peptide tyrosine tyrosine with 36 amino acid residues) is synthesized and secreted primarily by the enteroendocrine L cells in the small intestine and the colon in response to food intake (Steinert et al. 2017). PYY was named after the tyrosine residues at both the C and N terminals of the peptide. When the concentration of PYY in plasma is elevated, the binding of this peptide to the Y2 receptor in the ARC is increased to inhibit the AgRP/NPY neurons. A decrease in the production of AgRP/NPY leads to an inhibition of food intake. Thus, in experimental animals, either administration of PYY into the systemic circulation or microinjection of PYY into the paraventricular hypothalamic nucleus or the hippocampus caused a decrease in food intake (Alhadeff et al. 2015). Fats, fatty acids, and many other luminal nutrients, as well as the chyme in the proximal small intestine, induce PYY release by the small intestine. Thus, a large amount of food in the small intestine results in low appetite. Much evidence shows that PYY plays an important role in the short-term regulation of food intake (Steinert et al. 2017).

Cholecystokinin

Cholecystokinin (CCK) is produced by the enteroendocrine I cells of the duodenum in response to food intake and is also found in the brain (Steinert et al. 2017). Most CCK receptors are present in the DMH. Administration of CCK into the lateral ventricle of sheep inhibits feed intake (Baile and Della-Ferra 1984). Mechanistic studies indicate that the administration of CCK into the DMH of rats resulted in Fos activation in the entire brain and down-regulated NPY gene expression in the DMH (Chen et al. 2008). Peripherally administered CCK, which acts only for a short term, primarily activates neurons in the NTS but also stimulates neurons in the PVN and DMH. Thus, CCK is an afferent signal that acts on the NTS to reduce meal size. When gut motility is low, the release

of CCK from the intestine is increased as a peripheral satiety signal. This results in low appetite and low food intake. Thus, CCK contributes to short-term control of food intake by animals.

Glucagon-like Peptide-1

Glucagon-like peptide-1 (GLP-1) is released from enteroendocrine L-cells in the small intestine, particularly in the distal ileum and proximal colon, in response to food intake (Kaviani and Cooper 2017). It induces satiation (defined as the feelings of fullness in the GIT during a meal that lead to the termination of food consumption) through its inhibitory effects on NPY and AgRP neurons and stimulatory effects on POMC and CAR neurons (Stanley et al. 2004). GLP-1 is an acute satiety signal to inhibit food intake by animals. Thus, satiation results from signals from the GIT and neuroendocrine effectors in response to feeding.

Control of Food Intake by Nutrients, Metabolites, and Nonnutritive Substances

Dietary Energy Content

Dietary energy content is a major nutritional factor that affects food intake by animals (Table 12.3). Because energy is not matter, it does not influence the food intake of animals by itself. Rather, the nutrients (e.g., fats, carbohydrates, and protein) that contain energy, as well as their metabolites, affect the neurological network that controls food intake. Nutrient metabolism requires amino acids, vitamins, and minerals, as well as cellular signal transductions. Thus, the relationship between dietary energy content and food intake is complex. In both pigs and poultry consuming adequate nutrients (e.g., sufficient dietary intakes of amino acids, carbohydrates, lipids, vitamins, and minerals), food intake generally decreases as the dietary intake of metabolizable energy increases within a usual range but increases as the

TABLE 12.3
Nutrients That Affect Food Intake by Ruminants and Nonruminants

Nutrient	Feed Intake	Animals
Dietary content of protein and amino acids		
Low	Reduced	Ruminants and nonruminants
Medium	Increased	Ruminants and nonruminants
High	Reduced	Ruminants and nonruminants
Dietary imbalance of amino acids	Reduced	Ruminants and nonruminants
Dietary content of energy		
Low	Reduced	Ruminants and nonruminants
Medium	Increased	Ruminants and nonruminants
High	Reduced	Ruminants and nonruminants
Dietary content of total fatty acids		
Low	Reduced	Nonruminants
Medium	Increased	Nonruminants
High	Reduced	Ruminants and nonruminants
Low dietary content of PUFAs	Reduced	Ruminants and nonruminants
Glucose concentration in GIT		
High	Reduced	Nonruminants
Low	Increased	Nonruminants
High SCFAs in the rumen and blood	Reduced	Ruminants
High SCFAs and other weak OAs in diet	Reduced	Nonruminants (weanling)
GIT-fill		
Low	Increased	Ruminants and nonruminants
High	Reduced	Ruminants and nonruminants
High concentrations of KB in blood	Reduced	Ruminants and nonruminants
Dehydration status in the body	Reduced	Ruminants and nonruminants
Dietary minerals (e.g., Na, Ca, P, Fe, and Zn)		
Low	Reduced	Ruminants and nonruminants
High	Reduced	Ruminants and nonruminants
Dietary vitamins (e.g., B-complex vitamins)		
Low	Reduced	Nonruminants
High	Reduced	Nonruminants

Note: GIT, gastrointestinal tract; KB, ketone bodies; OAs, organic acids; PUFAs, polyunsaturated fatty acids; SCFAs, short-chain fatty acids.

dietary intake of metabolizable energy decreases within a usual range (Forbes 2007). Such a feeding behavior results in either the complete compensation of or a moderate increase in dietary energy intake. For example, in growing pigs, dietary DM intake is progressively reduced as the dietary content of digestible energy increases from 12.5 to 15.4 MJ/kg (Henry 1985). In laying hens, food intake progressively decreased from 127 to 107 g/day as the dietary content of metabolizable energy increased from 10.5 to 13.4 MJ/kg diet, but dietary intake of total metabolizable energy increased from 1.34 to 1.42 MJ/day (de Groot 1972).

Dietary Content of Nutritive Sweet Sugars

A small amount of sweet sugars (e.g., glucose and sucrose) in diets confers a sweet taste, therefore promoting food intake by animals. Sweet receptors, which are classified as the taste receptor type 1 (T1R, G-protein coupled receptor), are present in the apical membrane of taste cells in the taste bud of the tongue (Nelson et al. 2001). Interaction of sweet ligands with the T1R activates its coupled G-protein. The activated G_s α-subunit binds to and activates adenylate cyclase to produce cyclic adenosine monophosphate (cAMP). The latter activates protein kinase A to phosphorylate a voltage-gated K^+ channel, resulting in its closure, the depolarization of the taste cell, and the opening of Ca^{2+} channels on the basolateral membrane of the cell. An increase in intracellular Ca^{2+} concentration causes the release of neurotransmitter molecules (in the form of synaptic vesicles), which are received by receptors on the nearby primary sensory neurons. A resulting electrical signal is transmitted along nerve cells to reach the brain's feeding center that perceives and interprets the free-sugar stimulus in the tongue, thereby enhancing consumption of food by mammals, birds, and fish. In feeding practice, nutritive sweeteners for omnivores (including weanling pigs) are glucose and sucrose (e.g., 1–5% in feed or 10–30 mM in drinking water; Kennedy and Baldwin 1972), which are a source of energy and can increase the feed intake and weight gain of the animals.

Glucose Concentrations in Plasma

In contrast to dietary sweet sugars, Mayer (1953) reported that high circulating levels of glucose reduced appetite in monogastric animals. This author proposed a glucostatic theory in the control of food intake; namely, an increase or decrease in plasma glucose concentration can serve as a stimulus for satiety or hunger, respectively. Much evidence shows that it is the glucose concentration within the GIT or in the hepatic portal vein, but not in the systemic circulation, that regulates food intake by animals (Forbes 2007). For example, short-term infusions of a large amount of glucose into the GIT of growing pigs to increase its concentration in plasma also reduced food intake (Gregory et al. 1987). In contrast, neither hepatic portal nor jugular infusions of glucose affected short-term food intake by pigs (Houpt et al. 1979). Thus, glucose acts on sensors on the mucosae of the stomach or the small intestine to signal appetite inhibition. In poultry, infusion of glucose into the portal vein but not into peripheral veins suppressed food intake (Shurlock and Forbes 1981a, 1984). Similarly, duodenal infusion of glucose also inhibited food intake in chickens (Shurlock and Forbes 1981a). These results suggest that either glucose sensing by the small intestine or glucose uptake by the liver plays a role in controlling food intake. In dogs, the infusion of glucose into the hepatic portal vein inhibits food intake, but the infusion of the same amount of glucose into the general circulation through the jugular vein had no effect (Russek 1970). This finding further indicates a role of the liver in controlling food intake. Hepatic glucose oxidation may be required for this sugar to regulate food consumption of animals. An increase in glucose levels in the portal vein may change the rate of impulses generated in the afferent fibers of the autonomic nerves terminating in the liver (Forbes 2000; Shurlock and Forbes 1981b). When glucose and insulin concentrations in plasma are low, which occurs during fasting, the appetite of animals is stimulated. It should be borne in mind that elevated glucose concentrations in blood alone may not be sufficient to inhibit food intake, as shown in obese animals (including humans).

Protein and Amino Acids

Some amino acids themselves are neurotransmitters (Chapter 7). Amino acid levels in the brain and plasma affect the concentrations of the neurotransmitters and thus are expected to regulate food intake (He and Wu 2020). Results of extensive research have revealed that dietary protein content affects food intake by nonruminants. For example, a low or high content of dietary protein (e.g., 5% or 30%) reduces food intake by young rats when compared to a 20%-protein diet (Table 12.4). In addition, when fed ad libitum diets containing 12.5%, 17.4%, 21.3%, or 26.7% crude protein (CP; on an air-dried basis), food intake by pigs (12 to 30 kg BW) decreased gradually from 1001 to 971 g/day, but their growth rate increased gradually (i.e., 492, 627, 743, and 693 g/day for the four diets, respectively; Kyriazakis et al. 1990).

Much evidence shows that animals are very sensitive to dietary amino acid provision (Wu et al. 2014). For example, young rats fed an arginine-deficient diet consume less food per kilogram of BW than the rats fed an arginine-adequate diet (Table 12.5). When arginine is deficient from the diet, its ratio of arginine:lysine may be imbalanced for young animals, systemic NO synthesis may be impaired, and the intestinal absorption of nutrients may be reduced.

Poultry, pigs, and rats rejected a purified diet lacking one amino acid or a group of nonsynthesizable amino acids between 15 and 30 min after starting consumption of the diet (Edmonds and Baker 1987a,b; Edmonds et al. 1987; Harper et al. 1970). These animals continued to eat until satiation when the EAA-free diet was replenished with the missing EAA. Food intake of rats and pigs increased in response to a mild deficiency of dietary lysine, methionine, and threonine but decreased in response to a mild deficiency of tryptophan. In contrast, excess amino acids in diets also

TABLE 12.4
Effects of Dietary Protein Content on Food Intake by Male Sprague-Dawley Rats[a]

	Body Weight (BW, g)			Food Intake (g/100 g BW)		
Day of Feeding	**30% Casein**	**20% Casein**	**5% Casein**	**30% Casein**	**20% Casein**	**5% Casein**
d 0	98.2 ± 3.1	98.1 ± 3.2	98.3 ± 3.0	---	---	---
d 2	124 ± 3.3[a]	122 ± 3.2[a]	104 ± 3.1[b]	9.55 ± 0.39[c]	10.9 ± 0.43[b]	14.3 ± 0.53[a]
d 4	152 ± 3.7[a]	148 ± 3.5[a]	120 ± 3.3[b]	9.16 ± 0.37[c]	10.6 ± 0.42[b]	13.9 ± 0.52[a]
d 6	175 ± 4.0[a]	171 ± 3.8[a]	129 ± 3.3[b]	8.86 ± 0.35[c]	10.2 ± 0.42[b]	13.5 ± 0.49[a]
d 8	196 ± 4.2[a]	190 ± 4.2[a]	136 ± 3.4[b]	8.57 ± 0.34[c]	9.91 ± 0.45[b]	13.1 ± 0.47[a]
d 10	216 ± 4.8[a]	209 ± 4.5[a]	146 ± 3.4[b]	8.30 ± 0.32[c]	9.72 ± 0.41[b]	12.7 ± 0.48[a]
d 12	238 ± 4.9[a]	230 ± 4.6[a]	157 ± 3.6[b]	7.79 ± 0.31[c]	9.28 ± 0.38[b]	12.3 ± 0.48[a]
d 14	256 ± 5.1[a]	248 ± 4.9[a]	167 ± 3.5[b]	7.65 ± 0.32[c]	8.97 ± 0.36[b]	11.8 ± 0.44[a]

[a] The semi-purified diet was prepared as described by Wu et al. (1999). Male Sprague-Dawley rats had free access to their respective diets. Body weight and food intake were measured every 2 days.
d 0 = 30 days of age. Values are means ± SEM, n = 10.
[a-c:] Within a row, means not sharing the same superscript letters differ ($P < 0.05$).

TABLE 12.5
Effects of Dietary Arginine Content on Food Intake by Male Sprague-Dawley Rats[a]

	Body Weight (BW, g)			Food Intake (g/100 g BW)		
Day of Feeding	**1% Arg**	**0.3% Arg**	**0% Arg**	**1% Arg**	**0.3% Arg**	**0% Arg**
d 0	96.1 ± 3.4	96.0± 3.5	96.2 ± 3.4	---	---	---
d 2	114 ± 4.7[a]	102 ± 4.0[b]	99.0 ± 3.3[c]	10.6 ± 0.32[c]	11.8± 0.34[b]	13.9 ± 0.35[a]
d 4	139 ± 5.0[a]	111 ± 4.4[b]	105 ± 3.4[b]	10.1 ± 0.30[c]	11.2 ± 0.32[b]	13.3 ± 0.34[a]
d 6	160 ± 5.2[a]	122 ± 4.7[b]	112 ± 3.6[c]	9.77 ± 0.31[c]	10.8 ± 0.33[b]	12.8 ± 0.37[a]
d 8	179 ± 5.5[a]	135 ± 5.0[b]	120 ± 3.7[c]	9.46 ± 0.33[c]	10.5 ± 0.35[b]	12.2 ± 0.36[a]
d 10	199 ± 5.6[a]	149 ± 5.2[b]	128 ± 3.8[c]	9.15 ± 0.31[c]	10.1 ± 0.34[b]	11.8 ± 0.35[a]
d 12	217 ± 5.8[a]	161 ± 5.4[b]	134 ± 4.0[c]	8.87 ± 0.29[c]	9.82 ± 0.32[b]	11.4 ± 0.33[a]
d 14	232 ± 6.3[a]	171 ± 5.5[b]	140 ± 3.9[c]	8.55 ± 0.28[c]	9.48 ± 0.31[b]	11.2 ± 0.33[a]

[a] The semi-purified diet was prepared as described by Wu et al. (1999). Male Sprague-Dawley rats had free access to their respective diets. Body weight and food intake were measured every 2 days. d 0 = 30 days of age. Values are means ± SEM.
[a-c:] Within a row, means not sharing the same superscript letter differ ($P < 0.05$).

reduced food intake by animals. For example, young rats ate 25–60% less food if their diets contained 2% tryptophan; 3% histidine, methionine, phenylalanine, threonine, or lysine; or 4% leucine, isoleucine, or valine (Gietzen et al. 2007; Harper et al. 1970). Young pigs (8 kg BW) consumed 40%, 20%, and 30% less feed if their diets contained 4% DL-methionine, threonine, or tryptophan, respectively, but 4% leucine in the diet did not appear to affect their feed intake (Table 12.6). Interestingly, when young pigs were fed corn- and soybean meal (SBM)-based diets containing supplemental 4% lysine, threonine, arginine, DL-methionine or tryptophan, feed intake was reduced by 90–95% within the first 3 days (Table 12.7). Within 4–9 days, the pigs fed the supplemental 4% lysine or threonine diets consumed 38% of feed for the control group, the pigs fed the supplemental 4% arginine diet consumed 20% of feed for the control group, and the pigs fed the supplemental

TABLE 12.6
Effects of Amino Acids on Feed Intake by Young Pigs (8 kg) Fed a Corn- and Soybean Meal-Based Diet (20% CP)

	Supplemental Dose of Amino Acid (%)			
Amino Acid	**0**	**1**	**2**	**4**
	% of feed intake in the control group (no supplementation)			
DL-Methionine	100	95	83	61
L-Leucine	100	98	99	99
L-Threonine	100	95	80	80
L-Tryptophan	100	96	93	70

Source: Edmonds and Baker (1987).

TABLE 12.7
Self-Selection of Corn- and Soybean Meal-Based Diets (20% CP) by Young Pigs (8 kg of Body Weight)

Amino Acid Supplementation to Diet	Feed Intake (g/day)	
	Days 0–3	Days 4–9
Control	358	484
4% L-Lysine	30	224
4% L-Threonine	15	220
4% L-Arginine	27	130
4% DL-Methionine	8	18
4% L-Tryptophan	2.7	2.4

Source: Edmonds et al. (1987).

TABLE 12.9
Effects of Dietary Supplementation with Glutamine or Glutamic Acid on Feed Intake by Weanling Pigs[a]

	Dietary Supplementation (%)				
Amino Acid	0.0	0.5	1.0	2.0	Pooled SEM
L-Glutamine	348[a]	353[a]	342[a]	287[b]	19
L-Glutamic acid	352	356	361	358	22

[a] Values, expressed as g/day, are means with pooled SEM, n = 12. Pigs were weaned at 21 days of age (with an average body weight of 5.5 kg) to a corn- and soybean meal-based diet containing 21% crude protein (Wu et al. 1996) supplemented with 0.0 to 2% L-glutamine or L-glutamic acid. Feed intake was measured during the second week postweaning.

[a-b:] Within a row, means not sharing the same superscript letter differ ($P < 0.05$).

4% DL-methionine or tryptophan diet ate little feed (Table 12.7). Likewise, young chicks consumed 32% less feed if their diets contained 4% histidine, lysine, threonine, or tryptophan; 44% less feed if their diets contained 4% phenylalanine; or 50% less feed if their diets contained 4% methionine (Table 12.8). Finally, an imbalance among branched-chain amino acids (e.g., leucine:isoleucine:valine > 8:1:1) or basic amino acids (e.g., arginine:lysine:histidine > 2.5:1:0.4) greatly affected feed intake by all animals studied (including rats, pigs, and chicks; Harper et al. 1970; Smith and Austic 1978). In diets for lactating sows, optimum ratios of leucine:isoleucine:valine for milk production and piglet growth may be 2.4:1.0:1.7 (Rezaei et al. 2022a), an optimum ratio of arginine:lysine may be 2.0:1.0 (Mateo et al. 2008), and an optimum ratio of glutamate:aspartate may be 3.0:1.0 (Rezaei et al. 2022b). Thus, dietary protein or amino acid content influences the response of animals to an excess or deficiency of amino acids, and different animal species have a different sensitivity to an excess or deficiency of amino acids.

Most studies on food intake regulation have focused on amino acids that are not synthesized de novo in animal cells (Gietzen et al. 2007). However, relatively little is known about the role of biosynthesizable amino acids in the control of feed consumption by animals. Gatel and Guion (1990) found that the inclusion of 5 g of monosodium glutamate in 1 kg of creep feed (a starter diet) increased the feed consumption of sow-reared pigs by 36% on Day 18 after farrowing. In contrast, adding 0.5% or 1% glutamine to a corn- and SBM-based diet for 2 weeks did not affect feed intake by postweaning pigs. Likewise, adding 0.5% to 2% monosodium glutamate to the corn- and SBM-based diet (containing 21% CP) for 2 weeks did not affect feed intake by postweaning pigs (Rezaei et al. 2013). However, supplementing 2% glutamine (Table 12.9) or 4% monosodium glutamate (Rezaei et al. 2013) to a corn- and SBM-based diet reduced feed intake by postweaning pigs via different mechanisms. Excess glutamine increased the concentration of ammonia in plasma, whereas excess monosodium glutamate supplementation augmented sodium intake (Rezaei et al. 2013). For comparison, supplementing 2% glutamic acid to the same corn- and SBM-based diet did not affect food intake by pigs (Table 12.9). Furthermore, dietary supplementation with 2% glycine, but not 1% glycine, can stimulate the appetite and feed intake of omnivores (e.g., pigs; He and Wu 2020; He et al. 2023a) and aquatic animals (e.g.,

TABLE 12.8
Effects of Amino Acid Supplementation on Feed Intake by Young Male Chicks (8-Day-Old) Fed a Corn- and Soybean Meal-Based Diet (23% CP)

Amino Acid Supplementation	Feed Intake (g/day)	Amino Acid Supplementation	Feed Intake (g/day)
Control	175	Control	175
4% L-Leucine	175	4% L-Lysine	121
4% L-Arginine	165	4% L-Histidine	119
4% L-Valine	165	4% L-Tryptophan	118
4% L-Isoleucine	155	4% L-Phenylalanine	105
4% L-Threonine	125	4% L-Methionine	77

Source: Edmonds and Baker (1987).

hybrid striped bass; He et al. 2023b; Li et al. 2023b; Suehs et al. 2024). Thus, like biosynthesizable amino acids, different nonsynthesizable amino acids have different effects on feed intake by animals.

Mammals and birds respond similarly to dietary arginine:lysine imbalance. However, the species of fish must be borne in mind in discussing the effects of dietary arginine:lysine ratios on feed intake. For example, there is an antagonism between arginine and lysine in certain fish (e.g., Cobia) in that either a high or low ratio of arginine:lysine in the diet (e.g., 1.25/1.00 or 0.56/1.00 vs. 0.91/1.00) reduced their feed intake and growth (Van Nguyen et al. 2014). In contrast, some fish (e.g., *Midas Amphilophus citrinellus*) are not sensitive to a wide range of arginine:lysine ratios in diets (e.g., 0.27/1.00, 0.82/1.00, or 1.63/1.00; Dabrowski et al. 2007). Thus, supplementing up to 4% arginine to a casein–gelatin-based diet containing 28% CP for 6 weeks had no adverse effects on juvenile channel catfish (Buentello and Gatlin 2001).

Fatty Acids and Ketone Bodies

High levels of fatty acids in the GIT stimulate the release of intestinal peptide hormones CCK, PYY, GLP-1, gastric insulinotropic polypeptide, and gastric inhibitory peptide but suppress the release of ghrelin (Kaviani and Cooper 2017) and are therefore expected to inhibit feed intake. Similarly, elevated concentrations of fatty acids in plasma enhance the circulating levels of glucose and insulin, which further contributes to reductions in meal size and frequency. Usually, high-fat diets reduce gastric and intestinal emptying, increase the concentrations of free fatty acids in both the GIT and plasma, and promote the development of the fatty liver, thereby reducing food intake by animals. Such adverse effects of high-fat feeding occur despite no increase in dietary energy content. For example, in 4- to 16-week-old male rats fed ad libitum, food intake decreased from 36.3 to 29.6 g/kg BW/day because the fat content increased from 4.3 to 23.6% at the same dietary energy content of 4746 kcal/kg diet (Jobgen et al. 2009). In this case, the total dietary energy intake of the rats in the low- and high-fat groups was the same (145 kcal/kg BW/day; Jobgen et al. 2009).

High intake of dietary fats can result in increased production of acetoacetate and β-hydroxybutyrate (Chapter 6). In the fed state, elevated levels of these ketone bodies may also serve as a satiety signal to suppress food intake (Robinson and Williamson 1980). Intraperitoneal administration of β-hydroxybutyrate to 2-day-old chicks deprived of food for 3 h (0, 0.5, 1.0, or 1.5 g/kg BW) or intracerebroventricular administration of β-hydroxybutyrate (0, 0.25, 0.50, or 1.00 mg in 10 μl) under the same feeding conditions decreased subsequent food intake in a dose-dependent manner (Sashihara et al. 2001). Thus, in poultry, ketone bodies act as an inhibitory signal for food intake in both the central and peripheral nervous systems. In contrast, long-term intracerebroventricular infusion of β-hydroxybutyric acid to female rats for 28 days [fed either a high-fat/low-carbohydrate or a low-fat/high-carbohydrate diet (isocaloric)] reduced BW but not food intake (Sun et al. 1997). It is possible that peripheral oxidation of ketone bodies may be necessary for them to inhibit the centers of food intake control in the brain. Alternatively, there may be species differences in the response to exogenous ketone bodies.

Nitric Oxide

Nitric oxide (NO) is synthesized from arginine in nearly all cell types, including enterocytes and nitrergic neurons of the intestine (Chapter 7). By activating guanylate cyclase to produce cyclic guanosine monophosphate (cGMP), NO relaxes the smooth muscle of the GIT and stimulates the motility of the gut, thereby increasing food intake (Groneberg et al. 2016). Inhibition of NO synthesis impairs gastric accommodation and enhances meal-induced satiety (Tack et al. 2002). It is possible that NO plays a role in adaptive relaxation through a reflex response of the nonadrenergic, noncholinergic nerves to increases in intragastric pressure. Physiological levels of NO stimulate the food intake of many studied species, including chickens (Choi et al. 1994), mice (Czech et al. 2003), and rats (De Luca et al. 1995). These findings explain, in part, why dietary arginine deficiency reduces food intake by animals, including rats (Table 12.5).

Serotonin

Serotonin (a metabolite of tryptophan) is a neurotransmitter that mediates signal transduction between two neurons in the central (e.g., the PVN or VMH neurons for the food intake control) and gastrointestinal nervous systems (Abdalla 2017). Within the synapse, the first nerve cell releases serotonin into the space between the two neurons, and the second nerve cell has receptors that recognize serotonin and elicit a series of physiological responses (e.g., happiness when serotonin levels are high; Chapter 1). An inhibition of the reuptake of serotonin from the intercellular space prolongs its action. Intracerebroventricular or intraperitoneal administration of serotonin into animals, such as rats (Miryala et al. 2011) and fish (Pérez-Maceira et al. 2016), suppresses food intake by inhibiting the POMC/CART neurons in the ARC.

In animals, approximately 90% of serotonin is present in the GIT, and this neurotransmitter directly regulates intestinal contraction and appetite. When the intestinal concentration of serotonin is low, animals crave food. Conversely, a high concentration of serotonin, which can be brought about by activation of gastrointestinal serotonergic neurons, inhibits food intake by animals. Thus, drugs that stimulate serotonergic activity (e.g., fenfluramine, fluoxetine, and 3,4-methylenedioximetanfetamine) or block its uptake (e.g., Sibutramine and opioid antagonists) reduce meal size without affecting the initiation of feeding or meal frequency (Voigt and Fink 2015). Similarly, supplementing 4% tryptophan to the diets of young pigs and chicks substantially decreases their food intake (Edmonds and Baker 1987a,b).

Norepinephrine

Norepinephrine (a metabolite of tyrosine) is another neurotransmitter that regulates food intake by animals. The release of norepinephrine by the noradrenergic neurons (PVN or VMH) in the brain is stimulated by β-endorphin produced from the ARC neurons. Vagal inputs from peripheral tissues (e.g., the stomach and small intestine) can modulate norepinephrine release by neurons in the central nervous system, thereby integrating central and peripheral signals to control food intake (Voigt and Fink 2015). In general, norepinephrine inhibits food intake. Similar to serotonin, inhibition of norepinephrine reuptake by drugs (e.g., Sibutramine and GW320659) induces satiety and confers an antidepressant effect (Kintscher 2012). These findings explain, in part, why dietary supplementation with 4% phenylalanine (a precursor of tyrosine) reduces food intake by animals, including pigs and chicks (Edmonds and Baker 1987a,b).

Dietary Organic Acids

Weak organic acids (e.g., lactic acid, formic acid, acetic acid, propionic acid, butyric acid, citric acid, fumaric acid, and benzoic acid) are often supplemented to the diets of weanling mammals (e.g., swine) to enhance their feed intake during the first two weeks postweaning (Pluske 2013; Suiryanrayna and Ramana 2015). These acids can be both bacteriostatic and bactericidal, depending on the levels of their inclusion in the diet. Additionally, 0.8% lactic acid has also been added to drinking water for weanling piglets to improve their growth performance and feed efficiency, while reducing the number of *Escherichia coli* in the duodenum and jejunum (Cole et al. 1968). Organic acids can be provided from the microbial fermentation of feeds (Dai et al. 2020).

Dietary organic acids are beneficial for the intestinal health of weanling mammals. Of note, early weaned piglets (3–4 weeks of age) generally exhibit a limited digestive and absorptive capacity due to (a) insufficient production of gastric acid (HCl), as well as pancreatic and intestinal digestive enzymes; and (b) reductions in the small-intestinal villus area and the expression of intestinal transport proteins. Lowering dietary pH through the use of weak organic acids can alleviate the weaning-associated problems in young pigs. If possible, powder or pellet feeds should be stored under air-tight, dry, and cool conditions to maintain their good quality.

Dietary Nonnutritive Sweeteners

Like nutritive sugars, nonnutritive sweeteners can activate the sweet receptor (a T1R2/T1R3 heterodimeric protein) in the tongue and GIT of omnivores. One or more nonnutritive sweeteners are often added to the diets of weanling pigs to improve the palatability of the feed, as well as their feed intake, health, and growth performance. These sweeteners include sucram (550 times sweeter than sucrose; 20–100 ppm in feed), saccharin (e.g., 75 ppm in feed or 10 mM in drinking water), neotame (e.g., 10–50 ppm in feed), aspartame (e.g., 1 mM in drinking water), sucralose (e.g., 75–300 ppm in feed), stevia (e.g., 100–400 ppm in feed), stevioside (e.g., 100–300 ppm in feed), and neohesperidin dihydrochalcone (e.g., 30–75 ppm in feed; a derivative from citrus) based on published studies (Jansen and Kim 2024). At elevated inclusion levels in diets (e.g., 0.1–0.25%), nonnutritive sweeteners may beneficially alter the composition of the gastrointestinal microbiota to improve immunity and reduce the incidence of diarrhea in weanling mammals.

Other Chemical and Biochemical Factors

A dietary deficiency or excess of vitamins (e.g., the B complex of vitamins) and minerals (e.g., Na, Ca, P, Fe, and Zn) reduces food intake by nonruminants (Chapters 9 and 10). In addition, the color and physical form of food (e.g., hardness and particle size) can affect food intake (Forbes 2007). This is particularly important for weanling mammals such as pigs, calves, and lambs. These neonates can consume a large amount of liquid milk but only a small amount of solid food. For example, weanling piglets may eat < 10% of their preweaning DM intake during the first three days postweaning (Wu et al. 1996). Thus, highly palatable, digestible, and sweet foods (e.g., whey powder, blood meal, and sucrose), preferably in a liquid form, could be provided to weanling pigs. In addition, as both chemoattractants and enhancers of activation of the umami receptor on taste buds, dietary nucleotides (e.g., inosine 5'-monophosphate and guanosine 5'-monophosphate) can increase feed intake and improve growth in omnivores (e.g., pigs) and aquatic animals (e.g., fish). Interestingly, animals learn about the characteristics of their diets to develop conditioned preferences and aversions to meet their physiological needs in their life cycles (He and Wu 2020). Finally, animals with sepsis, fever, inflammation, cancer cachexia, and many other diseases exhibit low feed intake because endotoxins [e.g., lipopolysaccharide (LPS)] and inflammatory cytokines (e.g., IL-1α, IL-1β, IL-6, TNF-α, and interferon-γ) inhibit the AgRP/NPY neurons and stimulate the POMC/CART neurons to promote the release of the anorexic neurotransmitter α-MSH in the hypothalamus (Burfeind et al. 2016).

Control of Food Intake by Cytokines

Inflammatory cytokines (e.g., IL-1β, IL-6, and TNF-α) are soluble mediators released by the brain (e.g., astrocytes and microglia) and peripheral organs (including the liver and WAT) and cells (e.g., macrophages and lymphocytes) in response to infection, disease, and other stressors, including environmental and psychological factors (Table 12.10). Inflammatory cytokines potently reduce food intake by animals through synergistic and antagonistic interactions with the hypothalamus and other parts of the brain. In contrast, animals also produce anti-inflammatory cytokines (e.g., IL-2, IL-4, and IL-10) to maintain proper balances in physiological (including immunological) responses, thereby regulating feed intake and nutrient metabolism. Thus, trauma and heat stress promote the production of IL-1β, while decreasing the generation of IL-2. Our knowledge of this

TABLE 12.10
Release of Cytokines by the Central Nervous System and Peripheral Tissues in Animals

Cytokine	Source	Food Intake
Pro-inflammatory cytokines		
IL-1α, IL-1β, IL-6, and TNF-α	M1Φ/monocyte lineage (innate immunity), microglia, and astrocyte	Decrease
IL-2 and IFN-γ (T-helper-1)	T-helper lymphocyte, type-1 cell (Th1, cellular cytotoxic immunity)	Decrease
IFN-α	Many cell types (e.g., B-lymphocyte, M2Φ, and dendritic cell)	Decrease
Fibroblast growth factors (FGFs)	Many cell types (e.g., e.g., M1Φ, tumor cell, and osteocyte)	Decrease
IL-3 (during inflammation)	Immune and nonimmune cells	Decrease
Anti-inflammatory cytokines		
IL-1 receptor antagonist (IL-1Ra)	Many cell types (e.g., M2Φ, monocyte, neutrophil, fibroblast, and EP)	Increase
IL-4 and IL-10 (T-helper-1)	T-helper lymphocyte, type-2 cell (Th2, cellular cytotoxic immunity)	Increase
TGF-β (T-helper-3)	T-helper lymphocyte, type-3 cell (Th3)	Increase
IL-9	Many cell types (e.g., Th9, NKT, Th2, Th17, Treg, and mast cells)	Increase
IL-13	Many cell types (e.g., NKT, Th2, CD4, basophil, eosinophils, and mast cells)	Increase

Note: EP, epithelial cell; IL, interleukin; M1Φ. M1 macrophage; M2Φ. M2 macrophage; NKT, natural killer T-cell; TGF-β, transforming growth factor β; Th1, T-helper-1; Th2, T-helper-2; Th3, T-helper-3; Th9, T-helper-9; TNF-α, tumor necrosis factor-α; Treg, regulatory T-cell (formerly known as suppressor T-cell).

complex network is important for preventing and treating conditions like cachexia (a syndrome of weight loss) and anorexia (food refusal) that can occur under conditions of chronic diseases, infections, or other stresses. Antagonizing the actions of inflammatory cytokines can attenuate a decrease in food intake by animals.

Opioid peptides stimulates but naloxone (a synthetic opioid antagonist) inhibits, food intake by animals (Valbruna and Zvonarev 2020). Obese et al. (2007) reported that the intravenous administration of Syndyphalin-33 [SD33; Tyr-DMet (*o*)-Gly-methylphenylethylamide, a synthetic tripeptide alkylamide with prolonged analgesic activity] increased feed intake by sheep but did not overcome the LPS-induced suppression in feed intake. Interestingly, the oral administration of minocycline [a synthetic tetracycline family antibiotic with activity against neuroinflammation (Yong et al. 2004); 25 or 50 mg/kg BW/day] to LPS-challenged rats for 10 days substantially ameliorated the reduction in their feed intake (Abdo Qaid et al. 2022). In addition to pharmacotherapy, nutritional strategies to alleviate LPS-induced depression in feed intake include (a) the inclusion of highly palatable ingredients in diets; (b) dietary supplementation with specific nutrients (e.g., glutamine, glycine, arginine, vitamins E and C), prebiotics, and probiotics to support intestinal health and stimulate muscle protein synthesis; and (c) minimizing stress factors from the housing facilities.

REGULATION OF FOOD INTAKE BY RUMINANTS

The hormones, neurotransmitters, and neuropeptides in animals, as well as the physical, chemical, and nutritional characteristics of food that regulate feed intake by nonruminants (see above) influence feed intake by ruminants in a similar manner (Baile and Forbes 1974; Broderick et al. 2009; Daniel et al. 2013; Forbes 2007; Sartin et al. 2001, 2011; Whitlock et al. 2005). For example, the appetite neurotransmitters (e.g., NPY, orexin, and melanin-concentrating hormone) in the hypothalamus stimulate feed intake, whereas MSH inhibits feed intake in ruminants as expected (Sartin et al. 2011). In addition, obese sheep have markedly elevated concentrations of leptin in plasma but exhibit leptin resistance (Daniel et al. 2002) and the reduced transport of leptin from plasma into the brain (Adam and Findlay 2010). This may explain, in part, the inability of leptin to effectively signal satiety in obese sheep, leading to overeating and excess WAT gain. Note that kisspeptin induces GH release in short-term fasted (but not fed) sheep and has no effect on their DM or nutrient intake (Daniel et al. 2015; Foradori et al. 2017). This indicates that a change in the circulating level of only one factor may not be sufficient to affect feeding behavior. Like nonruminants, the productivity of ruminants is limited by the availability

of nutrients, particularly the dietary provision of energy, fat, and protein (Allen 2000; Forbes 2000). The following sections highlight these nutritional aspects.

Physical Limits of the Rumen

The bulk of food also affects the animal's appetite, particularly for ruminants, which typically depend on forages as a major source of nutrients (Ellis et al. 2000). Although the rumen has a very large capacity, the slow rates of digestion and passage of forage feeds mean that each particle of feed remains in the rumen for a very long time (typically 20–50 h) and that rumen capacity can be limiting to food intake. The more fibrous the forage is, the slower its digestion. There are stretch (tension) receptors in the smooth muscle layer of the rumen wall, as well as mechanoreceptors in the epithelium of the rumen. Of note, tension receptors and epithelial mechanoreceptors are also present in the reticulum, abomasum, the small intestine and the large intestine of ruminants (Forbes 2007). Thus, ruminal fill, acting through stretch receptors on the ruminal wall, is a major factor regulating the intake of feed (including forages) by ruminants. In addition, the entry of feed into the rumen and the increased amount of ruminal content can activate the ruminal epithelial mechanoreceptors. After feeding, physical distension and feed particles stimulate the tension receptors and mechanoreceptors in the ruminal wall, respectively, to generate chemical signals through the vagus nerve. These signals are received and interpreted by satiety centers in the brain to terminate a meal (Abdalla 2017). Any factors that decrease the residence time of the particle in the rumen, including reduced particle size of the food, increased activity of the ruminal microflora, and replacement of some of the forage with rapidly digested concentrate supplements, will increase voluntary food intake. In contrast, increased forage particle length, an increase in the dietary content of neutral detergent fiber (NDF), and a decrease in the digestibility of dietary DM (including NDF) will reduce feed intake by ruminants (Khan et al. 2014). Thus, food intake by ruminants is partially constrained by the physical limits of the rumen and other compartments of the GIT, as well as physical and chemical characteristics of dietary ingredients (e.g., particle size, particle fragility).

Control of Food Intake by Nutrients and Metabolites

Dietary Energy Content

Regarding feed intake, ruminants exhibit two-phase responses to dietary energy content (Baumgardt and Peterson 1971; Forbes 2007). Specifically, the feed intake of ruminants increases as dietary energy content increases within a physiological range, whereas their feed intake decreases as dietary energy content increases above the upper physiological level. For example, feed intake and dietary energy intake of growing sheep progressively increased when the dietary content of digestible energy was raised from 6 to 12.5 MJ/kg DM but gradually decreased when the dietary content of digestible energy was further raised from 12.5 to 14 MJ/kg DM (Baumgardt 1970). Similarly, feed intake and dietary energy intake of growing cattle increased with increasing dietary content of digestible energy from 8 to 13 MJ/kg DM, but their feed intake and dietary energy intake gradually decreased with further increasing dietary content of digestible energy from 13 to 15 MJ/kg DM (Baumgardt 1970).

Dietary Intake of Fats

The type and amount of dietary fats affect feed intake by ruminants (Palmquist and Jenkins 2017). Their feed intake and productivity are suboptimal when dietary fat content is low (e.g., ≤2.5% on a DM basis) possibly due to an inadequate provision of certain fatty acids (e.g., medium-chain, saturated, and monounsaturated fatty acids) for normal ruminal metabolism. Thus, supplementing 3.5% tallow (a kind of animal fat with relatively high amounts of medium-chain, saturated, and monounsaturated fatty acids) to an alfalfa hay-based diet (containing 2.1% crude fat) for dairy cows increased DM intake and milk yield by 8% and 15%, respectively (Table 12.11). In contrast, supplementing 3.9% plant-sourced oils [with high amounts of polyunsaturated fatty acids (PUFAs)] to a forage-based diet (containing 2.5% crude fat) for dairy cows decreased DM intake by 6.5%, without affecting milk yield (Table 12.11). These findings indicate that ruminal microbes are susceptible to the toxic effects of PUFAs as opposed to saturated fatty acids and monounsaturated fatty acids, possibly because the biohydrogenation of the former in the rumen requires H_2 or metabolic hydrogen ([2H]), thereby reducing their availability for ruminal metabolism, such as the production of methane and SCFAs. In addition, a high intake of dietary PUFAs increases the secretion of GLP-1 by the GIT, thereby inhibiting gastric emptying, reducing the motility of the small intestine (especially the duodenum and jejunum), and increasing satiety in ruminants (Relling and Reynolds 2007).

Like PUFAs, high levels of dietary fat also reduce feed intake by ruminants due to adverse effects of excess fatty acids on (a) the growth, activity, and population of ruminal microbes; (b) the function of the abomasum and the small intestine; and (c) the digestibility of fiber and other organic matter in the total GIT. In support of this view, supplementing 10% tallow in a grass silage-based diet (containing 2.0% crude fat) for dairy cows decreased DM intake by 22%, without affecting milk yield (Table 12.11). In addition, feeding a diet containing rumen-protected TAGs between late lactation and calving, as well as after parturition, reduced feed and energy intake by cows (Kuhla et al. 2016). The rumen-protected TAGs are hydrolyzed to fatty acids in the lumen of the abomasum and small intestine, leading to increases in the secretion of GLP-1 by the GIT

TABLE 12.11
Effects of Dietary Supplementation with Lipids (Including Fat) on Feed Intake and Milk Production by Lactating Cows with Free Access to Diets

	Nutrient Content in the Basal Diet (% of DM)				Supplemental Lipids						
Basal Diet	CF	CP	NDF	ADF	Source	Amount (% of DM)	Production Variables	Control Group	Lipids Group	SEM	*P*-Value
Forage + Conc[a]	2.5	17.1	30.9	18.3	Plants[b]	3.9	DM intake (kg/day)	18.6	17.4*	1.58	0.02
							Milk yield (kg/day)	26.9	26.5	2.55	NS
Hay + Conc[c]	2.1	17.1	34.6	21.1	Tallow	3.5	DM intake (kg/day)	18.9	20.4*	0.4	<0.05
							Milk yield (kg/day)	23.4	26.8*	0.5	<0.05
GS + Conc[d]	2.0	15.8	---	---	Tallow	10	DM intake (kg/day)	10.8	8.4*	0.22	<0.05
							Milk yield (kg/day)	13.9	13.7	1.7	NS

[a] Meta-analysis of 25 studies (Eugène et al. 2008). Dietary supplementation with lipids did not affect the content of fat (3.7%) or CP (3.1%) in milk, or methane production (19.3 vs. 18.9 g/kg of DM intake). Expressed as g/day, the rates of methane production by cows were 352 and 323 g/day in the control and lipid supplementation groups, respectively ($P = 0.03$).

[b] Oilseeds, either free oils (canola and soybean oils) or whole oilseeds (cotton, canola, and soybean), and calcium salts of palm oil.

[c] A study with 12 Holstein cows fed a basal diet containing 50% alfalfa hay (chopped), 26% corn (cracked), 10% beet pulp, 7.5% cottonseed meal, and 5% molasses (De Peters et al. 1989). The content of CP (3.21 vs. 3.03%) or fat (3.29 vs. 3.25%) in milk did not differ ($P > 0.05$) between the control and lipid supplementation groups (3.21 vs. 3.03%).

[d] A study with 4 Ayrshire cows fed a basal diet containing 88% rolled barley, 10% soybean meal, and 2% mineral mix (Clapperton and Steele 1983). The content of CP (3.15 vs. 2.98%) or fat (3.81 vs. 4.30%) in milk did not differ ($P > 0.05$) between the control and lipid supplementation groups (3.21 vs. 3.03%).

Note: CF, crude fats (crude lipids); Conc, concentrates; CP, crude protein; DM, dry matter; GS, grass silage; NDF, neutral detergent fiber; NS, not significant; ---, data are not available.

and its subsequent negative effect on reducing feed intake, as noted previously.

Dietary Nitrogen Content

In ruminants, food intake is reduced when they are fed diets containing either very low or very high content of nitrogen (N; Barker et al. 1988; Forbes 1996). A major function of dietary N (mostly protein and amino acids) is to fulfill the growth of ruminal microbes, which digest dietary carbohydrates to provide energy for both ruminal bacteria and the host. Moore and Kunkle (1995) reported that the dietary DM intake of growing cattle increases progressively from 0% to 2.5% of BW as dietary CP content increases from 0% to 8% (DM basis). Food intake and growth of ruminants (e.g., sheep and cattle) fed protein-deficient diets are increased by infusions of protein into the duodenum (Egan 1977). For example, growing beef cattle (135 kg BW) gain 0.23 kg BW when fed a 10%-CP diet (DM basis) but 1.36 kg BW when fed a 22%-CP diet. However, excess protein intake, which increases ammonia concentration in the rumen and plasma, reduces food intake by ruminants (Provenza 1996). Furthermore, in lactating cows, inadequate levels of protein and methionine in diets also reduce feed intake, and either increasing the dietary content of CP from 15.8% to 17.1% or dietary supplementation with 9 g of rumen-protected methionine per day for 4 weeks increased both feed intake and milk production (Table 12.12). Thus, adequate food intake is necessary for optimal ruminant production.

Glucose

In contrast to nonruminants, the concentration of glucose in plasma does not appear to play a role in regulating food intake by ruminants. For example, intraruminal, intravenous, or intracerebroventricular infusion of glucose did not influence food intake by cattle (Forbes 2007). An increase in plasma glucose concentration within a physiological range did not affect food intake by sheep or goats (Weston 1996). These results may be explained by a normally low circulating level of glucose in ruminants (2.3–3 mM), coupled with a low rate of glucose oxidation in the liver and other peripheral tissues.

Short-Chain Fatty Acids

SCFAs inhibit chemical receptors in the ruminal wall and play an important role in regulating feed intake by ruminants (Forbes 2007). When fed cereal grains that are highly digestible in the rumen, lactating cows consume 13% less feed (~3 kg DM/day; Allen 2000). Similarly, feeding more rapidly fermented starch to the cows increased the proportion of propionate in the SCFAs and the contribution of SCFAs as a fuel but reduced meal size by 17%, intermeal intervals by 10%, and overall food intake by 8% (Oba and Allen 2003). Much evidence shows that SCFAs have hypophagic effects on ruminants, and propionate is more potent in reducing feed intake than acetate or butyrate when infused into the portal vein of sheep or the mesenteric veins of steers (Allen et al. 2005). For example, compared

TABLE 12.12
Effects of Dietary Protein and Methionine Levels on Feed Intake and Milk Production by Lactating Cows[a]

	Crude Protein Level in Diet		Rumen-Protected Methionine in Diet		
Variable	**15.8%**	**17.1%**	**0 g/day**	**9 g/day**	**Pooled SEM**
DM intake	24.4	25.5*	24.6	25.3*	0.37
Milk yield	40.0	41.7*	40.0	41.4†	0.72

Source: Broderick et al. (2009).

[a] Values are kg/day. Holstein cows were fed corn silage-based experimental diets for 4 weeks.

* $P < 0.05$ and † $P = 0.10$ versus the corresponding control. DM = dry matter.

with the control, infusion of sodium acetate and sodium propionate (1 to 15 mol/3 h) into the rumen of cows and sheep reduces food intake in a dose-dependent manner (Grovum 1995). Some of these effects may be brought about by changes in osmotic pressure within the rumen. However, infusion of 1 mol of sodium acetate to the rumen depresses food intake more than the infusion of 1 mol of sodium chloride to the rumen (Forbes 2007). Interestingly, denervation of the liver prevents the effect of SCFAs on food intake in ruminants.

Extensive oxidation of propionate in the liver to produce ATP has been proposed as the major mechanism responsible for the hypophagic action of this SCFA. This further supports the view that feeding behavior is regulated, in part, by the hepatic oxidation of metabolic fuels (Allen and Bradford 2012). Of note, the hypophagic response to propionate is amplified by dietary intake of fats and long-chain fatty acids (particularly unsaturated long-chain fatty acids), hyperlipidemia during the periparturient period, as well as gut-derived hormones, such as CCK and GLP-1 (Ingvartsen and Andersen 2000; Litherland et al. 2005).

REGULATION OF FOOD INTAKE BY STRESS IN RUMINANTS AND NONRUMINANTS

Stress in animals can be defined as a state of physiological or behavioral adjustment in response to an adverse or challenging condition. In both ruminants and nonruminants, stress can be caused by physical (e.g., sleep deprivation and fatigue), physiological (e.g., weaning or pregnancy), pathological (e.g., infection and injury), behavioral (e.g., social mixing and long-distance transportation), or environmental factors (cold or elevated ambient temperatures). Physiological responses to stress include the activation of the hypothalamus-pituitary-adrenal axis and the sympathetic-adrenomedullary axis, followed by the increased release of glucocorticoids and catecholamines, respectively (Figure 12.5). These conditions are associated with changes

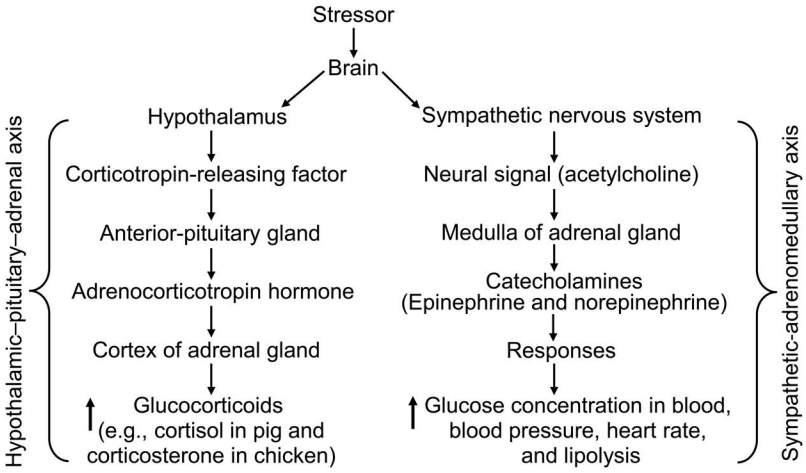


FIGURE 12.5 Physiological responses to stress in animals. The exposure of an animal to stressors causes the activation of the hypothalamus-pituitary-adrenal axis (HPA) and the sympathetic-adrenomedullary axis (SAM). This is followed by increases in the release of glucocorticoids and catecholamines from the cortex and medulla of the adrenal gland, respectively. Such hormonal changes result in alterations in nutrient metabolism and the activities of multiple systems (including cardiovascular, respiratory, digestive, and immune systems) in animals.

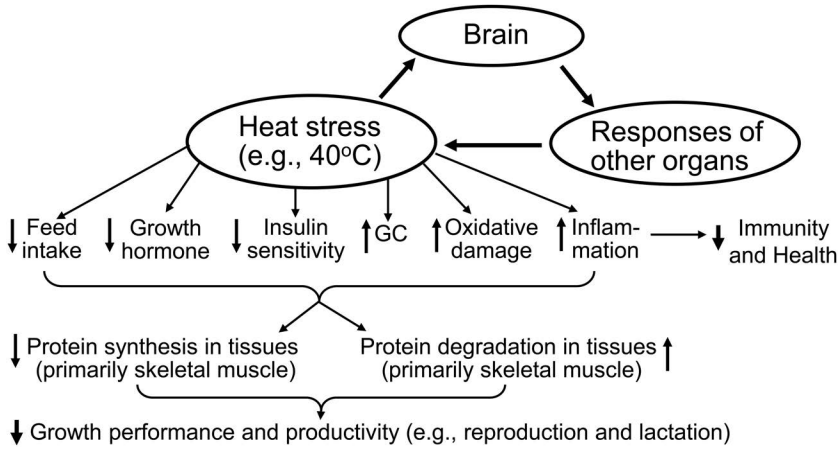


FIGURE 12.6 Relationships among stressors (e.g., elevated ambient temperatures), the brain, and responses in animals. Stress signals are transmitted to the brain, which regulates the activities (including metabolism and hormonal secretion) and coordination of other organs (including the liver, skeletal muscle, and lymphoid organs) via the release of modulatory factors (e.g., neurotransmitters and hormones). In response to heat stress, animals exhibit (a) reductions in feed intake and growth due to decreased protein synthesis and increased proteolysis in tissues (primarily skeletal muscle) and (b) impaired immune responses, increased susceptibility to infectious pathogens, and poor health.

in (a) circulating levels of regulatory factors [including hormones (e.g., leptin and CCK), cytokines (e.g., IL-1β and IL-6), and metabolites (e.g., glucose, fatty acids, and arginine)]; (b) heart rate, blood pressure, and respiratory rate; (c) the motility of the GIT in animals; and (d) the production of inflammatory cytokines and immune responses, depending on the type of stressors.

Stress may either increase or decrease food intake by animals, depending on its type. For example, exposure to endotoxins (e.g., LPS) or inflammatory conditions reduced food intake by ruminants via the enhanced secretion of (a) α-1-acid glycoprotein (an acute-phase protein) from the liver and (b) leukemia inhibitory factor (an IL-6 class cytokine) from the ARC of the hypothalamus in cows (Brown et al. 2021) and sheep (Daniel et al. 2016; Gregg et al. 2019). In addition, the exposure of cattle to low ambient temperatures (e.g., –20°C) elevates the circulating levels of not only glucocorticoids and catecholamines but also thyroid hormones, resulting in catabolic effects (e.g., muscle loss) as well as increases in feed and energy intake. By contrast, the exposure of cattle to 40°C suppresses their feed consumption and thus energy intake. Heat stress also promotes oxidative stress and inflammation and reduces insulin sensitivity, leading to protein losses from tissues (primarily skeletal muscle), as well as reductions in growth performance, productivity (e.g., reproduction and lactation), immunity, and health (Figure 12.6).

DIET SELECTION IN NONRUMINANTS AND RUMINANTS

Overview of Diet Selection by Animals

Animals can learn about the metabolic consequences of consuming diets with certain physical and sensory properties (e.g., appearance, flavor, and texture) and the content of energy and nutrients (e.g., protein, fats, sugars, and minerals) and choose preferentially or avoid diets that they have previously experienced (Forbes 2000). There are receptors for food flavors in the tongue and the GIT, which provides a biochemical basis for animals to reject, adapt to, or maintain food intake (Wu 2020). As demonstrated for laying hens, choice feeding, which allows self-adjustment of food intake to meet the requirements of animals for nutrients, may help to save the amount of the ration by 3–12% as compared with the conventional feeding (Henuk and Dingle 2002).

Animals prefer highly digestible foods that are abundant sources of energy and essential nutrients and dislike poorly digestible foods that are bulky and low in energy or protein (Baile and Forbes 1974). Thus, digestibility or gut-fill and food intake are positively correlated (Baile and Forbes 1974). In practice, a marginal deficiency of nutrients (particularly amino acids, vitamins, or minerals) leads to an increase in food intake, but a severe deficiency or excess of a nutritionally essential nutrient markedly reduces food intake. For example, animals choose diets containing a lower amount of protein as they get older to match the declining requirements for protein (Gietzen et al. 2007). Because most nutrients are toxic when present at very high levels in the diet, an excess of a nutrient may reduce food intake, growth performance, milk, and egg production. This is important for grazing animals and for animals offered different kinds of diets. In all animals, the quantity of their feed consumption and the content of nutrients in the self-selected diets reflect their nutritional requirements for maintenance, growth, and health. This involves the coordination of the GIT, the peripheral organs, and the central nervous system (San Gabriel and Uneyama 2013; Stanley et al. 2004). Diet selection is necessary for animals to survive during their evolution.

Nonruminant Omnivores

Rats, pigs, and chickens have an innate ability ("nutritional wisdom") to select a diet that contains adequate protein, amino acids, and mineral levels that are appropriate to their requirements (Baidoo et al. 1986; Baldwin 1985; Summers and Leeson 1979). For example, when rats had free access to diets containing 5, 25, 50, 100, or 200% of their lysine requirement, they foraged all the diets and selected the diet that contained 100% of their lysine requirement (Gietzen et al. 2007; Harper et al. 1970). When offered isocaloric 0.7%- and 1.0%-lysine diets, young pigs (7.5 kg) prefer a balanced diet (1.0%-lysine) over a lysine-deficient (0.7% lysine) one (Ettle and Roth 2009). Similarly, pigs and chicks are able to distinguish among diets differing in the content of methionine, threonine, or tryptophan and prefer a balanced diet over an amino acid-deficient one (Forbes 2007). In addition, given two choices of air-dried diets, growing pigs prefer one diet containing 17.4% CP (less limiting) rather than 12.7% CP (more limiting), or containing 21.3% CP (adequate) rather than 26.7% CP (excessive) (Kyriazakis et al. 1990). When 4- to 9-week-old broilers were given a choice between two foods, both of which contained higher protein content (28% or 32%) than that required for optimal growth, the animals preferred the lower protein (28%) diet and avoided the higher protein (32%) diet (Shariatmadari and Forbes 1993). Of note, when the broilers were offered a choice of two diets—6.5% vs. 11.5%, 6.5% vs. 22.5%, 11.5% vs. 22.5%, or 22.5% vs. 28% protein—they could differentiate successfully between two diets to meet their growth requirements for protein (Shariatmadari and Forbes 1993). Furthermore, poultry have an appetite for diets containing adequate levels of lysine, methionine, vitamin B1, vitamin B6, vitamin C, Ca, and zinc (Rose and Kyriazakis 1991). These findings demonstrate the "nutritional wisdom" of animals to choose between foods with adequate or low protein levels. The innate ability of animals to select food for optimal growth and health is orchestrated by changes in the concentrations of neurotransmitters in the central and peripheral nervous systems.

In addition to nutrients, nonruminants prefer to ingest specific feedstuffs at certain rates of inclusion in the diet. For example, Dove (1935) reported that, based on self-selection feeding, chicks consumed different amounts of various feed ingredients (% of total intake): yellow corn, 52.8; wheat bran, 21.3; fish meal, 11.4; oat meal, 8.9; bone meal, 2.9; dried skimmed milk, 2.1; and oyster shell, 0.6; the selected dietary mixture contained 17.9% CP, 2729 kcal ME/kg, 1.3% Ca, and 1.1% phosphorus. These values of nutrient content are similar to those recommended by the National Research Council (1994) for growing chicks: 18% CP, 2880 kcal ME/kg, 0.9% Ca, and 0.4% nonphytate phosphorus. In addition, at practical inclusion rates, postweaning pigs prefer to consume SBM containing 44% CP over SBM containing 48% CP, sunflower, or rapeseed meal; SBM over wheat gluten or potato; fish meal over spray-dried porcine plasma; dried skimmed milk over acid milk whey; and fresh feed ingredients over those stored for 10 months (Solà-Oriol et al. 2009).

Nonruminant Carnivores

As carnivores, dogs and cats have evolved differentially in some aspects of nutrition, metabolism, chemical sensing, and feeding behavior (Li and Wu 2024). Interestingly, dogs have adapted to omnivorous diets containing taurine-abundant meat and starch-rich plant ingredients, but domestic cats must consume animal-sourced foods for survival, growth, and development. Although dogs and cats have a relatively short digestive tract and a large stomach volume, they differ in the expression of certain taste receptors in the tongues (Li et al. 2006). For example, dogs, but not cats, possess sweet receptors. In addition, compared with dogs, cats have higher requirements for amino acids, some B-complex vitamins, and choline; greater rates of gluconeogenesis; a higher capacity to tolerate amino acid imbalances and antagonism; a more limited ability to synthesize arginine, taurine, and PUFAs from respective substrates; and cannot convert β-carotene into vitamin A. Thus, either food selection or feeding frequency differs between dogs and cats (Li and Wu 2024).

Food Selection of Dogs and Cats

Dogs can eat powdered, dry, semimoist, or canned diets. Dogs can rapidly consume a large meal that is sufficient to meet daily nutrient requirements, but they usually eat 4 to 8 meals/day and drink water during the light period. Dogs have evolved in their ability to digest a relatively large amount of cooked starch and select a diet lower in protein (30% of metabolizable energy from protein) than the diet of wild wolves (Buff et al. 2014). Thus, dogs have more latitude in the selection of food ingredients and more flexible adaptability to both animal- and plant-sourced diets. In addition, dogs have receptors for sweet substances and, therefore, have a preference for a sweet (e.g., 10% sucrose) solution compared to water. When offered complete and balanced diets with varying levels of protein, fat, and carbohydrate, dogs will choose a high-fat diet over a low-fat diet. Regarding dietary protein choices, dogs are more like rats than cats. When offered various levels of protein in diets, dogs will select diets containing 25–30% of energy as protein.

Unlike dogs, cats are picky eaters. Preferring meat with palatability factors (e.g., fats, amino acids, peptides, and nucleotides) as food, cats eat small prey, such as rats, mice, birds, lizards, and insects. Odor, taste, texture, particle size, and temperature of diets affect the food preference of cats. Cats may avoid a particular diet because of the textural difference but not the difference in its nutrient composition. Clearly, cats prefer (a) moist foods to dry foods, (b) warm foods to cold or hot foods, (c) high-protein foods to low-protein or protein-free foods, and (d) diets containing 25% total lipids to diets containing 10% or 50% total lipids (Li and Wu 2024). Thus, cats would select beef tallow over butter and chicken fat but have similar preferences for beef tallow,

lard, and partially hydrogenated vegetable oil. A diet consisting of high fat content and 5% hydrogenated beef tallow is particularly palatable to cats. By contrast, medium-chain fatty acids, present in either the free form (e.g., 0.1% caprylic acid) or triacylglycerols (e.g., 5% medium-chain triacylglycerols), and 25% hydrogenated coconut oil in diets can reduce food intake by cats (MacDonald et al. 1985). Of particular note, cats cannot taste sweetness because of the lack of sweet taste receptors due to the deletion of the *Tas1r* gene and, unlike dogs, do not select sweet substances such as sucrose (Li et al. 2006). For this reason, cats show no preference for sugar-rich foods such as fruits and juice.

When cats are offered complete and balanced diets with varying levels of protein, fat, and carbohydrate (without controlling palatability), cats will choose high-protein diets. Thus, high-starch diets over a prolonged period of time could be a major factor contributing to poor feline health and must be avoided in feeding both young and adult cats, particularly those with overweight or diabetes. Likewise, domestic cats select a macronutrient profile (52% of metabolizable energy from protein) similar to the diet of wild cats. Concerning amino acids, cats prefer proline, cysteine, ornithine, lysine, histidine, alanine, and leucine, but refuse arginine, isoleucine, phenylalanine, and tryptophan (Li and Wu 2024). This is consistent with the notion that cats are more sensitive to bitter taste than dogs. Although exposure of kittens to various flavors and textures early in life can affect their preference for foods later in life, adult cats can learn to select new and better flavors in addition to the ones early in life.

Healthy cats do not select sodium even if they are deficient in sodium, but acidotic cats will select diets containing excess sodium (Yu et al. 1997). Furthermore, cats can develop a learned taste aversion to diets that cause metabolic acidosis. Healthy cats are normally neophilic, namely selecting a new food (or flavor) if offered a variety of foods (or flavors) throughout their lives. Furthermore, cats do not consume a powdered diet but will ingest the same diet in a pelleted or gel form. If a diet is too dry or powdery, cats will eat very little even when no alternative food is offered.

Meal Frequency of Dogs and Cats

Dogs eat small and large prey, as well as plant-sourced foods. These animals can rapidly consume a large meal within 10 min that is sufficient to meet daily nutrient requirements, but they usually eat 4 to 8 meals per day (depending on breed). Growing puppies should have free access to food or be fed 2–3 times daily. Young and adult dogs generally ingest food and drink water during the light period and may eat at night if they are hungry or dehydrated, but some breeds of dogs also eat and drink during the dark period. On average, dogs drink more water than cats per kilogram of BW per day. Although adult dogs can do well on a regimen of eating a meal per day with possible health benefits, these animals should be offered food at least twice daily to ensure adequate nutrition and welfare. Multiple meals per day may help dogs to alleviate boredom while reducing the risk for the problem of gastric dilatation-volvulus in susceptible breeds of dogs (Bataller 1995). If overweight or obese, they should be fed either less or less energy-dense diets. Caloric restriction may help to reduce oxidative stress and delay aging in dogs (Lawler et al. 2008). Of particular note, intermittent fasting on a ketogenic diet containing medium-chain triglycerides may confer both metabolic and immunological effects in healthy dogs (Leung et al. 2020). Dogs with special health issues or dietary needs may require individualized feeding programs.

In contrast to dogs, cats do not have clear-cut circadian rhythms. However, some cats are nocturnal (e.g., resting during the daytime and going out or walking at night), and their existing rhythms can be altered by minor disturbances such as human noises. Cats generally eat frequent, small meals and drink water throughout the day and night and can voluntarily eat 12–20 meals/day, but fresh food should be offered daily. After they adapt to a new diet for 1 week, the size and number of meals are not affected by the type of diet. When cats eat a big portion of food at one time, they may immediately vomit it. Therefore, appropriate and frequent meals are critical for feline health. Pet owners can decide meal size and frequency for their dogs and cats rather than simply food availability.

Camara et al. (2020) recently reported that, compared with the feeding of the same canned food four times per day, adult cats fed only once daily had greater plasma concentrations of GLP-1, gastric inhibitory protein, and some amino acids within the initial postprandial hours (as expected because of more nutrient intakes at a single time), but a lower value of respiratory quotient (RQ) in the fasting state. These authors interpreted the data as showing that a reduced RQ value may result from a greater rate of whole-body fatty acid oxidation and that feeding once daily may be a beneficial feeding management strategy for indoor cats to promote satiation and lean body mass. However, a reduced RQ value in the fasting state may also result from a greater rate of the conversion of amino acids (released from the breakdown of muscle protein; possibly an adverse effect) into glucose, because this process has an RQ value of 0.333 (Chapter 8). Clearly, more studies are warranted to assess whether feeding once daily may have health benefits for cats.

Ruminants

Grazing ruminants must select forages wisely for growth and survival. They can discriminate among foods based on their color, taste, odor, texture, and shape; select nutritious foods from a diverse array of plant species to meet their nutritional requirements; and avoid foods with low nutrient content and toxins (Milne 1991; Provenza 1996). As explained in Chapter 1, the tongues of ruminants contain receptors for all five basic tastes, but some ruminants (e.g., cows) have fewer bitter receptors than other mammals and, therefore, are more tolerant to this taste. Interestingly, ruminants appear to have a distinct preference for the umami taste and the need-dependent preference for the salty taste (Ginane et al. 2011). Because the content of NaCl (salt) is

generally low in forages, grazing cattle are normally provided with salt lick blocks that also contain all required minerals and molasses (for masking the undesirable taste of some mineral supplements). For cows fed total mixed rations, the complete diets generally contain ~ 0.5% and 0.25% salt during the lactation and dry periods, respectively (on a DM basis).

Based on feedback from positive and negative consequences of foraging, as well as neurally mediated interactions among the sense, digestive, and central nervous systems, ruminants develop their habits of successful grazing. Like nonruminants, ruminants also have the "nutritional wisdom" to choose between foods with adequate, low, or high protein levels. For example, lambs acquire aversions to foods that are not balanced in amino acid composition (Egan and Rogers 1978). Furthermore, Kyriazakis and Oldham (1993) reported that growing sheep fed diets containing the same energy content (11 MJ ME/kg feed) but different CP contents (7.8%, 10.9%, 14.1%, 17.2%, and 23.5%; fresh feed basis) gained 273, 326, 412, 418, and 396 g/day, respectively. Specifically, when the sheep were given a choice between a 7.8%- or 10.9%-CP feed and a 23.5%-CP feed, they consumed different proportions of the 23.5%-CP diet (33.9% vs. 18.7% of total feed intake) such that the CP content in the mixed feeds was 13.1% and 13.3%, respectively. When the sheep were given a choice between a 14.1%- or 17.2%-CP feed and a 23.5%-CP feed, they consumed different proportions of the 23.5%-CP diet (17.6% vs. 9% of total feed intake), such that the CP content in the mixed feeds was 15.8% and 17.8%, respectively. Clearly, when the animals were offered diets containing CP content above the CP requirement, they preferred a diet with a lower CP content. Thus, sheep are able to select a diet that meets their CP requirement and avoid excessive protein intake. In addition to optimizing nutritional balance, ruminants also choose food with sufficient fiber content to stabilize ruminal fermentation (e.g., pH, SCFAs, and nonprotein N).

Foraging Behavior of Animals

Foraging behavior is an innate feeding strategy whereby animals acquire energy and nutrients to meet their nutritional and physiological needs. For example, when rats have free access to diets containing 5%, 25%, 50%, 100%, or 200% of their lysine requirement, they forage all the diets and then select the diet that contains 100% of their lysine requirement (Gietzen et al. 2007). Similar examples apply to methionine and tryptophan. Both dogs and cats avoid arginine-deficient diets while preferring flavor components in meat and peptides (e.g., protein hydrolysates) and free amino acids (e.g., alanine, histidine, leucine, lysine, and proline), as well as monosodium glutamate plus 5'-nucleotides (Li and Wu 2024). Honey bees forage pollens that are rich in protein and lipids for well-being, health, and productivity (Tsuruda et al. 2021). Foraging is mediated by complex interactions among nutrients (in both the GIT and plasma), gastrointestinal sensing and fill, hormones, neuropeptides, and neurotransmitters.

ECONOMIC BENEFITS OF FEED EFFICIENCY IMPROVEMENT

Feed efficiency refers to how effectively animals converts their diets into desired products (e.g., BW gain, fetal growth, milk yield, carcass yield, or litter size). Weight gain is easily measured in animal production and nutrition research. As noted previously, feed intake is the basis of livestock, poultry, and fish production. After food is ingested by animals, it is converted into protein and fats in tissues or other products (e.g., milk, wool, and eggs) through the metabolic transformations of nutrients and energy, as discussed in Chapters 5–10. By definition, optimal feed intake positively contributes to maximum feed efficiency. Koch et al. (1963) estimated that 25%, 38%, and 37% of the variation in the BW gain of cattle were attributed to genetic differences in feed consumption, genetic differences in feed efficiency, and environmental influences, respectively. Because metabolism is the foundation for the expression of potential genotypes as desirable phenotypes (e.g., BW gain), adequate knowledge about animal nutrition (including the regulation of food intake) is essential to maximum feed efficiency. In general, feed cost contributes to ~70% of the total cost of animal production (e.g., 66% in calf feeding and 77% in yearling feeding; Spangler 2013), and feed efficiency is a major determinant of farm profits. For example, in U.S. cattle production, a 10% improvement in feed efficiency results in a reduction of feed costs by U.S. $1.2 billion and a 43% increase in profits, whereas a 10% improvement in BW gain results in an 18% increase in profits (Spangler 2013). Furthermore, Taylor (2016) reported that, in the beef industry, a 1% improvement in feed efficiency has the same economic impact as a 3% increase in the rate of BW gain. Similar relationships either between feed efficiency and profits or between BW gain and profits also hold true for swine and poultry production (Williams 2012).

Although actual feed intake by pigs, poultry, or dairy cows housed in pens can be easily measured, actual feed intake by grazing ruminants is difficult to accurately quantify. Koch et al. (1963) recognized that rates of feed intake are related to the BW and weight gain of animals. These authors suggested that feed intake could be partitioned into two components: (a) the feed intake expected for the given level of production and (b) a residual portion. The residual portion of feed intake, namely RFI, could be used to identify animals that deviate from their expected level of feed intake. The lower the RFI value, the more efficient the animal is at converting feed into animal tissues or products. Thus, RFI, which is a measure of feed efficiency, is defined as the difference between an animal's actual feed intake (FI) and its expected FI (FI_{exp}) based on its size and growth over a specified period.

$$\text{Residual feed intake (RFI)} = \text{Feed intake (FI)} - \text{Expected feed intake } (FI_{exp})$$

Expected FI can be predicted from production data by using feeding standard formulas or by regression using actual feed test data; namely

$$FI_{exp} = b_0 + b_1 \times ADG + b_2 \times BW^{0.75} + e,$$

where ADG = average daily BW gain (kg/day), $BW^{0.75}$ = the metabolic weight of an animal, b_0 = intercept, b_1 = the partial regression coefficient of FI on ADG, b_2 = the partial regression coefficient of FI on metabolic BW, and e = the random error of FI (Connor et al. 2019). In cattle divergently selected for RFI, heat production plus body composition plus physical activity explained 73% of the variation in RFI: namely, protein turnover + tissue metabolism + stress, 37%; digestibility, 10%; heat increment and fermentation, 9%; physical activity, 9%; body composition, 5%; and feeding patterns, 2% (Herd and Arthur 2009). The mechanisms responsible for 27% of the variation in RFI are still unknown and may involve amino acid synthesis, gluconeogenesis, ion transport, and renal reabsorption. Because the heritability of RFI is higher than FI, the RFI is a useful variable for breeding and selection of domestic animals, including cattle (Herd and Arthur 2009) and pigs (Patience et al. 2015).

SUMMARY

Eating through the mouth is the ordinary way for animals to obtain energy and nutrients. Food intake is essential to the growth, development, and survival of mammals, birds, fish, and shrimp. A limited amount of ration is provided to some livestock species during a certain phase of production (e.g., gestating sows, gestating ewes, and breeding stocks) in modern farming systems so they will not become obese or overweight. However, most ruminants and nonruminants have free access to their foods under practical housing, grazing, or feeding conditions. Knowledge about physiological and nutritional control of food intake by animals is critical for animal productivity and welfare, as well as feed efficiency. The latter can be measured as RFI in ruminants. Research over the past 50 years has shown that the feed intake of animals is regulated by complex interactions among nutrients (in both the GIT and plasma), gastrointestinal sensing and fill, hormones, neuropeptides, and neurotransmitters (Figure 12.7). Either high dietary content of fats, protein, and energy or low dietary content of vitamins (e.g., B-complex vitamins) and minerals (e.g., Na, Ca, P, and Zn) reduces food intake by animals. The relationship between dietary energy content and food intake is

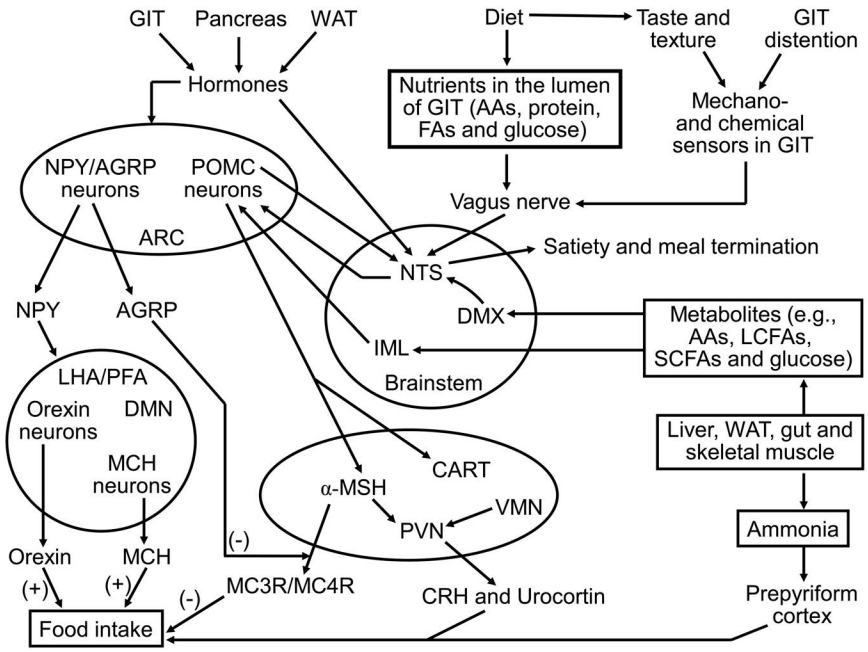


FIGURE 12.7 A summary of mechanisms whereby the food intake of animals is regulated by a complex interactive network among nutrients (in both the gastrointestinal tract and plasma), gastrointestinal sensing and filling, hormones, neuropeptides, and neurotransmitters. AAs, amino acids; AgRP, agouti-related peptide; ARC, arcuate; CART, cocaine-amphetamine regulated transcript; CRH, corticotrophin-releasing hormone; DMN, dorsal medial nucleus of the hypothalamus; DMX, dorsal motor nucleus of the vagus nerve; FAs, fatty acids; GIT, gastrointestinal tract; IML, intermediolateral cell column; LCFAs, long-chain fatty acids; LHA, lateral hypothalamic area; MC, melanocortin; MC3R, melanocortin-3 receptor; MC4R, melanocortin-4 receptor; MCH, melanin-concentrating hormone; α-MSH, α-melanocyte-stimulating hormone; NPY, neuropeptide Y; NTS, nucleus tractus solitarius; PFA, perifornical area; POMC pro-opiomelanocortin; PVN, paraventricular nucleus; SCFAs, short-chain fatty acids; VMN, ventromedial nucleus; WAT, white adipose tissue. (+), stimulation; (–), inhibition.

complex because nutrient metabolism depends on amino acids, vitamins, and minerals, as well as cellular regulatory machinery. In general, feed intake is reduced when dietary energy or nutrient content is low, increased when dietary energy or nutrient content is intermediate, and decreased when dietary energy or nutrient content is high.

Leptin (released from the WAT) and insulin (released from the pancreatic β-cells) inhibit food intake by activating the POMC/CART neurons and suppressing the AgRP/NPY neurons. These two hormones play an important role in both the acute and long-term energy balance of animals. Three important gastrointestinal peptides that regulate food intake are ghrelin, PYY, and CCK. These molecules act through different mechanisms. Specifically, PYY and CCK (released from the endocrine cells in the GIT) suppress AgRP/NPY neurons and reduce expression of the NPY gene, respectively, thereby inhibiting food intake. In contrast, ghrelin (another gastrointestinal peptide) stimulates food intake of animals by suppressing the POMC/CART neurons and activating the AgRP/NPY neurons through the Ca^{2+}-protein kinase C signaling pathway. As the major "hunger hormone", ghrelin opposes the actions of leptin (the major "satiety hormone"). In addition to the neuropeptides and hormones that are synthesized from amino acids, neurotransmitters are either amino acids (e.g., aspartate, glutamate, and glycine) or their metabolites (e.g., NO, serotonin, GABA, and norepinephrine). Nutrients and hormones communicate either directly or indirectly via signaling mechanisms to the hypothalamus and brainstem. Thus, dietary intake of protein or amino acids profoundly influences the neurological network, and therefore food intake by animals. This provides the biochemical basis for self-selection or "foraging" of foods as an innate ability of both ruminants and nonruminants to meet their nutritional and physiological needs. Collectively, food intake is regulated by dietary, genetic, physiological, pathological, and environmental factors, as well as management and behavior.

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13 Feed Additives

Improving the efficiency of utilization of dietary nutrients is essential to enhance animal productivity (Wu et al. 2014) and farm profits (Spangler 2013). Feed additives can play important roles in the quest to achieve this goal (Bedford and Apajalahti 2022). Digestion and metabolism of nutrients are two major targets of research and feeding practice for increasing nutrient utilization by livestock, poultry, companion animals, and aquatic species. Digestive enzymes are synthesized and secreted by the gastrointestinal tract (GIT), intestinal microbes, liver, and pancreas to digest feedstuffs (Chapter 1). However, in some circumstances (e.g., neonatal weaning, heat and cold stress, and enteric disease conditions), the amounts of digestive enzymes secreted into the GIT (e.g., proteases, carbohydrases, and lipases) may be insufficient for the optimal digestion of their targeted substrates (Beauchemin et al. 2001; Bedford 2000). In addition, nonruminant animals do not produce enzymes to degrade some dietary components (e.g., fiber and phytate) and thus supplementation with the appropriate enzyme can bring about significant benefits. Furthermore, nutrients that are ordinarily present in feed may require additional fortification in the form of concentrated additives if maximal growth or feed efficiency of animals is to be achieved (Tan and Yin 2017). Exogenous substances are often needed as antimold or antioxidative ingredients in rations under production conditions to maintain their shelf lives. Therefore, dietary supplementation with digestive enzymes, functional ingredients, growth-promoting agents, or mycotoxin absorbents can support a more economically viable utilization of raw materials by animals while reducing the output of undigested nutrients [e.g., protein, carbohydrates, calcium (Ca), and phosphorus (P)] and microbial fermentation products (e.g., ammonia, urea, and indoles) in the manure (Bedford and Schulze 1998; Phillips et al. 1995; Weaver and Kim 2014).

Any substance that is intentionally added in small quantities to feed to improve its "quality" is a feed additive. Over the past six decades, feed additives [exogenous enzymes, amino acids, fatty acids, sugars, vitamins, minerals, antioxidants, probiotics, prebiotics, postbiotics, and antibiotics] have received increasing attention from both nutritionists and producers (Bedford and Apajalahti 2022; Biely 2016; Cowieson and Roos 2016; De Oliveira et al. 2012; Hou et al. 2017; Jiang and Xiong 2016). Of note, animals are subject to various environmental challenges during their life cycles (Figure 13.1). How animals respond to a feed additive will depend on many factors, including (a) its source, form, and bioactivity; (b) age, physiological conditions, digestive structure, and health status (e.g., bacterial, viral, or parasite infection); (c) dietary ingredients, their interactions and nutritional composition, and their content of antinutritional factors; and (d) the environment in which animals are managed (e.g., indoors vs. outdoors) and fed. Thus, the efficacy of feed additives varies with animal species, farm management practices, feed composition, and methods of feed manufacture, and their economic value must be verified under practical production conditions. The main objective of this chapter is to highlight the use of feed additives in animal nutrition and the underlying mechanisms of action and to outline those conditions where their inclusion in diets is most likely to result in beneficial effects for livestock, poultry, companion animals, and aquatic species.

ENZYME ADDITIVES

Overview

Barley, oats, rye, triticale (a hybrid of rye and wheat), and wheat are common ingredients of poultry and swine diets in many regions of the world (Bedford 2000; Pettersson and Aman 1989). However, these cereals contain large amounts of high molecular weight, soluble nonstarch polysaccharides (NSPs), which can form viscous gels in the presence of water and under the conditions of the GIT (Chapter 2). The resistance of the luminal contents of the intestines to deforming is defined as intestinal viscosity. A major adverse effect of NSPs results from their ability to increase digesta viscosity in the GIT, which reduces

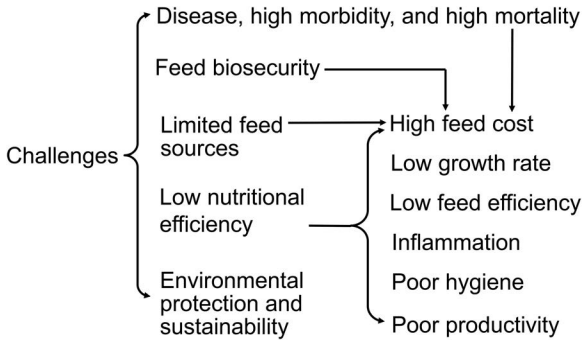


FIGURE 13.1 Environmental challenges faced by animals during their life cycles. Between birth and market weights, the health, well-being, growth, and production of livestock, poultry, and aquatic species are affected by a plethora of physiological (e.g., age and genes) and environmental factors (e.g., nutrition and pathogens) that can impact on economic profitability. These factors are also applicable to companion animals. Productivity includes spermatogenesis, pregnancy, lactation, plowing, and egg laying, depending on species and sex.

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diffusional rates of all molecules, especially large entities such as fat micelles. This is especially a problem in chickens compared with pigs, possibly due to the higher dry matter (DM) content of poultry compared with swine digesta, which effectively concentrates the viscous NSP fraction in the former (Bedford and Apajalahti 2022). Such conditions impair the digestion and absorption of nutrients in the host, and the undigested starch, protein, and lipids entering the large intestine can interact with the gut microflora to alter the morphology and function of the digestive system (Chapter 5). Intestinal viscosity is primarily driven by the presence of high molecular weight, water-soluble, viscous NSPs (e.g., β-glucan, guar gum, arabinoxylan, and pectin). This increases the volume of intestinal contents, interferes with nutrient digestion, and slows down nutrient absorption. In contrast to water-soluble fiber, water-insoluble NSPs do not significantly contribute to intestinal viscosity. Thus, significant quantities of NSP-degrading enzymes (NSPases) are added to almost all poultry diets based on wheat and, to a lesser extent, barley-, oats-, and rye-based diets (Bedford and Schulze 1998), and in the case of swine diets, the use rate is still very high but not as universal. Most animal feeds in the world are based on corn, however, and this grain is not viscous but still responds well to NSPase use in both chickens and pigs (Kerr and Shurson 2013; Liu et al. 2021). It is likely that such a benefit is derived from the production of soluble, fermentable fiber from the insoluble NSP, which drives a more favorable intestinal microbiome in poultry and swine (Bedford and Apajalahti 2022).

The feed enzymes used in nonruminant diets include (a) phytases, xylanases, proteases, glucanases, and mannanases in order of quantitative use; (b) a mixture of all these enzymes; (c) a mixture of proteases; and (d) a mixture of carbohydrases and proteases (Figure 13.2). In contrast, carbohydrases for ruminant diets generally fall into the classification of cellulases and hemicellulases (Kung 2001). Note that "hemicelluloses" is an old and inexact term and should be renamed generically "NSPases" or more specifically "xylanases and β-(1,3;1-4)-glucanases".

Although hundreds of enzyme products are marketed for livestock and poultry to date, they are manufactured primarily from four fungal species (*Trichoderma reesei, Aspergillus oryzae, Pichia pastoris*, and *Saccharomyces*

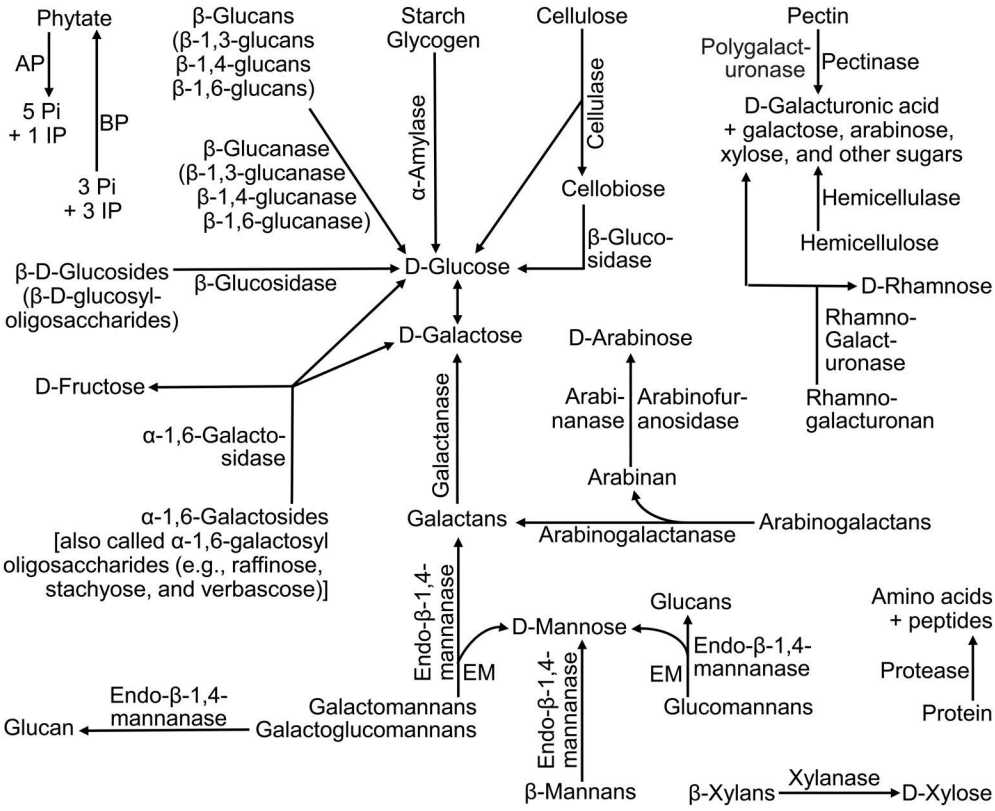


FIGURE 13.2 Use of carbohydrases and proteases to hydrolyze polysaccharides and protein in diets, respectively. All these enzymes have been used as feed additives to nonruminant diets, whereas cellulase and hemicellulase are generally used for ruminant diets. Complete hydrolysis products are shown. However, in the digestive tract of nonruminants fed the feed carbohydrases, plant-source polysaccharides are partially hydrolyzed. *A complex mixture of enzymes is required to fully degrade hemicelluloses. AP, acidic phytase; BP, alkaline phytase; EM, exo-mannanase; IP, inositol phosphate; Pi, inorganic phosphate.

cerevisiae) and four bacterial species (*Bacillus subtilis, Lactobacillus acidophilus, Lactiplantibacillus plantarum,* and *Streptococcus faecium* spp.; Meale et al. 2014). Note that the order of quantitative use is *Trichoderma*, *Aspergillus*, *P. pastoris*, then far less is *Bacillus*, and, to a much lesser extent, the rest of the microbes. In nonruminants fed barley- or rye-based diets, a reduction in the viscosity of the small-intestinal contents is primarily responsible for the beneficial effect of supplementation with carbohydrases on nutrient digestion and absorption. Intestinal viscosity is almost irrelevant in all corn-based diets, and most NSPases are fed corn- and SBM-based diets simply because corn grain accounts for ~70% of the nonruminant feed. In such cases, supplemental carbohydrases break down NSPs to generate fermentable, soluble fiber, along with the production of stimbiotic oligosaccharides (Bedford and Apajalahti 2022). In contrast, supplemental enzymes work mainly by facilitating the utilization of dietary fiber in ruminants (Beauchemin et al. 1997; Iwaasa et al. 1997). Thus, the use of enzyme additives depends on animal species and their developmental stages, with the goal of improving dietary nutrient digestibility and intestinal health (i.e., intestinal integrity and functionality, including digestion, absorption, metabolism, barrier and immunological defenses, and the maintenance of the intestinal microbiota).

Special Thermozymes (Thermotolerant Enzymes) for Feeds

Like any proteins, feed enzymes can be inactivated by heating, acidification, alkaline treatment, and by interaction with certain metals. In recent years, some thermostable enzymes (called hyperthermophilic enzymes or thermozymes) have been produced by hyperthermophiles (bacteria and archaea with optimal growth temperatures of >80°C) or by *Escherichia coli* through DNA-recombinant techniques (Vieille and Zeikus 2001). To date, thermozymes (thermotolerant enzymes) for feeds include xylanase, phytase, endoglucanase, and cellobiohydrolase (Fakruddin 2017). Their biochemical properties have important implications for the feed industry. Specifically, in the manufacturing of animal feeds, pelleting is often used to convert mash feeds into compressed pellets to confer several advantages, including convenient handling and transport, a reduction of dust and waste, a higher digestibility of carbohydrates, and a reduction or elimination of dietary pathogens (Amerah et al. 2011). First, this process involves a short conditioning period of the exposure of feed to high temperatures (e.g., 70 to 100°C for seconds to several minutes, depending on the type and formulation of the feed) and steam. Second, the feed undergoes extrusion through a pelleting die that temporarily raises the temperature of the feed because of friction. Because thermozymes are stable under pelleting conditions, it is now feasible to incorporate these enzymes into the mixer prior to manufacturing feed pellets. Some enzymes that are not thermotolerant are still used in the feed industry, but they have to be added as a liquid, sprayed onto the feed post-pelleting.

Enzyme Additives for Nonruminants

Dietary NSPs can reduce nutrient digestion by (a) increasing intestinal viscosity and thus decreasing the rate of digestion and (b) encapsulating nutrients (e.g., starch, protein, lipids, minerals, and vitamins) in the feed matrix, thereby preventing their exposure to digestive enzymes and the digestive medium (e.g., bile salts for fat emulsification). This ultimately decreases the availability of nutrients from grain feedstuffs for the animal host. Microbial NSPases can hydrolyze both water-soluble and insoluble fiber in diets (Chapter 5). These enzymes can improve the digestibilities of dietary carbohydrates and associated nutrients (e.g., protein and minerals) in the small intestine of nonruminants (e.g., swine and poultry). In addition, these enzymes can release insoluble fiber from the feed matrix and convert high-molecular-weight viscous fiber into smaller carbohydrate fractions that are more fermentable in the large intestine (Bedford and Apajalahti 2022). This is nutritionally significant as the fiber is fermented to provide short-chain fatty acids (SCFAs; major energy sources for the epithelial cells of the large intestine), while reducing fecal output and environmental pollution. Notably, viscosity effects and the benefits of hydrolysis of NSPs appear to be greater in the young than older nonruminants when fed plant-based diets, although the benefits related to increased fermentation in the large intestine are more relevant for older animals with more mature microbiota (Bedford 2000).

Endo-β-1,4-Xylanases

Pentosans [$(C_5H_8O_4)_n$] are homopolysaccharides consisting of β-(1-4)-linked pentose residues. Arabinans and xylans are two examples of pentosans and are hydrolyzed by arabinase and xylanase (collectively called pentosanases), respectively. Endo-β-1,4-xylanases (also known as endoxylanases or xylanases) that break down arabinoxylans are highlighted in this section for the following reasons. First, arabinans are absent from cereals, and the common oilseed meals where arabinans are present have little effect on intestinal viscosity in poultry and swine (Bedford and Apajalahti 2022). Second, arabinoxylans are significant antinutritional factors in corn grain and its products for nonruminants (Šimić et al. 2023). Third, corn-derived arabinoxylans are poorly fermented by the endogenous microbiota in poultry (including broiler chickens) and swine (Bach Knudsen 2014; Baker et al. 2021).

Xylanases are glycoside hydrolases that catalyze the random cleavage of the β-1,4-D-glycosidic linkages in the xylan backbone of arabinoxylans to generate constituent oligosaccharides such as xylo-oligosaccharide (XOS) and arabino-XOS, as well as esterified XOS and feruloylated

oligosaccharides. These enzymes are synthesized by a variety of fungi, bacteria, yeast, marine algae, protozoans, snails, crustaceans, insects, and plant seeds, but are not produced by mammalian or avian cells (Gírio et al. 2010; Kulkarni et al. 1999). Soluble arabinoxylans are relatively abundant in rye, wheat, and triticale (a hybrid of wheat and rye) and can induce viscosity in the small and large intestines of poultry and swine. When poultry and swine are fed diets that are based on cereals containing considerable amounts of soluble arabinoxylans, their weight gains and feed efficiency can be improved in response to dietary supplementation with xylanase (Baker et al. 2021; Bedford 2000; Gorenz et al. 2022; Pettersson and Aman 1989).

Poultry. Exogenous xylanases of microbial origin were initially introduced to wheat- or rye-based broiler diets in Europe about 40 years ago. The outcome was to decrease intestinal viscosity, risk for enteric disease, and fecal moisture content, as well as the incidence of footpad lesions, while improving intestinal health and growth performance in poultry. For example, dietary supplementation with xylanase improved feed efficiency and growth performance of broiler chickens fed rye-based diets (Grootwassink et al. 1989). Similarly, adding xylanase to rye- or wheat-based diets improved energy and protein digestibilities, as well as weight gain and feed conversion ratio in young broiler chicks (Table 13.1). Likewise, xylanase (e.g., 100 units/kg diet) can also increase the apparent digestibilities of crude protein (CP), DM, and energy in broiler chickens fed corn- and SBM-based diets that contain fewer water-soluble arabinoxylans than insoluble arabinoxylans (Stefanello et al. 2016). Furthermore, feeding high levels of rye to laying hens reduced egg production, but their productive performance was improved in response to dietary supplementation with xylanase. Compared with β-glucanase, xylanase appears to be less effective in reducing the adhesiveness of the fecal excreta in poultry, probably because (a) the arabinoxylan fragments remaining after the action of xylanase are relatively large and, therefore, contribute to the residual viscosity; and (b) there is continued arabinoxylan solubilization throughout the lower gut.

Choct (2006) reported that various microbial sources of supplemental feed xylanase improved body weight (BW) gains in broiler chickens, despite having different effects on small-intestinal viscosity (Table 13.2). The antinutritive effects of rye appear to be alleviated in older poultry when compared to young birds, likely due to the presence of a larger amount of microbial xylanase in the lumen of the crop as well as the small and large intestines of older birds. Furthermore, adding xylanase to rye-based broiler diets decreased the number of mucosa-associated enterococci and other Gram-positive cocci in the small intestine, and this effect was greater in tallow- than in soy oil-supplemented diets (Danicke et al. 1999). This may be explained by (a) unsaturated fats and fatty acids are more easily emulsified and better digested than saturated fats and fatty acids, and (b) the emulsification of fats and fatty acids in viscous diets is compromised under feeding conditions without supplementation with carbohydrases. Improvement in the digestion of saturated fat may minimize the incidence of intestinal inflammation (Bedford and Apajalahti 2022). Results of recent studies suggest that XOS (products of xylanase) may have prebiotic effects to beneficially alter the intestinal microbiota in poultry (Van Hoeck et al. 2021). Specifically, XOSs selectively stimulate the growth of beneficial bacteria (particularly *Bifidobacteria*) in the intestine, resulting in a favorable shift in the composition and activity of the intestinal

TABLE 13.1
Effects of Dietary Supplementation with Pentosanase Preparations on Growth Performance of Young Broiler Chicks

		Inclusion Levels of Enzyme (g/kg)						
Variable	**Days**	**0**	**0.11**	**0.22**	**0.44**	**0.88**	**SE**	***P*-value**
Body weight (BW, g)	15	282	347	350	364	373	5.22	<0.01
	27	810	893	914	941	951	9.50	<0.01
Weight gain (g)	15–27	524	545	564	576	577	7.74	<0.01
Feed intake (g)	1–15	413	470	464	477	482	7.71	<0.01
	1–27	1410	1532	1519	1533	1531	19.0	<0.01
	15–27	997	1062	1055	1056	1049	13.7	<0.01
Feed conversion[a]	1–15	1.46	1.36	1.33	1.31	1.29	0.02	<0.01
	1–27	1.74	1.72	1.66	1.63	1.61	0.02	<0.01
	15–27	1.89	1.94	1.87	1.83	1.82	0.03	<0.01

Source: Pettersson and Aman (1989). Chicks were fed a rye- or wheat-based diet between days 15 and 27 of age.
[a] Feed:BW gain (g/g).

TABLE 13.2
Effects of Dietary Supplementation with Different Sources of Xylanase on Small-Intestinal Luminal Viscosity and Carbohydrate Concentrations, and Growth Performance of Broiler Chickens[a]

	Viscosity (mPa•s)		Jejunal Luminal Carbohydrate[b]		Body Weight Gain[c]	Feed Conversion
Enzyme Supplementation	Jejunum	Ileum	Free Sugars	Soluble NSPs	(g/wk per bird)	Ratio (Feed:Gain, g/g)
Control (none)	9.4[B]	28.3[B]	1381[C]	445[B]	345[B]	2.16[A]
Xylanase A	5.2[C]	8.3[C]	1700[A]	689[A]	392[A]	1.97[B]
Xylanase B	18.4[A]	84.1[A]	1782[A]	696[A]	395[A]	2.01[B]
Xylanase C	3.4[C]	7.2[C]	1635[B]	426[B]	383[A]	2.04[A,B]

Source: Choct (2006).

[a] Xylanase A was isolated from *Thermomyces lanuginosus*, Xylanase B from *Humicola insolens*, and Xylanase C from *Aspergillus aculeatus*.

[b] mg/g marker.

[c] Broiler chickens were fed a wheat-based diet containing the normal content of metabolizable energy (13.7–14.5 MJ/kg dry matter).

[A-C:] Within a column, means not sharing the same superscript letters differ ($P < 0.05$).

Note: NSPs, nonstarch polysaccharides; mPa•s, millipascal•second; wk, week.

microbiota. This aids in promoting their fermentation to SCFAs (especially butyrate), which can improve gut health and overall well-being in animals.

Swine. Pigs fed a rye-based diet gained less BW compared with those fed a barley-based diet (Table 13.3). However, dietary supplementation with xylanase to young pigs fed rye-based diets did not have a consistent effect in enhancing growth performance (Bedford et al. 1992). Similarly, Thacker et al. (2002) reported that dietary supplementation with xylanase failed to improve the growth performance of pigs (21.5–100.7 kg BW) or their carcass traits. Most recently, Petry and Patience (2020) found that xylanase supplementation to corn-based diets for pigs increased the digestibility of dietary fiber in 50% of studies, as well as BW gain and feed efficiency in only 33% and 26% of studies, respectively. The lower efficacy of xylanase in swine than poultry diets is likely also a reflection of the ability of the fully established microbiome in older pigs to produce the enzymes required to degrade pentosans. Of note, there have been reports that dietary supplementation with xylanase improves intestinal barrier integrity, anti-inflammatory and antioxidative responses, and other health markers in growing pigs, while reducing mortality in both high- and low-health commercial herds (Baker et al. 2021; Petry and Patience 2020). Because corn and its industrial products [e.g., distiller's dried grains with solubles (DDGS)] that contain arabinoxylans as the major NSPs are commonly used in swine diets, further research on the mode of action of xylanase and its optimal supplemental doses is warranted.

TABLE 13.3
Effects of Dietary Pentosanase or β-Glucanase Supplementation on the Growth Performance of Weanling Pigs Fed Rye- or Barley-Based Diets

Treatment	Weight Gain (kg)	Feed Intake (kg)	Feed Efficiency (Gain:Feed, g/g)	Starch Digestibility (%)	Small-Intestine Luminal pH
High rye-based diet (Experiment 1; mean initial body weight = 10.7 kg)					
Control	4.24	6.94	0.608	81.9	6.04–6.10
Pentosanase	4.24	6.64	0.637	84.1	6.28–6.57
Hulless barley-based diet (Experiment 2; mean initial body weight = 12.1 kg)					
Control	5.24	9.05	0.572	73.8	5.86–6.19
β-Glucanase	6.14 *	9.57	0.646	71.5	5.76–6.19

Source: Bedford et al. (1992). Values are means, n = 6. Yorkshire × Landrace pigs were weaned at an average 26 days of age and housed in pairs in pens. Littermate pairs were randomly assigned to one of the two experimental treatments, providing 6 pens and 12 pigs per treatment. Following a 5-day period of acclimation, pigs had free access to their rye- or barley-based diets for 10 days. * $P < 0.05$ vs. the corresponding control group.

Phytase

As noted in Chapter 10, phytate is a phosphate-conjugated inositol and the major storage form of phosphate in plants. Phytate P accounts for >50% of total P in most plant-sourced feedstuffs, including barley, canola meal, corn, rice bran, soybean meal (SBM), and wheat bran (Table 13.4). Phytate P content is 140% higher in SBM than that in corn grain, but is similar between barley and corn grain. Phytate is often present as a protein-phytate complex in plants (Figure 13.3), rendering protein less susceptible to proteases in the small intestine and increasing the endogenous losses of nutrients (including amino acids and minerals). Furthermore, phytate has a capacity to chelate positively charged cations, especially Ca, iron, zinc, magnesium, potassium, and manganese (Figure 13.3). When phytate binds to digestive enzymes in the lumen of the GIT, these enzymes are less effective in hydrolyzing lipids, carbohydrates, proteins, minerals, and vitamins (Humer et al. 2015). By reducing the availability of dietary P and other nutrients, phytate has considerable antinutritive effects for animals and increases their requirements for dietary nutrients (Dersjant-Li et al. 2015; Woyengo and Nyachoti 2013).

Phytate itself is not a good source of P for nonruminants because of inadequate phytase activity in the GIT. In animals, the sources of phytase for phytate digestion may be endogenous mucosae, gut microflora, plant ingredients, and exogenous phytase preparations (Humer et al. 2015). Although phytase is present in the small-intestinal mucosa of broilers, laying hens, and swine, the enzyme activity may be insufficient to release phosphate from phytate under normal nutritional and physiological conditions. A deficiency of P may occur when inorganic sources of P are not added to nonruminant diets. Due to the uncertainty of the bioavailability of plant P, dietary formulations often rely on added

TABLE 13.4
Phytate Content in Plant-Sourced Foods and Feeds of Plants (on an As-Fed Basis)

Feed	Total P (%)	Phytate (%)	Phytate Pa (%)	Non-PT P (%)	Feed	Total P (%)	Phytate (%)	Phytate Pa (%)	Non-PT P (%)
Bakery meal	0.33	0.65	0.18	0.15	Rice (broken)	0.11	0.22	0.06	0.05
Barley	0.32	0.71	0.20	0.12	Rice (brown)	0.27	0.79	0.22	0.05
Canola expellers	1.09	3.15	0.89	0.20	Rice (white, polished)[b]	0.28	0.53	0.15	0.13
Canola meal	1.12	3.09	0.87	0.25	Rice bran (full fat)	1.91	5.94	1.66	0.26
Copra meal	0.52	0.79	0.22	0.30	Rice bran (DF)	2.35	7.52	2.11	0.23
Corn grain	0.25	0.65	0.18	0.06	Rice mill feed	0.63	2.01	0.56	0.07
Corn germ	1.13	2.97	0.84	0.29	Sorghum grain	0.30	0.80	0.23	0.08
Corn germ meal	0.79	1.90	0.54	0.26	Soy PC	0.62	1.41	0.39	0.23
Corn gluten meal	0.56	1.66	0.47	0.09	Soybean grain[b]	0.58	1.28	0.36	0.22
Corn DDGS (HO)	0.81	0.53	0.15	0.66	SBM (≥47% CP)	0.64	1.56	0.44	0.21
Corn DGGS (DF)	1.01	0.14	0.04	0.97	SBM (<47% CP)	0.63	1.66	0.47	0.16
Corn DDGS (LO)	0.82	1.14	0.32	0.50	SBM (fermented)	0.60	0.88	0.25	0.35
Corn (hominy feed)	0.70	2.07	0.58	0.12	Soybean expellers	0.78	1.36	0.38	0.40
Cottonseed meal	1.14	2.65	0.75	0.42	Sugar beet pulp	0.70	0.14	0.04	0.66
Field peas	0.42	0.73	0.17	0.25	Sunflower (full fat)	0.70	1.80	0.51	0.19
Hybrid rye	0.28	0.74	0.21	0.07	Sunflower expellers	0.65[c]	1.99	0.56	0.09
Lemna PC	0.51	0.15	0.04	0.47	Sunflower meal (PD)	0.93	2.88	0.81	0.13
Oat bran[d]	0.73	1.42	0.40	0.33	Sweet potato[b]	0.048	0.043	0.012	0.036
Oat grain	0.32	0.67	0.19	0.13	Triticale	0.38	0.74	0.21	0.17
Palm kernel expellers	0.53	1.25	0.36	0.17	Wheat	0.38	0.97	0.27	0.11
Palm kernel meal	0.53	1.18	0.33	0.20	Wheat (hard red)	0.36	0.89	0.25	0.11
Potato[b]	0.050	0.039	0.011	0.039	Wheat (soft red)	0.36	1.15	0.32	0.04
Potato PC (isolate)	0.13	0.33	0.09	0.04	Wheat bran[e]	1.16	3.44	0.97	0.19
Peas[e]	0.38	0.60	0.17	0.21	Yeast brewers	1.40	1.03	0.29	1.11
Pea (cowpea)[b]	0.35	0.99	0.25	0.10	Yeast (torula)	1.78	0.25	0.07	1.74

Source: Lee and Stein (2025); Lee et al. (2023a).

[a] Phytate-P was calculated by multiplying the total phytate by 0.282.

[b] Ravindran et al. 1994. The content of dry matter in pea (cowpea), potato, sweet potato, and soybean is 90%, 21%, 23%, and 96%, respectively (Hou et al. 2019).

[c] This value was taken from Ibagon et al. (2021).

[e] Eeckhout and De Paepe (1994).

[d] Gualberto et al. (1997).

Note: CP, crude protein; DF, defatted (<5% oil); DDGS, dried distillers grains with solubles; HO, high oil content (>9% oil); LO, low oil content (5% to 9% oil); PC, protein concentrate; PD, partially dehulled; SBM, soybean meal.

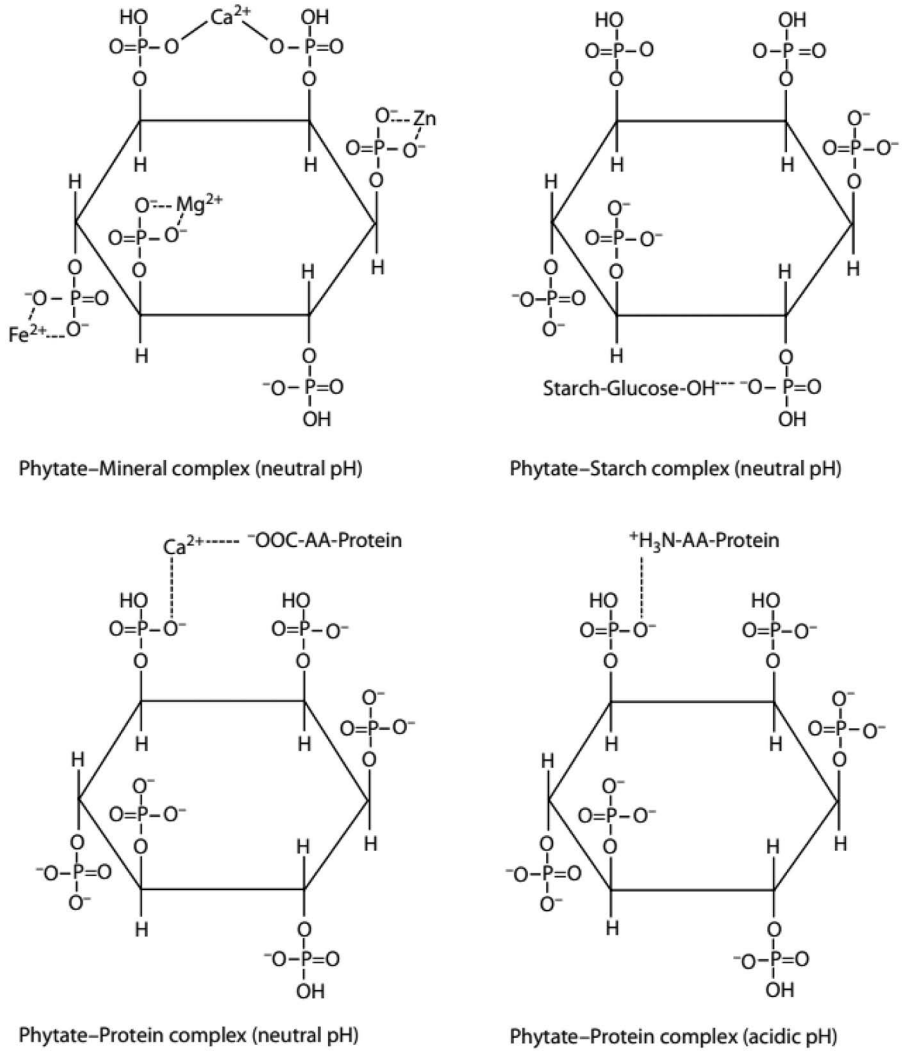


FIGURE 13.3 Interactions of phytate with protein, minerals, and starch/glucose in feedstuffs at a neutral pH. Note that the interaction of phytase with protein at an acidic pH differs from that at a neutral pH. In addition, monosaccharides or starches are bound to phytate via noncovalent hydrogen bonds. (Adapted from Humer et al. 2015.)

inorganic phosphates to meet most of the animal's needs. Therefore, most diets for nonruminants contain ~50% more P than the animal's daily requirements. Any excess P is excreted in feces and urine, which, if not managed properly, can cause environmental pollution. The need to reduce P and N excretion levels led to a market opportunity for the introduction of exogenous phytases.

There are two types of phytases: acidic phytases (optimum pH, 3.0–5.5) and alkaline phytases (optimum pH, 7.0–8.0). Acidic phytases from bacteria, fungi, or plants hydrolyze phytase to generate five inorganic phosphate and one inositol-phosphate molecule or even myo-inositol. However, none of the current commercial phytases produce inositol as an end product. Alkaline phytases hydrolyze phytate into inositol-triphosphates and three inorganic phosphate molecules. Like any enzyme, phytase activity is affected by pH (acidic pH in gastric and neutral in the small intestine), inhibitors (e.g., Ca, Fe, and Zn), and temperature. Through increasing phytate hydrolysis, supplementing phytase to diets for poultry and swine can enhance the bioavailability of dietary P by 20–30% or even up to 50% with higher doses (Humer et al. 2015). As a result, the amount of added inorganic P can be reduced accordingly without adverse effects on the health and growth performance of animals (Dersjant-Li et al. 2015; Humer et al. 2015). In nonruminants, the digestibility of amino acids is also positively correlated with the degree of phytate degradation (Amerah et al. 2014). In certain cases, the presence of natural phytase in some feed ingredients (e.g., wheat) may reduce the benefits of dietary phytase supplementation (Dersjant-Li et al. 2015).

Dietary Ca content affects phytase activity in the small intestine. The greater the Ca content of the diet, the less effective the phytase activity. This is because Ca not only coordinates with phytate in the gastric environment but also precipitates phytate and inhibits phytase activity at the pH

found in the lumen of the small intestine, thereby reducing the susceptibility of phytate to enzymatic hydrolysis. Use of chelators such as citric acid, which remove Ca from soluble phytate complexes, can be effective in increasing phytase activity in the small intestine. The ratio of Ca to P in diets is also important because an exceedingly high Ca:P ratio may reduce the nutritional benefits of phytase supplementation. There is evidence that phytase supplementation has beneficial effects in improving the digestion of dietary P, DM, and protein in broiler chickens fed diets with Ca to available P ratios of 1.43, 2.14, 2.86, and 3.57 (Amerah et al. 2014). Even though phytase is added to poultry and swine diets, they must still provide sufficient Ca and P to ensure proper skeletal (bone) growth and development, as well as whole-body metabolism and homeostasis.

β-Glucanases

β-Glucanases are proportionally very minor as compared to phytase and xylanase from a commercial perspective. β-Glucanases are also called β-D-glucan glucanohydrolases and, more accurately, β-(1,3;1,4)-glucanases. Poultry and swine do not produce animal-sourced enzymes to break down β-(1,3;1,4)-D-glucans in cereals (Campbell and Bedford 1992). In plants, these NSPs are unbranched polymers of β-(1,4)-glucans with interspersed β-(1,3)-linkages (Chapter 2). β-Glucans are present in higher plants (e.g., barley, oats, and wheat) as components of endosperm and its outermost layer (aleurone) and are particularly rich in barley and oats. When fed to poultry and swine, the viscous β-(1,3;1,4)-D-glucans in grains (e.g., barley and wheat) reduce digestion efficiency, as noted previously, and in some cases can pass through the entire GIT, where they manifest in the fecal excreta as sticky droppings (Burnett 1966).

Poultry. The use of enzymes for poultry diets dates back to the 1920s when Clicker and Follwell (1925) supplemented crude enzymes, called "protozymes", from *A. oryzae* into the diet of poultry to improve their growth performance and feed efficiency. Studies in the 1940s and 1950s demonstrated that adding a crude "amylase" preparation (which was later discovered to contain β-glucanase activity) to barley-based diets for chicks resulted in a significant improvement in BW gain and feed conversion ratio (Hastings 1946; Fry et al. 1957). These beneficial effects of β-glucanase on nutrient utilization and weight gains in young poultry fed barley-based diets have also been reported by other investigators (Edney et al. 1989; Grootwassink et al. 1989). However, the addition of a combination of xylanase and β-glucanase to corn- and SBM-based diets deficient in metabolizable energy (ME), CP, Ca, and P resulted in a significant increase in growth performance and utilization of DM, energy, P, and nitrogen (N) in broiler chickens (Lu et al. 2013; Liu et al. 2021), suggesting that intestinal-viscosity reduction is not always the mechanism most at play.

Interestingly, there are reports that supplementation with a β-glucanase/xylanase enzyme complex to barley-, oat-, or rye-based diets for laying hens does not consistently improve egg production (e.g., the number, weight, and specific gravity of eggs; Brenes et al. 1993). It is possible that in older birds, β-glucanase are produced by the microbes in the small intestine can digest β-glucans to some extent and thus limit the response noted above. In support of this view, mature, cellulolytic microflora are established in the digestive tract of older poultry. Reducing sticky droppings is important for cleanliness in group-raised broilers and for sanitation in egg production.

Swine. In general, the efficacy of dietary NSPases is somewhat lower in swine than in poultry. Adding β-glucanase to a barley-based diet can enhance the BW gain of 31- to 41-day-old postweaning pigs without affecting feed efficiency (Bedford et al. 1992). Importantly, enzyme supplementation may reduce diarrhea in weanling piglets. In contrast, dietary supplementation with β-glucanase does not result in a significant beneficial effect on growth performance or feed efficiency in 2-month-old or older pigs (19–100 kg BW; Graham et al. 1988; Thacker et al. 1988). Similarly, supplementation with β-glucanase to a corn- and SBM-based diet did not affect DM, energy, or CP digestibility in 6-kg pigs (Li et al. 1996). These findings may be explained by (a) denaturation of β-glucanase by the strong acid HCl in the stomach (with pH ~2) of older pigs; this will depend upon the enzyme employed because many are quite stable to gastric digestion; (b) relatively low contents of β-glucans in corn- and SBM-based diets; (c) the presence of β-glucanase in some feed ingredients; and (d) sufficient hydrolysis of β-glucans of such diets by microbial enzymes present in the small and large intestines of pigs. Thus, the most likely application of β-glucanase to the swine industry may be its addition to barley-based diets for weanling pigs in which the intestinal microbiome is not well developed.

Other Enzymes

β-Mannanase

Corn- and SBM-based diets, which are widely used in the United States, China, and many other countries to feed non-ruminants, contain high amounts of mannose-linked NSPs [e.g., 1.2% galactomannans (Kim et al. 2003)]. Microbial β-mannanases (glycoside hydrolases) are endo-acting enzymes that hydrolyze the internal glycosidic bonds of the mannan backbone chain, whereas β-mannosidases are exo-acting enzymes that hydrolyze the terminal ends of mannan oligosaccharides (MOSs) released by the action of β-mannanases. Thus, β-mannanases may be added to such diets for poultry and swine to improve their digestive function and productivity. Jackson et al. (2004) conducted an experiment to determine the effects of graded levels of β-mannanase (0, 50, 80, and 110 MU/ton) on the growth of 0- to 21-day-old male broilers fed antibiotic-free diets that were based on corn and SBM. Dietary inclusion of the enzyme at 80 or 110 MU/ton improved BW gain by 3.9–4.8% and feed efficiency by 3.5–3.8% over the control group, but the inclusion of 50 MU/ton had no significant benefit. Similarly, supplementation with β-mannanase (400 IU/kg diet) to a corn- and SBM-based diet enhanced the daily weight gain of 2- to 22-day-old broiler chickens by 3.5% (Kong et al. 2011). Likewise, Lv et al. (2013) found that

adding β-mannanase to a corn- and SBM-based diet (200, 400, or 600 U/kg diet) for growing pigs with an initial BW of 23.6 kg for 28 days increased (a) the digestibilities of dietary CP, fiber, Ca and P and (b) BW gain and feed efficiency in a dose-dependent manner. Moreover, dietary supplementation with 0.06% β-mannanase to postweaning pigs enhanced the apparent total tract digestibility of fats, jejunal villus height, the ratio of villus height to crypt depth, and the depth of the lower crypt depth, while tending to reduce the abundance of *E. coli* in the cecum (Jang et al. 2020). Note that not all studies have identified a positive effect of β-mannanase as a feed additive for pigs because supplementation with 0.05% β-mannanase to corn- and SBM-based diets had no effect on nutrient digestibility or growth performance of weanling and growing-finishing pigs (Pettey et al. 2002). Collectively, these findings indicate that the small-intestinal bacteria of poultry or young swine do not produce sufficient β-mannanase and that an exogenous source of this enzyme is necessary to fully digest corn- and SBM-based diets.

Enzyme Mixtures

Although some commercial enzymes employed for animal feeding are not pure preparations (with most being monocomponent products or their blends), the activities of associated enzymes may be much lower than those of the principal enzymes. Plant-sourced feed ingredients contain a variety of complex polysaccharides that are usually linked to proteins. Thus, a mix of enzymes may be more effective than a single enzyme in improving the digestibility of dietary nutrients. For example, Kim et al. (2003) reported that supplementation with a mixture of α-1,6-galactosidase, β-1,4-mannanase and β-1,4-mannosidase to a corn- and SBM-based diet enhanced DM and amino acid digestibilities, as well as feed efficiency in nursery pigs (a 35-day trial, 6.3–19.1 kg BW; and a 21-day trial, 8.0–15.2 kg BW). These authors also found that the use of the enzyme mixture decreased the concentrations of stachyose in the proximal and distal small intestine and of raffinose in the distal small intestine. Furthermore, supplementation with different multi-enzyme preparations to corn and SBM-based diets (small amounts of wheat, wheat screenings, barley, mill-run, canola meal, and peas) fed to 7 kg pigs for 28 days improved nutrient digestibility and growth performance (Omogbenigun et al. 2004). These mixed enzyme products contained xylanase, β-glucanase, amylase, protease, invertase, and phytase activities but differed in the type of plant cell wall-degrading activities (e.g., cellulase, galactanase, and mannanase; cellulase and pectinase; or cellulase, galactanase, mannanase, and pectinase). The use of protease is notable for enhancing the hydrolysis of plant-source proteins in the small intestine to augment the efficiency of their utilization for lean-tissue growth in nonruminants (Cowieson and Roos 2016). Thus, adding a mixture of β-glucanase and proteases to a corn- and SBM-based diet can increase the digestibilities of DM, energy, CP, and P in growing pigs (Ji et al. 2008). Likewise, 4-week dietary supplementation with a mixture of β-mannanase, α-amylase, and protease to growing barrows (mean initial BWs of 55.6–56.9 kg) augmented their daily BW gain by 3.3% and 2.6%, respectively, when fed a corn- and SBM-based or a complex diet (Jo et al. 2012). Results of recent studies indicated that adding 1% of an enzyme blend (glucoamylase from *Aspergillus niger*, α-amylase from *Bacillus stearothermophilus*, lipase, maltase, cellulose, and protease) to the diets of growing-finishing pigs increased BW, gain:feed ratios without affecting average daily feed intake, carcass weight, the apparent total track digestibilities of DM and gross energy, and the abundance of fecal *Lactobacillus*, while reducing back-fat thickness, the count of fecal *E. coli*, and NH_3 emissions (Balasubramanian et al. 2020). Collectively, these results indicate that the use of complex carbohydrases along with proteases can optimize the response of monogastric animals to feed enzymes. Despite these reports, there is evidence that blends of multicarbohydrase enzymes may not always outperform properly dosed monocomponent enzymes for nonruminants, depending on dietary composition, feeding regimen, animal age and health status, on-farm management, and the environment (Masey O'Neill et al. 2014).

Enzyme Additives for Ruminants

Efficient hydrolysis of cellulose and hemicelluloses by digestive enzymes in the GIT (mainly the rumen and large intestine) is essential for the utilization of dietary fiber by animals (Chapter 5). The resultant β-D-glucose is at equilibrium with α-D-glucose for metabolism. Over the past 60 years, many studies have been conducted to determine the effects of dietary supplementation with fiber-degrading enzymes on the growth and lactation performance of ruminants. The early enzyme preparations were poorly defined, and animal responses were variable (Burroughs et al. 1960; Perry et al. 1960; Rust et al. 1965). For example, when cattle were fed diets consisting of ground ear corn, oat silage, corn silage, or alfalfa hay mixed with an enzyme cocktail containing amylolytic, proteolytic, and cellulolytic activities (Agrozyme®), they gained 6.8–24.0% more BW and exhibited a 6.0–21.2% improvement in feed efficiency compared with the cattle fed untreated control diets (Burroughs et al. 1960). In contrast, Perry et al. (1960) reported that supplementation with Agrozyme reduced the daily weight gain of beef cattle by 20.4% when the enzyme product was fed with a corn silage diet. Clearly, interactions occur between dietary ingredients and enzyme preparations to affect their efficacy, and perhaps the product used varied from batch to batch, because it is unlikely that all claimed activities were measured in feed. Although many enzymes are required to fully degrade cellulose and hemicelluloses in the rumen and large intestine, not all of their preparations are currently available for additions to ruminant diets.

The opportunity to improve growth and lactation performance of ruminants through dietary supplementation with feed enzymes lies in complementing the rumen that already has an active microbiome to produce carbohydrases, proteases, peptidases, and lipases. The

TABLE 13.5
Effects of Adding Commercial Feed Enzymes on the Growth Performance of Feedlot Finishing Cattle[a]

	Inclusion Levels of Enzymes			
	Control	1 ×	2 ×	Change
Study 1 (no ionophore, no implant)[b]				
Initial weight (kg)	407	414	---	---
Dry matter intake (kg/day)	9.99	9.53	---	–5%
Live-weight gain (kg/day)	1.43	1.52	---	+6%
Dry matter intake/Live-weight gain	7.11	6.33	---	–11%
Study 2 (ionophore and implant)[c]				
Initial weight (kg)	477	477	477	---
Dry matter intake (kg/day)	11.1	11.5	11.5	+4%
Live-weight gain (kg/day)	1.70	1.87	2.01	+10% to +18%
Dry matter intake/Live-weight gain	6.50	6.18	5.70	–5% to –12%

[a] Feedlot cattle were fed high-concentrate diets consisting of barley grain, supplement and barley silage.
[b] Beauchemin et al. (1997).
[c] Iwaasa et al. (1997).

development of molecular biology techniques, reductions in fermentation costs, and the availability of better-defined enzyme preparations (e.g., cellulases and xylanases) have prompted re-examination of the potential use of exogenous enzymes in ruminant production (Meale et al. 2014). Some published studies have provided sufficient evidence for the responses of beef and dairy cattle to exogenous enzymes with consistent and predictable increases in weight gains (Tables 13.5 and 13.6). These findings argue against the previous view that the endogenous activity of ruminal microbes to break down plant cell walls could not be augmented by supplementary exogenous enzymes. The beneficial effects of exogenous enzyme preparations result mainly from improvements in ruminal fiber digestion, and ultimately an increase in the availability of digestible DM to the host. However, there are reports of various effects of dietary supplementation with exogenous fibrolytic enzymes on lactation and growth performance in ruminants (e.g., dairy cows, beef cattle, goats, sheep, or buffalo; Sujani and Seresinhe 2015). Clearly, much research is required to define the conditions under which consistent positive responses in ruminant production to enzyme preparations can be realized. With new knowledge, fibrolytic enzymes hold great potential to enhance feed utilization and productivity (e.g., growth and lactation performance) in ruminants.

NONENZYME ADDITIVES

Nonruminants

There is a long history of the use of nonenzyme additives in poultry and swine diets (Cromwell 2000; McDonald et al. 2021; Wang et al. 2012; Wang et al. 2016a; Yue et al. 2017). The additives include antibiotics, probiotics, prebiotics, mycotoxin absorbents, antioxidants, and crystalline amino acids. Animal producers have successfully improved weight gain and feed efficiency of nonruminants through the use of antibiotics and chemotherapeutics. Over the past 50 years, 17 antimicrobial agents (12 antibiotics and 5 chemotherapeutics) have been used as feed additives (Table 13.7). Nearly all antibiotics used for feeding nonruminants contain N, and some of them are aminoglycosides (Figure 13.4). The presence of N atoms in antibiotics alters the electronic configuration of the compounds and confers or enhances their antimicrobial activity (Patrick 1995). For example, in penicillin, the N atom of the β-lactam structure makes its carbonyl carbon highly electrophilic, thereby facilitating the binding of the antibiotic to transpeptidases (enzymes that catalyze the final step in cell-wall biosynthesis, i.e., the cross-linking of peptidoglycans) on the plasma membrane of bacteria (Sauvage et al. 2008). This results in an inhibition of the transpeptidases and the killing of susceptible bacteria. Without a cell wall, a bacterium cannot

TABLE 13.6
Effects of Supplementing Feed Enzymes to Diets for Cows in Early Lactation

	Rode et al. (1999)		Yang et al. (2000)		
Variable	Control	Enzyme	Control	Enzyme in Conc	Enzyme in TMR
Dry matter intake (kg/day)	18.7	19.0	19.4	19.8	20.4
Milk production (kg/day)	35.9	39.5	35.3	37.4	35.2
Milk composition (g/kg)					
Fat	38.7	33.7	33.4	31.9	31.4
Protein	32.4	30.3	31.8	31.3	31.3
Lactose	47.3	46.2	46.5	46.5	45.6
Live-weight change (kg/day)	-0.63	-0.60	0.15	0.04	0.14
Dry matter digestibility (%)	61.7	69.1	63.9	66.6	65.7
NDF digestibility (%)	42.5	51.0	42.6	44.4	45.9

Sources: Rode et al. (1999); Yang et al. (2000).
Note: Conc, concentrates; NDF, neutral detergent fiber; TMR, total mixed ration.

TABLE 13.7
Antimicrobial Agents Historically Used for Swine Diets

Antibiotics	Antibiotics	Chemotherapeutics
Apramycin*	Oxytetracycline*	Arsanilic acid†
Bacitracin methylene disalicylate	Penicillin*	Carbadox (synthetic)†
Bacitracin zinc	Sulfaquinoxaline*	Roxarsone†
Bambermycins*	Tilmicosin (Pulmotil)*	Sulfamethazine*
Chlortetracycline (CTC)*	Tiamulin	Sulfathiazole
Erythromycin*	Tylosin*	
Lincomycin*	Virginiamycin*	
Neomycin*		

* Use as in-feed antibiotics requires veterinary feed directive (VFD) approval according to the current regulations of the U.S. Food and Drug Administration (https://www.fda.gov/animal-veterinary/development-approval-process/drugs-veterinary-feed-directive-vfd-marketing-status).

† Use as in-feed antibiotics has been banned by the U.S. Food and Drug Administration.

TABLE 13.8
Efficacy of Antibiotics as Growth Promoters for Pigs[a]

Stage	Control	Antibiotic	Improvement (%)
Starting phase (7–25 kg)			
Daily weight gain (kg)	0.39	0.45	16.4
Feed:Weight Gain	2.28	2.13	6.9
Growing phase (17–49 kg)			
Daily weight gain (kg)	0.59	0.66	10.6
Feed:Weight Gain	2.91	2.78	4.5
Growing-finishing phase (24–89 kg)			
Daily weight gain (kg)	0.69	0.72	4.2
Feed:Weight Gain	3.30	3.23	2.2

Source: Cromwell (2000).

[a] Data collected from 453, 298, and 443 experiments involving 13,632, 5,783, and 13,140 pigs, for the starting, growing, and growing-finishing phases, respectively.

maintain its homeostasis and dies quickly. N-containing antibiotics generally have higher biological activity than non-N compounds (Bérdy 2012).

Antibiotics

Enhancers of feed efficiency. Antimicrobial agents were first used as additives in diets for chickens (Moore et al. 1946) and pigs (Brauder et al. 1953) in the 1940s and 1950s, respectively. Antimicrobial agents, used as feed additives to nonruminant diets at low concentrations (subtherapeutic), inhibit the growth of intestinal microbes, improve growth rate and efficiency of feed utilization, and reduce mortality and morbidity. Antimicrobial agents are also used at intermediate levels to prevent disease and at high (therapeutic) levels to treat diseases in animals (Cromwell 2002). This class of compounds includes antibiotics (which are naturally produced by yeasts, molds, and other microbes) and chemotherapeutics (which are chemically synthesized), as summarized in Table 13.7. Most antibiotics are naturally synthesized from amino acids in bacteria. In addition to organic substances, high concentrations of zinc and copper have antimicrobial activities and have been included in the diets of weanling pigs to prevent their intestinal dysfunction (e.g., diarrhea). The efficacy of antibiotics in improving growth performance and feed efficiency of pigs is well documented in the literature, and an example is shown in Table 13.8. The results are usually more pronounced on

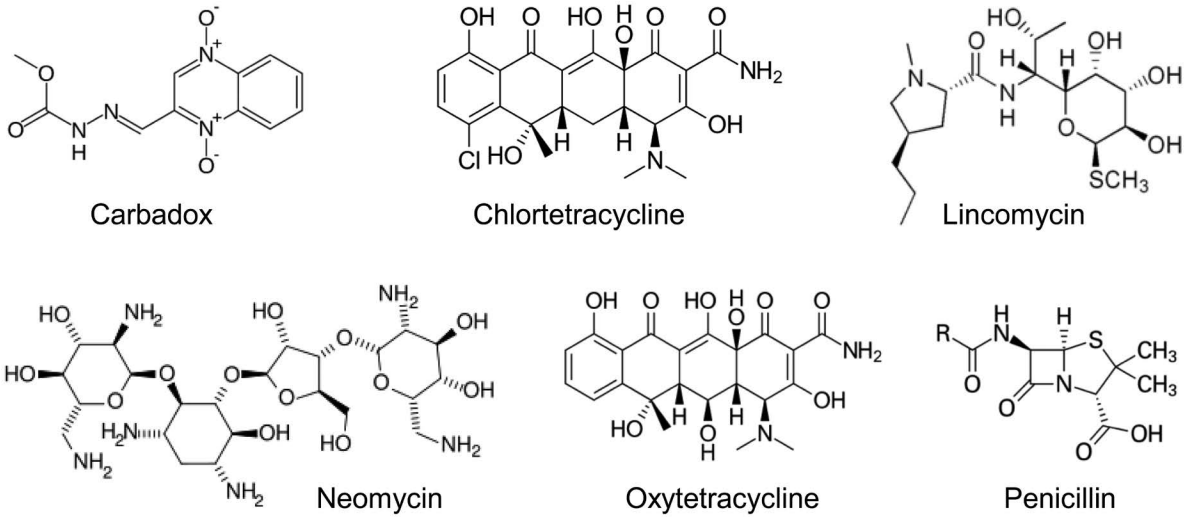


FIGURE 13.4 Antibiotics for feeding nonruminants. Nearly all antibiotics used for feeding nonruminants contain nitrogen, and some of them are aminoglycosides. In-feed antibiotics can improve the health and growth of livestock and poultry but can also result in antimicrobial resistance in humans and farm animals.

commercial farms than at research experiment stations, probably because of a cleaner environment in the latter. Antimicrobial agents play an important role in the efficient production of pork, beef, poultry meat, and other animal products worldwide.

Modes of action. The mechanisms responsible for the beneficial action of antibiotics are not well understood and may include (a) the inhibition of the growth of intestinal bacteria (including pathogenic microbes); (b) reduction in the thickness of the intestinal mucosa to enhance nutrient absorption and to decrease mucosal amino acid catabolism; (c) reductions in microbial use and catabolism of nutrients, particularly dietary amino acids; (d) the reduced production of growth-depressing microbial metabolites; and (5) the inhibition of subclinical infections (Gaskins et al. 2002; Visek 1978; Wu 1998). Because microbes in the small intestine are now known to play an important role in the catabolism of dietary amino acids, decreasing the numbers and activity of small-intestinal microbes with the use of antibiotics will reduce amino acid degradation in the intestinal lumen, thereby increasing the bioavailability of dietary amino acids for (a) the host and thus protein synthesis and (b) other synthetic pathways (e.g., the generation of polyamines and glutathione).

Factors affecting the pharmacokinetics of antibiotics. Consumers are concerned about residues of antibiotics left in animal products, such as meat, milk, and eggs. Thus, it is important to determine how long an in-feed, intramuscularly, or intravenously administered antibiotic is retained in the animal. The pharmacokinetics (i.e., maximum absorption, bioavailability, and half-life) of antibiotics (e.g., ceftiofur, penicillin, and amoxicillin) in an animal are affected by their rate of absorption, transport, and metabolism in the body, which are, in turn, dependent on physiological (e.g., age), pathological (e.g., viral infection, such as porcine reproductive and respiratory syndrome virus), nutritional (protein and mineral intakes), and environmental (e.g., heat stress) factors. For example, the excretion of many drugs requires glutathione; thus, adequate protein nutrition facilitates their conjugation and detoxification as water-soluble metabolites.

Concern over antibiotic-resistant bacteria. Bacteria naturally acquire new genes (including antimicrobial-resistant genes) to survive in a new environment or host. The antimicrobial-resistant genes produce enzymes (e.g., extended-spectrum β-lactamase in *E. coli)* to destroy or inactivate antibiotics (Figure 13.5). For example, penicillin-resistant bacteria (e.g., *Staphylococcus aureus* and *E. coli*) synthesize β-lactamase, which breaks down the β-lactam ring of penicillin to an inactive substance (Wu and Bazer 2019). Through this mechanism, the bacteria cannot be killed by penicillin, leading to antimicrobial resistance. The antimicrobial resistance genes in bacteria can be inherited from mother to daughter cells by division, as well as from one strain to another via plasmid transfer.

As early as the 1950s, concern was expressed that continued use of antibiotics to promote growth of poultry and other food animals might result in antibiotic resistance of pathogenic bacteria in humans (Starr and Reynolds 1951). The concern gained momentum in the late 1960s and subsequently developed into advocacy for governments to ban the use of antibiotics as feed additives. Antibiotic use in animals can have direct and indirect effects on human health due to (a) the presence of antibiotic residues in animal products (e.g., meat and milk) consumed by humans, (b) human contact with antibiotic-resistant bacteria from food animals, and (c) the spread of antibiotic-resistant bacteria to various components of the ecosystem (e.g., water and soil). The use of antibiotics as feed additives for growth promotion has been banned in member states of the European Union and is now being phased out in many countries. The United States has prohibited the inclusion of medically important antimicrobials in animal diets for growth promotion since January 2017 but now still permits the use of ionophores, tiamulin, bacitracin, and carbadox as feed additives. Alternatives to feed antibiotics must be actively developed (Swaggerty et al. 2022; Thacker 2013).

Direct-Fed Microbials (Probiotics)

Animals are sterile in the womb. Recent well-controlled studies do not support the hypothesis that microbiomes exist within the healthy fetal milieu (Perez-Muñoz et al. 2017). Neonates acquire some intestinal microbiomes during passage through the birth canal from the environment, and, in the case of poultry, from the eggshell at hatching (Coscia et al. 2021). After birth, with the consumption of milk (due to contact with the dam's skin) and other foods, the digestive tract is naturally colonized by a variety of microbes from the environment. Under healthy conditions, "beneficial" microbes colonize the intestine (and the

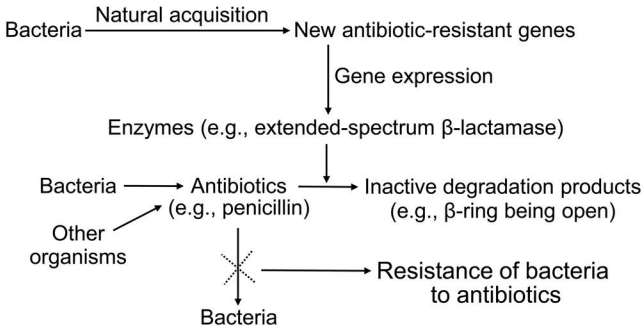


FIGURE 13.5 Mechanisms responsible for the development of antimicrobial resistance in bacteria. Bacteria naturally acquire new genes (including antimicrobial-resistant genes) to survive in a new environment or host. The antimicrobial-resistant genes produce enzymes (e.g., extended-spectrum β-lactamase in *Escherichia coli*) to destroy or inactivate antibiotics. For example, penicillin-resistant bacteria synthesize β-lactamase, which breaks down the β-lactam ring of penicillin to an inactive degradation product. Through this mechanism, the bacteria cannot be killed by penicillin, leading to antimicrobial resistance in infected animals and humans. The sign (X) denotes an inability to kill bacteria.

rumen of ruminants) in a symbiotic relationship with the host. Beneficial microbes compete with potential pathogens and are essential for the normal development and well-being of the host. For example, poultry and pigs raised in a sterile environment are more susceptible to subsequent bacterial infections, probably due to underdevelopment of the immune system and a poorly developed microbiota to compete with pathogenic organisms.

Direct-fed microbials (DFMs), commonly referred to as "probiotics," are live, naturally occurring microbes that are beneficial because they improve the intestinal environment for the host. Probiotics can be defined as dietary supplements containing beneficial live bacteria or yeast that confer a health benefit to the gut flora and the host. *Lactobacillus* and *Bifidobacterium* are the most widely used probiotic bacteria for maintaining the intestinal microbial ecosystem and mucosal integrity. Lactobacilli are the predominant lactic acid bacteria found in the small and large intestines of nonruminants (e.g., humans, pigs, and poultry) and constitute a major proportion of the small-intestinal microbiota but only make up a relatively small percentage of the overall bacterial population in the large intestine (Walter 2008). As such, they are of particular importance for the maintenance of gut health. Probiotics serve as sources of digestive enzymes and improve the balance of the intestinal microflora and have been used as feed additives since the 1970s. To date, DFMs approved for swine and other livestock are listed in Table 13.9 and should be used according to the conditions of permission. In poultry and pigs, the response to probiotics is considerably less consistent compared with the response to antibiotics.

The mechanisms proposed for the action of DFMs are summarized as follows (a) the production of antibacterial compounds (e.g., organic acids, bacteriocins, and antibiotics); (b) competition with undesirable organisms for colonization space and/or nutrients (competitive exclusion); (c) the formation of nutrients (e.g., amino acids and vitamins) and other factors that stimulate the growth of commensal microbes in the digestive tract, the host animal, or both; (d) the production and/or stimulation of enzymes for the digestion of dietary nutrients (e.g., protein, carbohydrates, and minerals); (e) the metabolism and/or detoxification of undesirable compounds; (f) the stimulation of immune response in host animals for self-defense; and (g) the increased absorption of amino acids into the portal circulation (Bergen and Wu 2009). This knowledge is fundamental to the development of new strategies to prevent enteric disorders. For example, when pigs are weaned at an early age (e.g., ≤21 days), they are at increased risk for enteric disease, diarrhea, and malnutrition, likely due to disruption in the establishment of a stable intestinal microbiota, thereby allowing pathogenic bacteria to flourish and cause disease.

TABLE 13.9
Direct-Fed Microbials Used in the Diets of Swine, Ruminants, and Poultry

***Aspergillus* (molds)**	*B. infantis*	*L. curvatus*
A. niger	*B. longum*	*L. delbruekii*
A. oryzae	*B. thermophilum*	*L. fermentum*
Bacillus	***Enterococcus***	*L. helveticus*
B. coagulans	*E. cremoris*	*L. lactis*
B. lentus	*E. diacetylactis*	*L. plantarum*
B. licheniformis	*E. faecium*	*L. reuteri*
B. pumilus	*E. intermedius*	***Leuconostoc***
B. subtilis	*E. lactis*	*L. mesenteroides*
Bacteroides	*E. thermophilus*	***Pediococcu***
B. amphophilus	***Lactobacillus***[a]	*P. acidilacticii*
B. capillosus	*L. acidolphilus*	*P. cerevisiae*
B. ruminocola	*L. acidophilus*	*P. pentosaceus*
B. suis	*L. brevis*	***Propionibacterium***
Bifidobacterium	*L. bulgaricus*	*P. freudenreichii*
B. adolescentis	*L. bunchner*	*P. shermanii*
B. animalis	*L. casei*	***Saccharomyces***
B. bifidum	*L. cellobiosus*	*S. cerevisiae*

Source: Buntyn et al. (2014); Cromwell (2000). The names of microbial genera are in bold, followed by their species.

[a] *Lactobacillus bunchneri* and *Lactobacillus farciminis* are also approved for use in cattle and swine diets, respectively.

Prebiotics

Prebiotics can be defined as food ingredients that are not digested by animal-sourced enzymes but can beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of the bacterial species in the small and large intestines. Oligosaccharides [mainly fructo-oligosaccharides (FOSs) and MOSs] are commonly used as prebiotics for livestock species, poultry, and humans (Halas and Nochta 2012). In current swine and poultry industries, FOSs are the leading prebiotics, but MOSs are not sufficiently defined despite their presence in yeast cell wall products. Of note, XOSs are new feed additives to wheat- or corn-based diets for nonruminants to improve intestinal immunity, health, and function (Ribeiro et al. 2018).

Dietary supplementation with MOS reduced the incidence of diarrhea in weanling piglets and enhanced their growth performance (Castillo et al. 2008). Similarly, adding chitosan oligosaccharides to the diets of young chicks and weanling pigs inhibited the growth of oral and intestinal pathogenic bacteria, improved immune status, and increased the density of small intestinal microvilli (Deng et al. 2007; Wang et al. 2003). Furthermore, dietary lactose has previously been shown to act as a prebiotic, promoting the growth of beneficial commensal bacteria, such as *Bifidobacteria* and *Lactobacillus*, and improving gut health and growth performance in weaning piglets (Daly et al. 2014; Pierce et al. 2006). Likewise, supplementing XOS at the dose of 0.1 g/kg diet to broiler chickens between days 0 and 42 of age increased weight gain and BW by 6.9% and 6.8%, respectively, without affecting feed intake (Ribeiro et al. 2018). The mechanisms responsible for the beneficial

effects of prebiotics include (a) improvements in the digestion of nutrients (protein, DM, and minerals), the absorption of amino acids and minerals by the small intestine, N economy in the intestine, and feed efficiency; (b) modulation of the metabolism of urea, ammonia, and amino acids in the lumen of the large intestine; and (c) changes in the intestinal microflora to selectively promote the growth and activity of beneficial bacteria (e.g., *Bifidobacteria*) and therefore the postnatal development of the intestinal and whole-body immune systems (Bergen and Wu 2009; Ribeiro et al. 2018).

Postbiotics

It has been recognized for more than a century that some nonviable microbes (e.g., lactobacilli and yeasts) or their cell components can beneficially impact health in humans. Over the past two decades, animal nutritionists and producers have paid growing attention to the use of such products to feed farm animals under stress conditions such as weaning, lactation, and long-distance transportation. Various terms describing these functional appear in literature, including cell lysates, ghostbiotics, heat-killed probiotics, nonviable probiotics, paraprobiotics, postbiotics, and tyndallized probiotics. Recently, the International Scientific Association of Probiotics and Prebiotics (ISAPP) defined a postbiotic as "a preparation of inanimate microorganisms and/or their components that confers a health benefit on the host" (Salminen et al. 2021). According to this definition, postbiotics must contain nonliving microbial biomass such as whole cells or their fragments and can include microbial metabolites. However, microbial metabolites (e.g., SCFAs) and macromolecules (e.g., proteins and peptides) alone are not considered postbiotics. In addition, a postbiotic may not necessarily be derived only from a probiotic. The health benefits of postbiotics primarily manifest in the gut but can extend to other tissues. For example, a postbiotic from *Saccharomyces cerevisiae* fermentation can decrease the number of *Salmonella enteriditis* in the ceca of laying hens (Gingerich et al. 2021); a *Lactobacillus*-derived postbiotic can diminish the severity of gut lesions caused by necrotic enteritis in broiler chickens (Abd El-Ghany et al. 2022), and a postbiotic from *L.* acidophilus fermentation can accelerate the development and establishment of microbiome clusters in nursery pigs (Khafipour et al. 2021). Furthermore, a postbiotic from bovine milk fermented with *Lactobacillus paracasei* CBA L74 can reduce the incidences of both gastrointestinal and respiratory infections in children (Corsello et al. 2017).

Alternatives to Feed Antibiotics

Improvements of intestinal health and immunity should serve as a guiding principle for the development of alternatives to feed antibiotics. The most widely researched alternatives include recombinant enzymes (e.g., feed enzymes as noted previously), probiotics, prebiotics, synbiotics (mixtures of prebiotics and probiotics), organic acidifiers (e.g., formate, propionic acid, butyric acid, fumaric acid, citric acid, and benzoic acid), lipids (e.g., lauric acid, 1-monoglyceride, and tributyrate), plant extracts, minerals [e.g., 250 ppm copper sulfate ($CuSo_4$) and 2500 ppm zinc oxide (ZnO)], antimicrobial peptides, clay minerals, egg yolk antibodies (e.g., IgY), essential oils (aromatic oily liquids obtained from plant material), eucalyptus oil-medium chain fatty acids [e.g., octanoic acid ($C_{8:0}$) and caproic acid ($C_{6:0}$)], rare earth elements (e.g., lanthanum-yeast mixture), spray-dried porcine plasma, yeast culture, oral vaccines, bacteriophages, lysozymes, bovine colostrum, lactoferrin, conjugated fatty acids, chito-oligosaccharides, seaweed extracts, and certain amino acids (Poppy et al. 2012; Ricke 2003; Skrivanova et al. 2009; Thacker 2013; Yang et al. 2015).

Essential oils usually have a characteristic odor or flavor and are mixtures of plant phenolic compounds (e.g., thymol, carvacrol, and eugenol), terpenes (e.g., citric and pineapple extracts), alkaloids (capsaicin), lectins, aldehydes (e.g., cinnamaldehyde), polypeptides, or polyacetylenes. Finally, certain protein hydrolysates from animal sources contain antimicrobial peptides (Chapter 4), which exert their actions by damaging the cell membrane of bacteria, interfering with the functions of their intracellular proteins, inducing the aggregation of cytoplasmic proteins, and affecting the metabolism of bacteria (Hou et al. 2017).

Zhitnitsky et al. (2017) reported a synergistic, broad-spectrum effect of a combination of an organic acid (acetate, propionate, butyrate, formate, or benzoate) plus a transition metal (zinc or copper) on inhibiting the growth of cultured bacteria (e.g., *E. coli* and *Salmonella enterica*). Either an inorganic acid at a high concentration (e.g., 150 mM acetate, propionate, or butyrate) or a transition metal at a nontoxic concentration (e.g., up to 2.4 mM zinc or copper) alone had only a weak effect on inhibiting the growth of bacteria. However, a combination of an organic acid plus a transition metal may fully suppress bacterial growth when the efflux of a transition metal from bacteria is blocked. The presence of an organic acid in cultures increased the uptake of a transition metal by bacteria (e.g., ~100% and 200% increases for zinc and copper, respectively). This work presents a novel, attractive idea for the new development of alternatives to antibiotics.

Chemical modifications of vancomycin may represent another means to manufacturing new antibiotics. Vancomycin, which is synthesized by the soil bacterium *Amycolatopsis orientalis*, has been used widely as an antibiotic to treat infections by Gram-positive bacteria since 1958. This glycopeptide inhibits the growth of bacteria by disrupting their cell membranes through binding to the D-alanine-D-alanine pocket of an outer-membrane peptidoglycan. However, pathogens (e.g., *Enterococci*) resistant to vancomycin were first reported in 1987. In addition, a vancomycin-resistant *S. aureus* strain was detected in 2003. Of note, the vancomycin-resistant bacteria contain a D-alanine-D-lactate pocket in their cell-membrane peptidoglycans. Okano et al. (2017) found that modifying vancomycin by introducing a C-terminal quaternary ammonium

salt (NR_4^+, R being an alkyl group) allowed it to (a) bind the D-alanine-D-lactate pocket on the vancomycin-resistant bacteria, thereby inhibiting their growth; and (b) maintain the ability to bind the D-alanine-D-alanine pocket of cell-membrane peptidoglycans, thereby effectively killing both the susceptible and mutated resistant strains simultaneously. The dual binding mechanisms of the structurally modified vancomycin permit its continued use as an antibiotic in clinical medicine. This study may aid in identifying a novel, effective strategy to chemically modify an existing antibiotic (e.g., vancomycin) to overcome antimicrobial resistance problems in human and animal medicine.

Over the past two decades, there has been growing interest in tributyrin as an alternative to feed antibiotics in nonruminant diets. This lipid is a triglyceride in which glycerol is esterified with three molecules of butyric acid. The latter is the major energy substrate for colonocytes, an activator of mitosis and differentiation of intestinal epithelial cells, an inhibitor of histone deacetylase (a regulator of epigenetics), and an antibacterial agent against Gram-negative and positive bacteria. However, butyric acid has a strong odor and its inclusion at high levels (e.g., 3%) in diets decreases the feed intake of animals. As an effective source of butyric acid, supplementation with tributyrin (e.g., 0.1–0.2% in diets for postweaning pigs) can beneficially modulate gut microbiota, alleviate intestinal injury, sustain energy provision in epithelial cells of the large intestine, and enhance nutrient digestibility, thereby improving the intestinal health and growth performance of animals (Hou et al. 2014; Tugnoli et al. 2020).

Agents to Remove or Absorb Mycotoxins in Feeds

Mycotoxins are secondary metabolites produced by organisms of the fungal kingdom, such as *Aspergillus*, *Penicillium*, and *Fusarium*. Mycotoxin-producing molds are distributed widely throughout the world and actively grow in different environmental conditions. Contamination with mycotoxins can arise on a farm throughout the harvest, drying, and storage processes, and the risk increases under conditions of high temperature and moisture (CAST 2003). The major classes of toxic mycotoxins in foods are aflatoxins, deoxynivalenol, zearalenone, trichothecenes, fumonisins, ochratoxin A, and ergot alkaloids. Aflatoxins are a family of toxins produced by certain fungi (*Aspergillus flavus* and *Aspergillus parasiticus*) present in agricultural crops such as corn, peanuts, cottonseed, and tree nuts. There are six common aflatoxins: B1, B2, G1, G2, M1, and M2 (Figure 13.6), which can bind to protein and DNA to cause aflatoxicosis (general poisoning to animals). Acute severe intoxication causes direct damage to the intestine and liver and subsequent illness or death, whereas chronic sub-symptomatic exposure results in inhibition of protein synthesis,

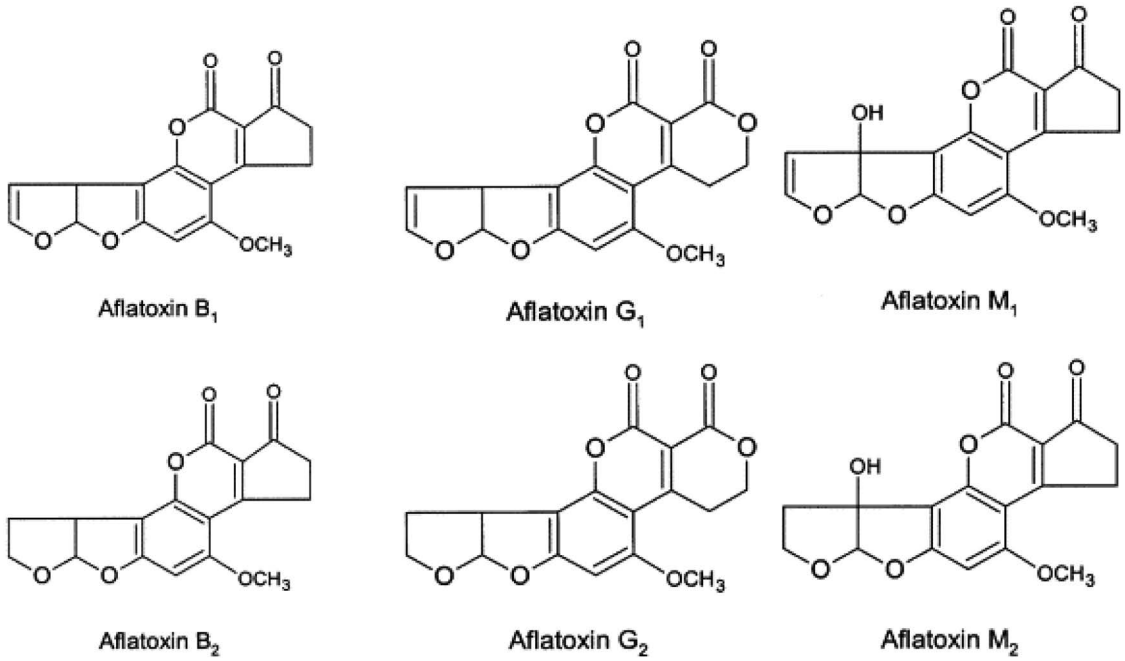


FIGURE 13.6 Aflatoxin structures. There are six common aflatoxins in animal feeds: B1, B2, G1, G2, M1, and M2. Aflatoxins B1, B2, and G1 are produced by *Aspergillus* molds, whereas aflatoxins M1 and M2 are the microbial metabolites of aflatoxins B1 and B2, respectively, and can be found in ruminant milk and its derived products. These toxins are divided based on their fluorescence under UV light and the presence or absence of a double bond at the 8, 9 carbons. Aflatoxins B1 and G1 have a double bond at the 8, 9 carbons, while aflatoxins B2 and G2 do not. This double bond leads to the formation of an epoxide, which is a more toxic form of aflatoxins B1 and G1. The B aflatoxins are named for their blue fluorescence, and the G aflatoxins for their green-blue fluorescence under UV irradiation on thin-layer chromatography plates. Of the four aflatoxins, aflatoxin B1 is the most potent natural carcinogen and the most prevalent one.

chronic disease, and poor growth performance. Among livestock species, swine are most sensitive to aflatoxins (Rezaei et al. 2013). These substances are usually present at varying concentrations in feed ingredients used for swine, poultry, and ruminant rations (Wu et al. 2016). The mean oral lethal dose (LD_{50}) for swine is 0.62 mg/kg, which is significantly lower than other livestock species (Pier 1992).

Methods for protecting animals from those toxic effects include grain testing, use of mold inhibitors, fermentation, microbial inactivation, physical separation, thermal inactivation, irradiation, ammoniation, ozonation, dilution, and the use of adsorbents (Rezaei et al. 2013). Adsorbents have been used as one of the best and most practical methods because they are relatively inexpensive, generally recognized as safe (GRAS), and can be easily added to animal feeds. Adsorbents added to aflatoxin-contaminated feeds can bind to aflatoxin during the digestive process and cause the toxin to be excreted safely from the animal body (Phillips et al. 1995). Possible adsorbent materials that have been studied so far include silicate minerals, activated carbons, complex indigestible carbohydrates (e.g., cellulose, polysaccharides in the cell walls of yeast and bacteria such as glucomannans, peptidoglycans, and others), and synthetic polymers (e.g., cholestyramine, polyvinylpyrrolidone, and derivatives).

Clay minerals consist of silicon, aluminum, and oxygen as in the natural extracted clays (bentonites, zeolite, and kaolin). These minerals can bind to toxic materials (e.g., aflatoxins, plant metabolites, heavy metals, and endotoxins) in the GIT of animals, thereby reducing their biological availability, absorption, and toxicity. Silicate minerals are the most widely studied mycotoxin-sequestering agents. There are two important subclasses of these minerals: phyllosilicates and tectosilicates. Mineral clays of the phyllosilicate subclass include the montmorillonite/smectite group, the kaolinite group, and the illite (also known as clay-mica) group. Tectosilicates consist of the highly studied zeolites. Montmorillonites, the major component of bentonite, can be categorized as Ca, magnesium, potassium, or sodium bentonites. These products have been widely used as mycotoxin sequestering agents due to their ion exchange effects (Abbès et al. 2008; Shi et al. 2007; Thieu et al. 2008). Zeolites are silicates that consist of interlocking tetrahedrals of SiO_4 and AlO_4. Large pores in zeolites have sufficient space for attracting and holding positive cations. Of the aluminosilicates, hydrated sodium Ca aluminosilicate is the most studied adsorbent, which is characterized as "aflatoxin-selective clay" and is therefore not considered as an effective adsorbent of other mycotoxins (Phillips et al. 1995).

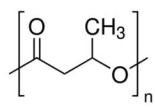
Microbial Polymers as Feed Additives

Poly-3-hydroxybutyrate (PHB) is a biodegradable polymer produced by many bacteria for energy storage and is an excellent alternative to conventional petroleum-based plastics. Thus, the use of PHB as a feed additive offers a sustainable solution to concerns over plastic waste as a source of environmental pollution in oceans and rivers as well as on land. In aquatic and terrestrial animals, intestinal epithelial cells and luminal microbes possess PHB depolymerase for hydrolyzing PHB to the 3-hydroxybutyrate monomer, which is further oxidized to acetoacetate (a ketone body) by nicotinamide adenine dinucleotide $(NAD)^+$-dependent 3-hydroxybutyrate dehydrogenase. Acetoacetate is converted into acetyl-CoA for either oxidation in virtually all tissues via the Krebs cycle to provide energy or the synthesis of fatty acids to store energy, depending on the physiological and nutritional states of animals. There are reports that dietary supplementation with 1% and 2% PHB increased growth and survival rates in marine large yellow croakers, but an inclusion level of 0.5% in rations did not affect weight or organ gain, feed intake, or feed efficiency (Wang et al. 2019). Similar growth-promoting effects of dietary supplementation with 2–5% PHB were observed in European sea bass (De Schryver et al. 2010) and Siberian sturgeon (Najdegerami et al. 2012). In addition, adding 2–8% PHB to diets alleviated lipopolysaccharide-induced oxidative stress, inflammation, and cell apoptosis in common carp (Li et al. 2023c). Likewise, dietary supplementation with 0.5–5% PHB to diets increased the survival of Nile tilapia larvae challenged with the pathogen *Edwardsiella ictaluri* gly09R by 20% (Situmorang et al. (2016). As a feed additive, PHB can play an important role in global circular bioeconomies.

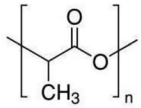
$$\text{Poly-(3-hydroxybutyrate)}_n + H_2O \rightarrow n\text{3-Hydroxybutyrate}$$
(Poly-3-hydroxybutyrate depolymerase)

$$\text{3-Hydroxybutyrate} + NAD^+ \rightleftharpoons \text{Acetoacetate} + NADH + H^+$$
(3-Hydroxybutyrate dehydrogenase)

Poly-lactic acid (PLA) is another biodegradable polymer that can replace petroleum-based plastics. PLA can be chemically synthesized from L-lactic acid in industry but is now produced by metabolically engineered microbes (Choi et al. 2019). They express two key enzymes for PLA biosynthesis: (a) propionyl-CoA transferase that converts lactic acid into lactyl-CoA and (b) PHA synthase that polymerizes lactyl-CoA into PLA. Of particular note, a PLA biofilm incorporated with caprylic acid has an antimicrobial property and is useful in the packaging industry (Her et al. 2024). The intestines of aquatic and terrestrial animals have PLA depolymerase to hydrolyze PLA into L-lactic acid, which can be a useful additive to the diets of farm animals, including livestock, poultry, fish, and crustaceans, for expanding circular bioeconomies. L-Lactic acid can beneficially acidify the lumen of the gut, serve as a metabolic fuel and a glucogenic precursor, modulate intracellular redox state, and regulate the metabolic network (Chapter 5).



Poly-3-Hydroxybutyrate



Poly-Lactic acid

Ruminants

Because ruminants position the bulk of their fermentation capacity at the anterior end of their digestive tract, the type of antibiotic-like substances (mainly ionophores) and DFMs employed in ruminant diets differ from those used for nonruminants. These ruminant-specific products modulate the production of SCFAs and microbial protein in the rumen to enhance the provision of both ME and a balanced mix of amino acids to enhance ruminant growth, lactation, and production (Cameron and McAllister 2016). To date, there are eight types of feed additives to improve ruminal function and ruminant production: antibiotics, ionophores, prebiotics, postbiotics, probiotics, protein and energy concentrates, amino acids and related substances, and phytochemicals (Baker et al. 2022; Golder et al. 2023; Hodge et al. 2024). The last two groups of feed additives will be discussed in the next section for both ruminants and nonruminants.

Ionophore Antibiotics

In ruminants, an inhibition of all microbes in the rumen is often counter-productive because of their essential roles in the fermentation of protein, fatty acids, as well as structural and nonstructural carbohydrates (Cameron and McAllister 2016). Likewise, there is also concern about the use of antibiotics as feed additives in ruminant diets due to the possibility of the evolution of resistant strains of disease-causing organisms. Ionophores (lipophilic compounds), which were originally used to control intestinal parasites in poultry (Bergen and Bates 1984), are now commonly added to ruminant diets. Examples for this group of polyether molecules include monensin, salinomycin, lasalocid, laidlomycin, and narasin (Figure 13.7). Ionophores do not induce resistance to medically important nonionophore antibiotics in farm animals or humans.

Unlike animal cells, the membranes of bacteria are relatively impermeable to ions and use ionic (both K^+ and Na^+) gradients as a driving force for nutrient uptake. Specifically, ruminal bacteria maintain high intracellular potassium concentration and low intracellular sodium concentration in the face of high extracellular sodium concentration and low potassium concentration. Because ruminal pH is frequently acidic but the intracellular pH of ruminal bacteria is neutral, these cells have an inwardly directed proton gradient (Russell and Strobel 1989). Ionophores stimulate the transport of ions across cell membranes of Gram-positive bacteria, dissipating ion (e.g., H^+ and Na^+) gradients and uncoupling energy expenditure from cell growth, thereby depleting intracellular ATP and killing these bacteria (Callaway et al. 2003). These antimicrobials specifically target Gram-positive ruminal bacterial populations and alter the microbial ecology of the intestinal microbial consortium to increase production efficiency. Gram-positive bacteria have the porous peptidoglycan layer that allows small molecules to reach the cytoplasmic membrane, where an ionophore rapidly dissolves into the cell membrane (Newbold et al. 1993). Conversely, Gram-negative bacteria (e.g., *E. coli*) are insensitive to ionophores.

Monensin is produced by *Streptomyces cinnamonensis*, whereas salinomycin is synthesized by *Streptomyces albus*. These two ionophore antibiotics (Figure 13.7) improve the feed efficiency of ruminants by modifying ruminal fermentation to enhance the production of propionate and reduce the production of acetate and butyrate. In addition, monensin and salinomycin reduce methanogenesis by up to 30% at least over short durations, which lessens the irreversible loss of dietary DM (Johnson and Johnson 1995). Note that ruminal methanogens are not directly inhibited by monensin; rather, the bacteria responsible for feeding metabolic hydrogen ([2H]) to methanogens are inhibited (Dellinger and Ferry 1984). Furthermore, these two ionophores inhibit

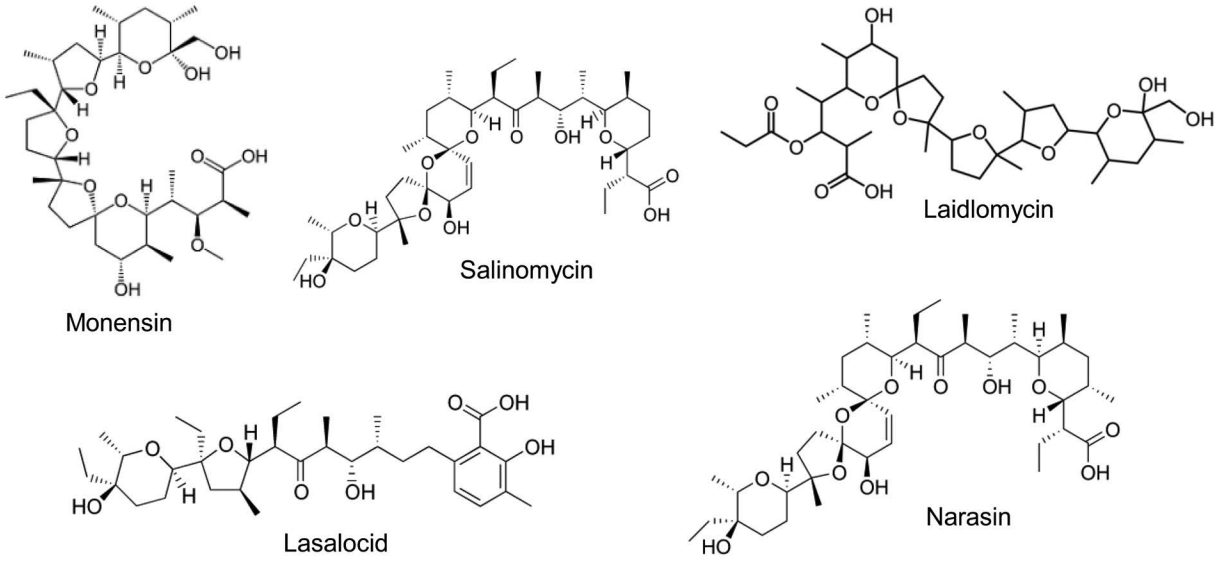


FIGURE 13.7 Ionophore antibiotics for feeding ruminants. These substances are polyether molecules that seldom contain nitrogen.

proteolytic and deaminating enzymes, thereby increasing the amounts of dietary proteins that escape the rumen to enter the abomasum and small intestine for digestion. Thus, monensin and salinomycin have gained wide acceptance as feed additives for growing and lactating ruminants.

Monensin, which was originally used to kill coccidia in poultry as noted previously, also kills protozoa to prevent coccidiosis in ruminants (including cattle and sheep; Noack et al. 2019). The contribution of protozoa to ruminal digestion and ruminant productivity has long been a matter of controversy (Chapter 7). Protozoa can digest polysaccharides and aid in the absorption of several minerals (e.g., Ca, magnesium, and P). However, protozoa are largely retained in the rumen and therefore reduce the amount of microbial protein that enters the abomasum and small intestine. This means that a continuous flow of protozoa from the rumen into the abomasum is required for optimal protein nutrition in ruminants. Thus, defaunation of the rumen reduces the digestion of fiber but increases the quantity of microbial protein reaching the duodenum by ~25% (Chapter 7).

Nonionophore Antibiotics

Tylosin phosphate (a macrolide) is the most common in-feed antimicrobial to control liver abscess (the primary hepatic abnormality) in feedlot beef cattle in North America (Amachawadi and Nagaraja 2016). This disorder, which is caused by *Fusobacterium necrophorum* (the primary pathogen, a Gram-negative bacterium) and *Trueperella* (formerly *Arcanobacterium*) *pyogenes* (the secondary pathogen, a Gram-positive bacterium), affects 12–32% of feedlot cattle on average and a higher percentage of dairy cows. A high intake of fermentable starch and a low intake of fiber, which contributes to ruminal acidosis and subsequent rumenitis, increases the susceptibility of cattle (including dairy cows) to develop the liver abscess. Although tylosin phosphate is not used in human medicine, this antibiotic may cause resistance to other antimicrobials within the macrolide superfamily (e.g., erythromycin) that are widely used to treat bacterial infections in humans. Thus, in the United States, the use of tylosin in both feedlot cattle and dairy cows for the prevention of liver abscesses has been under veterinary oversight since January 2017. An effective alternative to tylosin, together with sound nutritional management, should be established to control the liver abscess in both feedlot cattle and dairy cows.

Direct-Fed Microbials

Many producers and veterinarians have long inoculated sick ruminants (especially those that do not eat well) with ruminal fluid from healthy animals to (a) stimulate normal ruminal function (e.g., stabilizing ruminal pH, promoting ruminal fermentation and degradation, and enhancing feed digestion by the GIT); and (b) increase feed intake. However, there had been little scientific data to document the efficacy of dietary supplementation of microbes to ruminants until the 1980s. The proof of concept of DFMs for ruminants is provided by the following examples. First, the tropical forage *Leucaena leucocephala,* present in some regions of the world (e.g., Australia and India), contain mimosine (a nonprotein amino acid), whose metabolite [3-hydroxy-4(1H)-pyridinone] is a toxic, goitrogenic agent (Thompson et al. 1969). Interestingly, the rumen of goats raised in Hawaii contains a specific organism (*Synergistes jonesii*) capable of degrading 3-hydroxy-4(1H)-pyridinone and thus preventing its toxicity. When *S. jonesii* is inoculated into the rumen of Australian cattle that consume the forage *L. leucocephala*, this bacterium confers protection from 3-hydroxy-4(1H)-pyridinone toxicity (Allison et al. 1990). Second, some plants (e.g., Dichapetalaceae) in certain regions of the world (e.g., Australia and South Africa) contain a toxic substance called monofluoroacetate (an inhibitor of the Krebs cycle) (Peters et al. 1960). This substance, even at a low intake level (e.g., 0.3 mg/kg BW), can kill ruminants. When several strains of *Butyrivibrio fibrisolvens,* which contain an inserted gene encoding for fluoroacetate dehalogenase, are administered into the rumen of sheep, the toxicity of monofluoroacetate is markedly reduced (Gregg et al. 1998). Thus, exogenous microbes may play an important role in ruminal metabolism and ruminant health.

To date, there are many bacteria-based DFMs for use in ruminant diets. These products often contain lactate-producing bacteria (e.g., *L. acidophilus*, *Bifidobacterium*, *Enterococcus*, and *Streptococcus*) or lactate-utilizing bacteria (e.g., *B. subtilis*, *Propionibacterium*, and *Megasphaera elsdenii*). Milk-fed young calves, forage- or concentrate-fed weanling calves and finishing cattle, stressed cattle in long-distance transportation, and lactating cows have been shown to benefit from the use of DFMs (Ban and Guan 2021; Cangiano et al. 2020; Seo et al. 2000). For example, adding *Bacillus* (2×10^9 cfu/head/day) plus the anti-inflammatory lactate-producing bacteria *Enterococcus faecium* ID7 (1×10^9 cfu/head/day) to a nonmedicated liquid milk replacer (containing 20% CP and 20% fat, DM basis) enhanced BW gain by 5.9 kg between <7 days and 6 weeks of age without affecting food intake (Duersteler et al. 2012). Likewise, adding a supplemental powder (consisting of *Lactobacillus animalis* SB310, *Lactobacillus paracasei subsp. paracasei* SB137, and *Bacillus coagulans* SB117 in a 30:35:35 ratio, respectively; 1.8×10^{10} CFU/g of powder) to a nonmedicated liquid milk replacer for calves at the dose of 1 g powder/head/day between 2 and 28 days of life reduced the incidence of their diarrhea, while increasing their food intake and growth (Agazzi et al. 2014). In addition, supplementing a *B. subtilis* probiotic product (13 g/head/day) to postweaning calves reduced decreased *Salmonella* concentrations in their gut digesta and the biomarkers of systemic inflammation and increased their feed intake in response to a challenge with *Salmonella* (Broadway et al. 2020). Similarly, Crossbred-Angus steers fed a steam-flaked corn-based fishing diet supplemented with a mixture of direct-fed microbes (*L. animalis*, *Propionibacterium freudenreichii*, *B. subtilis*, and *B. licheniformis* in a 1:1:1:3 ratio, respectively) at a total of 6×10^9 CFU (50 mg)/head/

day had a 3% greater digestibility of DM, a 6.7% greater carcass-adjusted average daily gain, and a 6% greater feed efficiency (Lopez et al. 2024). Furthermore, dietary supplementation with *Lactobacillus spp.* (e.g., 6.5×10^{10} CFU/head/day) increased the quantity (27.8 vs. 25.3 kg milk per day) and quality (indicated by somatic cell counts; 5.0×10^{4} vs. 8.2×10^{4} cells/ml) of the milk produced by lactating dairy cows (Xu et al. 2017) and reduced the abundance of *Enterococcus* and *Streptococcus* (mastitis-causing pathogens) in the milk (Gao et al. 2020). Bacteria-based DFMs act through modulating ruminal pH and increasing the production of SCFAs and proteins by ruminal microbes.

There is also evidence that fungal DFMs (e.g., fungal species of *Saccharomyces* and *Aspergillus*) are beneficial to ruminants. For example, dietary supplementation with *S. cerevisiae* to lactating dairy cows (e.g., 5×10^{10} or 8×10^{10} cfu/head per day) increased DM intake, ruminal pH (e.g., 6.59 vs. 6.32) and total SCFA concentrations (e.g., 114 vs. 106 mM), and 4% fat-corrected milk yield (e.g., 29.6 vs. 26.5 kg/day), while alleviating the effect of subacute ruminal acidosis (AlZahal et al. 2014; Kumprechtová et al. 2019). The underlying mechanisms responsible for the effects of fungal supplements involve increases in the growth of cellulolytic microbes (e.g., *Fibrobacter succinogenes* and *Ruminococcus albus*) for fiber degradation, lactate-utilizing bacteria (e.g., *Selenomonas ruminantium*) for removing H^+, and amylolytic bacteria, which can all promote the production of SCFAs and microbial protein in the rumen (Jiang et al. 2017; Moya et al. 2018; Pinloche et al. 2013).

There are many discrepancies in the literature regarding the effectiveness and consistency of DFMs on the health, growth, and productivity of ruminants (Ban and Guan 2021; Seo et al. 2000). This may be explained by different experimental conditions, including the health status of animals, the composition of diets, and the environment. With the rapid development of molecular biology techniques, ruminal microbes and traditionally used DFMs may be modified to better improve growth performance and feed efficiency in ruminants.

Microbial Extracts

Microbial cultures contain substrates (e.g., B-complex vitamins, organic acids, ammonia, amino acids, and peptides) for the growth of ruminal bacteria and therefore may be beneficial for fermentation, SCFA production, and protein synthesis in the rumen. In support of this view, results of in vitro studies indicated that *Aspergillus* extracts promoted the growth of *S. ruminantium* (the predominant ruminal bacterium) in lactate medium, as well as the utilization of lactate and the production of SCFAs by this microbe (Martin and Nisbet 1992). Similarly, a yeast culture stimulated the growth of *F. succinogenes S85*, while reducing the lag time for *R. albus* 7 and *B. fibrisolvens* to grow (Baker et al. 2022). Results of feeding trials showed that adding a dried yeast culture (10 g/day) to lactating Holstein cows fed a high-concentrate diet (42.75% on a DM basis) increased DM intake (23.2 vs. 21.8 kg/day), the total tract digestibilities of CP (74.5 vs. 72.5%) and acid detergent fiber (ADF; 51.3 vs. 50.2%), and milk yield (20.1 vs. 18.9 kg/day), compared with the control group (Erasmus et al. 1992). However, some studies revealed no change in ruminal function or ruminant productivity in response to supplementation with yeast autolysates or hydrolysates (Baker et al. 2022). The inconsistent findings may be explained by different experimental conditions, including the composition of basal diets (e.g., the type and content of concentrates), the species of yeast (e.g., *Saccharomyces cerevisiae* or *Saccharomyces boulardii*), the methods of preparation (influencing the amount and composition of cell wall carbohydrates such as β-glucans and MOS), and the degree of protein hydrolysis (affecting the number, length, and type of peptides, as well as the amounts of free amino acids).

Inhibitors of Methanogenesis in Ruminants

In the rumen and large intestine, methanogenic archaea are responsible for methane production, which can be reduced by inhibiting key enzymes of its metabolic pathways and/or decreasing the availability of their substrates as discussed in Chapter 5. For example, both 3-nitrooxypropanol (a synthetic mononitrate ester of 1,3-propanediol) and bromoform [$CHBr_3$; a metabolite of red and brown seaweeds (marine algae such as those in those in the *Asparagopsis* genus)] are competitive inhibitors of methyl coenzyme M reductase, the enzyme that catalyzes the last step of methanogenesis. In addition, shifting ruminal fermentation towards H_2-consuming pathways (e.g., the synthesis of propionate as a hydrogen sink) can reduce methane production. Ideal inhibitors should selectively target methanogens without negatively affecting other beneficial ruminal microbes or the host's health and productivity.

Protein and Energy Concentrates

Grazing ruminants commonly consume forage that does not support their nutritional needs for maximal growth, reproductive performance, or lactation, particularly during dry and winter seasons. Likewise, high-producing lactating cows require adequate intakes of both protein and energy beyond those from conventional hays to sustain the production of large amounts of colostrum and milk. This warrants supplementation, to the rations of these animals, with protein and energy concentrates.

Protein feedstuffs generally contain 20–48% CP, such as distiller's grains, corn gluten feed, alfalfa hay, SBM, canola meal, whole cottonseed, and animal byproducts. The latter include animal protein blend, blood meal, fish meal, meat and bone meal, and feather meal (Li et al. 2021c). Unlike unprotected plant proteins, animal protein supplements undergo limited degradation in the rumen and largely enter the abomasum and the small intestine for digestion (Wu 2022a). This major advantage of animal byproduct feedstuffs should be taken to improve ruminant productivity.

Energy feed ingredients are usually grains (corn, barley, and oats) that are high in starch content, vegetable oils, and

animal fats. However, high starch or lipid intake can disturb ruminal function, such as reducing ruminal pH and interfering with forage digestion in the rumen (Chapters 5 and 6). Thus, the supplemental amounts of grains or lipids must be appropriate and adjusted according to ruminant health and productivity. Other energy supplements that can minimize the associated negative effects include high-fiber byproduct feeds, such as soyhulls, wheat middlings, and sugar beet pulp. It should be kept in mind that the cheapest ration is not necessarily the best or most cost-effective diet.

Urea

Because ruminal microbes can use urea as a source of ammonia for amino acid synthesis, urea is a low-cost and effective additive for diets of ruminants. Thus, urea (representing 15–25% CP in diets) has been fed to postweaning ruminants (e.g., cattle, sheep, and goats) as a nonprotein N supplement for more than 100 years (Kertz 2010). Efficacy and safety of supplementation with urea to ruminant diets depend on (a) the dosage (usually ≤1% in a concentrate-rich diet; ≤2% in low-CP hay) and frequency; (b) the percentages of dietary carbohydrates and CP, as well as their digestibilities in the rumen; and (c) an adequate supply of P, sulfur, and trace minerals. When good- or medium-quality hay is not available, low-quality roughages [such as wheat, oat, barley, and rice straw; corn stover; and very mature, low-quality grass hay (≤6% CP and ≤48% total digestible N; on a DM basis)] can be ammoniated for feeding ruminants (Gao 2000). The sources of ammonia may be (a) urea, with the ratios by weight (g) among straw, water, and urea being 100:40:4.5; (b) ammonium bicarbonate, with the ratios by weight (g) among straw, ammonium bicarbonate, and water being 100:12:40; (c) urea plus lime, with the ratios by weight (g) among straw, urea, lime, and water being 100:2.5:8:40; and (d) anhydrous ammonia, with the ratio by weight (g; on an air-dried basis) of anhydrous ammonia to straw being 3:100. Ammoniating low-quality roughages can increase their digestibilities in the rumen of ruminants (e.g., cattle) by 10–30% and can enhance their feed intake by 15–20%, depending on dietary composition and animals. In addition, the ammoniation can increase dietary N content by 100–200% to beneficially boost microbial protein synthesis in the rumen. It is noteworthy that dietary supplementation with excess urea or ammonia [e.g., ~30 g urea/100 kg BW in a single dose for cattle during a "store" period (e.g., winter) when consuming diets (e.g., low-quality forages such as hay and straw) where a plane of nutrition is slightly more than maintenance] can be toxic to ruminants (Wu and Benevenga 2023).

OTHER SUBSTANCES FOR RUMINANTS, PRERUMINANTS, AND NONRUMINANTS

Amino Acids and Related Compounds

Over the past 70 years, crystalline amino acids have been produced on an industrial scale as additives to animal diets. The manufacturing methods involve extraction from protein hydrolysates, chemical synthesis, and microbial fermentation (Wu 2022a). Feed-grade amino acids (e.g., arginine, citrulline, glutamate, glutamine, glycine, lysine, DL-methionine, threonine, and tryptophan) have been supplemented directly to nonruminant diets (including those for poultry, swine, fish, and crustaceans) in animal production. The resulting benefits include not only reductions in dietary protein content but also improvements in intestinal integrity, immune function, protein deposition, growth performance, lactation (in mammals), hatching (in birds), reproduction, and health (He et al. 2021a,b; Schwab and Broderick 2017; Li et al. 2021a,b, 2023d; Selle et al. 2020; Suehs et al. 2024; Wu et al. 2018; Zhang et al. 2021). For example, dietary supplementation with 1% glutamine or 2% glutamate to postweaning pigs and post-hatching broiler chickens improves the gut immune function and mucosal integrity, thereby reducing the incidence of diarrhea, enhancing nutrient digestion and absorption, and promoting weight gains. Likewise, rumen-protected protein, lysine, and methionine are widely used as feed additives for lactating cows and, to a limited extent, beef cattle (Awawdeh 2016). Dietary supplementation with unprotected citrulline and glutamate or rumen-protected glutamine and arginine is also effective in improving the productivity and health of ruminants, including gestating steers and goats as well as lactating cows and goats (Gilbreath et al. 2021; Lopez et al. 2025a; Newton et al. 2025; Wu et al. 2022, 2024). The nutritional concept of functional amino acids is now widely adopted to enhance the efficiency of animal (such as livestock, poultry, fish, and crustacean) production worldwide (Andersen et al. 2016; Chalvon-Demersay et al. 2021; Hodkovicova et al. 2023; Le Floc'h et al. 2018; Lee et al. 2023b; Li et al. 2021a,b; Martínez-Aispuro et al. 2023; McCoard et al. 2016; Wu et al. 2017, 2022, 2024). Because dietary amino acids undergo first-pass catabolism in the small intestine (mucosa and ruminal microbes) to various degrees [e.g., the active catabolism of arginine, glutamine, and proline in postweaning nonruminant mammals (Figure 13.8)], their bioavailabilities as feed supplements must be considered (Wu 2022a, 2024).

Reducing CP content in animal diets through supplementation with synthetic amino acids is an effective strategy to decrease feed costs and N excretion (Duarte et al. 2024; Lambert et al. 2023; Selle et al. 2020; Wang et al. 2018). Likewise, adding crystalline amino acids to diets may improve the survival and growth performance of animals (particularly those fed plant protein-based rations), thereby shortening the cycle of their production and diminishing the use of feeds, labor, electric power, and facilities. Glycine, which is the most abundant amino acid in vertebrates and is remarkably deficient in plant proteins (Li et al. 2021c; Suehs et al. 2024), is a prototype for such a nutritional strategy. For example, adding 1% or 2% glycine to low-fishmeal and high-SBM diets augments the growth performance of Phase I (5–40 g) and Phase II (110–240 g) hybrid striped bass and prevents intestinal damage (He et al. 2023b; Li et al. 2023a). In addition, dietary supplementation

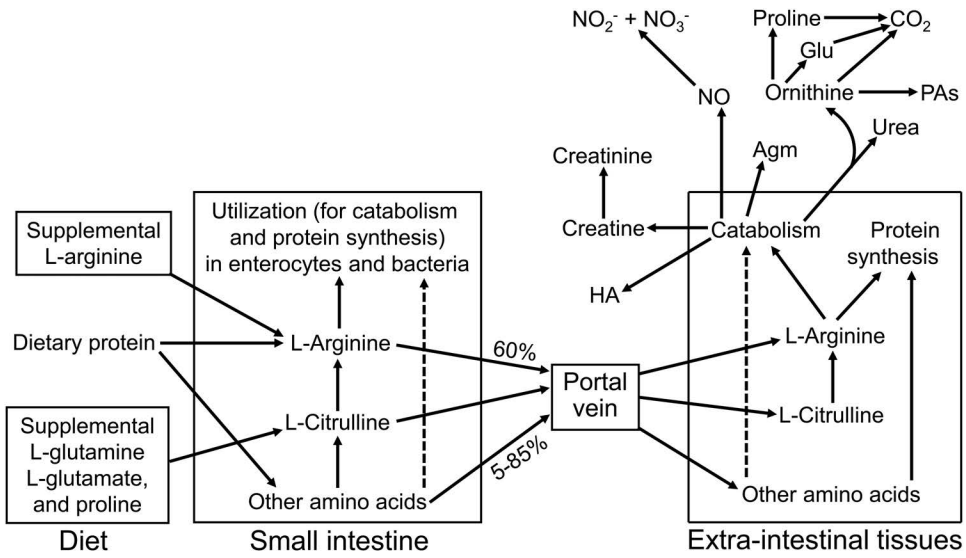


FIGURE 13.8 First-pass metabolism of L-arginine in postweaning nonruminant mammals. In the gastrointestinal tract, the hydrolysis of dietary protein by extracellular proteases and peptidases releases L-arginine, other amino acids, and small peptides into the lumen. Enterocytes take up these digestion products and degrade arginine, while synthesizing and releasing L-citrulline. Catabolism of amino acids also occurs in the luminal bacteria of the gut. In postweaning pigs as well as adult rats and humans, ~40% of free L-arginine and 15% (e.g., L-tryptophan) to 95–97% (e.g., L-glutamate) of other free amino acids present in the lumen of the small intestine are utilized by the gut through catabolism and protein synthesis during the first pass into the portal vein. Thus, 3–5% (e.g., L-glutamate) to 85% (e.g., L-tryptophan) of amino acids in the intestinal lumen enters the portal vein. In extraintestinal tissues, the L-arginine that is not used for protein synthesis participates in multiple catabolic pathways initiated by arginase, arginine:glycine amidinotransferase, arginine decarboxylase, and nitric oxide synthase. L-Arginine can be directly incorporated into swine, poultry, and aqua feeds, whereas either unencapsulated L-citrulline or rumen-protected L-arginine can be fed to post-weaning ruminants.

with 1% glycine enhances the growth of pigs (especially those with intrauterine growth restriction) between weaning and market weight while improving their meat quality (He et al. 2023a). Glycine exerts its beneficial effects through providing a building block for protein synthesis, activating the mechanistic target of rapamycin, and increasing the generation of glutathione (a potent antioxidant) and creatine (essential for muscle growth and function) in both fish and pigs (He et al. 2024a,b,c). Extending these findings to gestating mammals (e.g., supplementing 0.5%, 1%, or 2% glycine to conventional diets for swine beginning on day 0 of gestation) may improve placental growth (including vascular angiogenesis) and uterine antioxidative capacity, as well as embryonic/fetal survival, growth, and development (Figure 13.9). Dietary supplementation with glycine to pregnant swine has two advantages over dietary supplementation with arginine [an amino acid that can improve pregnancy outcomes in swine when supplemented to their diets beginning on day 14 of gestation (Wu et al. 2018)]. First, glycine supplementation in diets for pregnant swine can be beneficially started on day 0 of gestation to overcome the practical problem of initiating supplementation midway through the first 30-day phase of pregnancy. In contrast, dietary supplementation with 0.8% arginine between 0 and 25 days of gestation reduced the rate of ovulation by 17%, maternal progesterone concentration by 33%, and the number of live fetuses by 2.6 per liter because of excessive nitric-oxide production (Li et al. 2010). Second, the economic cost of dietary supplementation with glycine ($1/kg) is much lower than dietary supplementation with arginine ($24/kg) for swine producers.

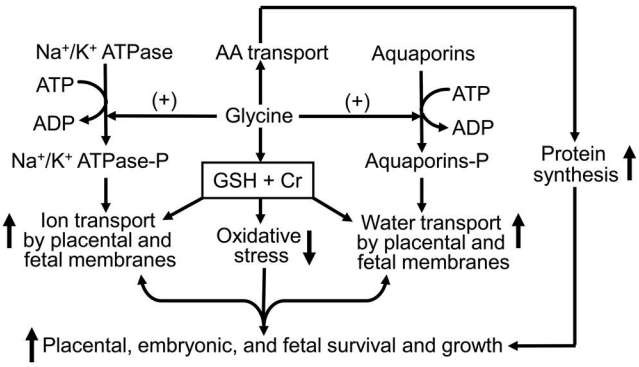


FIGURE 13.9 Possible mechanisms for glycine to enhance pregnancy outcomes in mammals, including swine (e.g., gilts and multiparous sows), cattle (e.g., beef and dairy cows), sheep, and goats. Glycine increases glutathione (GSH) and creatine (Cr) production in conceptuses, thereby increasing the placental transport of amino acids (AAs) and ATP availability for protein synthesis and other metabolic pathways that are essential for cell growth, development, and differentiation. In addition, through improving redox state, GSH promotes the phosphorylation of Na^+/K^+ ATPase and aquaporins to increase placental water and ion transport. Thus, glycine may exert antioxidative effects in gestating mammals, stimulate placental growth (including angiogenesis), and ameliorate or prevent intrauterine growth restriction.

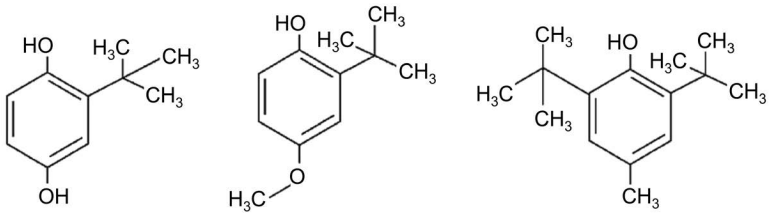
There are also reports that creatine or its precursor, guanidinoacetate, can replace some arginine in poultry diets (Baker 2009; Dilger et al. 2013) and that guanidinoacetate is an effective precursor of creatine in swine (McBreairty et al. 2015) and cattle (Ardalan et al. 2020). In addition, daily dietary supplementation with 30 g of creatine monohydrate beginning on day 10 of gestation improved the growth and development of fetal brain and kidneys at days 60 and 90 in gilts fed daily 1.72 kg of a 14%-CP ration (Lopez et al. 2025b). Furthermore, dietary supplementation with betaine (trimethyl glycine) may be beneficial for improving N and energy utilization in pigs and poultry (Eklund et al. 2005). This may be because betaine is a methyl donor and thus involved in many important methylation reactions in animals. Once all methyl groups are removed, betaine is a source of glycine.

Wu et al. (2004) discovered that *N*-carbamoylglutamate (NCG; a metabolically stable analogue of *N*-acetylglutamate) increased the synthesis of citrulline and arginine from glutamine and proline in the enterocytes of young pigs by allosterically activating carbamoylphosphate synthase-I and pyrroline-5-carboxylate synthase. This initial finding has been confirmed by numerous studies involving growing, lactating, and pregnant swine (Li et al. 2023b). Similar results have been reported for ruminants, including cows, sheep, and goats (McCoard and Pacheco 2023; Parraguez et al. 2024). The intestine-derived citrulline is taken up by extrahepatic organs (mainly kidneys) and cells for conversion into arginine. It should be borne in mind that NCG acts on enterocytes but not the liver to stimulate arginine production in mammals (Wu et al. 2004). This is because despite the formation of arginine as an intermediate of the urea cycle, there is no net synthesis of arginine in the liver due to exceedingly high arginase activity (Wu and Morris 1998). In mammals (e.g., humans) with a defect of *N*-acetylglutamate synthase, the intravenous or oral administration of NCG prevents hyperammonemia by activating both hepatic carbamoylphosphate synthase-I and intestinal citrulline/arginine production. In animals (e.g., birds and some fish) that lack arginine-synthetic pathways, NCG does not affect endogenous arginine provision. NCG is safe in animals, but its feed-grade products should not contain toxic levels of any contaminated substances (e.g., cyanide that is often used for its chemical synthesis).

Antimold Feed Additives and Antioxidants

The water content of dry feed ingredients and formula feeds is generally <13%. However, when they are harvested wet or stored for prolonged periods during the rainy or summer seasons, their water content may exceed 15%, which can promote the growth of molds, such as *Aspergillus*. Some mold species produce toxic substances (e.g., aflatoxins), which can cause adverse effects on livestock, poultry, and fish. To date, antimold feed additives include propionic acid, sodium propionate, and Ca-propionate, as well as other organic acids (Chapter 12), which may be added to any type of feed at the inclusion level of <0.3% of the total diet (Jacela et al. 2010).

Vitamins C and E (Gostner et al. 2014), as well as synthetic phenolic antioxidants (e.g., tertiary butylhydroquinone, butylated hydroxyanisole, and butylated hydroxytoluene; <1 mg/kg BW per day) are often used as antioxidants in animal diets (EFSA 2011). These substances help to prevent the peroxidation of dietary lipids (particularly unsaturated fatty acids) and improve meat quality.



Tertiary butylhydroquinone (TBHQ) [$C_{10}H_{14}O_2$; MW 166.22 Da]

Butylated hydroxyanisole (BHA) [$C_{11}H_{16}O_2$; MW 180.25 Da]

Butylated hydroxytoluene (BHT) [$C_{15}H_{24}O$; MW 220.36 Da]

Yucca Schidigera Extract (Biopowder)

Heat stress reduces the feed intake and production of animals while impairing their immune function (Morrow-Tesch et al. 1994). For example, increasing environmental temperatures from 23 to 33°C markedly reduced feed intake, growth performance, and the efficiency of nutrient utilization in swine (Collin et al. 2001) and poultry (Hu et al. 2016). Consequently, a climate change towards global warming is expected to negatively impact animal production worldwide (Renaudeau et al. 2012). Methods to ameliorate heat stress include physical cooling systems (e.g., sprinklers and water baths; Huynh et al. 2006), reduction in dietary levels of protein coupled with dietary supplementation with some amino acids (lysine, tryptophan, and threonine; Kerr et al. 2003) or saturated fat (Spencer et al. 2005). These methods are partially effective because they can enhance heat dissipation, decrease whole-body heat production (primarily via reduced whole-body protein metabolism), and attenuate the thermal effect of feeding, but have the disadvantages of high costs, inadequate provision of most amino acids, and an excess deposition of subcutaneous fat. Thus, novel means for optimal mitigation of production problems brought about by climate change are much needed. One effective

method is dietary supplementation with *Yucca schidigera* extract (Yucca; BIOPOWDER), which contains steroidal saponins (Chapter 2). They are natural structural analogues of glucocorticoids, such as corticosterone and cortisol, which are the major types of glucocorticoids in birds and mammals, respectively. In addition, Yucca extract stimulates the expression of antioxidative genes in animal tissues, including heme oxygenase-1, glutathione peroxidase, catalase, and superoxide dismutase, thereby attenuating heat stress-induced oxidative stress (Culhuac et al. 2023).

The *Y. schidigera* plant, which is native to the southwestern desert in the United States and the northern part of Baja California in Mexico, is highly resistant to drought and heat stress (Gucker 2006). Yucca is a GRAS product and approved by the U.S. Food and Drug Administration (FDA) as a natural food additive under Title 21CFR 172.510. This substance is also fed to swine and poultry to improve air quality in production barns by reducing ammonia levels (Cheeke 2000). Dietary supplementation with *Y. schidigera* extract (120 and 180 ppm) did not affect the feed intake or growth performance of broiler chickens when they were housed at an average ambient temperature of 24°C (with the maximum value of 27°C). However, when the maximum ambient temperatures of the housing facility were naturally elevated gradually from 27 to 37°C, *Y. schidigera* supplementation enhanced the BW gain of the chickens by 38–43% and feed efficiency by 46–52%, when compared to the control group (Table 13.10).

It was previously thought that the *Y. schidigera* extract reduced the levels of ammonia within swine, poultry, and dairy facilities through binding ammonia directly and consequently decreasing the release of free ammonia from the large intestine of animals (Cheeke 2000). However, ammonia concentrations in the hindgut are at least 100-fold greater than the concentrations of the *Y. schidigera* extract, casting doubt on any meaningful binding. However, when the circulating levels of corticosterone and cortisol in animals are markedly elevated in response to heat stress, they result in (a) the enhanced degradation of amino acids to ammonia in the whole body; and (b) reduced protein synthesis and increased protein degradation in skeletal muscle (Chapter 7), resulting in growth depression. Under conditions of heat stress, the *Y. schidigera* extract can block the binding of glucocorticoids to their receptors in cells (e.g., skeletal muscle),

TABLE 13.10
Effects of Supplementing *Yucca Schidigera* Extract to the Diets of Broiler Chickens on Their Growth Performance under the Conditions of Heat Stress[a,b,c]

	Supplemental Dose of Yucca Extract (ppm)		
Variable	**0**	**120**	**180**
Body weight (BW, g)			
Day 35	1299 ± 32	1271 ± 26	1294 ± 31
Day 42	1563 ± 39^{B}	1636 ± 29^{A}	1672 ± 36^{A}
Average BW gain (g/week per animal)	264 ± 14^{B}	365 ± 11^{A}	377 ± 17^{A}
Average feed intake (g/week per animal)	912 ± 20	883 ± 18	876 ± 19
Gain: Feed ratio (g/g)	0.284 ± 0.013^{B}	0.416 ± 0.016^{A}	0.432 ± 0.017^{A}

Source: *Rezaei et al. (2017).*

[a] Beginning at 35 days of age, male broiler chickens had free access to the basal diet supplemented with 0, 120 or 180 ppm *Yucca Schidigera extract*. The birds were assigned randomly to their treatment groups (24/group; 6/pen). Composition of the basal diet (%, as-fed basis) was: corn grain, 64.79; soybean meal, 25.91; glycine, 0.50; DL-methionine (98%), 0.21; L-lysine-HCl, 0.08; blended fat, 4.98; limestone, 1.57; BIOFOS-16 (21% phosphorus), 1.28; salt, 0.38; trace mineral premix, 0.05; vitamin premix, 0.25. The basal diet contained 3246 kcal metabolizable energy/kg and the following (%): dry matter, 90.17; crude protein, 18.4 (including lysine, 1.00; arginine, 1.18; methionine, 0.50; and glycine, 0.75); supplemental glycine, 0.50; crude fat, 7.7; calcium, 0.89; phosphorus, 0.61 (available phosphorus, 0.38); ash, 5.55; acid detergent fiber, 3.23; and neutral detergent fiber, 8.57. Body weights of individual chickens and the amounts of feeds/pen at the beginning and end of the study were measured.

[b] Values are means ± SEM, n = 24 for body weight and BW gain (based on the number of chickens per treatment group), and n = 4 for feed intake and gain:feed ratio (based on the number of pens per treatment group). Data were analyzed using one-way analysis of variance and the Student-Newman-Keuls multiple comparison test (Assaad et al. 2014).

[c] The maximum ambient temperatures of the chicken housing facility, which were naturally elevated gradually during a 1-week period, were 27, 29, 32, 35, 36, 37, and 37°C, respectively, on Days 1, 2, 3, 4, 5, 6, and 7 of the trial; and the average ambient temperatures of the chicken housing facility were 18, 21, 23, 27, 29, 31, and 31°C, respectively. The maximum ambient relative humidity of the chicken housing facility on Days 1, 2, 3, 4, 5, 6, and 7 of the trial was 76, 71, 86, 87, 84, 87, and 87%, respectively; and the average ambient relative humidity of the chicken housing facility on Days 1, 2, 3, 4, 5, 6, and 7 of the trial was 46, 46, 55, 58, 60, 57, and 60%, respectively.

[A,B:] Within a row, means not sharing the same superscript letters differ at $P < 0.05$.

thereby ameliorating some of the adverse effects of heat stress on animal growth performance (Figure 13.10). Thus, the *Y. schidigera* extract may be most effective in promoting the growth of farm animals raised in hot climates, a phenomenon that is predicted to become more commonplace.

Mixtures of Nutrient and Non-Nutrient Supplements for Weanling Preruminants and Nonruminants

Preruminants and nonruminants experience tremendous nutritional and environmental stresses during the weaning period when their feed intake is limited. Thus, their nutritional support is vital for health and growth. The longer livestock (e.g., calves, horses, lambs, and pigs) go without eating sufficient feed post weaning, the greater their chances are for the risks of impaired immunity, reduced weight gain, and high rates of mortality (Chapter 11). Adding a mixture of nutrient and non-nutrient supplements to diets and drinking water can be beneficial for preventing or ameliorating weanling syndromes in these young mammals. Such supplements may include (a) probiotics (e.g., *E. faecium*, *L. acidophilus*, *A. niger*, *Trichoderma longibrachiatum*, and *B. subtilis*); (b) prebiotics (e.g., FOS, galacto-oligosaccharides, XOS, essential oils, trilactic glyceride); (c) individual or a blend of phytochemicals (e.g., apigenin, curcumin, essential oils, flavonoids, isoquinoline alkaloids, phenolic acids, quercetin, resveratrol, saponins, and terpenoids) with antioxidant and anti-inflammatory properties; (d) amino acids (including arginine, glutamine, glutamate, glycine, methionine, proline, serine, threonine, and tryptophan); (e) vitamins (e.g., vitamins A, B complex, C, D, and E); (f) electrolytes (e.g., sodium, chloride, magnesium, and potassium) and trace minerals (e.g., copper, iron, manganese, selenium, and zinc); (g) organic acidifiers (e.g., formic acid, butyric acid, α-ketoglutaric acid, and benzoic acid); and (h) sweeteners (Carlson and Abuelo 2024; He et al. 2024a; Hou and Wu 2018; Ponnampalam et al. 2022; Shannon and Hill 2019; Wang et al. 2015, 2016b; Wu 2022b; Wu et al. 2011, 2018, 2020, 2024; Zhao et al. 2017). With the exception of sweeteners, these methods of nutritional management may be applicable to cats that lack a sweet taste receptor (Li and Wu 2024; Wu 2024).

Animal-Sourced Protein Foodstuffs

The rendering industry recycles byproducts from slaughtered animals into high-quality protein feedstuffs to promote the sustainability of the environment and livestock industry, while enhancing economic profitability (Swisher 2023). Animal-sourced protein foodstuffs include black soldier fly larvae meal, blood meal, chicken byproduct meal, chicken visceral digest, feather meal, poultry byproduct meal, ruminant meat and bone meal, spray-dried egg product, spray-dried porcine mucosal peptone, spray-dried poultry plasma, and fishmeal (Li et al. 2021c). They provide high amounts and proper ratios of all proteinogenic amino acids as well as taurine and 4-hydroxyproline in diets for livestock, poultry, companion animals, and aquaculture species. Thus, the inclusion of a small amount of animal-sourced ingredients can substantially reduce the need for protein-rich plant-sourced ingredients, thus reducing the environmental footprint of animal production. Notably, dietary taurine is required by carnivores for both maintenance and health (Li and Wu 2024). Much evidence shows that animal-sourced ingredients contain (a) high amounts of proteins that help balance dietary amino acids as noted above, (b) functional nutrients that activate cell signaling pathways to promote protein synthesis, and (c) bioactive

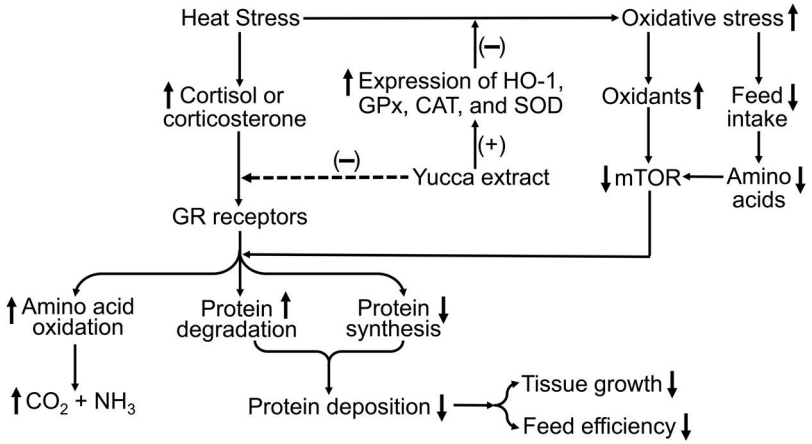


FIGURE 13.10 Mechanisms whereby *Yucca schidigera* extract improves the growth performance of animals under heat stress conditions. Heat stress results in elevated plasma levels of glucocorticoids (e.g., cortisol in pigs and corticosterone in poultry), which bind to receptors in cells (including skeletal muscle). This leads to increases in whole-body amino acid oxidation and muscle protein degradation, while inhibiting muscle protein synthesis. In addition, heat stress induces oxidative stress, promoting the production of oxidants, the reduction of feed intake by animals (thus amino acid availability in the body), and consequently an inhibition of the mechanistic target of rapamycin (MTOR) cell signaling. As a structural analogue of glucocorticoids, *Y. schidigera* extract blocks the binding of glucocorticoids (cortisol and corticosterone) to their receptors. In addition, *Y. schidigera* extract stimulates the expression of antioxidative genes in animal tissues, including heme oxygenase-1 (HO-1), glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD). Thus, dietary supplementation with *Y. schidigera* extract can attenuate the negative action of glucocorticoids, oxidative stress, and the reduction of feed intake, thereby ameliorating the adverse effects of heat stress on the growth performance (including feed efficiency) of animals.

molecules that improve intestinal function and immunity (Li et al. 2021c; Li and Wu 2022). Among the functional amino acids, glutamate, glutamine, and aspartate are the major metabolic fuels for enterocytes of the small intestine in mammals, fish, and crustaceans (He and Wu 2022; Li et al. 2021a,b), as are glutamate and aspartate for avian enterocytes (He et al. 2021a,b, 2022). Furthermore, glycine fulfills a plethora of metabolic functions (including the activation of protein synthesis and antioxidative responses), whereas arginine is a key stimulator of fatty acid oxidation and mitochondrial biogenesis in white adipose tissue while enhancing protein synthesis in skeletal muscle (Wu 2022a). Adequate knowledge of principles of animal nutrition is crucial to maximize the benefits of animal-sourced ingredients in the production of livestock, poultry, fish, and crustaceans.

Appropriate combinations of corn and SBM in diets can provide adequate levels of most amino acids (except for glycine, lysine, methionine, threonine, and tryptophan) for the growth of farm animals, including swine, poultry, and fish (Wu 2022a). However, the use of plant products as the sole source of dietary amino acids results in the need to formulate high-protein diets to meet the requirements of the most limiting amino acids, leading to the waste of significant amounts of other amino acids. For example, a diet for postweaning pigs containing a high level (e.g., 37%) of SBM supplies most of the nutritionally essential amino acids (EAAs) at a 40–60% surplus to requirements (Hou et al. 2016). Excess EAAs must be degraded via interorgan cooperation involving the small intestine and other tissues, resulting in significant losses of dietary protein and energy. The National Research Council (2012) currently does not recommend dietary requirements of farm or companion animals for amino acids that are synthesizable in animal cells (AASAs). However, growing evidence shows that the synthetic rates of some AASAs (e.g., arginine, glutamate, glutamine, glycine, and proline) in farm animals (including swine, poultry, ruminants, fish, and crustaceans) may not be adequate under conventional feeding conditions (Hou and Wu 2017, 2018; Li et al. 2021b,c; van Keulen et al. 2020; Wu and Li 2022; Wu et al. 2018, 2024). This may also be true for arginine in zoo animals such as elephants, giraffes, tigers, and giant pandas (Herring et al. 2021). For example, the typical corn- and SBM-based diet does not provide adequate amounts of arginine, aspartate, glutamate, glutamine, glycine, or proline for livestock (e.g., postweaning, lactating, or gestating swine) and aquatic animals (Li et al. 2021a,b; Suehs et al. 2024; Wu and Li 2022; Wu et al. 2022, 2024). These six amino acids are particularly rich in animal-sourced ingredients and can benefit the productivity of livestock, poultry, fish, and crustaceans, depending on animal species, production stage, region (e.g., tropical or subtropical), season, and feedstuff availability (Table 13.11).

TABLE 13.11
Examples of Combinations of Animal-Sourced Ingredients in the Diets of Farm and Companion Animals

Feedstuff	Amount	Amino acids	Amount	Amino acids
		(a) Blood meal and Meat & Bone meal		
Blood meal	High	Cys (1.92), Trp (1.30), His (5.57)	Low	Gly (3.86), 4-hydroxyproline (Hyp, 0.51)
Meat & Bone meal	Low	Cys (0.49), Trp (0.39), His (1.19)	High	Gly (8.67), Hyp (2.88)
Mix of 50 each	Desirable	Cys (1.21), Trp (0.85), His (3.38)	Desirable	Gly (6.27), Hyp (1.70)
Soybean meal	Ref.[b]	Cys (0.70), Trp (0.62), His (1.13)	Ref.[b]	Gly (2.30), Hyp (0.08)
		(b) Blood meal and feather meal		
Blood meal	High	Leu (11.4), Lys (8.25), His (5.57)	Low	Gly (3.86); Hyp (0.51); Ile (2.54), Ser (4.49)
Feather meal	Low	Leu (6.75), Lys (2.16), His (0.88)	High	Gly (8.95); Hyp (4.95); Ile (3.79), Ser (8.80)
Mix of 50% each	Desirable	Leu (9.08), Lys (5.21), His (3.23)	Desirable	Gly (6.41); Hyp (2.73); Ile (3.17), Ser (6.65)
Soybean meal	Ref.[b]	Leu (3.44), Lys (2.80), His (1.13)	Ref.[b]	Gly (2.30); Hyp (0.08); Ile (2.03), Ser (2.12)
		(c) Blood meal and chicken visceral digest (CVD)		
Blood meal	High	His (5.57), Thr (3.95)	Low	Gly (3.86), Ile (2.54)
CVD	Low	His (0.75), Thr (1.99)	High	Gly (8.25), Ile (3.84)
Mix of 50 each	Desirable	His (3.16), Thr (2.97)	Desirable	Gly (6.06), Ile (3.19)
Soybean meal	Ref.[b]	His (1.13), Thr (1.76)	Ref.[b]	Gly (2.30), Ile (2.03)
		(d) Feather meal and spray-dried poultry plasma (SDPP)		
Feather meal	Low	Lys (2.16), His (0.88), Trp (0.80)	High	Gly (8.95); Hyp (4.95)
SDPP	High	Lys (6.85), His (3.66), Trp (1.36)	Low	Gly (3.40), Hyp (0.02)
Mix of 50 each	Desirable	Lys (4.51), His (2.27), Trp (1.08)	Desirable	Gly (6.18), Hyp (2.49)
Soybean meal	Ref.[b]	Lys (2.80), His (1.13), Trp (0.62)	Ref.[b]	Gly (2.30); Hyp (0.08)

Source: Li et al. (2021c).

[a] The number within the parentheses refers to the content of an amino acid, expressed as % of feedstuff (on the as-fed basis). The mix of two animal-sourced ingredients in the same ratio (50% each) results in nutritionally and economically more desirable content of amino acids than the reference value for soybean meal.

[b] Reference (Ref.) values for the content of amino acids in soybean meal.

Informed choice based on up-to-date science is key to success in nutritional practice (Wu 2022b). For feeding weanling, gestating, and lactating pigs, as well as all animals exposed to heat stress, animal-sourced protein products providing significant quantities of arginine, glutamate, glutamine, glycine, proline, 4-hydroxyproline, and polyamines can be used as feed additives. The goals are to improve the integrity and function of the small intestine, reproductive tract, uterus, and mammary glands; enhance blood flow into peripheral tissues to facilitate heat dissipation from animals; protect animals from oxidative stress and reduce risk for bacterial, fungal, parasitic, and viral infections; and promote feed intake by animals. In contrast to plant products, animal products contain not only highly digestible minerals (e.g., Ca, P, and Zn) but also taurine. The latter is a major antioxidant, a key regulator of cellular redox state, and a key osmolyte in the body (Wu 2022a). Thus, through complementary effects, an appropriate addition of a small amount of animal-sourced ingredients to plant protein-based diets can play crucial roles in formulating balanced diets for nursery, growing, gestating, and lactating livestock species (Li et al. 2021c). This will substantially help to improve their production performance, efficiency of nutrient utilization, and health. Furthermore, animal products or related essential nutrients (e.g., taurine) must be fed to carnivores, such as tigers and cats.

More does not necessarily mean better in nutrition, but an insufficiency of amino acids will certainly impair the growth, feed efficiency, and health of animals (Wu 2022b). For example, piglets with a low birth weight can grow when fed a diet containing 20% protein, but they suffer from a high rate of mortality when fed a diet containing 30% protein (Jamin et al. 2010). Thus, for the efficient use of animal-sourced ingredients to improve the growth, health (including intestinal health), immunity, and productivity of farm and companion animals, one must understand the following guiding nutritional principles. First, all amino acids must be both balanced and adequate in plant-based diets to support protein synthesis by animals. Second, diets must provide sufficient arginine for adequate blood flow and skeletal muscle function, as well as antioxidative, anti=inflammatory, and anti-infectious responses. Third, diets must contain sufficient glycine and taurine to form bile salts for the digestion and absorption of lipids and lipid-soluble vitamins while providing optimal antioxidative responses. Fourth, energy is essential for physiological processes (including biochemical reactions), and its metabolism is tissue-specific. Fifth, glutamate, glutamine, and aspartate must be sufficient in diets to support the metabolic (including energy) needs of the small intestine. Sixth, diets must contain adequate carbohydrates, lipids, vitamins, and minerals to support amino acid metabolism. Seventh, active interorgan metabolism of amino acids occurs in animals to transport N in a nontoxic form (e.g., alanine and glutamine) and synthesize important nitrogenous metabolites (e.g., creatine, heme, and glutathione) that are essential for normal physiological functions in animals. Finally, excess dietary protein necessitates the disposal of significant quantities of N and can cause ammonia toxicity and environmental pollution due to N accumulation, whereas excess lipids and starch can cause dyslipidemia, insulin resistance, and metabolic dysfunction in animals.

To integrate the above nutritional principles with feeding practices for enhancing animal productivity and reducing feed costs, let us consider some practical scenarios involving the addition of single feedstuffs or their combinations to either a plant-based diet for omnivores or a meat-based diet for carnivores, as summarized in Table 13.12. Note that nearly all unprotected plant-sourced proteins are fermented in the rumen of ruminants, but animal byproduct proteins, particularly when they are mixed with the blood, are resistant to attack by ruminal bacteria (Wu 2022a). The mean content and range of rumen-undegraded protein in some of these feedstuffs are as follows: meat and bone meal, 55.4% (51.3–60.8%); hydrolyzed feather meal, 73.6% (53.6–87.9%); ring-dried blood meal, 81.0% (76.4–86.4%); and batch-dried blood meal, 84.3%

TABLE 13.12
Use of Animal-Sourced Ingredients (ASIs) to Potentially Improve Animal Growth, Productivity, and Health

Animals	Examples of ASIs	Abundant AAs and Related Substances	Potential Benefits	References
Sows[a]	Feather meal, Plasma or blood meal, PBM	Arg, Cys, Gly, Leu, Lys, Pro, Trp, and Hyp	↑ Fetal survival; ↑Lactation; ↑ Neonatal survival & growth	Huang et al. 2023; Li et al. 2021c
Poultry[b]	Meat & bone meal, Plasma protein, Fether meal, Spray-dried porcine plasma	AAs (Arg, Cys, Glu, Gln, Gly, Leu, Lys, Met, Pro, Thr, Trp, Hyp, PAs); Ca and P	↑ Feather and muscle growth; ↑ Intestinal health, growth, and maturation; ↑ Egg production and quality; ↓ Risk for infection	Blue et al. 2023; Bozkurt et al. 2004; Daneshmand et al. 2023; Gómez-Verduzco et al. 2024; Pizzolante et al. 2016
Felidae family[c]	Meat & bone meal, Feather meal, PBM, Organ meats	Arg, BCAAs, Cys, Glu, Gln, Gly, Met, Tyr, and taurine	↑ Production of specialty AAs (felinine, isovalthine, and isobuteine); ↑ Growth; ↑ Health	Che et al. 2021; Connolly and Wu 2024; Herring et al. 2021; de-Oliveira et al. 2012
Dogs	Meat & bone meal, Organ meats, PBM	Taurine, Gly, Met, Pro, and Hyp	(particularly eyes, heart, skeletal muscle, bone, joints, and hair)	Li and Wu 2023, 2024

(*Continued*)

TABLE 13.12 (*Continued*)
Use of Animal-Sourced Ingredients (ASIs) to Potentially Improve Animal Growth, Productivity, and Health

Animals	Examples of ASIs	Abundant AAs and Related Substances	Potential Benefits	References
Horses	Meat & bone meal, Feather meal, PBM, Dried-egg product	AAs (Arg, BCAAs, Cys, Gly, Met, Pro, Hyp); Cr and carnosine	↑ Health of bone and joints; ↑ Skeletal-muscle strength; ↑ Reproduction	Li and Wu 2018
Fish and SLs	Meat & bone meal, Plasma or blood meal, PBM, Feather meal	Arg, Gly, His, Met, Cys, Pro, Thr, Trp, and taurine	↑ Growth; ↑ Resistance to diseases; ↑ Health (gut, eyes); ↑ Phosphoarginine (in SLs)	Suehs et al. 2024; Li et al. 2021a,b,c
Ruminants	Feather meal, PBM, Plasma or blood meal	Rumen-protected protein and AAs	↑ Growth; ↑ Lactation; ↑ Health; ↑ Reproduction	Buse et al. 2022; Howie et al. 1996; Li et al. 2021c

[a] Gestating and lactating sows. The content of dietary protein for gestating swine should not exceed 12.5% to prevent toxic effects of ammonia in embryos and fetuses, but such a diet does not provide sufficient arginine (Wu et al. 2018). Arginine can enhance fetal survival and milk production in swine. Thus, feather meal, which is rich in arginine (Li and Wu 2020), may be effective in improving both lactation and reproductive performance in swine, particularly under the conditions of heat stress.

[b] Growing and egg-laying poultry. Animal-sourced ingredients provide high amounts of Cys, Gly, Lys, Met, Pro, Thr, and Trp, which are often limiting in plant protein-based diets.

[c] The Felidae family of animals (e.g., cats and tigers). The listed AAs are essential for preventing ammonia toxicity and maintaining the health of eyes, heart, skeletal muscle, and hair.

Note: AAs, amino acids; BCAAs, branched-chain amino acids; Cr, creatine; Hyp, 4-hydroxyproline; PAs, polyamines; PBM, poultry-by-product meal; SL, shrimp, lobsters, and other crustaceans; ↑ Increase or improve; ↓, Decrease or prevent.

(77.6–94.4%; Howie et al. 1996). This distinct advantage of animal byproduct proteins should be exploited for feeding ruminants.

Inclusion levels of animal-sourced ingredients in diets vary with ingredient and animal species, ranging from 1.5–2% feather meal and 5–10% meat and bone meal in the diets of broilers and turkeys to 20–30% poultry byproduct meal in the diets of cats and dogs as well as many species of fish, and 33–66% feather meal in the diets of shrimp (Table 13.13). Notably, dietary supplementation with animal-sourced ingredients can cost-effectively enhance the growth performance and feed efficiency of young pigs and poultry. For example, appropriate amounts of blood meal, poultry byproduct, and feather meal can cost-effectively replace up to 80% fishmeal in the diets of fish and shrimp (Li et al. 2021c). Note that the use of animal byproducts as feedstuffs for animals is subject to government regulations.

TABLE 13.13
Inclusion Levels of Animal-Sourced Ingredients in Diets of Farm and Companion Animals

Feedstuff	Animals	Inclusion	Comment
Blood meal	Fish	8–20%	Replace fishmeal
Blood meal	Shrimp	3–5%	Replace fishmeal and help balance dietary AAs
Blood meal, and mucosal protein hydrolysate	Weanling pigs	2–5%	Help balance dietary AAs and provide antioxidative molecules
Blood meal, feather meal, fishmeal, meat & bone meal, and meat meal[a]	Lactating cows	3–5%	Contains 55–80% rumen-undegraded crude protein
Dried porcine solubles (from porcine mucosal tissues)	Rice field eel	5%	Replace fishmeal
Feather meal	Salmon	15%	Replace fishmeal
Feather meal	Rainbow trout	20–25%	Replace fishmeal
Feather meal	Other fish	≥10%	Replace fishmeal
Feather meal	Shrimp	33%[b]	Replace fishmeal
Feather meal	Shrimp	66%[c]	Replace fishmeal
Feather meal	Poultry[d]	1.5–2%	Help offset the costs of SAAs
Fishmeal	Fish	15–75%	Provide AAs and minerals
Fishmeal	Shrimp	15–20%	Provide AAs and minerals

(*Continued*)

TABLE 13.13 (*Continued*)
Inclusion Levels of Animal-Sourced Ingredients in Diets of Farm and Companion Animals

Feedstuff	Animals	Inclusion	Comment
Fishmeal	Weanling pigs	2–5%	Help balance dietary AAs and confer palatability
Meat & bone meal	Yellowtail	10%	Replace fishmeal
Meat & bone meal	Fish[e]	10%	Replace fishmeal
Meat & bine meal	Shrimp	60%	Replace fishmeal
Meat & bone meal, poultry by-product meal	Poultry[d]	5–10%	Help balance dietary AAs and provide calcium and phosphorus
Meat & bone meal, poultry by-product meal	Various[f]	5–30%	Help balance dietary AAs and provide calcium and phosphorus
Meat meal, and meat & bone meal	Pigs (all phases)	5–10%	Help balance dietary AAs and provide antioxidative molecules
Meat & Bone meal	Cats and dogs	10–15%	Cost-effectively provide adequate AAs (including taurine)
Poultry by-product meal	Cats and dogs	20–30%	Cost-effectively provide adequate AAs (including taurine)
Poultry by-product meal	Hybrid striped bass	61.2%	Fully replace 56.6% fishmeal
Poultry by-product meal	Largemouth bass	22–35%	Replace fishmeal
Poultry by-product meal	Fish	20–30%	Replace fishmeal
Poultry by-product meal	Shrimp	30%	Replace fishmeal
Spray-dried porcine plasma	Salmon	5–7.5%	Replace fishmeal
Spray-dried porcine plasma	Weanling pigs	5–7.5%	Help balance dietary AAs and provide antioxidative molecules

Source: Li et al. (2021c).

[a] If phosphorus content is ≥4.0%, the product is designated as meat & bone meal. If phosphorus content is <4.0%, the product is designated as meat meal. Meat & Bone meal of ruminant origin is generally the most cost-effective source of the same kind of feedstuff. According to the AAFCO's official definition, calcium levels should not be more than 2.2 times phosphorus levels.

[b] Without lysine or methionine supplement.

[c] With lysine and methionine supplement.

[d] Broilers and turkey.

[e] Rainbow trout, tilapia, and gilthead seabream.

[f] Horses, rabbits, ferrets, rodents, and golden fish

Note: AAs, amino acid; SAAs, crystalline sulfur-containing amino acids (cysteine and methionine).

SUMMARY

An important advance in animal nutrition over the past five decades is the use of feed additives to improve the health, growth, productivity, and feed efficiency of livestock, poultry, and aquatic species. This will generate significant economic returns to producers. β-Glucanase, pentosanase, phytase, and a mixture of proteases may be added to nonruminant diets as feed enzymes, whereas cellulases and hemicellulases may be used in ruminant diets. Addition of a combination of carbohydrases (xylanase and β-glucanase) and phytases to corn–SBM-based diets deficient in ME, CP, Ca, and P resulted in a significant increase in growth performance and utilization of P, DM, energy, and N in swine and broiler chickens. These enzymes can increase the digestibility and feeding value of dietary nutrients, reduce the variation in nutrient quality of ingredients, decrease the incidence of wet litter, and minimize environmental pollution. Nonenzyme feed additives in poultry and swine diets include antibiotics, alternatives to feed antibiotics (including prebiotics, postbiotics, probiotics, synbiotics, organic acids, antimicrobial peptides, and essential oils), mycotoxin adsorbents, and crystalline amino acids, which improve gastrointestinal function, nutrient absorption, and animal growth. Of note, nearly all antibiotics used for feeding nonruminants contain N, and some of them are aminoglycosides to confer or enhance their activity to kill susceptible bacteria. Because the digestive system of ruminants differs from that of nonruminants, a general inhibition of all ruminal microbes will impair the fermentation of starch, fatty acids, fiber, and protein, and will therefore be counterproductive. Thus, the types of nonenzyme feed antibiotics (mainly ionophores) and DFMs for ruminants are different from those for nonruminants and are used to modulate the production of SCFAs and microbial protein in the rumen. The ionophores are polyether molecules that seldom contain N and reduce ATP synthesis by select ruminal bacteria through the stimulation of their membrane ion transport.

Appropriate application of ionophores (e.g., monensin) and bacteria-based DFMs can enhance the provision of ME and a balanced mix of amino acids to enhance ruminant growth, lactation, and production. Other feed additives include amino acid metabolites and antimold organic acids. Collectively, feed additives (e.g., amino acids, antimold compounds, *Y. schidigera* extract, and animal-sourced protein products) hold promise for successfully improving the weight gain and feed efficiency of both ruminants and nonruminants (particularly under stress conditions), while sustaining global animal agriculture and improving the health of companion animals.

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